Section A7.1.3-01 **Annex Point IIA7.7**		**Adsorption / desorption screening test**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Reynolds J.L. (2001): Adsorption and Desorption of (^{14}C)-RH-893 in Three Soils and One Sediment. XenoBiotic Laboratories. Unpublished report,▬ ▬▬▬▬	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Thor GmbH Rohm and Haas Company	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; OECD guideline 106 (1989) and OPPTS guideline 835.1220. However, the study design follows essentially the OPPTS guideline with regard to terminology and study conduct.	
2.2	**GLP**	Yes	
2.3	**Deviations**	No (with regard to OPTTS guideline)	
		3 MATERIALS AND METHODS	
3.1	**Test material (radiolabelled)**	RH-24893 (which is identical to OIT) labelled with (^{14}C) ▬▬▬ ▬▬▬▬▬▬ . In the test report, the last three digits of the RH-number are commonly used as an abbreviation for the test substance, i.e. RH-24893 = RH-893. However, in this summary document the denomination OIT is used to be consistent within the dossier submitted.	
3.1.1	Lot/Batch number	▬▬▬	
3.1.2	Specification	 • denotes position of [^{14}C]-radiolabel	
3.1.3	Purity	Assigned purity from the provider Rohm and Haas was ▬▬ . Re-analysis at the performing laboratory at various study stages revealed radiopurities of ▬▬▬▬	
3.1.4	Further relevant properties	▬▬▬▬▬▬▬▬▬▬▬▬	
3.1.5	Method of analysis	HPLC with UV and radioactivity detector for the test substance; biological sample oxidiser and liquid scintillation counter for combustion and radioanalysis	
3.2	**Test material (non**	RH-24893, common name: isothiazolone (2-n-octyl-4-isothiazolin-3-	
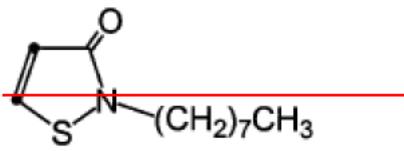
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	radiolabelled)	one) serving as reference chemical in HPLC. RH-24893 is structurally identical to substance OIT	
3.2.1	Lot/Batch number	▬▬▬▬▬	
3.2.2	Specification	Empirical formula: $C_{11}H_{19}NOS$	
3.2.3	Purity	▬▬▬▬	
3.2.4	Further relevant properties	Not given	
3.3	**Degradation products**	No degradation products tested	
3.3.1	Method of analysis for degradation products	Not applicable	
3.4	**Soil types**	See Table A7.1.3-1. Before use, all soils were sieved through a standard 2 mm mesh screen.	X
3.5	**Testing procedure**		
3.5.1	Test system	The test was performed in 30 or 50 mL Teflon centrifuge tubes with screw caps as sample containers filled with the desired amounts of soil and dosed 0.01 M $CaCl_2$ solution (there were also some soiless as well as untreated control samples). The samples were either placed in radial or wrist action shakers at ambient temperature and shaken continuously for a certain time period (up to 48 h, depending on the aim of the sub-test, see 3.5.2). All soil-containing tubes were centrifuged at the respective sampling interval, the supernatant being submitted to HPLC analysis and LSC, the residual soil extracted and combusted (for mass balance purposes at the highest test concentration) and radioanalysed. Where needed, samples were weighed and the volume of supernatants was determined.	
3.5.2	Test solution and Test conditions	The water used for the study was CO_2 - free boiled deionised water processed through a water purification system. The 0.01 M $CaCl_2$ solution was prepared by adding 4.44 g of $CaCl_2$ into 4 L of CO_2 - free boiled deionised water. Different treatment solutions were prepared at the various stages of the study. In principle, in order to obtain the stock solution a certain amount of the test chemical was dissolved in methanol and usually diluted with unlabelled test substance in a 1:1 solution of methanol/water. The nominal specific activity was determined. The stock solution was then dosed to the 0.01 M $CaCl_2$ solution in order to achieve the desired final test concentration. The amount of cosolvent methanol employed in the test system accounted for less than 1% by volume in all cases. Sterilised soils were used throughout the study since it was found in a pre-test that the test substance was almost completely degraded in air-dried soil/$CaCl_2$ solution. There were three tiers of testing: a) the *preliminary test* was conducted to assure that the selected analytical method was adequate to quantify the test chemical in the system (one soil, one concentration: ~ 5 ppm) b) the *screening test* was used to prove the adsorption/desorption potential of the test chemical and the effect of 0.01 M $CaCl_2$ on	

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	adsorption/desorption (all 4 soils, one concentration: ~ 5 ppm)
	
	c) the *advanced test* consisted of two parts:
	    - in the *soil kinetics* part, the appropriate soil-to-solution ratio was determined and also the time to achieve equilibrium partitioning of the test chemical between the liquid and solid phases (all soils, one concentration: ~ 5 ppm)
	    - in the *Freundlich isotherm* part, the adsorption of the test chemical was examined as to its concentration dependency (all soils, 4 concentrations: 0.04, 0.2, 1 and 5 ppm)	

3.6 **Test performance**		
3.6.1 Preliminary test	The silt loam soil was equilibrated with the aqueous phase in duplicates in a soil-to-solution ratio of 1:5 (5 g dry soil/25 mL 0.01 M $CaCl_2$), shaken continuously for 16 hours, and then centrifuged for 10 min at 2500 rpm. The supernatants were removed, their volume measured and dosed with the stock solution to achieve a concentration of ca 5 ppm. After vortexing, the dosed $CaCl_2$ solutions were removed for LSC or directly analysed by HPLC.	
3.6.2 Screening test: Adsorption and desorption	Teflon centrifuge tubes were prepared in duplicates each containing 5 g soil in 25 mL of 0.01 M $CaCl_2$ (1:5 soil-to-solution ratio) except for the sandy loam soil which was prepared with 5 g soil in 10 mL aqueous phase in order to meet the > 25% sorption criteria (1:2 ratio). Tubes were dosed with stock solution to provide a test concentration of about 5 ppm. In addition, duplicate soiless controls, containing 5 ppm of test chemical only and also two untreated soil-solution control tubes per soil for background radioactivity were set up. All tubes were shaken for 16 h at ambient temperature (adsorption step), thereafter centrifuged at 2500 rpm for 10 min. Supernatants were removed and duplicate aliquots analysed by LSC. An equivalent volume of untreated 0.01 M $CaCl_2$ solution was added to all tubes, which were again shaken for 16 hours and centrifuged etc. as above (1st desorption). This procedure was repeated once (2nd desorption).	
3.6.3 Advanced test (soil kinetics)	Several soil-to-solution ratios (1:5, 1:10, 1:100, i.e., 5g soil:25mL $CaCl_2$, 2.5g soil:25mL $CaCl_2$, 0.3g soil:30mL $CaCl_2$) were prepared in duplicates in Teflon tubes (30 or 50mL capacity) for all soils, except for the sandy loam soil which had ratios of 1:1, 1:2 and 1:5, i.e., 10g soil:10mL $CaCl_2$, 5g soil:10mL $CaCl_2$ and 5g soil:25mL $CaCl_2$, respectively. All tubes were dosed at ca 5 ppm with a stock solution and shaken continuously for 48 hours at ambient temperature. All tubes were sampled at 0, 4, 8, 24 and 48 hours (i.e.: centrifugation, removal of aliquots from supernatant for LSC analysis and returning the tubes to the shaker). At the end of the shaking period, the supernatants were analysed by LSC again and, in addition, also chromatographically.	
3.6.4 Advanced test (Freundlich adsorption isotherms)	A soil-to-solution ratio of 1:5 (5g soil:25mL $CaCl_2$) was used for all soils except for the sandy loam soil for which a 1:1 ratio (10g soil:10mL $CaCl_2$) was taken. A total of 10 Teflon tubes for each soil type were prepared, i.e., two tubes per treatment rate (0.04, 0.2, 1 and 5 ppm) and two untreated soil-solution controls. In addition, duplicate blank tubes with only the treatment solution (soiless control) for each dose rate were prepared. The tubes were shaken continuously at ambient temperature for their respective equilibration time (24 or 48 hours). After shaking, the tubes were centrifuged (10 min, 2500 rpm) and duplicate aliquots were taken for LSC analysis. For mass balance purposes, the high dose rate tubes (5 ppm) of all soils	

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		were subjected to extraction of the solids after decantation of the supernatant. Methanol was used repeatedly to extract retained radioactivity. The extracts were combined and analysed via LSC and HPLC. Subsamples of the residual soil residues were air-dried and then analysed by combustion after mixing with ca 30 mg cellulose in a biological sample oxidiser.	
3.6.5	Chromatographic analysis	Reversed-phase HPLC was used to analyse the supernatant layers for the presence and quantity of the test chemical. Characterisation was performed either by co-chromatography with unlabelled reference substance or by comparison with the retention time of the standard. In addition, the methanol extracts from the solids from the isotherm determination phase were examined the same way. Samples were monitored by collecting fractions (0.5 min per fraction) followed by counting with LSC. HPLC eluates (fractions) from each sample analysis were mixed with a cocktail, counted by LSC and a chromatogram was then reconstructed for recovery and area percent quantitation (a technique which is referred to as 'reconstructed' HPLC chromatogram).	

Two HPLC systems were used. They consisted of a ▮▮▮▮▮▮▮ ▮▮▮▮▮▮▮▮▮, equipped either with a photodiode array detector and a flow-through radioactivity cell or with a UV 254 nm absorbance detector plus the flow-through cell. The reversed phase column had the dimensions ▮▮▮▮▮▮ and the mobile phase was a gradient composed of water and methanol run with a flow rate of either ▮▮▮▮▮▮.

The stability of the test substance under the given test conditions was examined by HPLC analyses of the $CaCl_2$ solutions from the soil kinetics test in all four soil types treated at 5 ppm following 48 hours of shaking. Also the methanol extracts from the solids were analysed as to their (unchanged) test substance presence.

3.6.6	Calculations	The following equations were used to describe the sorption phenomena of the investigated soils:

Freundlich adsorption isotherm:

$Log\ X/m = Log\ K + 1/n * Log\ C_e$,

Where:

X = amount of test chemical adsorbed to the solid phase (μg), m = dry weight of soil (g), C_e = equilibrium concentration of test chemical remaining in solution after the adsorption step. K = Freundlich adsorption constant (calculated from the y-intercept $Log\ K$ at $C_e = 1$ $\mu g/mL$), $1/n$ = slope of the straightline. All adsorption data were fitted using linear regression analysis (a plot of $Log\ X/m$ versus $Log\ C_e$).

The **adsorption constant K_{OC}** (based on the organic carbon content of each soil) is then obtained by:

$K_{OC} = 100 * K / (\%organic\ carbon)$,

Where: % organic carbon = (% organic matter) / 1.72

		4 RESULTS	
4.1	**Preliminary test**	The applicability of the analytical HPLC method chosen was ensured. On an average, greater than 97% of the radioactivity injected was detected in the OIT region of interest (% ROI).	
4.2	**Screening test: Adsorption/**	Adsorption and desorption data from the screening test are presented in Table A7.1.3-2.	

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	Desorption		
4.3	**Definitive study**		
4.3.1	Advanced test: soil kinetics	According to the guidelines the sorption level should preferably range between 20% and 80% sorption of a chemical from solution. Soil kinetics data indicated that a soil-to-solution ratio of 1:5 was appropriate for the silt loam, the sand and the loamy sand soil, whereas a 1:1 ratio seemed favourable for the sandy loam soil. The equilibrium period for the silt loam and the sandy loam soil was determined to be 24 hours, whereas a 48 hour equilibrium period was required for both the sand and the loamy sand soil.	X
4.3.2	Advanced test: Freundlich sorption isotherms	See Table A7.1.3-3 for the linear regression analysis equations. See Table A7.1.3-4 for the Freundlich adsorption constants and K_{OC} values (mobility classification according to McCall: $500 < K_{OC} < 2000$ indicates low mobility).	
4.3.3	Mass balance	See Table A7.1.3-5	
4.3.4	Stability over duration of study	The study results confirmed that the test chemical was relatively stable using sterilised soils in the test system. The majority of radioactivity in the chromatograms of all soils (after 48 h shaking at 5 ppm) was found to represent the test substance (> 95% ROI), while minor peaks ranged from 0.69% in the silt loam soil to 3.46 % ROI in the loamy sand soil.	
4.4	**Degradation product(s)**	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Adsorption/desorption of test substance RH-893 (= OIT) was investigated on three different sterilised soil types and one sediment at ambient temperature on a shaker in 30 or 50 mL Teflon centrifuge tubes, essentially according to OPPTS guideline 835.1220. The test concentration for the so-called preliminary and the screening test was ca 5 ppm (= 5 mg/L). For the advanced test and the calculation of the Freundlich isotherms, 4 concentrations (0.04, 0.2, 1 and 5 ppm) were used. Optimum soil-to-solution ratios were found to be 1:1 or 1:5 (g soil : mL 0.01 M $CaCl_2$ serving as aqueous phase). The time to achieve equilibrium partitioning of the test chemical between the solid and the liquid phase was determined. Analyses of tube supernatants and soil extracts were done using reversed-phase HPLC and LSC. Freundlich adsorption constants were determined based on the empirical Freundlich equation. A mass balance was established for the highest test concentration of 5 ppm in each soil. Stability of OIT under the given test conditions was ensured.	
5.2	**Results and discussion**	The adsorption coefficients of OIT related to the organic carbon content of the four soil types (K_{OC} values) were obtained to range between 604 and 1297 mL/g organic carbon. The results indicate that OIT has only a low mobility potential in soils and sediment.	
5.2.1	Adsorbed a.s. [%]	The screening test revealed sorption of OIT to range from 22.6% to 55.2% under the selected test conditions.	
5.2.2	Soil distribution	Based on the screening test (i.e., based on one concentration of 5 ppm),	

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	(partition) coefficient, K_D	this coefficient (termed K' in the report) was calculated via the formula $K' = (X/m)/ C_e$ with the following results: K'$_{mean}$ = 5.25, 3.16, 0.59 and 6.17 mL/g for the silt loam, the sand, the sandy loam and the loamy sand, respectively. This data correspond to K'$_{OC\ mean}$ values of 477, 791, 591 and 561 mL/g OC, respectively.	
5.2.3	Freundlich soil adsorption coefficient, K_F	The Freundlich adsorption constant (termed K in the report) varied from 1.21 to 10.77 mL/g soil, depending on soil type	
5.2.4	Freundlich soil adsorption coefficient normalised for organic carbon content, K_{OC}	K_{OC} values varied from 604 to 1297 mL/g organic carbon, depending on soil type. Regardless of the soil type, the K_{OC} values indicate that OIT shows a low mobility potential.	X
5.2.5	Freundlich exponent, 1/n	Ranging from 0.78 to 0.92, depending on soil type, indicating that the sorption process is slightly non-linear with increasing test substance concentration in the aqueous phase.	
5.3	**Conclusion**	Based on the McCall (1981) classification scheme, the test chemical demonstrated only a low mobility potential in all four soil types studied (K_{OC} > 500 mL/g organic carbon)	
5.3.1	Reliability	2	
5.3.2	Deficiencies	Yes; soil selection: one soil with very low OC content (0.1% organic carbon); no soil with OC content greater than 1.1% organic carbon. Desorption K_{OC} values missing (but not required acc. to OPPTS guideline). However, the study results confirm essentially the data (K_{OC} ~240) obtained in the available adsorption test relying on the HPLC method (OECD guideline 121) and thus corroborate the assessment of a medium to low mobility potential of the test substance in soil, sewage sludge and sediment.	X
		Evaluation by Competent Authorities	
		EVALUATION BY RAPPORTEUR MEMBER STATE	
Date		06 Nov 2009	
Materials and Methods		Applicant's version is considered acceptable.	

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Results and discussion	Applicant's version is considered acceptable noting the following:
	3.4 As stated in the technical guidance documents for biocides five soils should be tested, instead of four.
	All have a low organic carbon content (<1.1 %) and they also all have a low clay content. This implies that a wide range of soil conditions has not been investigated. As such it may not therefore be appropriate to state that OIT has a low mobility 'regardless of the soil type'. However the UK CA accepts these soils to be suitable as the selection will produce conservative values since an increase in either organic matter or clay is likely to increase the amount of sorption occurring.
	4.3.1: It is not clear that equilibrium was reached. A graph should be included to demonstrate that this was the case. The table below shows the % change in radioactivity between the time the applicant states equilibrium was reached, and the previous time point.

Soil	Applicant's Equilibrium	Last Time Period	Percent Change in Radioactivity	Equilibrium Reached
Soil 1 Pennsylvania 00012-1	24 h	8 to 24 h	7.49	No
Soil 2 New Jersey 00012-3	48 h	24 to 48 h	16.90	No
Soil 3 New Jersey 00012-4	48 h	24 to 48 h	4.49	Yes
Soil 4 (sediment) Pennsylvania 00012-5	24 h	8 to 24 h	11.19	No

It is the CA's opinion that equilibrium was not reached in 3 of the soils, however it is the opinion of the UK CA that the stated equilibrium times should be considered acceptable, as the adsorption rate has increased and is relatively stable, and if the test was for longer a natural increase in adsorption may occur and hence the sorption values provided are likely to be conservative.

The applicant also states that the silty loam originally did not reach equilibrium on account of it not being properly sterilised. However, the test was subsequently repeated and chromatographic data from HPLC demonstrates that no appreciable degradation of parent occurred in any of the soils (2 metabolites were found, but always at <5 % combined of original radioactivity level).

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Conclusion	Applicant's version is considered acceptable noting the following: **5.2.4:** It may not be appropriate to state that OIT has a low mobility 'regardless of the soil type' due to the rather limited range of soil and sediment types tested. **5.3.2:** The K_{OC} values of ~240 (from the HPLC study) and 604 to 1297 mL/g (from this study) are not similar. In addition, this study indicates a low mobility of OIT, whereas the HPLC study indicates a medium mobility. Therefore, it is not appropriate to imply that the 2 studies have generated similar results. However, as the results from this study imply a lower mobility of the test substance to the HPLC study, this is acceptable. Guideline 121 states that it is possible for this method to be only 0.5 log units away from the batch equilibrium method; therefore the results presented for the 2 methods may count as 'close'. In addition, the results of the batch sorption methodology should be considered higher tier in comparison with the HPLC methodology.
Reliability	1 Study conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and /or minor methodological deficiencies, which do not affect the quality of relevant results.
Acceptability	Acceptable
Remarks	A wider range of soil types could have been investigated; however the soils studied approximately meet those required by guideline OPPTS 835.1220. The UK CA note that sorption correlated well with orgabic carbon content over the limited range tested. All endpoints and data presented have been checked against the original study and are correct.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A 7.1.3-1: Classification and physico-chemical properties of soils used as adsorbents

Parameter / Soil name	Soil 1 Pennsylvania 00012-1	Soil 2 New Jersey 00012-3	Soil 3 New Jersey 00012-4	Soil 4 (sediment) Pennsylvania 00012-5
Source	██████	██████	██████	██████
Textural classification, USDA	Silt loam	Sand	Sandy loam	Loamy sand
Sand (%)	17	89	73	77
Silt (%)	66	6	18	18
Clay (%)	17	5	9	5
Organic matter (%)	1.9	0.7	0.2	1.9
Organic carbon (%) [1]	1.10	0.41	0.12	1.10
pH	6.6	5.1	6.4	7.4
Cation exchange capacity (MEQ/100 g)	9.0	3.6	3.7	6.1
Bulk density (g/mL)	1.09	1.54	1.56	1.25
Field capacity (FC, 1/3 bar)	28.8	6.7	7.9	14.1

[1]   Calculated from organic matter content: OC = OM/1.72 (as specified in the study report).

Table A 7.1.3-2: Percent adsorption/desorption from $CaCl_2$ solutions (screening test)

Soil	Replicate	Soil-to-solution ratio	Percent adsorbed (%)	Percent desorbed (%)
Pennsylvania silt loam	A B Average	1 : 5	52.08 50.33 51.21	28.68 33.77 31.23
New Jersey sand	A B Average	1 : 5	38.86 38.65 38.76	52.61 57.13 54.87
New Jersey sandy loam	A B Average	1 : 2	18.60 26.59 22.60	88.52 61.26 74.89
Pennsylvannia loamy sand	A B Average	1 : 5	54.52 55.94 55.23	42.28 43.77 43.03

Table A 7.1.3-3: Linear regression equations (from advanced test: Freundlich isotherms)

Soil	Equation	Log K	Exponent 1/n	Coefficient of determination (r^2)
Pennsylvania silt loam		0.82253	0.80575	0.99880
New Jersey sand	Log X/m = Log K + 1/n * Log C_e	0.71515	0.87255	0.99995
New Jersey sandy loam		0.08306	0.91879	0.99860
Pennsylvannia loamy sand		1.03233	0.78074	0.98886

Table A 7.1.3-4: Adsorption constants in four soils and mobility classification (from advanced test: Freundlich isotherms)

Soil	% organic carbon	K* (mL/g soil)	K_{OC} [&] (mL/g OC)	Mobility class [&&]
Pennsylvania silt loam	1.1	6.65	604	low
New Jersey sand	0.4	5.19	1297	low
New Jersey sandy loam	0.1	1.21	1211	low
Pennsylvannia loamy sand	1.1	10.77	979	low

[&] rounded values; [&&] according to McCall et al. (1981): $500 < K_{OC} < 2000$ = low mobility

Table A 7.1.3-5: Mass balance (radioactivity recovery) in four soils (from advanced test: Freundlich isotherms)

Soil	Soil-to-solution ratio	Interval (hours)	Replicate	Soiless controls (%)	Untreated control (%)	5 ppm treatment (%)
Pennsylvania silt loam	1:5 (25 mL)	24	A B Average	108.06 109.19 108.63	0.01 0.16 0.10	101.94 102.51 102.23
New Jersey sand	1:5 (25 mL)	48	A B Average	99.39 99.42 99.41	0.24 0.07 0.16	95.27 96.77 96.02
New Jersey sandy loam	1:1 (10 mL)	24	A B Average	96.50 99.40 97.95	0.05 0.07 0.06	91.08 94.13 92.61
Pennsylvannia loamy sand	1:5 (25 mL)	48	A B Average	99.39 99.42 99.41	0.16 0.09 0.13	96.75 93.93 95.34