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Title

Long-term experiments to investigate irreversibility in sorption of pesticides to soil

Authors

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Abstract

Experiments investigated irreversibility in pesticide sorption to soil. Sorption behaviour under abiotic conditions was quantified for chlorotoluron, prometryn and hexaconazole in three soils over periods of up to 274 days. An isotope-exchange procedure was used whereby sorption of ^{12}C- and ^{14}C-pesticide isotopes in shaken suspensions of three soils (56-168 days shaking) was followed by substitution of the isotopes in the liquid phase and a 14-day exchange phase. This was followed by forced isotope exchange where the sorbed ^{14}C material was exchanged by adding an excess of non-radiolabelled compound. Experiments were concluded with solvent extraction and soil combustion to determine remaining radioactivity. Under conditions of continuous shaking, the pesticide-soil systems took around four months to approach sorption equilibrium, resulting in strong asymmetry between the profiles of exchange for isotopes of all three compounds. Physically entrapped residues were released back into solution under the steep concentration gradient of forced isotope exchange and

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small amounts of radioactivity were still being released at the termination of the experiment. The profiles of exchange did not deviate markedly from ideal behaviour based on the assumption that sorption is fully reversible. Whilst the timescales for release of sorbed residues back into solution were very long, soil combustion at study termination only yielded <1-2% of applied radioactivity; this confirms that sorption processes under abiotic soil conditions were overwhelmingly reversible for this set of compounds and soils.

Key words

Pesticide, sorption, desorption, irreversible sorption, bound residue, non-extractable residue

Introduction

Sorption is a key process controlling the fate of a pesticide in soil as it determines the distribution between soil solid phase and soil water phase. Sorption (both adsorption and absorption) and desorption can be kinetically-controlled, typically involving multiple domains of sorption kinetics ranging from instantaneous to very slow with true equilibrium approached over months or years [1]. Pesticide sorption to soils is often reported to be only partially reversible, with a fraction of the sorbed pesticide apparently resistant to desorption at the tails of kinetically-controlled release [2]. The formation of non-extractable (bound) residues is important for the fate and transport of organic contaminants in environmental systems as it limits the bioavailability of pesticides in surface soil systems and has potential to reduce pesticide mobility in the environment [3].

Physical entrapment within the soil solid phase and covalent bonding to soil organic matter are two processes known to give rise to bound residues [4, 5]. Some parent compounds will be directly subject to covalent bonding [6, 7], but in many cases, the formation of bound

residues has been found to arise as a result of biodegradation of the parent compound to one or more metabolites that are prone to covalent bonding to organic matter. Several studies have demonstrated a large reduction in formation of bound residues in sterile soil where biodegradation to metabolites is inhibited [8]. Recent work has demonstrated that biodegradation of parent compound can also be followed by assimilation into biogenic material by soil microorganisms (*e.g.* as fatty acids and amino acids) [9]. Such biogenic material is excluded from conventional definitions of bound residues, but is indistinguishable in studies based solely on recovery of radiolabelled chemical from treated soil. Whilst the formation of bound residues effectively reduces bioavailability and transport of pesticide in soil, questions have been raised about long-term reversibility and possible implications for human and ecosystem health. In this context, the parent compound is generally most toxicologically active and thus of greatest concern [10]. For chemicals that are not prone to covalent bonding to soil organic matter, bound residues of the parent compound will be most frequently associated with physical entrapment. The current paper investigates whether or not there is an irreversibly bound fraction for pesticide residues sorbed to soil over extended periods of time.

Experimental approaches and instrumental methods used to characterise bound residues in soil were reviewed by Northcott and Jones [11]. Classical extraction procedures aimed to recover as much of the pesticide as possible using exhaustive extraction techniques [12]. A modification to the IUPAC definition of bound residues by Führ *et al*. [13] stipulated that "the extraction method must not substantially change the compounds themselves or the structure of the matrix", meaning that bound residues now typically refer to those residues that cannot be extracted from soil by an organic solvent and without alteration to the chemical structure of the compound.

71 Celis and Koskinen [14, 15] presented an isotope exchange technique using ^{14}C to
72 characterise pesticide exchange kinetics. The principle of the approach is pre-equilibration of
73 duplicate tubes with soil-water slurry and dosed with either ^{12}C- or ^{14}C-pesticide; the tubes
74 are centrifuged and the supernatants switched to allow characterisation of the kinetics of
75 pesticide exchange and estimation of amounts of sorbed pesticide that do not participate in
76 the exchange. The authors proposed that their method eliminated inherent experimental
77 artefacts of other approaches such as the specific effectiveness of the extracting method and
78 changes to the soil solid phase. Despite the promise of the approach, it is unlikely that true
79 irreversible sorption could be measured during Celis and Koskinen's [14, 15] three-day tests.
80 Sander and Pignatello [16, 17] used equilibration times of up to 140 days with the objective
81 of ensuring sorption equilibrium in their forward isotope exchange experiment with the
82 persistent hydrocarbons naphthalene and 1,4-dichlorobenzene. Therefore, sorption and
83 desorption periods should be extended to widen the scope and environmental applicability of
84 isotope exchange studies.
85 An isotope exchange study was carried out using three test compounds in three soils to
86 characterise irreversibility in pesticide sorption-desorption to soils over time and to determine
87 whether any irreversibly bound fraction of pesticide changes over time and/or differs for
88 differing pesticides and soil types. The three pesticides were selected to have a range in
89 sorption properties; they comprised chlorotoluron [*N'*-(3-Chloro-4-methylphenyl)-*N,N*-
90 dimethylurea], prometryn [*N,N'*-bis(1-methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-
91 diamine], and hexaconazole [α-butyl-α-(2,4-dichlorophenyl)-1*H*-1,2,4-triazole-1-ethanol].

92 ## Materials and methods

93 **Soils.** Three soils from the UK with differing physico-chemical properties were selected for
94 study. Fresh soils from 0-15 cm depth of a Blackwood loamy sand, Andover clay loam and

95 Salop sandy clay loam were collected, air-dried and passed through a 2-mm sieve (sampling
96 sites and dates are given in Table 1). Soil characterisation was carried out by NRM
97 Laboratories Ltd (Bracknell, UK; Table 1). Soils were gamma irradiated at 35.40 kGy by
98 Isotron Ltd (Bradford, UK) to sterilise and thus inhibit microbial degradation. Irradiated soils
99 were subsequently stored under sterile conditions (at 4°C in darkness) until use. It is assumed
100 throughout that any impact of irradiation on the soil constituents did not materially influence
101 pesticide sorption behaviour.
102 **Chemicals.** Analytical-grade ^{12}C-pesticides were purchased from Sigma-Aldrich Ltd (Dorset,
103 UK). All ^{14}C-pesticides were supplied by Syngenta Ltd (Jealott's Hill International Research
104 Centre, Bracknell, UK). Details of purity and specific activity are given in Table S1 in the
105 Supporting Information. Preliminary batch-slurry experiments with ^{12}C-pesticides quantified
106 sorption isotherms in the three soils after 24-h shaking and for seven initial concentrations of
107 each pesticide. All chemicals were either received sterile or sterilised prior to use by
108 autoclave.
109 **Isotope exchange.** Experiments comprised a sequence of adsorption (periods from 7 to 168
110 d), isotope exchange and re-equilibration over 14 days, forced isotope exchange (periods
111 from 145 to 204 days), then finally solvent extraction, analysis for metabolites and soil
112 combustion. These different procedures are described in turn below and any differences in
113 methodology for experiments with the three pesticides are given in Table S1 in the
114 Supporting Information. Long-term experiments commenced with the isotope exchange
115 technique based on the method described by Celis and Koskinen [14, 15], but longer
116 timescales were used here. The experiment involved independent application of two isotopes
117 of pesticide (^{12}C and ^{14}C) to the three study soils. The study was performed in triplicate and
118 over four sorption periods (7, 14, 28 and 56 d for chlorotoluron; 28, 56, 112 and 168 days for
119 prometryn and hexaconazole). Soil was irradiated and all materials were sterilised prior to use

120 in order to inhibit microbial degradation, but no additional sterility controls were applied
121 during subsequent experimental manipulations.
122 Preliminary experiments determined that the optimal soil:solution ratio (*i.e.* giving 30-70%
123 sorption over 96 hours) varied between 1:4 (chlorotoluron) and 1:50 (hexaconazole). Test
124 vessels were 50 mL Teflon® centrifuge tubes, irradiated to sterilise for 20 min prior to use in
125 a CL-1000 ultraviolet cross-linker (UVP Ltd, CA, USA). Tubes had screw-topped lids and
126 we assumed negligible losses of liquid via evaporation between experimental steps. Soils
127 were weighed into vessels to give the appropriate soil:solution ratio on an oven-dry basis
128 (Supporting Information Table S1) and then 19.0 mL of sterile 0.01M $CaCl_2$ was added (24.0
129 mL for hexaconazole). The experiment was performed in the dark in an incubator at 4°C
130 (Sanyo Fitotron RS232 incubator) to minimise degradation, with continuous shaking at 150
131 rpm (HS 501 Digital IKA®-Werke reciprocal shaker). Soil suspensions were pre-equilibrated
132 by shaking overnight. Sterile pesticide treatment solutions (^{12}C and ^{14}C) were prepared in
133 0.01M $CaCl_2$, ensuring equivalence in concentration. This was applied (1.0 mL) to the pre-
134 equilibrated soils to give 20 mL total pesticide solution (25 mL for hexaconazole) with an
135 initial concentration of 0.70, 0.78 and 0.77 μg mL^{-1} for chlorotoluron, prometryn and
136 hexaconazole, respectively. For each soil-pesticide combination, half of the samples
137 contained only ^{12}C-pesticide and half contained only ^{14}C-pesticide.
138 After the respective sorption period, samples were centrifuged at 3500 rpm for 10 min
139 (Hermle Z513K, LaborTechnik, Bench Top Centrifuge). The supernatant from each sample
140 was removed by weight (Supporting Information Table S1) using a Pasteur pipette, taking
141 care not to disturb the soil. A 200-μL aliquot was taken from each ^{12}C supernatant to
142 determine percentage sorption by HPLC analysis. The same sample volume was also
143 removed from each ^{14}C supernatant but instead mixed with 10 mL of EcoScint A for
144 quantification by LSC. Supernatants were then exchanged between corresponding tubes, with

initially ^{12}C samples receiving the parallel ^{14}C supernatant and vice versa. This substitution did not disturb sorption equilibrium when expressed for the combination of the two isotopes as the same sorption equilibrium was reached in both tubes prior to the exchange, only with different carbon isotopes. Samples were then shaken for a further 14 days for isotope exchange to occur and sampled during this time on days 1, 3, 7 and 14 (centrifuged and a 200-µL aliquot of supernatant removed for LSC quantification) to measure exchange between ^{12}C- and ^{14}C-pesticide isotopes over time.

Forced isotope exchange. A forced isotope exchange procedure involving the addition of a high-concentration ^{12}C-pesticide solution in 0.01M $CaCl_2$ was carried out following the 14-day isotope exchange phase. Repeated influx of high-concentration ^{12}C-pesticide over time ensures that competition for sorption to soil between ^{12}C- and ^{14}C-pesticide is increasingly biased towards the former; supply of ^{12}C-pesticide to the soil solid phase surfaces to take part in sorption is essentially instantaneous with exchange with ^{14}C-pesticide the rate-limiting step. Thus, as ^{12}C-pesticide occupies all available sorption sites by out-competing any available ^{14}C-pesticide, it is possible to identify, through measurement of ^{14}C-pesticide in solution, the proportion of sorbed ^{14}C-pesticide available for desorption and hence the proportion of ^{14}C-pesticide not taking part in the sorption-desorption process.

Only initially ^{14}C samples (three soils, three replicates) adsorbed for 56 days (all pesticides) or for 112 and 168 days (prometryn and hexaconazole) were used in this part of the study as these had the greatest mass of ^{14}C-pesticide sorbed to soil after the 14-day exchange phase. There were 12-17 sampling points over periods of 145-204 days (Supporting Information Table S1). Samples were shaken between sampling points (150 rpm, 4°C). At each sampling point, samples were centrifuged and the supernatant was removed by weight (Table S1). A 250-µL aliquot was taken for quantification by LSC to measure release of ^{14}C from soil over

time. The supernatant that was removed was then replaced with fresh, high-concentration (Table S1) ^{12}C-pesticide solution to maintain the competition for sorption sites.

At the end of forced isotope exchange, soils were extracted with acidified methanol (0.1% H_3PO_4) to determine whether the ^{14}C-pesticide residue remaining sorbed to the soil was available by harsher extraction. Extractions involved addition of 20 mL of methanol acidified with 0.1% H_3PO_4 before shaking at 250 rpm for 24 hours at room temperature. The process was repeated until further removal of ^{14}C-pesticide was insignificant (<1% of initial-applied). Supernatants and soil extracts were analysed by radio-HPLC for parent compound and metabolites, and soils were finally combusted to complete the mass balance.

Theoretical development for isotope exchange and forced exchange. Theoretical development is provided for treatments with initially ^{14}C-pesticide as these were taken through the full procedure of isotope exchange followed by forced exchange. Equivalent relationships (equations 1 to 3) for isotope exchange of treatments with initially ^{12}C-pesticide can be obtained by reversing the isotope nomenclature. At the end of the equilibration phase prior to isotope exchange and for tubes with initially ^{14}C-pesticide, the partition coefficient (K_d, mL g^{-1}) can be defined as:

$$K_d = \frac{^{14}C_s}{^{14}C_e} = \frac{^{14}M_s}{^{14}M_e} \cdot \frac{V}{S} \quad (1)$$

where $^{14}C_s$ and $^{14}C_e$ are the concentration of ^{14}C-pesticide in soil and solution (µg g^{-1} and µg mL^{-1}), respectively; $^{14}M_s$ and $^{14}M_e$ are the mass of ^{14}C-pesticide in soil and solution (µg), respectively; V is the volume of solution (mL); and S is the mass of soil (g). At this point, the supernatants are exchanged between initially ^{14}C and initially ^{12}C tubes. The total mass of ^{14}C-pesticide present in the initially ^{14}C tube after exchange is now $^{14}M_s$ and all of this will be sorbed at the point of exchange. We can define the quantity of ^{12}C-pesticide as $^{12}M_e$ which

will be numerically equivalent to ${}^{14}M_e$ and will be wholly in the solution phase at the point of exchange.

It is assumed that sorbed and equilibrium pesticide concentrations are identical for tubes with the same treatment and differing only in having initially ^{14}C- or initially ^{12}C-pesticide and that the sorption equilibrium is maintained after isotope exchange when expressed on the basis of the total pesticide (*i.e.* ^{14}C and ^{12}C) in the system. This assumption is supported by the research of Sander & Pignatello [17] who showed complete reversibility of sorption at low concentrations in their long-term isotope exchange experiments. In this circumstance and assuming that all sorbed ^{14}C-pesticide is available for exchange, the K_d will be maintained at the pre-exchange value and there will be a net transfer of ^{12}C-pesticide out of solution into the sorbed phase and a numerically equivalent net transfer of ^{14}C-pesticide in the opposite direction. Theoretically, these net fluxes will continue until the point where the two isotopes each attain the characterising equilibrium (nomenclature as previously, with equilibrium for each individual isotope designated with prime symbols (*′*):

$$K_d = \frac{{}^{12}M_s'}{{}^{12}M_e'} \cdot \frac{V}{S} = \frac{{}^{14}M_s'}{{}^{14}M_e'} \cdot \frac{V}{S} \qquad (2)$$

The amount of ^{14}C-pesticide released into solution following isotope exchange is given by ${}^{14}M'_e$. As it is assumed that K_d is constant throughout the exchange process and no degradation occurs, and as ${}^{14}M'_s$ is given by $({}^{14}M_s - {}^{14}M'_e)$:

$${}^{14}M_e' = ({}^{14}M_s - {}^{14}M_e') \cdot (\frac{{}^{14}M_e}{{}^{14}M_s})$$

which can be rearranged to:

$${}^{14}M_e' = \frac{{}^{14}M_s.{}^{14}M_e}{({}^{14}M_s + {}^{14}M_e)} \qquad (3)$$

For the condition where there is no irreversible sorption, we can thus calculate theoretical values for ${}^{14}M'_e$ as a function of the partitioning measured immediately prior to isotope exchange. This relationship is plotted as the dashed line in Figure 3.

The sorption equilibrium is completely changed during forced exchange due to the addition of concentrated ^{12}C-pesticide solution. At this point in the experiment, we are interested in how much of the ^{14}C-pesticide that is still sorbed after isotope exchange is participating in reversible sorption equilibrium. Assuming zero irreversible sorption, all of the ^{14}C-pesticide should be released back into solution over successive exchange steps due to the huge imbalance in presence of the two isotopes. The ^{14}C released during forced exchange is thus defined as:

$$ {}^{14}M''_s = ({}^{14}M_s - {}^{14}M'_e) \quad (4) $$

For the condition where there is no irreversible sorption, we can thus calculate theoretical values for ${}^{14}M''_e$ as a function of the partitioning measured immediately prior to isotope exchange. This relationship is plotted as the solid line in Figure 3.

Testing for degradation. Degradation was measured in parallel to the isotope exchange studies. The three soils were treated with ^{12}C-pesticide (chlorotoluron) or ^{14}C-pesticide (prometryn and hexaconazole); there were again three replicates, but this time with five sorption periods (7, 14, 28, 56 and 70 days for chlorotoluron; 28, 56, 112, 168 and 182 days for prometryn and hexaconazole) to include the isotope exchange period. Soil suspensions were prepared and shaken exactly as in the main study using the same soil:solution ratio and initial mass of pesticide. After each sorption period, samples were centrifuged, the supernatant was removed by weight (Supporting Information Table S1) and a 1-mL aliquot was taken for analysis by HPLC or radio-HPLC. The soils were then extracted with solvent by adding 20 mL methanol (acidified with 0.1% H_3PO_4) and shaking at 250 rpm for one hour

238 at room temperature. After centrifuging to separate the soil extract, a 1-mL aliquot was taken
239 for analysis by HPLC or radio-HPLC to derive the mass balance and assess presence/absence
240 of any degradation products. Supernatants and soil extracts taken from chlorotoluron
241 experiments at 28, 56 and 70 days were analysed by LC-TOF-MS to confirm that mass
242 balances were solely attributable to parent compound and not to any degradation products.
243 This step was not necessary for the other two compounds due to use of radio-HPLC.
244 **Chemical analysis.** All ^{12}C-pesticide samples in solution were analysed by HPLC (Agilent
245 1100 Series, Agilent Technologies UK Ltd); full details are given in Table S2 in the
246 Supporting Information. ^{14}C-pesticide samples in solution were analysed by LSC (LS 6500
247 Beckman Coulter Inc., Fullerton, USA). The LSC method counted each sample three times
248 for a total of 15 min (5 min each). Duplicate blanks were used to account for the background
249 radioactivity and results were corrected for quench and luminescence. Limits of
250 quantification were 0.00011, 0.00012 and 0.00022 Bq mL^{-1} for chlorotoluron, prometryn and
251 hexaconazole, respectively. Radio-HPLC analysis was carried out using a Hewlett Packard
252 1100 Series HPLC with Perkin-Elmer Radiomatic 625TR Flow Scintillation Analyser (see
253 Table S3 in the Supporting Information for details).
254 To prepare soil samples for combustion, soils were air-dried (7 days) and then ground using a
255 pestle and mortar. Approximately 200 mg of dry, ground soil was weighed into a combustion
256 cone sandwiched between two combustion caps. Soil samples were then oxidised in a Perkin-
257 Elmer Oximate 80, Model 370 and finally analysed by LSC (Perkin-Elmer Tri-Carb 2810TR
258 Liquid Scintillation Analyser). Limit of quantification was 2.5 Bq g^{-1} sample.
259 LC-TOF-MS analysis of supernatants and soil extracts containing chlorotoluron was
260 performed using an Agilent 1200 Series LC with G120 Time of Flight Mass Spectrometer
261 (Santa Clara, CA, USA). LC was performed using a Waters Acquity BEH C_{18} (2.1 x 50 mm,
262 1.7 µm) column (at 35.0°C) with 0.2 µm in-line filter. Mobile phases were 5 mM ammonium

263 acetate in water (Channel A) and methanol (Channel B). A gradient method and flow rate of
264 0.6 mL min^{-1} was used. The initial ratio of ammonium acetate:methanol was 98:2, changing
265 to 2:98 over 5 min, held for 3.1 min before then returning to original conditions after 8.1 min
266 (total run time 9 min). Retention time of parent chlorotoluron was 3.01 min. Injection volume
267 was 3 µL in acetonitrile. TOF-MS analysis was carried out in positive or negative mode
268 electrospray with a nebulizer pressure of 45 psi, capillary of 4000 V, gas temperature of
269 450°C, drying gas flow at 15 L min^{-1}, skimmer of 60 V, fragmentor of 150 V and octopole
270 RF voltage of 250 V. The mass range measured was 100-1100 m/z with resolving power of
271 5000. Total ion chromatographs were generated using Agilent Masshunter.

272 **Results and discussion**

273 **Degradation and 24-h sorption isotherms.** LC-TOF-MS analysis did not identify
274 chlorotoluron metabolites in any supernatants or soil extracts analysed following 28, 56 or 70
275 days of sorption; ^{12}C-chlorotoluron samples did not differ (except for chlorotoluron peak)
276 from the blank supernatants and soil extracts. Hence, it was assumed that negligible
277 degradation of chlorotoluron occurred during the 70-day experiment. Radio-HPLC analysis
278 confirmed that there was no quantifiable degradation of hexaconazole over the 182 days
279 investigated. Small amounts of degradation of prometryn occurred with the proportion of
280 radioactivity attributable to parent decreasing from 97 ± 0.3%, 97 ± 0.1% and 98 ± 1.9% after
281 56 days to 92, 94 and 97% after 182 days in the Blackwood, Andover and Salop soils,
282 respectively; there were no replicates at the latter date due to combination and concentration
283 of supernatants. Table S4 in the Supporting Information characterises sorption isotherms for
284 24-hour batch-slurry experiments with all pesticide-soil combinations to act as a reference
285 point for data from long-term sorption experiments. These batch values are in line with
286 ranges reported in regulatory databases (e.g. www.sitem.herts.ac.uk/aeru/ppdb).

287 **Isotope exchange.** Figure 1 characterises isotope exchange in the study soils during the 14-
288 day exchange phase for the three compounds subjected to 56 days of sorption. This sorption
289 period is discussed in detail as it was common for all pesticides; corresponding results for
290 other sorption periods are shown in Figures S1-S3 in the Supporting Information. For initially
291 ^{12}C samples, Figure 1 shows a significant reduction of ^{14}C-pesticide in solution over the first
292 day after exchange. Meanwhile, for the initially ^{14}C samples, a significant increase of ^{14}C-
293 pesticide in solution is evident during the same period. The influx of ^{12}C- (initially ^{14}C
294 sample) or ^{14}C- (initially ^{12}C sample) pesticide at 0 days (supernatant exchange) gave large
295 differences between the ratios of the two isotopes in the soil and solution in both tubes. Thus,
296 rapid sorption (initially ^{12}C sample) and release (initially ^{14}C sample) of ^{14}C-pesticide in one
297 direction dominates as the system responds to accommodate for the new conditions.
298 The remainder of the isotope exchange phase (between 1 and 14 days) sees any further net
299 exchange between ^{12}C- and ^{14}C-pesticide become much slower. For both initially ^{12}C and ^{14}C
300 samples, the disparity in isotope concentration has lessened as the majority of isotope
301 exchange has already occurred. For initially ^{12}C samples, there is an overall decrease of ^{14}C-
302 pesticide in solution over time as this sorbs to the soil, displacing ^{12}C-pesticide. For initially
303 ^{14}C samples, the anticipated increase in ^{14}C-pesticide in solution over time is short-lived.
304 After the initial (0 to 1 day) increase due to release of sorbed material, there is a net decrease
305 (chlorotoluron) or relatively constant amounts (prometryn, hexaconazole) of ^{14}C-pesticide in
306 solution between 1 and 14 days. Any decrease in concentration of ^{14}C-pesticide in solution is
307 seen most strongly in those instances when the shaking period prior to isotope exchange was
308 shorter (*e.g.* for shaking periods of 7-56 days; Supporting Information Figures S1-S3). This
309 phenomenon is likely the result of continued sorption, demonstrating that the system had not
310 reached equilibrium at the point of supernatant exchange, even after 56 days (Figure 1). None
311 of the pairs of isotope exchange curves shown are symmetrical around a horizontal line,

312 indicating that none of the systems reached true equilibrium during the experimental period,
313 even where the sorption phase lasted 168 days for prometryn and hexaconazole (Supporting
314 Information Figures S1-S3). There is no trend of different behaviour for the different soils.
315 If sorption equilibrium had been reached and all pesticide was in exchange between the soil
316 and solution, meaning sorption was a fully reversible process, then Figure 1 would show the
317 measured radioactivity in solution (solid lines) reach the expected radioactivity in solution
318 (dashed lines) during the 14-day isotope exchange phase. Expected radioactivity lines were
319 calculated based on the proportion of initial ^{14}C-pesticide in solution after the respective
320 sorption period, so the same proportion of ^{14}C-pesticide was expected to be in solution after
321 the 14-day exchange phase if all sorbed pesticide was participating in exchange. This is
322 clearly not the case for the initially ^{14}C samples, as continued sorption of ^{14}C-pesticide that
323 was released into solution immediately after supernatant exchange means the measured
324 radioactivity in solution actually deviates further from that expected over time (Figure 1).
325 Central to the explanation of Figure 1 is that the sorption of pesticide to soil is time-
326 dependent. Extent of sorption increased between 28 and 56 days for all pesticide and soil
327 combinations except prometryn in the Blackwood and Andover soils (Supporting Information
328 Table S5). There were no measurements of sorption of chlorotoluron beyond 56 days, but
329 data for prometryn in the Salop soil and hexaconazole in all three soils show further increases
330 in sorption between 56 and 112 days. All systems with prometryn and hexaconazole were
331 apparently very close to true sorption equilibrium after 112 days as there was little or no
332 increase in sorption through to 168 days (Supporting Information Table S5). Gao *et al.* [18]
333 also found chlorotoluron sorption to soil to be time-dependent and suggested that the time-
334 range to reach equilibrium was likely to be months or years. In contrast, Celis and Koskinen
335 [14, 15] considered that triadimefon and imidacloprid-guanidine had reached sorption
336 equilibrium during their three-day isotope exchange tests. However, the authors observed

337 asymmetry in the behaviour of initially ^{12}C and ^{14}C samples which was similar to that
338 observed here and may suggest that true equilibrium was not attained [19]. The fact that
339 sorption was not at equilibrium at the point of isotope exchange meant that results from this
340 phase of experimentation could not be used to calculate the irreversibly sorbed fraction of
341 pesticide as proposed by Celis and Koskinen [14]. This is because exchange patterns for the
342 two isotopes should be symmetrical in Figure 1.
343 Overall, the patterns of asymmetry exhibited in Figures 1 and S1-S3 are relatively consistent
344 between the different pesticides and across the three different soils. One exception is for
345 chlorotoluron in the Salop soil for shorter equilibration periods (Figure S1, 7- and 14-d
346 equilibration) where the effect of continued increase in strength of sorption after isotope
347 exchange is particularly marked. This indicates that sorption of chorotoluron was further
348 from the equilibrium in Salop soil after 7-14 days than in the other two soils at the equivalent
349 time; however, the reasons for this deviation are unclear.
350 **Forced isotope exchange.** Figure 2 shows the result of the forced isotope exchange over time
351 for samples sorbed for 56 days with ^{14}C-chlorotoluron then subjected to isotope exchange
352 over 14 days (data for prometryn and hexaconazole showed a similar form and are given in
353 the SI as Figures S4 and S5). The bulk of total recoverable ^{14}C-chlorotoluron was extracted
354 with the first addition of ^{12}C-chlorotoluron (average 25% Blackwood, 42% Andover and 21%
355 Salop after 1 day; Figure 2). This was followed by a gradual decline in ^{14}C-chlorotoluron
356 recovery over time, which finally reached <1% recovery of initial for the Blackwood and
357 Andover soils and 2% for the Salop soil between 161 and 204 days.
358 Although exposure to high concentrations of pesticide in solution is far from normal field
359 conditions, the approach is anticipated to leave the soil solid phase essentially unchanged and
360 thus may be a useful indication of long-term availability of residues to be biodegraded or
361 leached in soils. Undoubtedly, there is some effect on the soil solid phase from shaking with

362 aqueous solution over a protracted period (*e.g.* a possible increase in activity of the sorption
363 sites) and this has not been quantified here.
364 The amount of ^{14}C-pesticide released into solution from the solid soil phase following both
365 isotope exchange and forced exchange phases was 79-87%, 93-96% and 88-96% of that
366 sorbed immediately prior to exchange for chlorotoluron, prometryn and hexaconazole,
367 respectively. It should be noted that in each case the forced exchange was still releasing small
368 amounts of ^{14}C-pesticide from the soil solid phase at the end of the process, albeit at very
369 slow rates (Figure 2; Figures S4 and S5 in the Supporting Information).
370 **Mass balance.** The complete mass balance of the three ^{14}C-pesticides for an initial sorption
371 period of 56 days is given in Table 2, whilst those for prometryn and hexaconazole sorbed for
372 112 and 168 days are given in the Supporting Information (Table S6). Repeated solvent
373 extraction after the forced isotope exchange released small amounts of the initially applied
374 ^{14}C-pesticide from the three soils (2-11%, 0.6-1.9% and 0.5-2.5% of initial radioactivity for
375 chlorotoluron, prometryn and hexaconazole, respectively). Whilst this material had not been
376 released by forced isotope exchange, it would not be included within a standard definition of
377 bound residues [13]. Radio-HPLC analysis of forced exchange solutions identified that a
378 small proportion of the initial ^{14}C-chlorotoluron applied to the soils had degraded to
379 transformation products during this phase of the study. The extent of degradation was 4.0 ±
380 1.6% (Blackwood), 3.9 ± 2.3% (Andover) and 1.6 ± 1.5% (Salop) of initial ^{14}C-chlorotoluron
381 mass per sample. The half-life of chlorotoluron under laboratory conditions has been reported
382 by Gao *et al.* [18] to be 30 days, but soil sterilisation and experimental conditions of 4°C in
383 the dark effectively inhibited degradation despite the long duration of the study (274 days).
384 Combustion of the extracted soils released radioactivity equivalent to 1.1-2.3%, 0.3-1.4% and
385 0.1-0.5% of initial radioactivity for chlorotoluron, prometryn and hexaconazole, respectively.
386 These small fractions could not be identified, but fit with a classical definition of bound

387 residues. It should be noted that these percentages would not reflect environmentally-relevant
388 irreversible fractions, which are likely to be greater than those reported here; this is because
389 these figures were obtained through alteration of the soil solid phase via solvent-extraction
390 and because the microbial community was eliminated. Finally, 0.6-4.6%, 0.2-1.4% and 0.9-
391 3.6% of initial radioactivity was unaccounted for chlorotoluron, prometryn and hexaconazole,
392 respectively. This may result from mineralisation to $^{14}CO_2$, volatilisation or cumulative errors
393 over the course of the experiment due to the multiple solute exchanges undertaken.
394 **Comparison with ideal behaviour.** Under conditions of ideal, fully reversible sorption of
395 pesticides to soil, the fraction of ^{14}C-pesticide that is in the solid phase after the initial period
396 of sorption will exchange completely with ^{12}C-pesticide and be released into solution by the
397 combination of isotope exchange and forced exchange. Perfect isotope exchange will be
398 maximal at 50% initial sorption where 25% of the initial ^{14}C-pesticide (*i.e.* 50% of that
399 sorbed) should exchange with ^{12}C-pesticide and be released back into solution (Equation 3);
400 release of ^{14}C-pesticide will be a smaller proportion of initial radioactivity for both weaker
401 and stronger sorption (dashed line in Figure 3). In contrast, ^{14}C released by forced exchange
402 will increase non-linearly with proportion of initial radioactivity sorbed (Equation 4; solid
403 line in Figure 3). Figure 3 compares this ideal behaviour with measurements for the three
404 pesticides in the three soils following 56-days sorption. The measurements closely mirror the
405 expected form of relationships indicating that there is no major deviation from ideal
406 behaviour. ^{14}C-pesticide released by isotope exchange is smaller than predicted in all cases
407 and this can be attributed to continued increase in sorption during the exchange phase and/or
408 some small component of sorbed pesticide that does not participate in exchange. Presence of
409 dissolved organic carbon and/or colloidal material in solution is a potential confounding
410 factor as in most sorption studies. Any such material would be directly exchanged during

411 isotope exchange, whereas presence of dissolved organic carbon and colloids would be
412 reduced during forced exchange due to repeated replacement of the soil solution.
413 In conclusion, study of the long-term fate of pesticide residues in soil provides complex
414 experimental challenges. This study demonstrates that earlier proposals for use of isotope
415 exchange are constrained by the extremely long timescales needed to approach true sorption
416 equilibrium. Chlorotoluron and prometryn have typical degradation half-lives in soil in the
417 range of one to a few months which is of the same order as the times needed to approach
418 sorption equilibrium [20]. Time-dependence in pesticide sorption changes the availability of
419 residues for leaching over time and it is important that this effect is included within models
420 used to characterise risks to the environment from pesticide use [21]. The steep concentration
421 gradient established within the forced isotope exchange element of this study acted to
422 accelerate release of sorbed pesticide from the soil solid phase. Nevertheless, pesticide was
423 still being released from the soil solid phase after more than six months exposure to this
424 process. The forced exchange procedure was intermediate in efficiency of releasing sorbed
425 pesticide between field conditions, where desorption would occur more slowly [22], and a
426 conventional solvent extraction. This is confirmed by the release of further sorbed
427 chlorotoluron on extraction with methanol. The accepted definition of pesticide bound
428 residues excludes any fraction that can be extracted by a solvent [13]. Thus, the radioactivity
429 released by soil combustion at study termination provides the purest measure of truly
430 irreversible sorption (1-2% of applied radioactivity). This implies that abiotic processes of
431 bound residue formation are overwhelmingly reversible for the three compounds and the set
432 of soils studied here.

433 **Acknowledgements**

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435 acknowledged for funding this research.

436 **Supporting Information Available**

437 Supplementary data are provided relating to experimental methods (Table S1), analytical
438 methods (Tables S2 and S3), classical sorption isotherms measured after 24 h (Table S4),
439 pesticide sorption over time (Table S5), mass balances for initial sorption periods of 112 and
440 168 d (Table S6 supplementary to Table 2), isotope exchange for different sorption periods
441 (Figures S1-S3 supplementary to Figure 1), and forced exchange over time (Figures S4 and
442 S5 supplementary to Figure 2).

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Tables and Figures

443 **TABLE 1**. Characterisation of the three study soils.

Soil type	Texture	Sample location (OS grid ref.)	Date collected	pH (in $CaCl_2$)	Content (% w/w)			
					Sand	Silt	Clay	Organic matter
Blackwood	Loamy sand	SE50917051	29/05/2009	5.6	83	8	9	5.6
Andover	Clay loam	SE99105892	28/05/2009	6.9	49	32	19	5.1
Salop	Clay loamy sand	SP26616611	28/05/2009	5.8	54	26	20	5.2

444 **TABLE 2**. Mass balances for tubes containing initially ^{14}C-pesticide and with a 56-day sorption period (all values are % of initially applied

445 radioactivity with one standard deviation in parentheses)

Pesticide	Soil	In solution after sorption	Released by isotope exchange	Released by forced exchange	Released by solvent extraction	Residue remaining	Degradation	Unaccounted for
Chlorotoluron	Blackwood	23.58 (1.05)	11.21 (1.04)	50.79 (1.68)	5.48 (0.66)	2.27 (0.36)	3.96 (1.60)	2.71 (0.93)
	Andover	39.23 (1.93)	14.19 (1.22)	38.98 (0.66)	2.03 (0.23)	1.07 (0.12)	3.90 (2.31)	0.60 (0.86)
	Salop	10.84 (0.71)	5.09 (0.37)	64.99 (0.72)	10.77 (0.73)	2.19 (0.22)	1.57 (1.49)	4.55 (0.09)
Prometryn	Blackwood	46.96 (0.95)	18.72 (0.35)	32.37 (0.99)	1.09 (0.06)	0.29 (0.10)	2.94 (0.32)	0.57 (0.39)
	Andover	67.92 (1.16)	16.19 (0.56)	14.60 (0.29)	0.56 (0.05)	0.33 (0.06)	2.76 (0.11)	0.40 (0.57)
	Salop	63.10 (1.11)	14.80 (0.28)	19.49 (0.34)	1.21 (0.11)	0.34 (0.01)	1.95 (1.93)	1.06 (0.44)
Hexaconazole	Blackwood	42.94 (1.70)	20.74 (0.39)	34.18 (1.70)	0.89 (0.04)	0.18 (0.03)	0 (-)	1.07 (0.72)
	Andover	68.64 (0.76)	18.44 (1.67)	11.47 (0.49)	0.45 (0.09)	0.14 (0.01)	0 (-)	0.87 (2.58)
	Salop	64.36 (0.60)	18.05 (0.31)	13.36 (0.48)	1.23 (0.11)	0.19 (0.02)	0 (-)	2.81 (0.58)

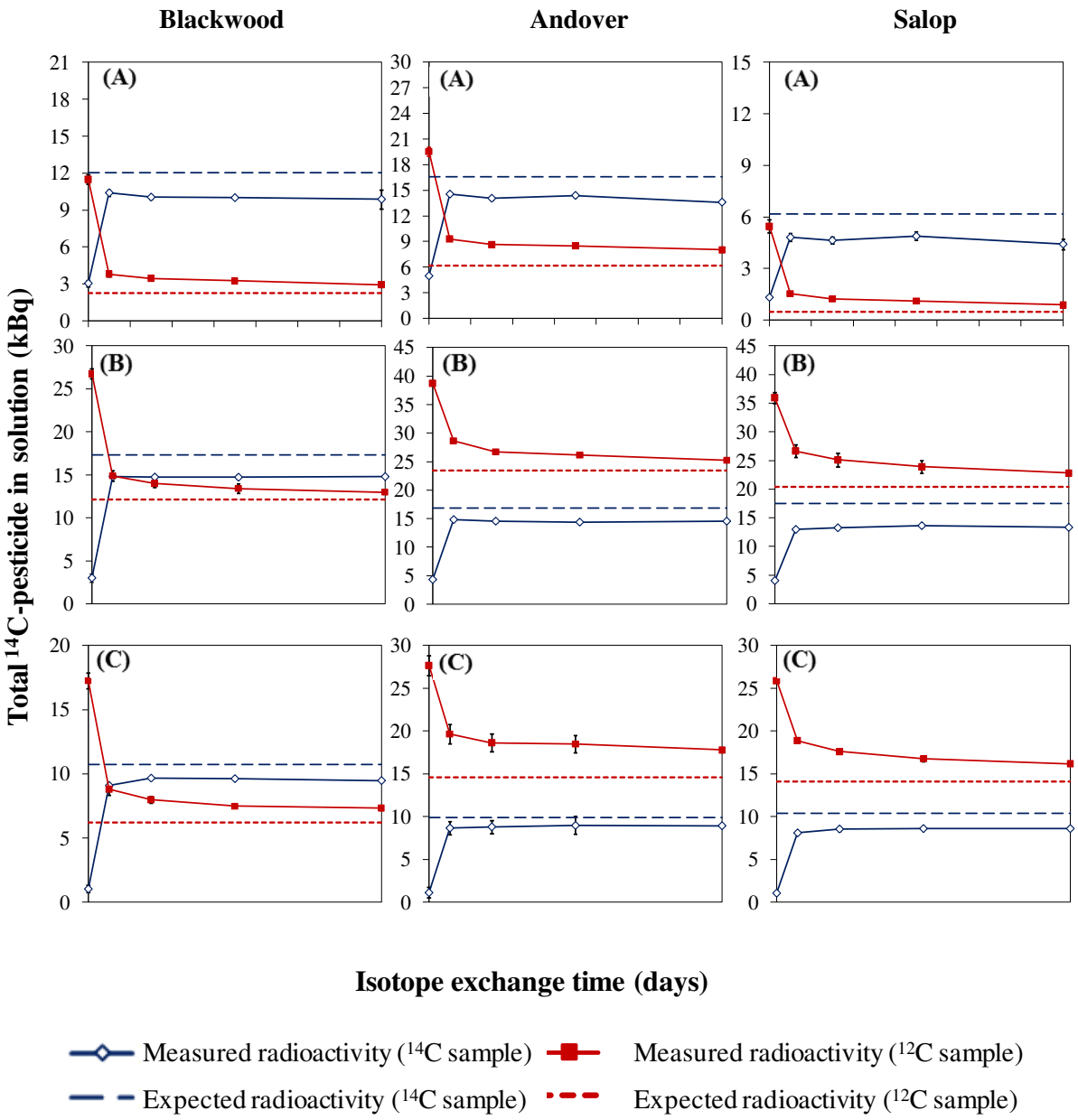


FIGURE 1. Pesticide isotope exchange over time following 56 days of sorption. The solid lines show the measured change in total ^{14}C-pesticide in solution over 14 days for the Blackwood, Andover and Salop soils for (A) chlorotoluron; (B) prometryn; and (C) hexaconazole. Diamonds represent the initially ^{14}C-pesticide samples, squares represent the initially ^{12}C-pesticide samples. Data are shown as the mean value with standard deviation of three replicates included. The dotted lines represent the radioactivity in solution that the measured radioactivities are expected to reach if there is perfect exchange, *i.e.* if sorption is a fully reversible process.

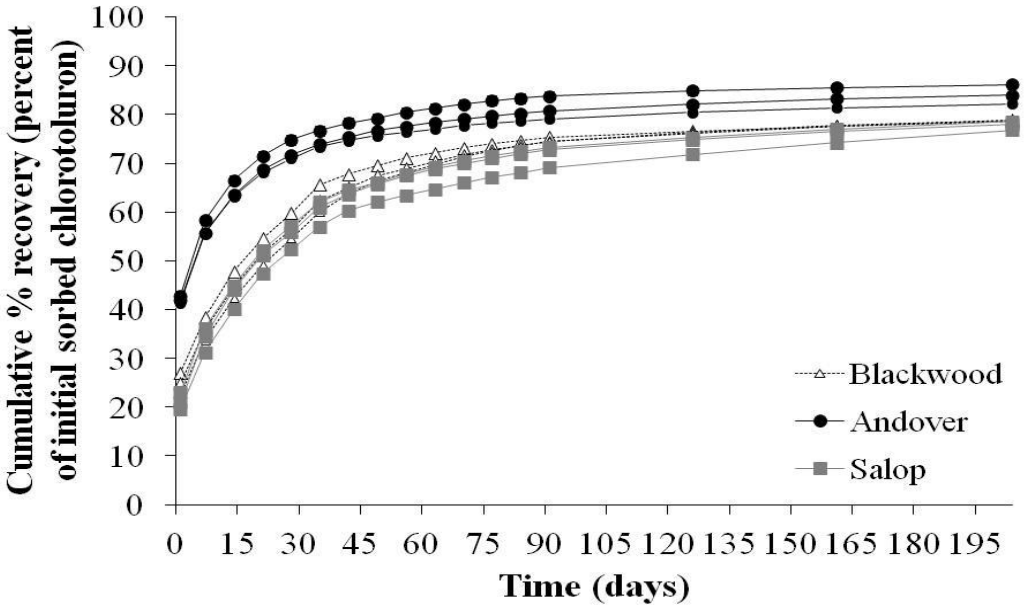
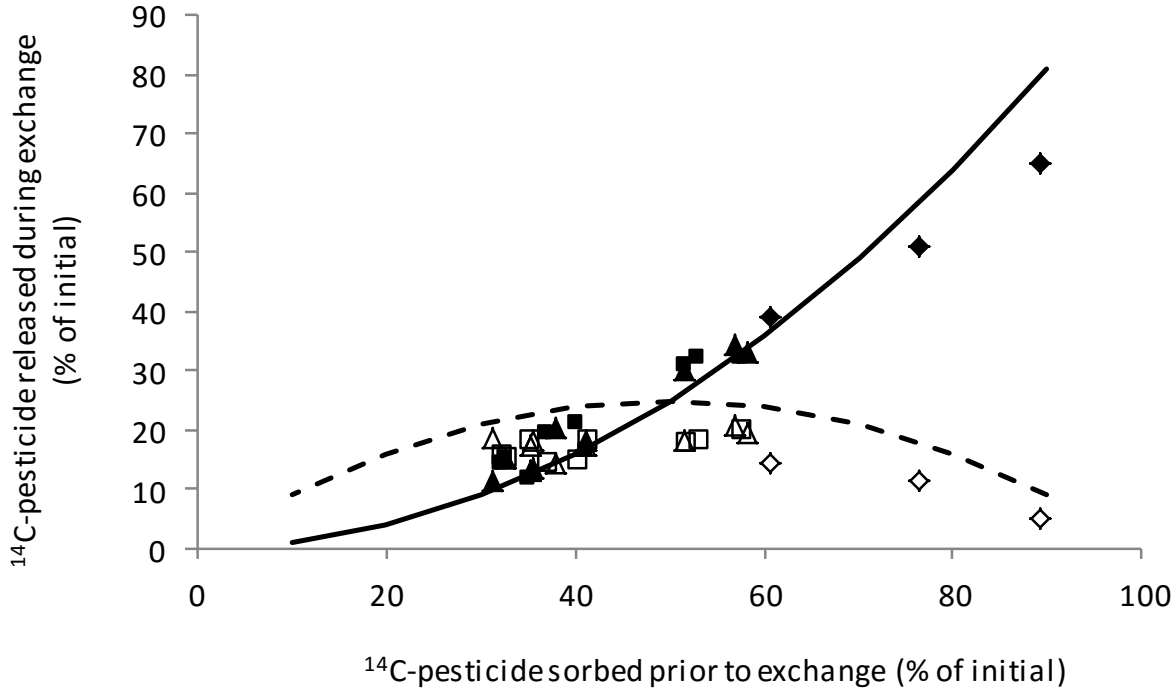


FIGURE 2. Cumulative percentage recovery of ^{14}C-chlorotoluron during forced isotope exchange. Recovery is expressed as a percentage of the 14C-chlorotoluron that is sorbed at the start of the forced exchange procedure. Samples are initially ^{14}C with 56-days sorption and isotope exchange for 14-days (3 replicates per soil).

454



455 **FIGURE 3**. Relationship between the amount of ^{14}C-pesticide sorbed immediately prior to
456 isotope exchange (i.e. after 56, 112 or 168 d) and the amount of ^{14}C-pesticide released during
457 subsequent phases of isotope exchange (open symbols) and forced exchange (solid symbols).
458 Data are averages of triplicate measurements in three soils and for the three equilibration
459 times for chlorotoluron (diamonds), prometryn (squares) and hexaconazole (triangles);
460 standard deviations are given in Tables 2 and S6. The lines show theoretical behaviour
461 assuming ideal and fully reversible exchange for the isotope exchange (dashed line) and
462 forced exchange (solid line), respectively.

463

Title

Long-term experiments to investigate irreversibility in sorption of pesticides to soil

Authors

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491 **Tables and Figures**

492 TABLE S1. Points of difference in experimental methodology for the three compounds

Parameter	Chlorotoluron	Prometryn	Hexaconazole
^{12}C pesticide grade	Fluka® analytical; 99.7% pure	Riedel-de-Haën® analytical; 99.2% pure	Fluka® analytical; 99.7% pure
^{14}C label position	Phenyl-U-^{14}C	Triazinyl-U-^{14}C	Triazolyl-U-^{14}C
^{14}C pesticide purity and activity	99.2% pure; 4.57 MBq mg^{-1}	98.1% pure; 4.16 MBq mg^{-1}	99.6% pure; 2.26 MBq mg^{-1}
Initial pesticide concentration (µg mL^{-1})	0.70	0.78	0.77
Soil:solution ratio	1:4 (5 g soil, 20 mL solution)	1:10 (2 g soil, 20 mL solution)	1:50 (0.5 g soil, 25 mL solution)
Initial mass of pesticide per tube (µg)	13.90	15.55	19.53
Initial radioactivity per tube (kBq; ^{14}C-pesticide only)	62.87	64.60	42.34
Initial sorption periods (d)	7, 14, 28, 56	28, 56, 112, 168	28, 56, 112, 168
Volume of supernatant exchanged (mL)	17.00	18.00	24.00
Isotope exchange period (d)	1, 3, 7, 14	1, 3, 7, 14	1, 3, 7, 14
Forced exchange period	17 sampling points over 204 days	14 sampling points over 175 days	12 sampling points over 145 days
Concentration of ^{12}C pesticide solution for forced exchange (µg mL^{-1})	40.01	29.85	14.91

493 TABLE S2. HPLC methods for analysis of ^{12}C samples

HPLC method parameter	Chlorotoluron	Prometryn	Hexaconazole
Method type	Gradient (Initial proportion of methanol at 65%, increasing to 100% after 10 minutes)	Gradient (Initial proportion of methanol at 20%, increasing to 100% after 15 minutes)	Gradient (Initial proportion of methanol at 20%, increasing to 100% after 15 minutes)
Mobile phases	A: HPLC-grade water (0.1% H_3PO_4)* B: HPLC-grade methanol	A: HPLC-grade water (0.1% HCOOH)[a] B: HPLC-grade methanol	A: HPLC-grade water (0.1% HCOOH)[a] B: HPLC-grade methanol
Flow rate (mL min^{-1})	0.8	1.0	1.0
Total run time (min)	22	23	23
Column	C_{18} Supelco Discovery (15 cm x 4.6 mm x 5 µm)	C_{18} Supelco Discovery (15 cm x 4.6 mm x 5 µm)	Agilent Technologies CN Zorbax (25 cm x 4.6 mm x 5 µm)
Column temperature (°C)	30.0	30.0	30.0
Injection volume (µL)	25.0	25.0	25.0
Retention time (min)	8.91	11.79	12.59
Wavelength (nm)	210	254	205
Limit of detection (µg mL^{-1})	0.01 (in 0.01M $CaCl_2$) 0.03 (in MeOH)	0.003 (in 0.01M $CaCl_2$) 0.006 (in MeOH)	0.18 (in 0.01M $CaCl_2$) 0.21 (in MeOH)

494 *Acidified with ortho-phosphoric acid (H_3PO_4)
495 [a]Acidified with formic acid (HCOOH)

496 TABLE S3. Radio-HPLC methods for analysis of ^{14}C samples

HPLC method parameter	Chlorotoluron	Prometryn	Hexaconazole
Method type	Gradient (initial proportion of acetonitrile at 20%, increasing to 100% after 15 minutes)	Gradient (initial proportion of acetonitrile at 20%, increasing to 100% after 15 minutes)	Gradient (initial proportion of acetonitrile at 20%, increasing to 100% after 15 minutes)
Mobile phases	A: HPLC-grade water (0.1% HCOOH)* B: HPLC-grade acetonitrile + Flowlogic 1:1 scintillation cocktail	A: HPLC-grade water (0.1% HCOOH)* B: HPLC-grade acetonitrile + Flowlogic 1:1 scintillation cocktail	A: HPLC-grade water (0.1% HCOOH)* B: HPLC-grade acetonitrile + Flowlogic 1:1 scintillation cocktail
Flow rate (mL min^{-1})	1.0	1.0	1.0
Total run time (min)	20	20	20
Column	Agilent Eclipse XDB-C_{18} (5 µm x 4.6 mm x 150 mm)	Agilent Eclipse XDB-C_{18} (5 µm x 4.6 mm x 150 mm)	Agilent Eclipse XDB-C_{18} (5 µm x 4.6 mm x 150 mm)
Column temperature (°C)	30.0	30.0	30.0
Injection volume (µL)	100.0	100.0	100.0
Retention time (min)	9.42	9.54	12.42
Wavelength (nm)	210	254	205
Limit of detection (µg mL^{-1})	0.22	0.24	0.44

497 *Acidified with formic acid (HCOOH)

TABLE S4. Freundlich sorption coefficient (k_f) and Freundlich exponent (n_f) from preliminary experiments to determine 24-h sorption isotherms for all pesticide-soil combinations

Pesticide	Blackwood soil		Andover soil		Salop soil	
	K_f (ml g^{-1})	n_f	K_f (ml g^{-1})	n_f	K_f (ml g^{-1})	n_f
Chlorotoluron	5.07	0.81	2.81	0.82	4.50	0.76
Prometryn	9.13	0.88	3.81	0.84	3.47	0.85
Hexaconazole	43.5	0.79	21.8	0.82	21.7	0.72

All experiments used ^{12}C-pesticide and the soil:solution ratios given in Table S1. Initial concentrations in solution were 0.5, 2.5, 5, 10, 20, 40 and 50 µg mL^{-1} for chlorotoluron, 0.3, 0.6, 1.25, 2.5, 5, 10 and 15 µg mL^{-1} for prometryn, and 0.2, 0.5, 1, 2, 4, 8 and 12 µg mL^{-1} for hexaconazole. All data were fitted to the Freundlich isotherm: $C_s = k_f \cdot {C_e}^{n_f}$, where C_s is concentration in the solid phase (µg g^{-1}) and C_e is concentration in the liquid phase (µg mL^{-1}).

507 TABLE S5. Sorption of ^{14}C-pesticide to the three soils over time (values expressed as % of initially applied pesticide ± 1 s.d.; n = 3)

Pesticide	Sorption period (d)	Blackwood soil	Andover soil	Salop soil
Chlorotoluron	7	71.10 ± 0.48	54.61 ± 1.57	77.14 ± 0.34
	14	70.28 ± 0.85	57.38 ± 1.38	81.39 ± 0.86
	28	71.19 ± 0.54	57.90 ± 0.84	85.72 ± 0.11
	56	76.42 ± 1.05	60.77 ± 1.93	89.16 ± 0.71
Prometryn	28	52.33 ± 1.63	31.90 ± 1.33	35.30 ± 1.70
	56	53.04 ± 0.95	32.08 ± 1.16	36.90 ± 1.11
	112	51.71 ± 1.13	32.61 ± 0.68	40.00 ± 1.34
	168	51.46 ± 0.18	32.76 ± 1.20	38.09 ± 0.33
Hexaconazole	28	53.58 ± 1.13	32.40 ± 1.88	33.33 ± 1.55
	56	57.06 ± 1.70	31.36 ± 0.76	35.64 ± 0.60
	112	57.34 ± 0.30	35.00 ± 2.40	41.22 ± 2.86
	168	58.26 ± 1.11	35.24 ± 0.50	41.28 ± 6.03

TABLE S6. Mass balances for tubes containing initially ^{14}C pesticide and with 112- and 168-d sorption period (all values are % of initially applied radioactivity as mean of three replications and with one standard deviation in parentheses)

Pesticide	Sorption period (d)	Soil	In solution after sorption	Released by isotope exchange	Released by forced exchange	Released by solvent extraction	Residue remaining	Degradation	Unaccounted for
Prometryn	112	Blackwood	48.29 (1.13)	18.35 (0.20)	30.93 (0.91)	1.14 (0.07)	0.54 (0.11)	0 (-)	0.75 (1.10)
		Andover	67.39 (0.68)	15.47 (0.30)	15.36 (0.48)	0.72 (0.07)	0.58 (0.07)	0 (-)	0.48 (0.61)
		Salop	60.00 (1.34)	15.12 (1.32)	21.12 (1.40)	1.89 (0.19)	0.48 (0.06)	0 (-)	1.39 (0.86)
	168	Blackwood	48.54 (0.18)	18.19 (0.27)	30.06 (0.56)	1.27 (0.03)	0.83 (0.27)	0 (-)	1.11 (0.22)
		Andover	67.24 (1.20)	15.13 (1.12)	15.19 (1.06)	0.89 (0.13)	1.35 (0.60)	0 (-)	0.20 (0.44)
		Salop	61.91 (0.33)	14.32 (0.42)	20.23 (0.26)	1.78 (0.14)	0.77 (0.04)	0 (-)	0.99 (0.44)
Hexaconazole	112	Blackwood	42.66 (0.30)	20.43 (0.34)	32.16 (0.40)	1.28 (0.04)	0.29 (0.00)	0 (-)	3.18 (0.25)
		Andover	65.00 (2.40)	18.76 (1.38)	11.77 (0.70)	0.68 (0.23)	0.25 (0.03)	0 (-)	3.55 (0.49)
		Salop	58.78 (2.86)	18.59 (0.73)	16.33 (1.93)	2.47 (0.62)	0.36 (0.03)	0 (-)	3.45 (0.46)
		Blackwood	41.74 (1.11)	19.48 (0.63)	33.08 (0.82)	1.89 (0.26)	0.39 (0.11)	0 (-)	3.43 (0.35)
		Andover	64.76 (0.50)	17.54 (0.63)	13.21 (1.30)	0.70 (0.06)	0.28 (0.01)	0 (-)	3.52 (0.63)
		Salop	58.72 (6.03)	17.22 (1.36)	18.07 (4.34)	2.18 (0.67)	0.50 (0.06)	0 (-)	3.31 (0.35)

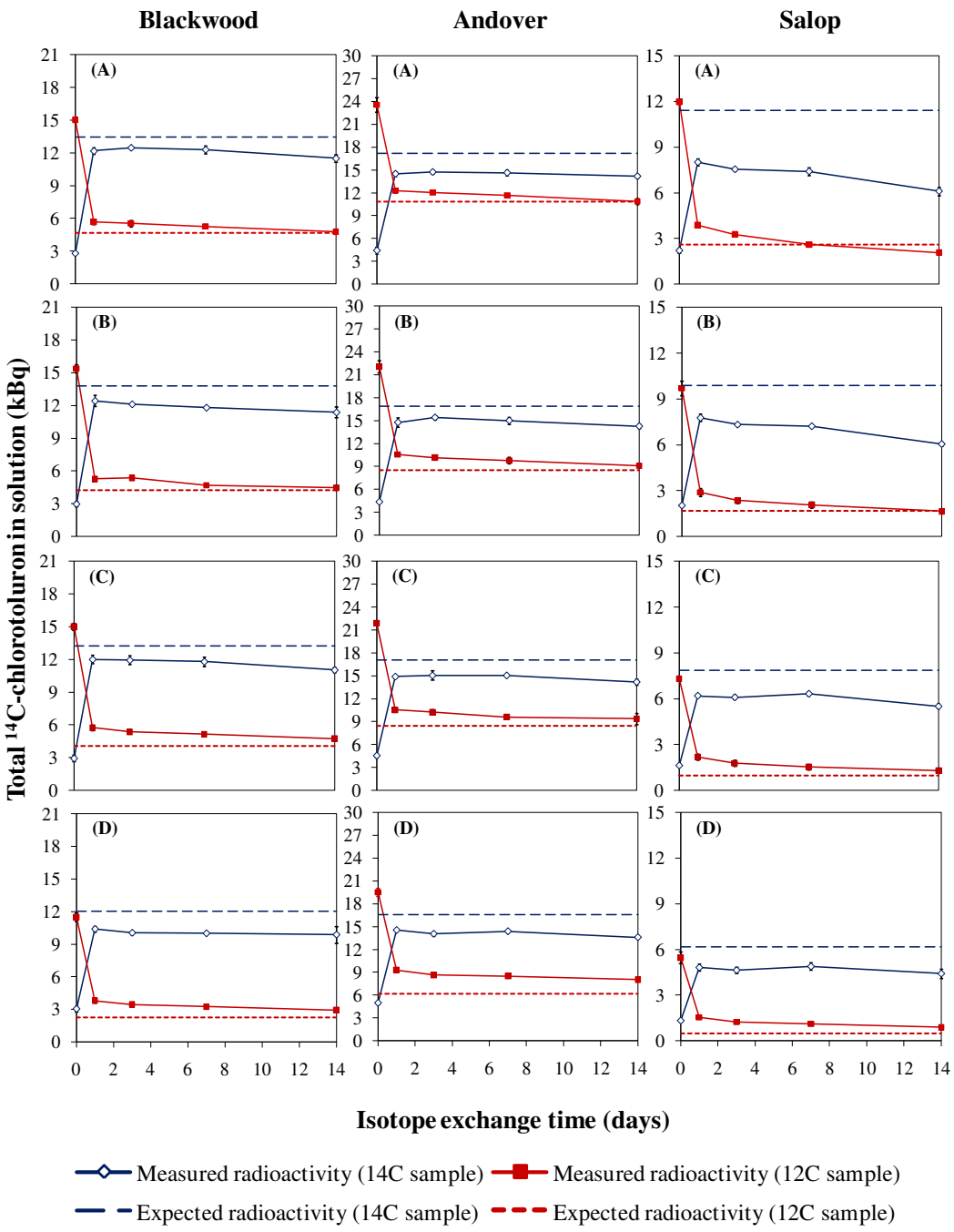

FIGURE S1. Isotope exchange over time for chlorotoluron. The solid lines show the change in total ^{14}C-chlorotoluron in solution over 14 days for the Blackwood, Andover and Salop soils after adsorption times of (A) 7 d; (B) 14 d; (C) 28 d; (D) 56 d. Diamonds represent the initially ^{14}C-chlorotoluron samples, squares represent the initially ^{12}C-chlorotoluron samples. The dotted lines represent the radioactivity in solution that the measured radioactivities are expected to reach if there is perfect exchange, i.e. if sorption is a fully reversible process.

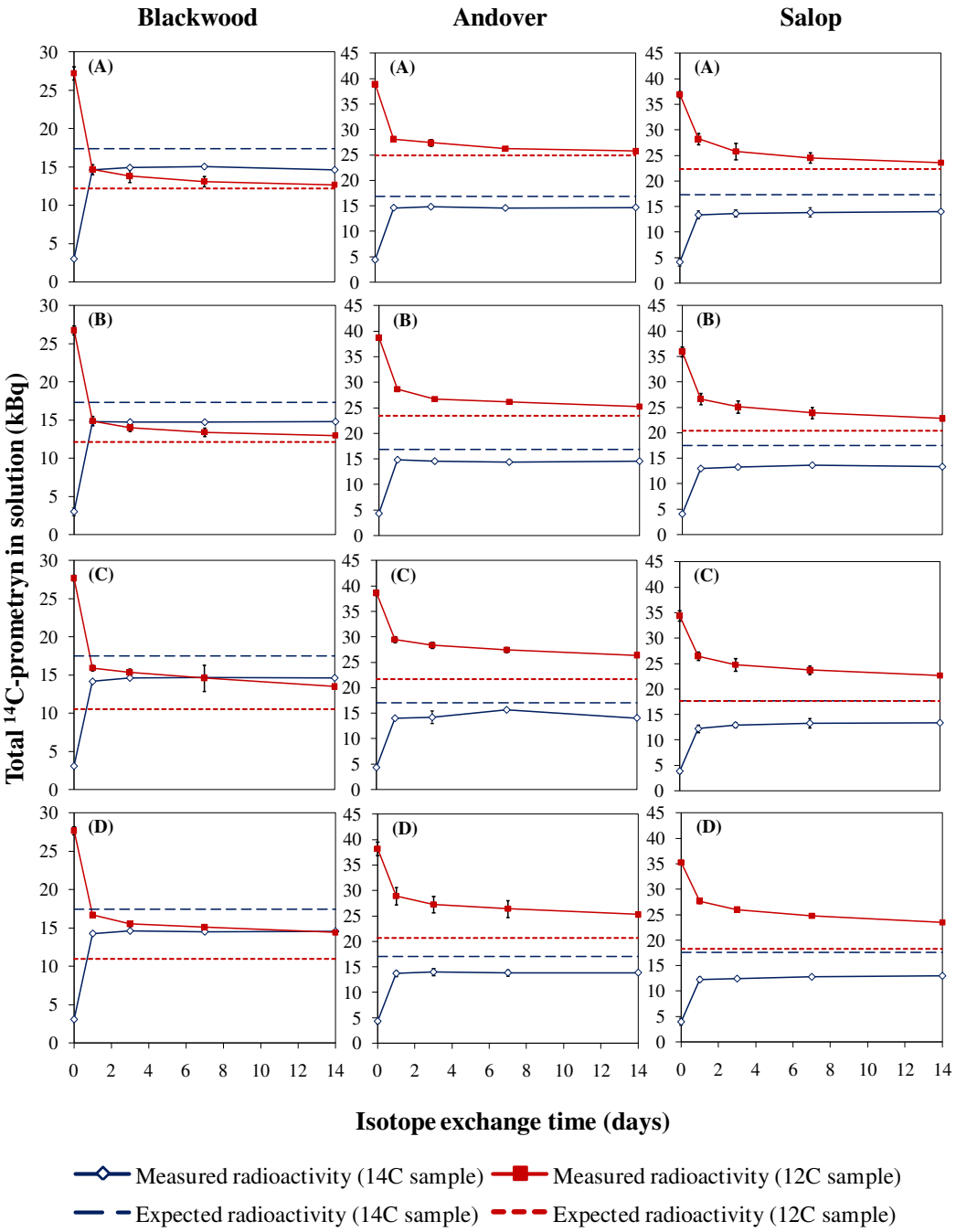

FIGURE S2. Isotope exchange over time for prometryn. The solid lines show the change in total ^{14}C-prometryn in solution over 14 days for the Blackwood, Andover and Salop soils after adsorption times of (A) 28 d; (B) 56 d; (C) 112 d; (D) 168 d. Diamonds represent the initially ^{14}C-prometryn samples, squares represent the initially ^{12}C-prometryn samples. The dotted lines represent the radioactivity in solution that the measured radioactivities are expected to reach if there is perfect exchange, i.e. if sorption is a fully reversible process.

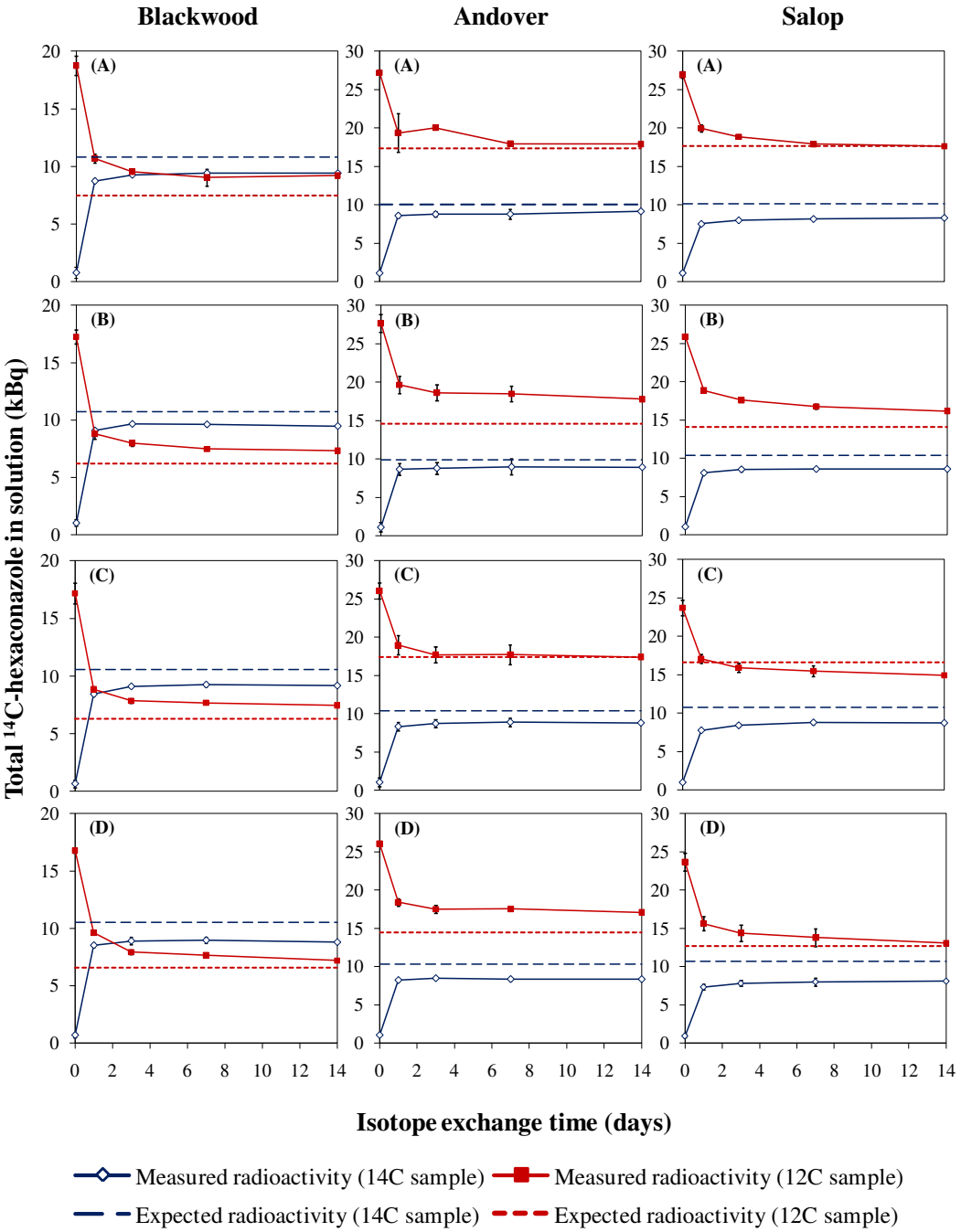

FIGURE S3. Isotope exchange over time for hexaconazole. The solid lines show the change in total ^{14}C-hexaconazole in solution over 14 days for the Blackwood, Andover and Salop soils after adsorption times of (A) 28 d; (B) 56 d; (C) 112 d; (D) 168 d. Diamonds represent the initially ^{14}C-hexaconazole samples, squares represent the initially ^{12}C-hexaconazole samples. The dotted lines represent the radioactivity in solution that the measured radioactivities are expected to reach if there is perfect exchange, i.e. if sorption is a fully reversible process.

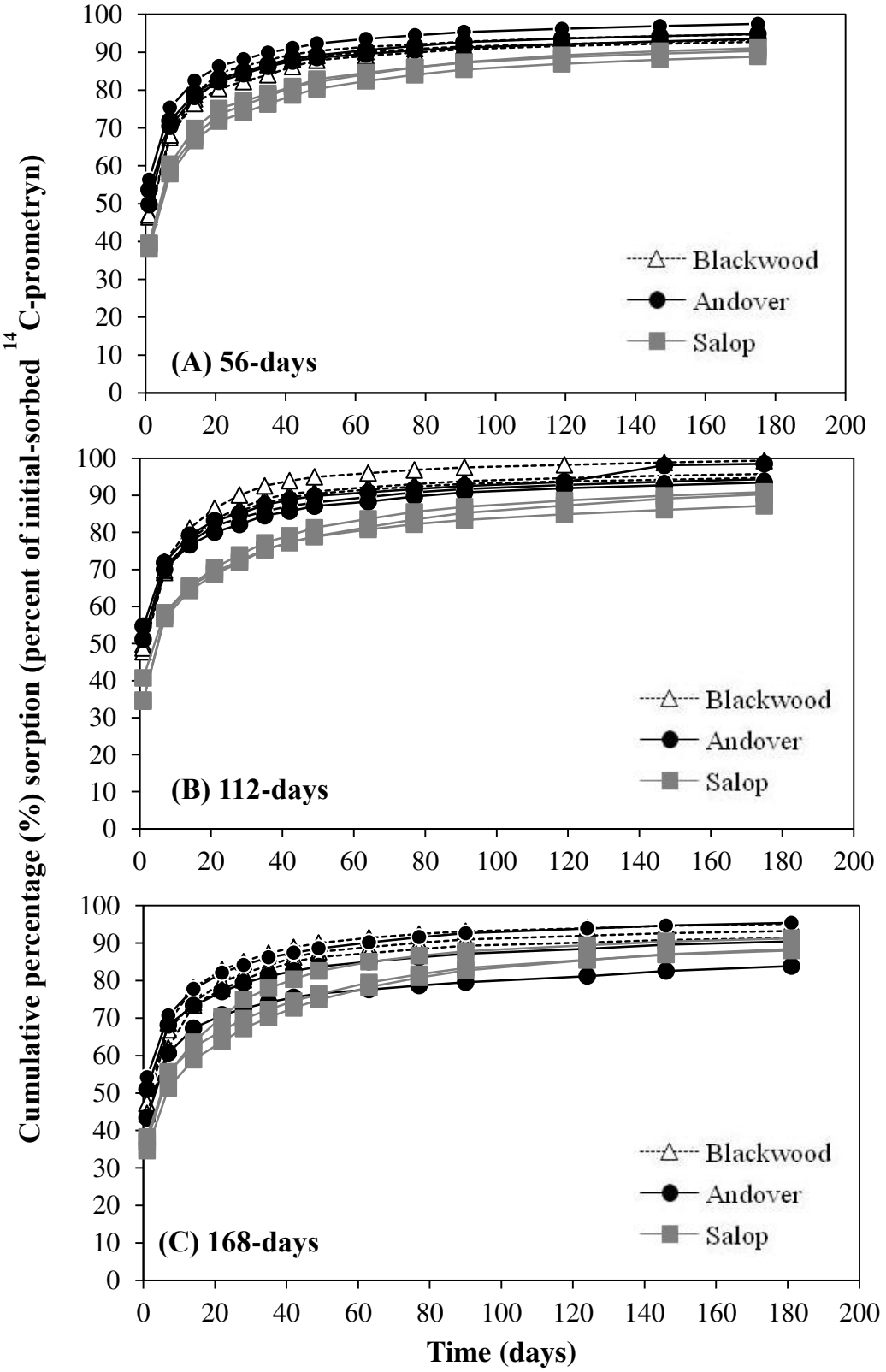

FIGURE S4. Cumulative recovery of ^{14}C-prometryn (% of initial-sorbed) using the forced isotope exchange method. Samples are initially ^{14}C with sorption for (A) 56 d, (B) 112 d, and (C) 168 d followed by isotope exchange for 14-days (three replicates per soil).

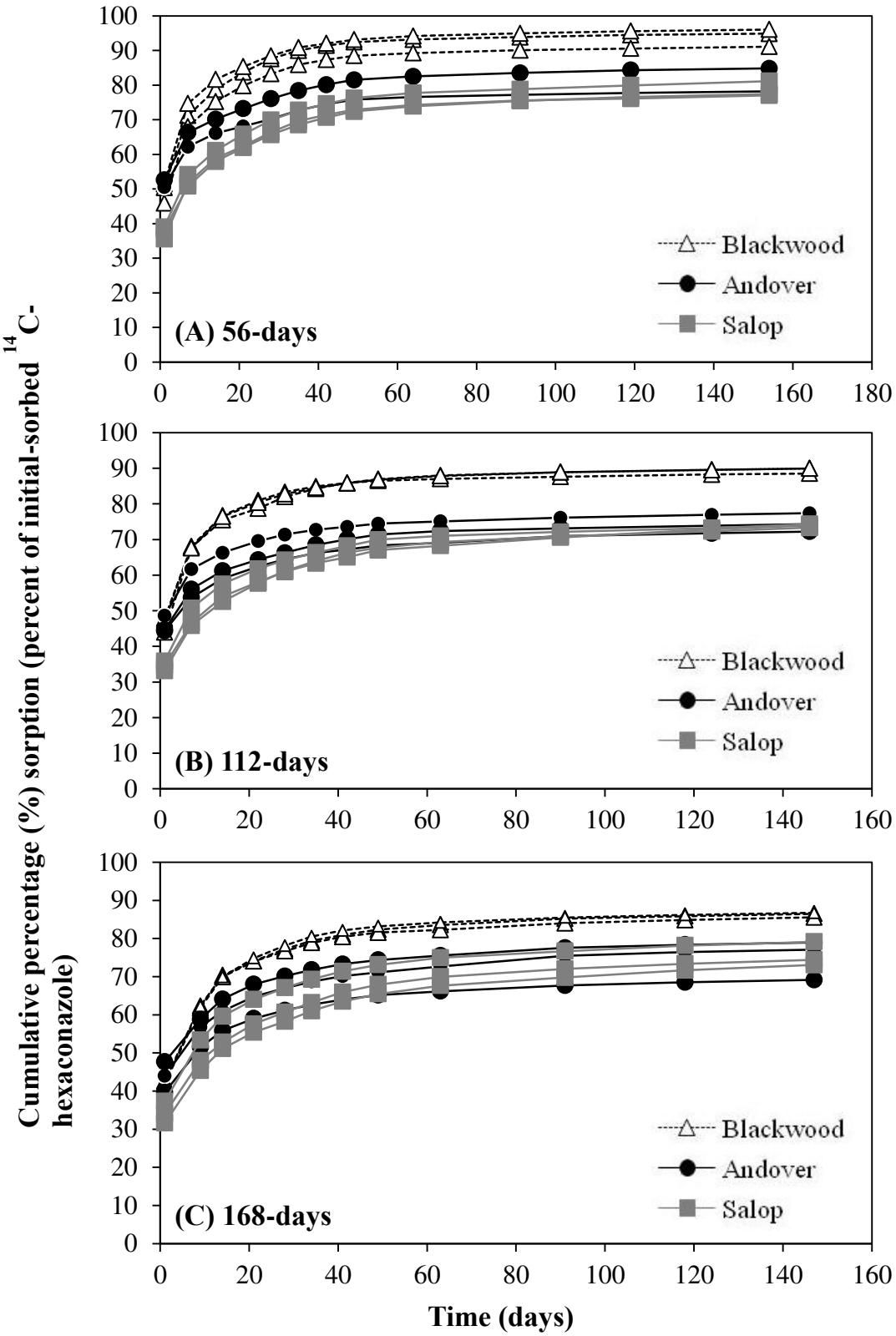

FIGURE S5. Cumulative recovery of ^{14}C-hexaconazole (% of initial-sorbed) using the forced isotope exchange method. Samples are initially ^{14}C with sorption for (A) 56 d, (B) 112 d, and (C) 168 d followed by isotope exchange for 14-days (three replicates per soil).

Highlights

- Isotope exchange method is extended to study sorption over periods up to 9 months
- Three pesticides in three soils took ca. 4 months to approach sorption equilibrium
- Release of sorbed pesticide after exchange approximated ideal behaviour
- Sorption processes under abiotic conditions were overwhelmingly reversible