

**INVESTIGATION INTO
IRREVERSIBLE SORPTION OF
PESTICIDES TO SOIL**

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ABSTRACT

The importance of extremely slow retention and release has superseded the notion that sorption of pesticides to soil is an instantaneous and reversible process. A fraction of sorbed pesticide is also often reported to bind irreversibly to the soil matrix. This has important implications for pesticide mobility and bioavailability. It is essential to understand sorption phenomena to allow accurate prediction of pesticide fate within the soil environment.

This thesis describes the result of applying a sequential extraction procedure, based on the principles of isotope or “self-exchange”, to nine pesticide/soil systems. The significance of irreversible sorption in controlling pesticide mobility was assessed using isotope exchange (^{12}C and ^{14}C) to characterise pesticide exchange kinetics *in-situ* over protracted time-scales. Sequential extraction increased in harshness in the order: isotope exchange < forced isotope exchange < solvent extraction. Three pesticides (one neutral, one basic, one acidic) and three temperate, arable soils (ranging in texture and pH) were studied. A three-site sorption model was developed to further interpret the data obtained. Although results showed the experimental design of the isotope exchange technique was not powerful enough to identify whether remaining sorbed pesticide was participating in slowly reversible or irreversible sorption, the forced isotope exchange procedure was able to provide an indication of amounts of pesticide not participating in exchange between the soil and solution. Under abiotic conditions, only minimal amounts of initial-applied pesticide were found to take part in irreversible binding. Soil combustion quantified irreversible sorption in the order: chlorotoluron ($\leq 2.27 \pm 0.36\%$) > prometryn ($\leq 1.35 \pm 0.60\%$) > hexaconazole ($\leq 0.50 \pm 0.06\%$). Varying the soil composition had little effect on amounts of irreversibly sorbed pesticide, probably due to the small amounts of irreversible sorption observed overall. These results suggest that the vast majority of sorbed chlorotoluron, prometryn and hexaconazole (in the parent form) participated in very slow but reversible binding, a result also confirmed by the three-site sorption model.

Pesticide sorption behaviour is a complex process. Although sorption phenomena are still not fully understood, these results provide a greater insight into the significance of irreversible binding for predicting pesticide fate.

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AUTHOR'S DECLARATION

The work in this thesis was undertaken as a PhD student in the EcoChemistry team at the Food and Environment Research Agency (FERA), and also in part at the University of York (October 2008 – May 2012). The research was funded by the Engineering and Physical Sciences Research Council (EPSRC) and also by Syngenta Ltd. (Jealott's Hill International Research Centre). The work presented in this thesis is original to the best knowledge of the author. Where this is not the case, appropriate citations and acknowledgements have been given.

CHAPTER 1

INTRODUCTION

A diverse range of substances is encompassed by the term pesticide, though the vast majority are typically both synthetic and organic in nature. Thousands of synthetic pesticide products are currently in existence, containing in excess of 1000 different chemicals and combinations thereof (Karabelas *et al.*, 2009). Pesticide products are thus categorised according to shared properties, with classification frequently by target, mode (or period) of action or chemical structure as tabulated by Arias-Estévez *et al.* (2008). Pesticides dispersed within the environment prevent, eliminate, repel or mitigate approximately one million species of harmful insects, ten thousand phytophagous species, 1800 weed species and 1500 plant diseases, all of which would otherwise have the potential to cause serious economic loss and crop failure throughout the world (Cremlyn, 1979; Arias-Estévez *et al.*, 2008; Karabelas *et al.*, 2009). There is no doubt, therefore, that the use of pesticides plays a vital role in optimising food productivity. The enormity of their role becomes ever more crucial when considering recent predictions that by 2030 the world will need to produce 50% more food with the total world population expected to reach 9 billion by 2050, and life expectancy also anticipated to increase (Carvalho, 2006; Beddington, 2009). Such a rising demand for food, which has to be met within heightened constraints on land, poses a great challenge for science and technology (Beddington, 2009). However, though accomplishing such a feat is possible with pesticide use, doing so in a sustainable manner, without detriment to the environment, is where the greatest challenge lies.

Whilst the idea of using chemicals to control pests is not new, pesticides are now used to a much greater extent than in the past as the quantity, concentration and frequency of pesticide use have all increased (Wilson and Tisdell, 2001). In the European Union several million tons of pesticides are applied to land each year, which has led to the frequent detection of pesticides in both surface and groundwaters (Sattler *et al.*, 2007; Shriks *et al.*, 2010). Research from the Environment Agency (2006) found pesticides in over a quarter of groundwater monitoring sites in the UK in 2004. As groundwater represents approximately 98% of available fresh water on the planet (de Wilde *et al.*,

2008), the diffuse pollution of ground and surface water resources caused by pesticides poses a significant risk to the quality of drinking water (Van Dijk-Looijaard and Van Genderen, 2000; Lapworth and Gooddy, 2006; Lerner and Harris, 2009). Of the twelve substances identified as persistent organic pollutants by the Stockholm Convention (2004), nine were pesticides. When the Convention was amended in 2009 to include an additional nine persistent organic pollutants, six of these were also pesticides. The listed compounds possessed a particular range of physical and chemical properties such that once released into the environment they were found to: (i) persist for exceptionally long periods of time; (ii) bioaccumulate; (iii) become widely distributed throughout the environment (including detection in areas where they had never been used); and, (iv) were toxic to both humans and wildlife. One well known example was that of dichlorodiphenyltrichloroethane (DDT), which was the cause of worldwide environmental non-target pesticide poisoning involving a variety of organisms, both aquatic and terrestrial due to its persistent and lipophilic nature (Carson, 1962; Nakamaru *et al.*, 2003; Mispagel *et al.*, 2004; Beard, 2006; Bachetta and Mantecca, 2009; Barnhoorn *et al.*, 2009; Ssebugere *et al.*, 2009). Although not all pesticides exhibit such extreme environmental effects, many of the problems created by pesticides have received substantial attention (Galt, 2008).

The continuous detection of pesticides in UK surface waters is a major concern for environmental agencies and may jeopardise their ability to meet targets set within the European Water Framework Directive. Legal limits concerning pesticides in drinking water were introduced by the Drinking Water Directive (80/778/EEC) (Anon., 1980), which stipulated that a single pesticide should not be present in drinking water in quantities above $0.1 \mu\text{g L}^{-1}$, and total pesticide concentrations should not exceed $0.5 \mu\text{g L}^{-1}$. These limits were later reconfirmed by the European Water Framework Directive (2000/60/EC) (Anon., 2000). The recognition of groundwater as a valuable natural resource (as it is the main source of drinking water in many regions across the European Union) led to the establishment of the Groundwater Directive (2006/118/EC) (Anon., 2006), in which the same limit of $0.1 \mu\text{g L}^{-1}$ per pesticide was also applied to groundwaters. The total number of legally marketed active substances in the European Union at present is 276, of which 194 existed before 1993 and 82 are new active substances (Karabelas *et al.*, 2009). There are also another 31 active substances under evaluation at present. The list that is currently authorised includes

24% insecticides, 32% herbicides and 28% fungicides (Karabelas *et al.*, 2009). To ensure that legal standards are met, it has become increasingly important that the fate and behaviour of active substances applied within terrestrial systems are understood in order to prevent detrimental impacts to environmental systems.

All pesticides detected in groundwater and most residues present in surface water enter via the soil. It is thus vital that the dynamics of pesticide interaction with soil are studied in order to accurately predict their environmental fate (Führ *et al.*, 1998; Arias-Estévez *et al.*, 2008). Sorption is recognised as a key retention mechanism influencing pesticide fate by reducing pesticide mobility as well as limiting bioavailability (Gao *et al.*, 1998; Spark and Swift, 2002; Boivin *et al.*, 2005; Wang and Keller, 2009). Fate, transport and risk assessment models all contain terms for sorption; therefore, an understanding of pesticide sorption dynamics is also crucial to their success (Pignatello and Xing, 1996). For many pesticides, it is generally accepted that sorption to soil increases as a function of pesticide-soil contact time, a phenomenon often termed aged or time-dependent sorption (Gevao *et al.*, 2000; Reid *et al.*, 2000; Beulke *et al.*, 2004; Regitano *et al.*, 2006). Furthermore, there is substantial evidence to suggest that a fraction of aged pesticide residues can become irreversibly bound to the soil matrix (Dec *et al.*, 1997; Mordaunt *et al.*, 2005; Wanner *et al.*, 2005). Two extreme examples of pesticides exhibiting irreversible sorption are paraquat and diquat, both of which are non-selective contact herbicides. On reaching the soil, paraquat and diquat become rapidly and strongly sorbed to clay minerals within the soil matrix; as a result, they become biologically and chemically inert and are thus, effectively removed from the environmental system (INCHEM, 1984). Irreversible sorption has also been observed for many other pesticides and is often suggested as a cause of hysteresis in laboratory studies (Celis and Koskinen, 1999a).

There is currently however, no standard laboratory method available with the ability to measure irreversibility in pesticide adsorption to soil. Difficulty arises from the fact that it is hard to discriminate experimentally between kinetically-controlled (time-dependent) sorption and irreversible sorption, both of which also require prohibitive experimental times to be observed. On a mechanistic basis, bound residues are formed by abiotic interactions that operate between the pesticide and soil. The two mechanisms directly facilitating the formation of bound residues are: (i) covalent bonding; and, (ii) physical entrapment (Dec *et al.*, 1997; Huang and Weber, 1997;

Kan *et al.*, 2000). Both the parent molecule and/or its metabolite(s) have the ability to bind to the soil via these mechanisms. Biotic interactions are also known to play a significant role in bound residue formation, though this occurs on an indirect basis (Rice *et al.*, 2002). By the microbial route of bound residue formation, microbial action acts as a transitional process, transforming the parent molecule into its more reactive chemical forms (metabolites) (Barriuso and Benoit, 2006). Due to their higher chemical reactivity, metabolites are able to take part in additional covalent bonding and physical entrapment interactions and thus augment the formation of bound residues. The definition of bound residues includes the parent molecule and its metabolite(s) (Roberts *et al.*, 1984). However, as most studies are carried out using ^{14}C -labelled pesticide, the chemical identity i.e. the relative proportions of parent and non-parent material forming bound residues is often uncertain (Kästner *et al.*, 1999; Barraclough *et al.*, 2005).

The work in this PhD explicitly focuses on bound residues formed under abiotic conditions. This is achieved via elimination of the microbial community and enables the long-term study of parent pesticide molecules (true hysteresis) in the test soil systems. Since the direct mechanisms of formation remain uninhibited, it is anticipated that the quantities of irreversible sorption, specifically involving the parent molecule, will be revealed. The methods used to measure bound pesticide residues are based on Celis and Koskinen's (1999a; 1999b) isotope exchange technique, described in detail in Chapters 4 and 5. Their method uses both ^{12}C - and ^{14}C -pesticide to measure the quantity of pesticide not participating in exchange between the soil and solution *in-situ*. The main objectives of this work are:

- (i) review the current knowledge concerning pesticide sorption phenomena;
- (ii) determine the significance of irreversible sorption for a range of parent pesticide molecules;
- (iii) develop an experimental method(s) with the ability to discriminate between pesticide taking part in slow, reversible and irreversible sorption; and,

- (iv) use the data from such experiments to gain an insight into how to best account for the process within mathematical models for leaching and ground water exposure.

This thesis comprises seven chapters; brief descriptions of each chapter's contents are given below.

Chapter 2 synthesises existing knowledge of pesticide sorption phenomena. The mechanisms responsible for, and factors that influence, sorption are discussed. Equilibrium, non-equilibrium (time-dependent sorption or ageing) and irreversible (bound residues, non-extractable residues) sorption phenomena are considered, as well as approaches used to measure and model the processes. This chapter also identifies gaps in the existing knowledge.

Chapter 3 provides some background information concerning the experimental test systems, analytical methods to quantify the study compounds and preliminary work carried out prior to beginning the experimental procedures. The key chemical and environmental fate properties of the studied pesticides are identified as well as the main properties of the studied soils. The methods and results for selection of optimal soil:solution ratios and determination of Freundlich distribution coefficients and Freundlich exponents are presented for all three pesticides and soil types.

Chapter 4 presents the results of implementing an isotope exchange technique, after increasing lengths of adsorption time (7, 14, 28 and 56 days), in order to differentiate between slowly reversible sorption and irreversible sorption of chlorotoluron to the three test soils. A forced isotope exchange technique and solvent extraction procedure were then used sequentially to assess desorption (by "self-exchange") of chlorotoluron from the study soils after 56-days adsorption and 14-days isotope exchange. A full mass balance is included.

Chapter 5 reports the results of performing the isotope exchange technique after significantly longer periods of adsorption (28, 56, 112 and 168 days) for the pesticides prometryn and hexaconazole. Forced isotope exchange results are also presented after increasing periods of adsorption time (56, 112 and 168 days) and 14-

days isotope exchange for both pesticides. Full mass balances are presented for both pesticides after 56, 112 and 168 days of adsorption for each.

Chapter 6 describes the development and implementation of a three-site model to interpret Celis and Koskinen's (1999a; 1999b) isotope exchange data, as well as the chlorotoluron, prometryn and hexaconazole isotope exchange and forced isotope exchange data. Model estimates of the irreversible fractions are included and compared with the measured values.

Chapter 7 summarises the main conclusions and describes the wider implications of the reported findings. Recommendations for further research in the area are also proposed.

CHAPTER 2

SORPTION OF PESTICIDES TO SOIL AND ITS RELEVANCE FOR PESTICIDE FATE IN THE ENVIRONMENT

2.1. Introduction

In recent years a great deal of research has been carried out in order to assess the environmental fate of pesticides in soil systems. The focus has largely been centred on sorption phenomena due to their important role in control of both mobility and bioavailability of pesticides in soil systems. This chapter reviews the adsorption-desorption process in detail and includes mechanisms of binding, measurement of sorption, factors that control sorption, non-ideal sorption behaviour (time-dependent sorption, non-equilibrium sorption, ageing) and irreversible sorption phenomena (bound residues, non-extractable residues) with an attempt also made to identify gaps and research needs.

2.2. Mechanisms of sorption

Adsorption is defined by Koskinen and Harper (1990) as:

“...the attraction and accumulation of molecules at the soil-water or soil-air interface, leading to the formation of molecular layers on the surface of soil particles.”

According to the properties of solutes and substrates, there are various molecular binding mechanisms potentially responsible for interactions taking place between organic compounds and soil components (Calvet, 1989). Mechanisms of binding may include van der Waals forces, hydrophobic bonding, hydrogen bonding, charge transfer, ligand exchange, covalent bonding and ionic bonding.

Isolating the specific binding mechanism(s) responsible for pesticide retention in a given soil system is difficult however (Calvet, 1989; Kah and Brown, 2006). Most binding mechanisms arise from an interaction of forces and factors, which incorporate

a diverse range of solute molecular structures and adsorbent properties of soil constituents (Calvet, 1989; Kah and Brown, 2006). Direct experimental evidence of a particular mechanism is also quite rare, often confining many to propose a hypothesis and thus only postulate the intermolecular interactions involved (Calvet, 1989). Furthermore, although the retention reactions themselves tend to be relatively rapid, a pesticide may be initially retained by rapid low-energy bonding mechanisms and subsequently converted to more stable high-energy bonding mechanisms over time (Koskinen and Harper, 1990). Nevertheless, the extensive volume of published results, continually enhanced by new research, allows some partial conclusions to be drawn on the subject (Calvet, 1989). As a consequence, retention mechanisms have been reviewed in detail during recent decades (Hamaker and Thompson, 1972; Calvet, 1989; Koskinen and Harper, 1990; Senesi, 1992; Gevao *et al.*, 2000; Kah and Brown, 2006) so only their principal features will be summarised here.

2.2.1. Van der Waals interactions

Van der Waals forces are weak interactions that occur and exist in addition to stronger binding forces in all adsorbent-adsorbate interactions (Senesi, 1992; Gevao *et al.*, 2000). Since they are short-range dipolar or induced dipolar interactions, van der Waals forces decay rapidly with distance and their contribution to adsorption is thus greatest for those ions in closest contact with the adsorbent's surface (Gevao *et al.*, 2000). Van der Waals forces are also additive; their contribution increases with the size of the interacting molecule and with its ability to adapt to the adsorbate surface (Senesi, 1992; Kah and Brown, 2006). They are of particular importance in the adsorption of non-ionic and non-polar pesticides on suitable sites of soil constituents (Senesi, 1992). Although scarce experimental evidence is available, this mechanism was proposed as contributing to the adsorption of benzonitrile and DDT (Pierce *et al.*, 1971).

2.2.2. Hydrophobic bonding

Hydrophobic retention may be regarded as a partitioning between a solvent and a non-specific surface, rather than an active adsorption mechanism (Gevao *et al.*, 2000; Kah and Brown, 2006). Hydrophobic bonding is considered the main mechanism responsible for retention of non-polar pesticides by active hydrophobic sites of humic

substances or clay (Kah and Brown, 2006). Hydrophobic sites include: (i) aliphatic side-chains or lipid portions; (ii) lignin-derived moieties with high carbon content; and, (iii) a small number of polar groups of macromolecules contained within humic substances (Senesi, 1992). Hydrophobic adsorption by soil organic matter and humic substances is suggested as an important bonding mechanism for DDT and other organochlorine pesticides (Pierce *et al.*, 1971), as well as some ionisable pesticides in their molecular form, such as the weakly basic sterol herbicide prometryn (Khan, 1982).

2.2.3. Hydrogen bonding

Hydrogen bonds (or H-bonds) are formed by the interaction between the chemical structure of organic compounds and the nature of soil constituents they are adsorbed to (Calvet, 1989). Hydrogen bonds are able to form, for example, between the numerous oxygen and hydroxyl-containing functional groups present within humic substances and pesticides that contain suitable groups (Senesi, 1992). Pesticide molecules may however, compete strongly with water for these binding sites (Gevao *et al.*, 2000). Hydrogen bonding is suggested to play a vital role in the adsorption of several non-ionic polar pesticides, as discussed by Gevao *et al.* (2000).

2.2.4. Charge transfers

Charge transfer processes are formed via electron donor-acceptor mechanisms; electrons are transferred from an electron-rich donor to an electron-deficient acceptor (Gevao *et al.*, 2000). Humic substances contain both electron-deficient moieties e.g. quinines and electron-rich centres e.g. diphenols within their chemical structure (Senesi, 1992). The formation of charge-transfer complexes with soil humic substances has been reported for the bipyridilium pesticides, paraquat and diquat; evidence for this interaction comes from infra-red (IR) spectroscopy (Gevao *et al.*, 2000). Likewise, a shift towards lower frequencies in the IR spectrum observed between several *s*-triazines and humic acid also provides experimental evidence for the formation of charge-transfer complexes between methoxytriazines and soil organic matter (Gevao *et al.*, 2000).

2.2.5. Ligand exchange

Adsorption by ligand exchange involves the replacement of relatively weak ligands e.g. H₂O partially holding polyvalent cations associated with soil organic matter, by suitable adsorbent molecules such as *s*-triazines and anionic pesticides (Senesi, 1992; Gevao *et al.*, 2000). The substitution may also be facilitated by an entropy change, if a pesticide molecule succeeds in replacing several H₂O molecules associated with one or several complexed metal ion(s) (Gevao *et al.*, 2000).

2.2.6. Covalent bonding

The formation of covalent bonds between pesticides (and/or their metabolite(s)) and soil humic substances is often mediated by chemical, photochemical or enzymatic catalysts and leads to stable, mostly irreversible incorporation of the adsorbed pesticide into the soil (Senesi, 1992; Gevao *et al.*, 2000). Covalent bonding is thus regarded as the principal binding mechanism responsible for the formation of bound residues. Loiseau and Barriuso (2002) and Mordaunt *et al.* (2005) both propose that a ‘truly bound’ residue is one that is covalently bonded to the soil, usually through C-C, C-O, C-N or N-N bonding between the pesticide and humic substances. The subject of bound residues is dealt with further in section 2.6. Pesticides with the greatest tendency to bind covalently to humic substances have similar functionalities to the components of the soil humus (Gevao *et al.*, 2000). For instance, pesticides that structurally resemble phenolic compounds are able to covalently bind to soil humic materials (Gevao *et al.*, 2000). Compound classes that can bind covalently to soil humic material without the involvement of microbial activity include acylanilides, phenylcarbamates, phenylureas, dinitroaniline herbicides, nitroaniline fungicides and organophosphate insecticides (Senesi, 1992; Gevao *et al.*, 2000).

2.2.7. Ionic bonding (ion exchange)

Ionic bonding is a nonspecific electrostatic interaction that can involve either anionic or cationic pesticide forms. Ionic bonding involves ionised or easily ionisable carboxylic and phenolic hydroxyl groups of humic substances (Senesi, 1992).

2.2.7.1. Cation exchange

Adsorption by cation exchange applies only to those pesticides which are in cationic form or can accept a proton and become cationic e.g. basic compounds at $\text{pH} < \text{p}K_a$ (Senesi, 1992; Kah and Brown, 2006). Cation exchange can occur at negatively charged sites on clay mineral surfaces occupied by a metal ion (Kah and Brown, 2006). Cation exchange can also occur between positively charged bipyridilium pesticides (e.g. diquat and paraquat), which bind to soil humic substances by ion exchange via their cationic group (Gevao *et al.*, 2000). They form highly stable and unreactive bonds with the carboxyl groups of the humic substances (Gevao *et al.*, 2000). However, not all negative sites on organic matter seem to be positionally available to bind large organic cations, probably due to steric hindrance (Senesi, 1992).

2.2.7.2. Anion exchange

Anion exchange is the attraction of an anion to a positively charged site on the soil surface and involves the exchange of one ion for another at the binding site (Kah and Brown, 2006). Anion exchange is not usually significant in temperate soils as clays and organic matter are generally either non-charged or negatively charged; however, it is an important binding mechanism for podzols, which contain positively charged sesquioxides of aluminium and iron and are able to adsorb ions such as phosphate (PO_4^{3-}) (Saunders, 1965). Anion exchange is also a significant binding mechanism occurring in tropical soils that contain significant quantities of aluminium and iron (hydr)oxides (Kah and Brown, 2006).

2.3. Measurement and characterisation of equilibrium sorption

The adsorption of pesticides to soils, by one or more of the various mechanisms described above, controls the availability of pesticide in the soil solution. It is thus essential that the partitioning of a pesticide between solid and aqueous phases is determined as accurately as possible in order to assess the potential mobility and bioavailability of a pesticide in a given soil system (Kah and Brown, 2007). It is often assumed that a characteristic and reversible ratio between the chemical in solution and the chemical adsorbed onto the soil is established instantaneously. This is referred to as sorption equilibrium. Levels of pesticide retention by soils are most commonly determined using the batch-equilibrium method, outlined by OECD

guideline 106 (OECD, 2000; Cooke *et al.*, 2004; de Wilde *et al.*, 2008). This method quantifies both the proportion of the initial mass of pesticide that has partitioned to the aqueous phase and that which has partitioned onto the solid phase, at final equilibrium (Weber *et al.*, 2004; Cryer, 2005; Kah and Brown, 2007). This is achieved by shaking a soil with an aqueous pesticide solution for a specific time (the adsorption step, typically 24 hours). Samples are then centrifuged and the supernatant is removed and analysed to determine the proportion of the initial pesticide in the supernatant. Using mass balance, the percentage sorption is established by assuming the pesticide which was not measured in the supernatant, is sorbed to the soil. The supernatant removed at the adsorption step is then replaced with fresh 0.01M CaCl₂ (to minimise soil mineral balance disruption) and shaken again for specific time intervals to determine how much of the adsorbed pesticide can be desorbed. The study can also be carried out in parallel at different concentrations to fit a Freundlich isotherm (OECD, 2000).

Soil sorption is characterised by a partition constant K , conventionally written with a subscript 'd', for distribution (Hamaker and Thompson, 1972). The ratio between the pesticide adsorbed to soil ($\mu\text{g g}^{-1}$) to the pesticide remaining in solution ($\mu\text{g mL}^{-1}$) is thus characterised by the distribution coefficient K_d (Weber *et al.*, 2004). K_d values are ideally determined at pesticide concentrations that would occur in soils when the compounds are applied at recommended rates followed by enough rainfall to bring the soil to field capacity (Weber *et al.*, 2004). The K_d is calculated using the equation:

$$K_d = C_s \div C_{aq}$$

where C_s refers to the concentration of pesticide in the soil or solid phase ($\mu\text{g g}^{-1}$) and C_{aq} refers to the concentration of pesticide in the aqueous phase ($\mu\text{g mL}^{-1}$) at equilibrium, if C_s varies linearly with C_{aq} . Most often however, the ratio of sorbed to dissolved pesticide is not a linear relationship and the distribution coefficient is instead better expressed in terms of the empirical Freundlich relationship:

$$K_f = C_s \div C_{aq}^{1/n}$$

where K_f refers to the Freundlich distribution coefficient and $1/n$ refers to the Freundlich exponent. Though thousands of K_d and K_f measurements have been

generated, the only generalisation made is that there is usually a high correlation observed between the organic matter content of the soils and K_d (Wauchope *et al.*, 2002). Measuring the soil organic matter content of a soil is usually achieved by determining the amount of organic carbon present using digestion or combustion techniques (Wauchope *et al.*, 2002). Since the ratio of soil organic matter mass to soil organic carbon mass is inconsistent (ranges from 1.72 to 2.0), the organic carbon fraction itself is usually reported (Wauchope *et al.*, 2002). The soil organic carbon sorption coefficient (K_{oc}) of a pesticide is calculated by dividing a measured K_d in a specific soil by the organic carbon fraction (F_{oc}) of the soil (Wauchope *et al.*, 2002):

$$K_{oc} = K_f \text{ (or } K_d) \div F_{oc}$$

The simple soil adsorption coefficient K_d or the concentration dependent K_f are generally normalised by the soil organic carbon content for two reasons: (i) to improve the ability to compare partitioning behaviour between compounds measured in different soils; and, (ii) to use the K_{oc} and organic carbon content to assess pesticide sorption in a soil where it has not been measured (Gawlik *et al.*, 1997). Furthermore, despite the simple calculation, K_{oc} values are universally used as measures of the relative mobility of pesticides in soils and in “fugacity” models describing the partitioning of pesticides in soil and water systems (Gramatica *et al.*, 2000; Wauchope *et al.*, 2002).

The appropriateness of the batch-equilibrium method to measure pesticide retention has been questioned due to concerns regarding: (i) whether the soil:solution ratios and intense mixing required to reach equilibrium are atypical of field soil moisture conditions such that results may not adequately reflect sorption processes in field-moist or unsaturated soil (Kah and Brown, 2006; Folberth *et al.*, 2009); (ii) important experimental features which have not been standardised e.g. temperature, type of vessel, type of shaking, centrifugation speed and soil:solution ratios, making results from different studies difficult to compare (Kah and Brown, 2007); and (iii) whether an equilibrium is actually reached, since research suggests this is not likely to be met within a reasonable timeframe (Altfelder and Streck, 2000). The usefulness of the batch-equilibrium approach however, lies within its ability to establish a common ground from which comparisons between pesticide adsorption to soil can be made (Koskinen and Harper, 1990). Further advantages include that the soil and solution

can be separated effectively, a large volume of solution is obtained for analysis, and the method can be easily used for routine laboratory work following OECD guideline 106 (OECD, 2000).

Alternatives to the batch-equilibrium approach include the centrifugation technique (Walker and Jurado-Exposito, 1998; Kah and Brown, 2007), soil extraction with an excess of water (EEW) or other solutes (Hawthorne *et al.*, 2000; Latawiec *et al.*, 2008) and soil column experiments (Fouqué-Brouard and Fournier, 1996), all of which have been applied to assess the leaching potential of chemicals in soils (Folberth *et al.*, 2009). Furthermore, thin-layer chromatography (TLC) methods (Johnson and Sims, 1998), gel filtration chromatography methods (Madhun *et al.*, 1986) and supercritical fluid extraction methods (Berglöf *et al.*, 2003) have also been used to assess levels of pesticide retention by soils.

2.4. Factors influencing sorption

The level of pesticide retention by a given soil is dependent on a large number of factors. These may be grouped broadly into two categories: (i) pesticide properties (e.g. dissociation, lipophilicity); and, (ii) soil properties (e.g. pH, ionic strength, organic matter content, clay content, microbial activity). They will be discussed individually in the following sections.

2.4.1. Influence of pesticide chemistry

2.4.1.1. Dissociation

Ionisable compounds possess either weak acidic and/or basic functional group(s) (Kah and Brown, 2006). As a consequence, they may be partially ionised within the range of normal soil pH. Dissociation strongly affects the soil reactivity of an ionisable compound since the neutral and ionic species exhibit different polarities (Kah and Brown, 2006). A weakly acidic compound dissociates in water to produce protons (H_3O^+), thus it exists in both anionic (negatively charged) and neutral forms in aqueous solutions. A weakly basic compound dissociates in water to produce OH^- or is a compound that can accept a proton; thus it exists both in cationic (positively charged) and neutral form in solution.

The extent to which ionisation of a pesticide occurs is described by the dissociation constant K_a or K_b , which refer to either the acid or basic equilibrium constant, respectively. As well as the pH of the aqueous solution, these determine the relative amounts of the undissociated and dissociated forms present. An indication of the dissociation of a compound is given by the pK_a , which is the pH value at which a compound is present at equal amounts of its neutral and ionic species. At applicable environmental pH ranges pesticides with a very low or very high pK_a will be present primarily as one type of species, thus their sorption behaviour is unlikely to be dependent on soil pH as the pH value at which they would dissociate would not occur in the environment (Kah and Brown, 2006). The protonated form of an ionisable compound generally has a greater propensity for adsorption than the dissociated form. Three sorption behaviours in particular have been observed for ionisable pesticides under changing pH conditions, and these are discussed further in section 2.4.2.1 (influence of soil pH).

2.4.1.2. Lipophilicity (hydrophobicity)

The lipophilicity (or hydrophobicity) of a pesticide also influences sorption behaviour. It is a measure of the chemical's tendency to partition into lipids and hence indicates the propensity of a compound to bioconcentrate in organisms. Lipophilicity is often described by the octanol-water partition coefficient (K_{ow}), typically expressed as $\log K_{ow}$ or $\log P$. The K_{ow} is defined as the ratio of a chemical's concentrations in the octanol-phase and water-phase at equilibrium. Higher $\log K_{ow}$ or $\log P$ values indicate greater lipophilicity. For example, most organochlorine pesticides have a $\log K_{ow}$ or $\log P$ between 3.5 and 6, which explains their high solubility in lipids and tendency to bioconcentrate in organisms (El Bakouri *et al.*, 2009). Furthermore, Gao *et al.* (1998) found that with increasing hydrophobicity (higher K_{ow} , lower solubility) pesticide adsorption was stronger and faster, and their desorption much less effective and incomplete even after a long equilibration time. Alternatively, low $\log K_{ow}$ or $\log P$ values indicate a compound that tends to be hydrophilic; therefore, the compound is likely to be soluble in water, increasing the possibility of its transport to surface and ground waters. The persistence of certain organic pollutants in soil has thus been proposed to be positively correlated to compound hydrophobicity (Reid *et al.*, 2000). The lipophilicity of ionisable organic compounds will be affected by pH; as the polarities of the neutral and ionic forms differ, so does their partitioning behaviour (Kah and Brown, 2006). Therefore, the

lipophilicity of ionisable compounds can be expressed as $\log D$, which is the $\log K_{ow}$ or $\log P$ corrected for dissociation at a given pH value (Kah and Brown, 2008).

2.4.2. Influence of soil properties

2.4.2.1. Soil pH

As discussed in section 2.4.1.1, the sorption behaviour of ionisable pesticides is strongly dependent on the soil pH. The pH ranges found in natural soil systems can vary significantly; the sorption behaviour of ionisable pesticides will therefore be affected by the extent of dissociation and proportions of ionic and neutral species present. For instance, Kumar and Philip (2006) found pH to be the main factor controlling sorption of endosulfan in soil systems. Sorption increased greatly when the pH was reduced and desorption rates were reported to be faster at both acidic and alkaline pH ranges compared to a neutral pH. There are three main types of sorption behaviour that have been observed to occur as a function of soil pH. With increasing soil pH, sorption most often either: (i) decreases; (ii) initially increases, reaches a maximum and then decreases; or, (iii) increases. These behaviours have been both illustrated and discussed in detail by Kah and Brown (2006) so the reasons for these differing sorption behaviours will only be summarised here.

The first behaviour (when sorption decreases with an increasing pH) may occur for both weak acids and weak bases. For weak bases, the explanation is relatively simple; attraction by negatively charged soil particles results in stronger retention of the cationic form than its dissociated form (cation exchange) (Kah and Brown, 2006). The weak acid mesotrione has also exhibited this type of sorption behaviour (Dyson *et al.*, 2002), though there are several reasons why the neutral form of a pesticide is much more strongly sorbed than the anionic form (Kah and Brown, 2006). Some are direct consequences of the molecular dissociation as: (i) anionic species undergo repulsion created by the negatively charged surfaces of soil particles; (ii) the anionic form is less hydrophobic than the neutral form; and, (iii) the anionic form has a greater solubility limit in water than the neutral form. Other reasons are instead consequences of the pH-dependent characteristics of soil: (iv) in variable-charge soils, anionic exchange capacity increases at lower pH values thus, as pH decreases sorption of the anion increases by ionic interactions; (v) organic matter dissociation may result in conformational changes and may account for low adsorption under

alkaline pH ranges; and, (vi) at alkaline pH ranges, an increased presence of hydroxyl ions (HO^-) are present to outcompete other ions for remaining positively charged sites (Kah and Brown, 2006).

The second sorption behaviour (where sorption increases to a maximum and then subsequently decreases) is usually only observed for weak basic compounds. The pH corresponding to the adsorption maximum is sometimes close to the $\text{p}K_a$ of the molecule; however this should not be considered a general rule (Calvet, 1989). The decrease in sorption at more acidic pH ranges is generally attributed to: (i) competition for anionic sorption sites between the cationic form and other cations (H^+ and Al^{3+}); and, (ii) an increase in the cationic species leads to a lower hydrophobic interaction between the pesticide and humic acid (Calvet, 1989; Kah and Brown, 2006). The third and last sorption behaviour, where sorption increases as a function of increasing soil pH, sometimes occurs for weak bases that are adsorbed as neutral molecules or by molecules that form complexes with cations (Calvet, 1989). However, this is unlikely since the protonated form has a greater propensity for sorption than the undissociated form (Kah and Brown, 2006).

2.4.2.2. Ionic strength

The ionic strength of the soil solution has been observed to influence the sorption behaviour of ionisable compounds; variations in ionic strength appear to have a limited effect on the sorption behaviour of neutral compounds (Clausen *et al.*, 2001). The ionic strength of a natural soil solution rarely exceeds 10^{-3}M , though experimental electrolyte concentrations may range from 0 to 1M (Kah and Brown, 2006). Different salt solutions, including CaCl_2 , KCl , NH_4Cl , NaCl , $\text{Ca}(\text{H}_2\text{PO}_4)_2$, $\text{Na}_4\text{P}_2\text{O}_7$ and KH_2PO_4 , have also been used to assess the effect of electrolyte composition on pesticide sorption behaviour (Kah and Brown, 2006). These variations can strongly affect the sorption behaviour of ionic molecules.

The effect of increasing ionic strength is usually found to positively affect pesticide sorption behaviour. For example, with an increasing CaCl_2 electrolyte concentration Clausen *et al.* (2001) observed that adsorption of mecoprop and 2,4-D to kaolinite also increased. This phenomenon may be partly explained by the increase in ionic strength causing replacement of protons from the soil surface; the resultant slight decrease in pH causes the amounts of neutral species present to increase, and these

are sorbed more strongly than their anionic form (Regitano *et al.*, 1997; de Jonge and de Jonge, 1999). It is also possible for complexes to form between the pesticide molecule and surface-exchanged multivalent cations; these may contribute to the stronger sorption observed at higher ionic strengths as the diffuse double layer is compressed and Ca^{2+} is able to bind more strongly to the clay surfaces (de Jonge and de Jonge, 1999; Clausen *et al.*, 2001).

A negative relationship between ionic strength and sorption has also been observed. For instance, in a variably charged soil Hyun and Lee (2004) observed a fivefold decrease in prosulfuron adsorption as the ionic strength of the soil solution increased from 0.0015 to 1.5M CaCl_2 . Also with increasing CaCl_2 concentration, Clausen *et al.* (2001) noted a decrease in the adsorption of ionic pesticides to calcite and α -alumina. The authors postulated that the decreasing adsorption of anionic pesticides to mineral surfaces with a net positive charge was caused by three solution effects: (i) increased competition for adsorption sites by electrolyte anions; (ii) the increasing electrolyte concentration results in increasing aqueous complexation with the anionic pesticides; and, (iii) the increasing electrolyte concentration additionally causes a decrease in the activity of the charged ions.

As ionic strength does not normally exceed 10^{-3}M in natural soil systems, the effects of changes in electrolyte concentration can usually be discounted (Lee *et al.*, 1990). Using 0.01M CaCl_2 in standardised soil sorption experiments (OECD, 2000) is a decision that will however affect the environmental-applicability of sorption coefficients generated by these tests, particularly for ionisable compounds (de Jonge and de Jonge, 1999; Kah and Brown, 2006). Therefore, using such data may restrict the ability to accurately predict the sorption behaviour of ionisable compounds under field conditions (Kah and Brown, 2006).

2.4.2.3. Soil organic matter

The fact that the soil organic matter is a significant site for the binding of pesticides and is the predominant factor influencing the retention of non-ionic pesticides in soils has been extensively documented (Reid *et al.*, 2000; Spark and Swift, 2002; Li *et al.*, 2003; Barraclough *et al.*, 2005). The soil organic matter itself is constituted of humic substances, which are mostly composed of humic and fulvic acids (Senesi, 1992). These acids have many intrinsic chemical properties that significantly augment their

interaction with organic pesticides including: (i) a polydispersed nature and polyelectrolytic character; (ii) surface activity properties and presence of various chemically reactive functional groups e.g. carboxylic acids, phenols, amines, amides, alkoxy, hydroxyl, quinines, ethers and esters; (iii) free radical moieties; and (iv) hydrophilic and hydrophobic sites in their molecular structure (Senesi, 1992; Barraclough *et al.*, 2005).

Organic compounds contain many of the same functional groups and structural units; on entering the soil they thus associate closely with the organic matter as a result of involvement in many of the same microbial and chemical transformations (Barraclough *et al.*, 2005). Over time organic compounds become indistinguishable from soil organic matter (Loiseau and Barriuso, 2002; Barraclough *et al.*, 2005). As a consequence, soil organic matter is a significant factor influencing the formation of bound residues (section 2.6). The nature of the organic matter may also influence pesticide sorption behaviour (Ahangar *et al.*, 2008). Soil organic matter is thought to be composed of two different types: (i) organic material of a glassy (condensed, rigid) nature; and, (ii) a rubbery (expanded, flexible) structure (Pignatello and Xing, 1996). The two types are thought to have different sorptive properties and the implication of this is discussed further in section 2.5.2.

For soils that have a low organic matter content, the sorption behaviour of a pesticide is instead often related to the active components of the inorganic fraction, predominantly the clay-sized fraction (Spark and Swift, 2002). In addition, soil organic matter is often associated with soil clay minerals and so simple relationships between adsorption and organic matter are not often observed; this is discussed further in the following section.

2.4.2.4. Clay content

An increase in the soil clay content is often observed to reduce pesticide mobility in the environment and is also an important factor to consider when assessing pesticide retention by soils. A study by Walker *et al.* (1985) investigating napropamide residues in soil found a strong positive correlation between adsorption and clay content. Their adsorption capability is a result of their small particle size, large surface area per unit weight and negative surface charge (Gilchrist *et al.*, 1993). Clay minerals may be grouped into two broad categories: swelling and non-swelling clays.

Swelling clays such as montmorillonite, smectite and vermiculite (2:1 clays) undergo interlayer expansion upon wetting, generating internal surface areas as high as $800 \text{ m}^2 \text{ g}^{-1}$ (Pu and Cutright, 2006). On the other hand, non-expandable clay minerals such as kaolinite have much smaller internal surface areas of approximately $10 - 20 \text{ m}^2 \text{ g}^{-1}$ (Pu and Cutright, 2006). Thus, though organic compounds are able to bind to clay minerals in either category, the clay type will clearly affect pesticide retention. Torrents and Jayasundera (1997) reported that the herbicides alachlor, metolachlor and linuron all sorbed to kaolinite clay to a lesser extent than to Na^+ montmorillonite.

Many pesticide sorption studies have investigated the influence of clay and soil organic matter contents simultaneously (Lesan and Bhandari, 2003; Kumar and Philip, 2006; Rama Krishna and Philip, 2008). This is because clay and organic matter are often found to be associated with one another in soils, thus the relationship between adsorption and clay content (or organic matter content) is not a simple one. This was confirmed in a study by Pu and Cutright (2006) who found that the sorption of pentachlorophenol was a function of both soil organic matter content and clay content. However, the clay content was found to be the dominant constituent for resisting desorption. Gao *et al.* (1998) positively correlated the hysteresis phenomenon observed in their study with sediment particle-size and organic matter fractions and noted that the distribution exhibited a bimodal behaviour; both the smaller particles (clay, fine silt, $< 6.3 \mu\text{m}$) as well as the larger particles (fine sand, $63 - 200 \mu\text{m}$) showed the most effective sorption of pesticides.

2.4.2.5. Microbial activity

Pesticide sorption behaviour is also influenced by microbial activity. The metabolism of organic chemicals in soil is highly dependent on soil microbial populations (Burauel and Führ, 2000). Degradation rates are consistently reported to be significantly greater in non-sterile soils where microbially-mediated metabolism processes are operating (Rice *et al.*, 2002; Gevao *et al.*, 2005). Microorganisms are able to assimilate the derived carbon into their cellular components (e.g. fatty acids and amino acids), which in turn become stabilised within the soil organic matter (Nowak *et al.*, 2010). Biodegradation is therefore often strongly associated with bound residue formation (see also section 2.6).

Pesticide incubation experiments have documented that the vast majority of bound residues are formed almost exclusively in the top layer of the soil profile, where microbial activity is concentrated, with little or no formation in deeper soil samples (Kruger *et al.*, 1997; Burauel and Führ, 2000). A study by Rice *et al.* (2002) found that metolachlor bound residue formation was significantly reduced in unsaturated sterile soils compared to non-sterile soils, for both surface soils (16.7% vs. 27.6%) and subsurface soils (0.0% vs. 8.3%). The increased strength of sorption usually observed for non-parent material may be explained by its greater chemical reactivity than the parent compound. Parent compounds are often relatively inert and mostly unable to covalently bind with soil components to any significant extent (Gevao *et al.*, 2005). Microorganisms provide the metabolic activation required to transform the parent compound into more reactive microbial metabolites; these transformation products are able to take part in additional binding interactions with the soil organic matter (due to their greater chemical reactivity) and augment the formation of bound residues (Richnow *et al.*, 2000; Rice *et al.*, 2002; Lerch *et al.*, 2009).

The microbial role in the release of soil-bound residue has not yet been investigated to any significant extent. Some published data suggest that amounts of soil-bound residue released via microbially-mediated processes is negligible with their liberation instead controlled by mainly physico-chemical factors (Hayar *et al.*, 1997). Barriuso *et al.* (2008) reported that data consistently show only small percentages of total amounts of bound residue can be released. Nevertheless, several biodegradation studies using ^{14}C -pesticide residues have shown that upon reincubation, the bound residues of different pesticides can be released via mineralisation to $^{14}\text{CO}_2$ (Hsu and Bartha, 1974; Roberts and Standen, 1981; Gevao *et al.*, 2005; Lerch *et al.*, 2009) or otherwise rendered extractable (Khan and Ivarson, 1981; Gevao *et al.*, 2005).

2.5. Non-ideal sorption behaviour (time-dependent sorption, non-equilibrium sorption and ageing)

2.5.1. Slow sorption kinetics

It is now known that the adsorption-desorption process is not a simple instantaneous equilibrium process as previously thought, or assumed by contaminant transport models (Kan *et al.*, 1994; Cox and Walker, 1999). Sorption hysteresis is characteristic

of laboratory batch-equilibrium studies; desorption solid/aqueous phase distribution coefficients have frequently been reported to significantly exceed those measured for adsorption in the same solid/solute systems, carrying the implication that pesticide adsorption to soils occurs with only partial reversibility (Huang and Weber, 1997). The strength of binding has also frequently been shown to increase with residence time in soil, a phenomenon that may be referred to as either ageing, time-dependent sorption, non-equilibrium sorption or kinetic sorption (Huang and Weber, 1997; Cox and Walker, 1999; Lesan and Bhandari, 2003; Lesan and Bhandari, 2004; Villaverde, 2007; Shareef and Shaw, 2008). Although it is often assumed in batch experiments that partitioning between the solid and liquid phases reaches equilibrium after 24 hours, there is now abundant evidence to suggest that equilibrium for many compounds is reached after periods of days, months or even years, and the partition coefficient can increase several fold as the time of exposure increases (Ball and Roberts, 1991; Beulke *et al.*, 2004; Renaud *et al.*, 2004). Thus, the importance of extremely slow retention and release of organic compounds to and from soil is now well established (Altfelder and Streck, 2006).

The tendency of sorbed organic compounds to become more strongly associated with the sorbent over time has a significant impact on the desorption of aged residues (Lesan and Bhandari, 2003). The desorption of organic compounds from soil was previously generally accepted to be at least biphasic, with a “fast desorbing fraction” and “slow desorbing fraction” of sorbed chemical described in the literature (Gao *et al.*, 1998). Later, the second “slow” phase was shown to be comprised of two fractions which differ in their first-order rate constants by at least two orders of magnitude (van den Heuvel and van Noort, 2006). Thus, it has been recognised that the kinetics of desorption are better described in terms of three empirically distinct kinetic fractions: a fast desorbing fraction, a slowly desorbing fraction, and a very slowly desorbing fraction (van den Heuvel and van Noort, 2006; Chai *et al.*, 2007; Sormunen *et al.*, 2008; Yang *et al.*, 2010).

2.5.2. Mechanisms

Gevao *et al.* (2000) clarify the processes that account for pesticide ageing in soils to include diffusion into spatially remote areas, such as soil macro- and micropores accompanied by equilibrium sorption to the organic pore surface (Ball and Roberts,

1991; Burgos *et al.*, 1996; Pignatello and Xing, 1996) and entrapment within soil organic matter (Brusseau *et al.*, 1991; Huang and Weber, 1997; Park *et al.*, 2004). Shor *et al.* (2003) have proposed that it is a combination of these processes that is responsible. Detailed reviews on the phenomena involved in slow sorption have been published by Brusseau *et al.* (1991), Pignatello and Xing (1996) and Luthy *et al.* (1997), therefore, the mechanisms involved will only be summarised here.

There are three different processes involving diffusive mass transfer which can cause sorption-related non-equilibrium: (i) film diffusion through the water “film” that extends a few nanometres from the soil particle surface; (ii) pore diffusion (retarded intra-particle diffusion) through pores within the soil particle; and, (iii) matrix diffusion (retarded intra-sorbent diffusion) in permeable solid phases (Brusseau *et al.*, 1991; Pignatello and Xing, 1996).

The relative importance of each interaction and their contribution to slow sorption kinetics in soils is still a matter of discussion in the literature; it is generally accepted however, that liquid diffusion processes (e.g. film diffusion) are not expected to be an important time-limiting factor compared to pore (intra-particle) and matrix (intra-sorbent) diffusion (Brusseau *et al.*, 1989; Pignatello and Xing, 1996). Liquid diffusion is only considered a potential rate-limiting factor for the initial fast stage of sorption (i.e. instantaneous sorption onto soil particle surfaces) (Pignatello and Xing, 1996). Pore (intra-particle) diffusion can occur in pore liquids or along pore wall surfaces; as liquid and surface diffusion may act simultaneously, they are difficult to distinguish (Pignatello and Xing, 1996).

Matrix (intra-sorbent) diffusion could involve intra-organic as well as intra-mineral diffusion; however the former is considered to be of significantly greater importance than the latter for its role in the adsorption/desorption or diffusion of hydrophobic compounds (Weber *et al.*, 2001). The “glassy” and “rubbery” types of organic matter characterised by Pignatello and Xing (1996), which were described in section 2.4.2.3, are assumed to show different behaviour relating to time-dependent sorption and sorption capacity (dual mode sorption). Slow sorption is assumed to occur mainly in the glassy domain, causing non-linear sorption due to its limited sorption capacity; faster sorption predominantly occurs in rubbery-type soil organic matter, and usually exhibits linear sorption (Pignatello and Xing, 1996).

2.6. Bound residues, non-extractable residues and irreversible sorption

2.6.1. Nomenclature

The descriptive terms “free” and “bound” residue are used to differentiate between pesticide residues that can be readily extracted from the soil and those that remain resistant to such an extraction (Gevao *et al.*, 2000). The most widely used definition of bound residues provided to date was proposed by Roberts *et al.* (1984) and also adopted by the International Union of Pure and Applied Chemistry (IUPAC); they defined bound residues as:

“...chemical species originating from pesticides, used according to good agricultural practice, that are unextracted by methods which do not significantly change the chemical nature of these residues...”

Although this definition has been modified many times, it has not changed substantially (Northcott and Jones, 2000). The most notable addition was provided by Calderbank (1989) who introduced the concept that the environmental significance of bound residues hinges on their bioavailability and biological effect, rather than the extent to which they can or cannot be extracted or released by chemical methods:

“...clearly the important matter is not so much how the residue is defined but the question of its biological availability...”

The last agreed definition for bound residues was provided by Führ *et al.* (1998) who also considered the structure of the soil matrix:

“Bound residues represent compounds in soil, plant or animal, which persist in the matrix form of the parent substance or its metabolite(s) after extraction. The extraction method must not substantially change the compounds themselves or the nature of the matrix. The nature of the bond can be clarified in part by matrix altering extraction methods and sophisticated analytical techniques. To date, for example, covalent, ionic and sorptive bonds as well as entrapments have been identified in

this way. In general the formation of bound residues reduces the bioaccessibility and bioavailability significantly.”

Although the aforementioned definitions have been widely acknowledged, there is a tendency in the literature to describe bound pesticide residues using alternative terminology; therefore some clarifications will be made as to their use and meaning.

Firstly, the term non-extractable residue (sometimes referred to as unextractable residue) is used commonly throughout the literature. The term reflects the operationally-defined nature by which the majority of bound residues are measured experimentally (Burauel and Führ, 2000; Gevaio *et al.*, 2005; Mordaunt *et al.*, 2005; Wanner *et al.*, 2005; Barriuso *et al.*, 2008). Thus, when considering that the definition of a bound residue relates to the fraction of sorbed pesticide resistant to extraction, a bound residue is a non-extractable residue (Barriuso *et al.*, 2008). Conversely, a non-extractable residue is not necessarily a bound residue. The fraction of non-extractable residue quantified is entirely dependent on the experimental conditions, method and solvent used to perform the extraction and obtain the reported results (Gevaio *et al.*, 2000). The decision to discontinue a given extraction is usually an arbitrary choice and additional amounts of the bound chemicals can normally be recovered by increasing the time or intensity of extraction (Alexander, 1995). The classification of compound residues into these definitions can be misleading due to the range of methods use to quantify such fractions (Northcott and Jones, 2000). Thus, the non-extractable fraction can result in overestimation of the true bound residue fraction due to its operationally-defined nature.

Secondly, in certain circumstances the term irreversible sorption is also used. Further complicating matters are the numerous variations of this term that exist within the literature, the meanings of which are essentially equivalent to that of irreversible sorption, though worded differently (Table 2.1). “Irreversible” implies that this fraction of sorbed pesticide is irretrievably bound to the soil matrix, i.e. it can never be recovered. In some studies, the authors have instead defined their own terms. For instance, Sander and Pignatello (2005) have used the term irreversible sorption in a thermodynamic context:

“...that does not necessarily imply an irretrievably bound state.”

Furthermore, in their later paper (Sander and Pignatello, 2009) the authors also put into context their use of the term irreversible:

“Note that the term “irreversible” does not necessarily imply the generation of irretrievable (unextractable) residues, although it does not exclude this possibility.”

It is clear that this inconsistency in choice of descriptors, and study-dependent definitions of irreversible sorption, limits the ability to make direct comparisons between studies and results in confusion or misinterpretation of their meaning. There is a need therefore, for the standardisation of the terms and definitions used to describe compound residues.

Table 2.1. Variation for the term irreversible sorption in the literature.

Terminology used	Reference
Irreversibly sorbed Irreversibly bound Irreversible behaviour Irreversibility in pesticide adsorption-desorption Irreversible sites	Celis and Koskinen (1999a)
Sorption irreversibility Irreversible sorption behaviour Non-desorbable Irreversible component	Celis and Koskinen (1999b)
Irreversible compartment	Chen <i>et al.</i> (2004)
Irreversible binding	Burgos <i>et al.</i> (1996)
Desorption irreversibility	Yu <i>et al.</i> (2010)
Irreversible effects Desorption resistant	Sander and Pignatello (2009)

Use of the term irreversible sorption does appear to be generally associated with a permanent change in the adsorbate/adsorbent system i.e. a mechanical or structural rearrangement (Kan *et al.*, 1994). For instance, Yu *et al.* (2010) consider the highly irreversible sorption of pyrethamyl to soils amended with high-microporosity biochar to be the result of adsorption and desorption occurring from different physical environments. Chen *et al.* (2004) believe the hysteresis phenomenon observed in their study was partially caused by the irreversible sorption (by physical entrapment) of pentachlorophenol to lipids. Furthermore, Sander and Pignatello (2005; 2009) have discussed in detail the irreversible deformation of micropores by the sorbate such that

adsorption and desorption follow different pathways. This mechanism therefore, is also thought to contribute to the irreversible sorption of pesticides to soils (Sander and Pignatello, 2009).

The following definitions are provided for the meanings of the terms used in this thesis: (i) the term irreversible sorption is used to refer to pesticide residues that even after destruction of the soil matrix, cannot be retrieved; (ii) the term non-extractable residue is used to define the fraction of pesticide resistant to desorption by the chosen extraction method; and (iii) the term bound residue is used to describe the irreversibly sorbed fraction of pesticide in addition to a fraction of non-equilibrium sorbed pesticide, which cannot be extracted within the time-scale of the experiment. Irreversible sorption and bound residues are hence differentiated in terms of time-scale and potential for remobilisation. Irreversible sorption implies that the pesticide residue is unlikely to be released from the soil matrix under any circumstance, and may be thought of as essentially removed from the environmental system. Bound residues do not carry such a strict meaning as they are not considered permanently bound. Using the terms non-extractable residue and bound residue is therefore, less strict than irreversible sorption. This is the result of the experimental difficulty in reliably establishing desorption endpoints, which may require a prohibitive experimental time-scale to be observed.

The formation of soil-bound pesticide residues has gained significant attention for its importance for the fate and transport of organic contaminants in environmental systems through limiting the bioavailability of pesticides in surface soil systems and having the potential to reduce pesticide mobility in the environment (Huang and Weber, 1997). As a consequence, bound residue phenomena have been reviewed in detail on several occasions (Gevao *et al.*, 2000; Reid *et al.*, 2000; Barraclough *et al.*, 2005; Mordaunt *et al.*, 2005; Barriuso and Benoit, 2006; Barriuso *et al.*, 2008). The subject is explored in greater detail in the following sections.

2.6.2. Mechanisms

The main two mechanisms involved in the formation of bound residues are: (i) covalent bonding; and, (ii) physical entrapment (Dec *et al.*, 1997; Huang and Weber, 1997; Kan *et al.*, 2000; Loiseau and Barriuso, 2002). Both mechanisms of formation

may involve the parent molecule and/or its metabolite(s). Covalent bonding and physical entrapment are the direct result of abiotic interactions (i.e. physical and chemical binding). Indirectly or artificially however, biotic interactions (microbial action) have the ability to significantly augment the formation of bound residues. As discussed in section 2.4.2.5, several publications have shown that soil microbial activity positively influences the formation of bound residues; a relationship particularly apparent in experiments where bound residue formation is assessed in sterile and non-sterile soils. The reason for this positive relationship is the partial degradation of the parent molecule, leading to the production of metabolites with a higher chemical reactivity higher than the initial pesticide (Barriuso and Benoit, 2006). As bound residues can only be quantified using ^{14}C -labelled compounds, little knowledge of their chemical identity is ever revealed (Kästner *et al.*, 1999; Barraclough *et al.*, 2005). The work in this thesis focuses on the abiotic formation of bound residues (via the study of sterilised soils) to assess whether the long-term sorption of pesticide to soil is reversible or irreversible in the absence of microbial activity, since the direct mechanisms of formation remain uninhibited.

2.6.3. Regulatory significance of bound residues

The definitions of bound residues described above have been made more quantitative for regulatory purposes by Council Directive 91/414/EEC (Anon., 1991), which has been recently superseded by Regulation 1107/2009/EC (Anon., 2009). The Uniform Principles stipulate that in laboratory tests, if non-extractable soil residues are formed at $> 70\%$ of initial dose after 100 days, with mineralisation to CO_2 at $< 5\%$ after 100 days, then no authorisation shall be granted, *unless*, it is scientifically demonstrated that under field conditions there is no accumulation in soil at such levels that unacceptable residues in and/or unacceptable phytotoxic effects on succeeding crops occur, and/or that there is an unacceptable impact on the environment (Anon., 1991). If a pesticide triggers these criteria then it requires the full range of environmental fate, long term effects and crop residue tests to identify whether any risks are posed by the bound residues (Craven and Hoy, 2005). However, although non-extractable residue must be measured, it is ambiguous as to which solvents and extraction conditions should be used in order to assess the formation of such residues (Mordaunt *et al.*, 2005). UK studies have tended in the past to refer to non-extractable residues as those which are not removed from soil by exhaustive extraction with polar and non-

polar solvents (Craven and Hoy, 2005). A range of techniques have been employed to assess both the magnitude and nature of pesticide bound residues in soils, as discussed in the following section.

2.6.4. Methods of extraction and measurement of bound residues

At present there are a range of techniques available to assist the characterisation of soil-bound residues (Table 2.2). These techniques may be separated into the following groups: (i) quantitative methods e.g. solvent extraction procedures that aim to extract pesticide residues from the soil and provide numerical estimates of the irreversible fraction; (ii) diagnostic methods e.g. electron spin resonance (ESR), fourier transform infra-red (FTIR) and nuclear magnetic resonance (NMR) that aim to qualitatively determine the nature of bound residue interactions rather than the amount; and, (iii) indirect experimental methods e.g. hydrolysis methods, derivatisation extraction, model compound investigations, pyrolysis and thermal desorption techniques used for isolation and fractionation of soil humic substances in order to indicate the circumstances of bound residue formation (Northcott and Jones, 2000). Although the combination of the above techniques is clearly a powerful tool for the analysis, characterisation and further study of bound residues, factors such as cost are always an important consideration, as are time and availability of the technique (Mordaunt *et al.*, 2005). The aforementioned techniques for determination of organic bound residues have been discussed in detail by Northcott and Jones (2000).

2.6.4.1. Experimental examples

A great deal of research has been carried out using the methods of extraction and measurement of bound residues described in the previous section. Some examples of such studies will be summarised here, along with their main results. Burauel and Führ (2000) used outdoor lysimeter studies to observe the formation and long-term fate of non-extractable residues. They separately applied three pesticides, two polycyclic aromatic hydrocarbons (PAHs) and two polychlorinated biphenyls (PCBs) to orthic luvisols that contained either post-emergent winter wheat, pre-emergent maize or post-emergent summer wheat. They then used an optimal extraction method for each compound, always starting with a 24-hour extraction using 0.01M CaCl₂ and then using progressively harsher solvent extractions. A microcosm study carried out by

Table 2.2. Methods of extraction and measurement of bound residues; summarised from Northcott and Jones (2000) *.

Technique	Description
Quantitative methods	
Solvent and advanced solvent extraction	Assesses quantity of adsorbed pesticide extracted using a specific solvent. Often accomplished using batch solvent shaking extraction or Soxhlet extraction. Other extraction technologies include ultrasonication, microwave extraction, supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE).
Indirect experimental methods for the isolation and fractionation of soil humic substances	
Hydrolysis methods	Methanolic hydrolysis (saponification) used to release non-solvent extractable organic contaminants. Achieved by: (i) hydrolysing organic matter to split labile ester bonds, causing limited breakdown of macromolecular humic network to improve solvent accessibility; and, (ii) introduction of alkaline conditions to cause an extension of the humic macromolecular polymeric structure due to mutual repulsion between negatively charged carboxyl, phenolic and hydroxyl functional groups.
Derivatisation extraction	Used to enhance extraction of soil humic substances. Trimethylchlorosilane common choice of reagent for silylation of humic substances. Involves substitution of a silyl group for active hydrogen atoms in –OH, =NH, –NH ₂ , –SH and –COOH functional groups. Silylation of functional groups in soil organic matter results in disaggregation of the humic macromolecules into smaller fragments that are normally held together by hydrogen bonding and other non-covalent interactions.
Analytical pyrolysis (or high-temperature distillation, HTD) and thermal desorption (TD)	Used to characterise the chemical structure of soil organic matter by analysis of the evolved pyrolysis of thermal products. Pyrolysis and TD differ in the way the sample is heated. Pyrolysis and TD methods used to distinguish differences in binding regimes/energies of organic contaminants in soil and soil organic matter fractions. They have been combined with mass spectrometry to determine the structure of evolved bound residues and differentiate between parent compound and altered/covalently bound metabolite(s).

Diagnostic methods

Electron Spin Resonance (ESR) spectroscopy	Used to detect paramagnetic species or free radicals in samples. Useful for studying charge transfer reactions involving pesticides but limited to compounds that undergo these reactions, therefore it has limited application to bound residue studies.
Fourier Transform Infra-Red (FTIR) spectroscopy	Powerful tool for identifying and determining the structure of organic, inorganic, and biochemical species. Provides evidence at the molecular level for binding mechanisms by measuring the relative intensity of the compound infra-red absorption bands.
Fluorescence spectroscopy	Used to determine equilibrium constants for the association of fluorescing hydrophobic organic compounds with dissolved humic substances.
NMR spectroscopy	Used for structural characterisation of soil humic substances. Most popular technique used for the study of humic materials and other geochemical/geological solids used a combination of cross polarisation (CP) and magic angle spinning (MAS) on solids. Most specific advantage is that it allows the observation of the chemical environment of atoms within a molecule. In combination with ^{13}C -labelling (^{13}C -NMR) it is a powerful technique for examining the covalent and non-covalent interactions of specific molecules.

*Northcott, G.L. & Jones, K.C. (2000). Experimental approaches and analytical techniques for determining organic compound bound residues in soil and sediment. *Environmental Pollution*, 108(1), 19-43.

Mordaunt *et al.* (2005) characterised the formation of bound residues over 91 days for atrazine, dicamba, isoproturon, lindane, paraquat and trifluralin. The extractability of the adsorbed pesticide was determined using a sequential extraction procedure, with increasing strength of solvent (0.01M CaCl₂ < acetonitrile:water (9:1) < methanol < dichloromethane) to simulate readily available and potentially available fractions. The soils were finally combusted to complete the mass balance.

Mordaunt *et al.* (2005) did discuss however, that although the results of such solvent-extraction studies reflect potential behaviours of the compounds and their metabolites in the environment, the fraction of non-extractable compound still remains operationally-defined by the chosen extraction procedure. Thus, it will always be possible to obtain better recoveries of compound where availability of technique, time and cost is of no consideration. The soil used in the experimental system no longer represents natural soil as the use of organic solvents in soil extractions dramatically changes the appearance of the soil structure (Mordaunt *et al.*, 2005). This occurs due to the removal of both soil water and a large portion of organic matter (usually the more soluble humic acids) from the soil matrix. Therefore, the non-extractable fraction determined using such methods may only be of limited relevance to natural soils. A more sensible approach would adopt a more “natural” extraction (i.e. one that mimics solutions likely to be present in the soil), giving a resultant bound residue likely to be representative of field conditions (Gevao *et al.*, 2001).

Gevao *et al.* (2001) assessed the bioavailability of non-extractable (bound) pesticide residues to earthworms using a Soxhlet extraction. Soils treated with ¹⁴C-labelled atrazine, dicamba and isoproturon were incubated for 100 days and then subjected to exhaustive Soxhlet extractions with methanol and dichloromethane. Clean soil was then added to the extracted soil in the ratio of 7:1 to increase the volume. After earthworms had lived in these previously extracted soils for 28 days, 0.02 - 0.2% of previously bound ¹⁴C activity was adsorbed into earthworm tissue, showing that soil-bound residues can be bioavailable to earthworms, albeit at very low percentages. This supports speculation that soil-bound residues are not excluded from environmental interactions and processes. The authors also found that the presence of earthworms in soils suppressed the formation of bound residues.

A study by Dec *et al.* (1997) used a silylation procedure and ^{13}C -NMR-spectroscopy to measure bound residue formation for the fungicide cyprodinil. Soils were incubated with either a low-concentration ($3\ \mu\text{g mL}^{-1}$) or high-concentration ($500\ \mu\text{g mL}^{-1}$) of cyprodinil for 6 months; the bound residue fractions amounted to approximately 50% and 18% of the initial radioactivity, respectively. The isolated humic acid fraction and the NaOH-extracted soil (the humin fraction) were suspended in chloroform and silylated by overnight shaking with trimethylchlorosilane. Analysis of the silylated extracts by ^{13}C -NMR revealed that the formation of bound residue in the $500\ \mu\text{g mL}^{-1}$ samples involved: (i) sequestration of the unaltered or slightly altered fungicide in the humin fraction; and, (ii) cleavage of the cyprodinil molecule between the aromatic rings with subsequent covalent binding of the separated moieties to humic acid.

Loiseau and Barriuso (2002) characterised the formation of bound residues for atrazine using fractionation techniques for soil organic matter. A laboratory incubation period of 56 days with either the presence or absence of microflora meant that bound residue fractions ranged from 10 - 40%. Soil size fractionation was followed by alkaline extraction, before and after treatment with hydrogen fluoride; acid hydrolysis with 2M hydrochloric acid in reflux conditions was then applied to the soils containing bound residues. Most of the bound residue was found to be in the finest fraction ($< 20\ \mu\text{m}$) that contained the humified organic matter (from 61 - 77% of the total bound residue), and between 78 and 89% was made soluble during the different steps of the chemical fractionation procedure. Between 20 - 50% of the bound residue fraction was identified as intact atrazine and its main derivatives, indicating that this proportion of the bound residue was probably formed by entrapment in voids of the soil organic matter. Between 13 and 30% of the bound residue was associated with humic acids. The ability of microorganisms to mineralise the triazinic ring augments the formation of bound residues via generation of reactive transformation products. A soil $\text{pH} < 6$ favours the formation and stabilisation of hydroxylated derivatives of atrazine and a high content of humic acids favours the formation of chemically bound residues.

In addition to those methods discussed by Northcott and Jones (2000) other methods have also been used to determine the relative quantities of bound residue. Celis and Koskinen (1999a; 1999b) and Sander and Pignatello (2005) both used an isotope exchange technique to assess the reversibility of pesticide sorption to soil *in-situ*. The

isotope exchange technique involves the use of both ^{12}C - and ^{14}C -labelled pesticide, which are initially applied to soils separately. Following the adsorption period however, ^{12}C - and ^{14}C -pesticide supernatants are then exchanged between corresponding samples. The subsequent exchange between ^{12}C - and ^{14}C -pesticide is then observed during a specific length of time in order to characterise the kinetics of pesticide exchange and provide an estimation of the amount of sorbed pesticide that was not taking part in the exchange. This method is anticipated to leave the soil matrix essentially unchanged as the composition of the soil solution is not disturbed, therefore it is a more “natural” method by which bound residues may be determined. Sander and Pignatello (2005) also advocate the use of isotope exchange techniques, which they consider a promising method to unequivocally establish whether sorbate entrapment occurs during a sorption-desorption cycle.

2.6.5. Potential for release

An important question to consider is whether released pesticide residues are of ecotoxicological significance (Gevao *et al.*, 2000). Some reviews have stated that available data have consistently shown that only negligible amounts of bound residues are released by microbial action (Gevao *et al.*, 2000; Barriuso *et al.*, 2008). The existing knowledge of the mechanisms by which residues bind to soil organic matter suggests that release will be closely dependent on soil organic matter breakdown/turnover (Barraclough *et al.*, 2005). Craven and Hoy (2005) discussed whether bound residues can be re-released over time under natural conditions and what their possible long term effects may be; they concluded that the issue requires further study and debate. They also suggested that a possible direction for future developments to experimental approaches is accelerated laboratory tests that mimic longer term field exposure to the bound material. However, the authors also go on to say that it is perhaps questionable whether any amounts of bound residue released are of importance compared with the level of pesticide originally added to the soil during agricultural application in the field. It is generally accepted that exposure should be less than that resulting from initial use of a compound, even if all the bound residue was remobilised by some mechanism (Craven and Hoy, 2005).

2.7. Modelling approaches

Predicting the fate of pesticides released into the environment is necessary to anticipate, and thereby minimise, potentially adverse impacts to non-target environmental compartments. The environmental fate of a pesticide is dependent on a large number of factors, which have been discussed previously. Although many of the interactions occurring between the pesticide and soil have been measured experimentally either in laboratory or field studies, for many situations experimental data are not available. Under these circumstances, mathematical modelling provides an important means by which experimental results may be extrapolated to unstudied scenarios. Estimates of the potential risks to surface and groundwater quality and/or toxicity to aquatic and terrestrial organisms may then be generated.

The development and validation of mathematical models with the power to accurately assess pesticide fate in the environment is a complicated process. Here, in keeping with the specific focus of the thesis, attempts to model the sorption of pesticides to soils will be assessed. The sorption of organic chemicals in soils is kinetically controlled by rate-limiting processes (section 2.5). As a consequence, a number of attempts have been made to model these non-equilibrium sorption processes mathematically. The most common non-equilibrium model approaches include mass transfer models and diffusion models, though other model approaches also exist. They will be summarised in the following sections, building on the literature reviews of Ma and Selim (1997), Scow and Johnson (1997), Pignatello (2000) and Maraqa (2001) and the report for DEFRA (project PS2235) by van Beinum *et al.* (2010).

2.7.1. Mass transfer models

Mass transfer models assume that the sorbent is comprised of one or multiple sorption sites or domains (Fortin *et al.*, 1997). Solute movement between the different sites or domains is usually assumed to follow first-order reaction kinetics, though second-order kinetics have also been used (Selim and Amacher, 1988). Rate-limited transfer processes are generated by either: (i) transport-related (physical) non-equilibrium; or (ii) sorption-related non-equilibrium (Brusseau *et al.*, 1991). Transport-related (physical) non-equilibrium is caused by heterogeneous flow domains (e.g. aggregates and macropores); their rate-limited effect on solute transport has been well

documented (Brusseau and Rao, 1990; Brusseau *et al.*, 1991; Jarvis, 2007). Sorption-related non-equilibrium results from either chemical non-equilibrium (e.g. chemisorption) or rate-limited diffusive mass transfer processes (see section 2.5.2) (Brusseau *et al.*, 1991). Since the sorption of organic compounds is usually driven by partitioning between the soil solution and soil organic matter, chemical non-equilibrium effects are usually disregarded (Brusseau *et al.*, 1991).

Two major mass transfer modelling approaches have been established: (i) mobile-immobile (two-region) models; and, (ii) two-site and multi-site models. Stochastic models, hybrid models and attempts to model irreversible sorption will also be considered here.

2.7.1.1. Mobile-immobile (two-region) models

Mobile-immobile (two-region) models explain non-equilibrium behaviour by assuming that the soil matrix consists of two types of regions: (i) a region where the soil water is mobile; and, (ii) a region where the soil water is immobile (van Beinum *et al.*, 2010). Deans (1963) was amongst the earliest to conceptualise the soil matrix in terms of mobile and immobile regions. van Genuchten and Wierenga (1976) also proposed a model where the soil water is divided into mobile and immobile regions. Convective-dispersive solute transport is limited to the mobile soil water region and diffusive mass transfer processes control the rate of pesticide adsorption-desorption within the immobile soil water region (Rao and Jessup, 1982). Ma and Selim (1997) have also provided equations to describe solute movement in mobile and immobile regions of the soil water. The difficulty in using mobile-immobile (two-region) models however, is the uncertainty involved in defining the relative amounts of mobile and immobile regions (Ma and Selim, 1997).

2.7.1.2. Two-site models and multi-site models

Two-site models (or two-stage models) are the simplest form of a multi-site model. In a two-site model, the soil matrix is divided into two types of sorption sites: (i) sites where sorption reactions occur instantaneously (equilibrium sorption); and, (ii) sites where sorption reactions are rate-limited and occur kinetically, proceeding as a first-order reaction (van Genuchten and Wagenet, 1989; Maraqa, 2001). The faster sorption reactions are assumed to occur on easily accessible sites e.g. the outer surface of soil aggregates, with the slower sorption reactions postulated to occur on

less accessible sorption sites situated within the soil organic matter (van Beinum *et al.*, 2010). Solute transfer between the fast and slow sorption sites is described by kinetically-controlled reactions (van Genuchten and Wagenet, 1989; Streck *et al.*, 1995; Heistermann *et al.*, 2003). Degradation may also be integrated into two-site models, operating at either one or both types of sorption sites (Guo *et al.*, 2000).

The two-site approach has been used in the leaching model PEARL (Leistra *et al.*, 2001) and in the PEARLNEQ software for predicting parameters to describe long-term sorption kinetics (Boesten *et al.*, 2007). Solute sorbed at equilibrium sites is assumed to be constantly at equilibrium and the solute sorbed at non-equilibrium sites is described by a pseudo first-order sorption rate equation (Boesten *et al.*, 2007). Degradation is only assumed to affect the solute sorbed at equilibrium sites. A similar approach to non-equilibrium sorption has also been implemented into the pesticide leaching model MACRO (Larsbo and Jarvis, 2003), though degradation in this model is described by four separate first-order kinetic rate coefficients (solid and liquid, micro- and macropores).

The assumption of only two sorption sites oversimplifies soil systems. Boesten *et al.* (1989) for example, found it was necessary to include three sorption sites in order to adequately describe their data: (i) sites where sorption reactions occur instantaneously; (ii) sites where sorption reactions occur kinetically using a time-scale of days; and, (iii) sites where sorption reactions occur kinetically using a time-scale of hundreds of days. Saffron *et al.* (2006) considered three types of desorption regimes: (i) a fast or instantaneous regime, where desorption occurs at rates not captured by the first few sampling points; (ii) a dynamic regime in which rates are well measured by the sampling scheme; and, (iii) a slow regime where rates are slower than can be measured given the combination of data uncertainty and duration of sampling. They found that although naphthalene desorption was best described by two regimes, all three regimes were required to adequately describe the desorption behaviour of atrazine. Although in reality there are multiple sorption sites, each with different rates of sorption, obtaining experimental data with sufficient data to adequately parameterise such models is challenging (van Beinum *et al.*, 2010).

2.7.1.3. Irreversible sorption models

In an irreversible sorption model, the rate of desorption from one type of sorption site is set to zero. Selim and Amacher (1988) proposed the use of a second-order kinetic approach to describe solute retention during transport in soils; their model incorporated three different sorption sites, two reversible (S_1 , S_2) and one irreversible (S_{irr}). They used five adsorption-desorption rate constants in total (though the rate of desorption from the irreversible site (S_{irr}) is zero). Prata *et al.* (2003) fitted atrazine breakthrough curves with a three-site chemical non-equilibrium convective-dispersive transport model considering irreversible sorption. The three-site non-equilibrium model predicted that around 40% of the applied atrazine was irreversibly sorbed at the end of the leaching experiment, which corresponded with the sum of their measured extractable and non-extractable fractions. Celis and Koskinen (1999a; 1999b) also considered irreversible sorption in their two-site model. They assumed that sorption occurred on either easily desorbable sites or irreversible sites; their model estimated that approximately 10% of sorbed pesticide was irreversibly bound.

Irreversible binding is not however, considered in many of the existing mass-transfer models due to difficulties in deriving the parameters. Slowly reversible sorption and irreversible sorption are difficult to separate experimentally (van Beinum *et al.*, 2010). Very slow desorption kinetics can give the impression that the sorbed pesticide is irreversibly bound, though it is instead an effect of an experimental time-frame that is not long enough to observe desorption end-points (van Beinum *et al.*, 2010).

2.7.1.4. Stochastic models

Since soil aggregates exhibit considerable heterogeneity in terms of their size, shape and composition, a stochastic approach has been adopted a number of times to describe pesticide sorption kinetics (Ahn *et al.*, 1999). Stochastic models assume that a continuum of possible diffusion coefficients, sorption equilibrium constants, and/or first-order mass transfer rate constants exist (Ahn *et al.*, 1999). Connaughton *et al.* (1993) were able to effectively describe the desorption of naphthalene from contaminated soil using a gamma distribution of first-order mass-transfer-limited processes.

2.7.1.5. Hybrid model

The hybrid model described by Ahn *et al.* (1999) is a combined two-site (or two-region) non-equilibrium and gamma model. Ahn *et al.* (1999) conceptually divided the soil organic matter into two compartments, with fast and slow kinetic desorption. Naphthalene sorbed in the rapid compartment is assumed to be in instantaneous equilibrium with the aqueous phase, while its release from kinetically-mediated sorption sites is assumed to be governed by a gamma distribution of rate coefficients (Ahn *et al.*, 1999). Their hybrid model successfully described both the initial rapid release and following slow release of naphthalene over 25 days.

2.7.2. Diffusion models

These models are based on the assumption that sorption can be described in terms of diffusion through the spherical geometry of the sorbent, usually based on Fick's law (Fortin *et al.*, 1997; Maraqa, 2001). Ma and Selim (1997) have established complex diffusion equations based on several aggregate geometries (rectangular, solid and hollow cylindrical aggregates) by introducing a time-dependent phase transfer constant in addition to the diffusion coefficient. van Beinum *et al.* (2006) and Altfelder and Streck (2006) have both used diffusion models to simulate time-dependent sorption. van Beinum *et al.* (2006) were able to effectively describe the radial diffusion of pesticide into lignin particles and its following desorption. Altfelder and Streck (2006) compared a first-order and spherical diffusion model to describe and predict the long-term sorption and desorption processes of chlorotoluron in two soils; they found that the spherical diffusion model performed better than the first-order model. Diffusion models based on well-defined geometry are difficult to apply to the field situation, since they require information relating to the geometry of structural units, which are rarely available (Fortin *et al.*, 1997).

2.7.3. Evaluation of modelling approaches

van Beinum *et al.* (2010) have explained that the selection of a regulatory model to describe non-equilibrium sorption behaviour must reach a compromise between: (i) the ability of the model to describe aged sorption under a range of situations; and, (ii) the possibility to determine the model parameters from experiments with reasonable

effort. In addition, it must also be possible to implement the time-dependent sorption routine into models that simulate the transport of pesticides through the soil profile.

Currently, only the simplest form of the multi-site model (two-site model with identical adsorption and desorption rates and only a single degradation rate) is considered to be a viable option (van Beinum *et al.*, 2010). The two-site model already has six unknown parameters; the addition of any further sorption sites increases the number of unknown parameters by two, thus also increasing the difficulty of accurately estimating the model parameters (van Beinum *et al.*, 2010). Although two-site models have been used successfully to describe experimental systems (Brusseu *et al.*, 1991; Streck *et al.*, 1995; Heistermann *et al.*, 2003), many of the other modelling approaches described have performed better in comparison.

Over long time intervals, Altfelder and Streck (2006) found that the spherical diffusion model performed better than the two-site model. A number of authors have also found stochastic gamma distribution models to out-perform diffusion and two-site models (Connaughton *et al.*, 1993; Ahn *et al.*, 1999). Saffron *et al.* (2006) found that although naphthalene desorption was best described by two regimes, all three regimes were required to adequately describe the desorption behaviour of atrazine (section 2.7.1.2). Alternatively, Johnson *et al.* (2001) compared six modelling approaches in their ability to describe phenanthrene desorption data: (i) three-parameter kinetic model; (ii) five-parameter kinetic model; (iii) gamma-distribution model; (iv) one-parameter pore-diffusion model; (v) two-parameter pore-diffusion model; and, (vi) three-parameter biphasic polymer diffusion model. They concluded that all desorption profiles were at least biphasic and that models composed of two-regimes provide a good basis for describing desorption profiles.

In summary, non-equilibrium sorption cannot be perfectly predicted for all documented pesticide/soil scenarios using a specific set of model assumptions (van Beinum *et al.*, 2010). This is due to the inherent heterogeneities observed in field soils and subsequent difficulties in studying soils, in addition to their complex interaction with organic compounds. Thus, no model has been universally accepted and/or validated. The two-site model is considered to be the best option for use in regulatory leaching models (van Beinum *et al.*, 2010). Although they may not have the ability to describe sorption at all time-scales, this approach is appropriate for

describing the long-term sorption behaviour associated with leaching over an agricultural season (van Beinum *et al.*, 2010). Robust parameter values can be determined for many situations from well-designed experiments (van Beinum *et al.*, 2006).

2.8. Summary of current knowledge

The current knowledge available on the mechanisms, processes, influencing factors and approaches to measure and model sorption phenomena have been reviewed. The main conclusions are as follows:

- (i) The mechanisms responsible for pesticide sorption to soils include van der Waals interactions, hydrophobic bonding, hydrogen bonding, charge transfers, ligand exchange, covalent bonding and ionic bonding. Isolating a specific binding mechanism accountable for pesticide retention in a given soil system is difficult however, and direct experimental evidence of a particular mechanism is also quite rare.
- (ii) Sorption is a key process limiting the mobility and bioavailability of pesticide in a soil system. It is often assumed that a characteristic and reversible ratio between the chemical in solution and the chemical sorbed to the soil is instantaneous. This is referred to as equilibrium sorption and is typically measured using the batch-equilibrium method outlined by OECD guideline 106. The ratio between the pesticide sorbed to the soil and the pesticide remaining in solution is characterised by a distribution coefficient (K_d or K_f).
- (iii) Many factors have been observed to influence pesticide retention in soils. Partitioning between the aqueous and solid phase may be partly controlled by the chemistry of the pesticide itself (dissociation of ionisable compounds, lipophilicity/hydrophobicity). Once released into the environment however, the properties of the soil (pH, ionic strength, organic matter content, clay content, microbial activity), as well as hydrological and climatic conditions, play a significant role in pesticide fate.

- (iv) The adsorption-desorption process is not a simple instantaneous equilibrium process as previously thought, or assumed by many contaminant transport models. Time-dependent interactions have been observed to occur between the pesticide in the aqueous phase and sorbed phase, in which the strength of sorption increases with increasing pesticide-soil contact time. Such a phenomenon is referred to as time-dependent sorption, non-equilibrium sorption or ageing. The mechanisms responsible for this process are proposed to include diffusion into spatially remote areas e.g. soil micro- and macropores (film diffusion, pore diffusion, matrix diffusion) and entrapment within soil organic matter.
- (v) Further to time-dependent interactions, some sorbed pesticide has been observed to exist in an irretrievable, soil-bound state. This fraction has important implications for pesticide leaching and bioavailability. Although this fraction of sorbed pesticide is termed irreversible sorption in principle, many variations of this term exist within the literature. This is the result of the operational definition of such residues, which means that their quantification is dependent on the method of extraction used. Therefore, the terms bound residue and non-extractable residue are commonly used in order to express uncertainty in the true fraction of irreversibly sorbed pesticide. The mechanisms responsible for irreversible sorption are proposed to include covalent bonding and physical entrapment.
- (vi) Techniques used to measure/assess the nature bound residues include: (i) quantitative methods e.g. solvent extraction procedures that aim to extract pesticide residues from the soil and provide numerical estimates of the irreversible fraction; (ii) diagnostic methods e.g. electron spin resonance (ESR), fourier transform infra-red (FTIR) and nuclear magnetic resonance (NMR) that aim to qualitatively determine the nature of the bound residue rather than the amount; and, (iii) indirect experimental methods e.g. hydrolysis methods, derivatisation extraction, model compound investigations, pyrolysis and thermal desorption techniques used for isolation and fractionation of soil humic substances in order to indicate the circumstances of bound residue formation.

- (vii) Finally, a number of models exist to predict pesticide fate in soil systems. Sorption phenomena are an important component of such contaminant transport models. The main approaches implemented to model sorption behaviour are mass transfer models (two-region or mobile/immobile models, two-site and multi-site models, irreversible sorption models) and diffusion models. Models used within pesticide registration use instantaneous sorption as a standard assumption. Time-dependent sorption can be used at higher tiers of assessment but no harmonised methodology exists. Irreversible sorption is often not considered.

2.9. Gaps and research needs

During recent decades the literature has seen significant advances in the understanding of pesticide sorption phenomena, and this has contributed to a more accurate assessment of pesticide fate in the environment. Despite this progress, there are still many questions that remain unanswered, which necessitate further investigation.

As bound residues or non-extractable residues are currently operationally-defined, comparability between results obtained from individual studies is often restricted. The majority of bound residue and non-extractable data obtained thus far, may only be reproducible when implementing the same specific experimental conditions and methods of extraction used in the initial study. Therefore, the meaning of such data may be limited. The standardisation of laboratory extraction procedures would improve the collection of data and make comparisons between studies more feasible. A shift towards more “natural” methods of extraction would also increase the environmental applicability of these types of studies. This also leads on to the need for standardisation of terminology used to refer to bound residues, non-extractable residues and irreversible sorption. If definitions of the terms were used consistently, this would also facilitate comparability of data between studies. Finally, there is also a need for the development of a well-designed experiment with the ability to differentiate between non-equilibrium and irreversible sorption. Longer-term experiments are required to allow the correct evaluation of time-dependent sorption kinetics. Alternatively, an experimental design that enabled the acceleration of

pesticide desorption from soil would be equally valuable, in order to increase the practicality of carrying out such studies.

Further gaps in current knowledge include equating extractability more closely with bioavailability and availability for movement to water bodies. More information is required on the possible effects bound residues may have on the environment, and whether these effects are significant. Finally, incorporating irreversible sorption phenomena into the current modelling approaches would enable a more accurate prediction of the fate and behaviour of pesticides in soil systems. However, this would rely on development of an experimental method with the power to adequately distinguish between slowly reversible and irreversible sorption in order to support the additional parameters.

CHAPTER 3

TEST SYSTEMS, ANALYTICAL METHODS AND PRELIMINARY EXPERIMENTS

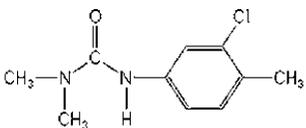
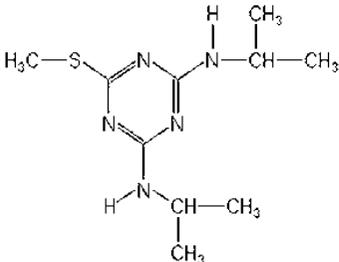
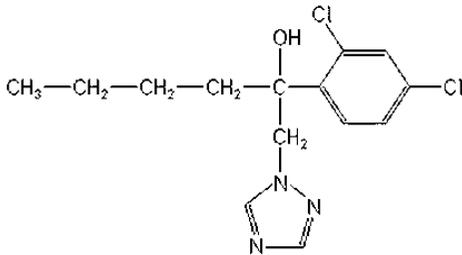
3.1. Introduction

Three pesticides and three soils were selected for study in this thesis. The three pesticides were: (i) the urea herbicide chlorotoluron; (ii) the triazine herbicide prometryn; and, (iii) the triazole fungicide hexaconazole. The three arable UK soils were: (i) a Blackwood loamy sand; (ii) an Andover clay loam; and, (iii) a Salop loam. The key chemical and environmental fate properties of the studied pesticides are outlined below, as well as the main properties of the studied soils. Preliminary tests were carried out prior to commencing the main experimental work; these initial tests involved: (i) the selection of an optimal soil:solution ratio for each pesticide; and, (ii) subsequent running of batch Freundlich sorption studies for each pesticide and soil type in accordance with OECD guideline 106 (2000). The methods used to carry out these tests and the results obtained are explained in detail below. Furthermore, the analytical methods used to analyse the ^{12}C - and ^{14}C -pesticide samples generated by the work carried out in this thesis are also described.

3.2. Pesticide properties

The key chemical and environmental fate properties of the pesticides studied are summarised in Table 3.1. The three pesticides (chlorotoluron, prometryn and hexaconazole) were chosen based on their differing chemical and environmental fate properties. One neutral, one basic and one acidic compound were studied. Pure ^{12}C analytical grade chlorotoluron (purity 99.7%, Fluka®), prometryn (purity 99.2%, Riedel-de Haën®) and hexaconazole (purity 99.7%, Fluka®) were purchased from Sigma-Aldrich Ltd (Dorset, UK). [^{14}C -Phenyl-U- ^{14}C] chlorotoluron (purity 99.2%, specific activity 4.57 MBq mg $^{-1}$), [Triazinyl-U- ^{14}C] prometryn (purity 98.1%, specific activity 4.16 MBq mg $^{-1}$) and [Triazolyl-U- ^{14}C] hexaconazole (purity 99.6%, specific activity 2.26 MBq mg $^{-1}$) were supplied by Syngenta Ltd (Jealott's Hill International Research Centre, Bracknell, UK).

Table 3.1. Key chemical and environmental fate properties of the study pesticides*.

Property	Chlorotoluron	Prometryn	Hexaconazole
Chemical properties:			
Pesticide type, substance group	Herbicide, urea	Herbicide, triazine	Fungicide, triazole
Mode of action	Selective, non-systemic adsorbed by roots and foliage. Acts by the inhibition of photosynthetic electron transport.	Selective, systemic, contact and residual triazine. Photosynthetic electron transport inhibitor at the photosystem II receptor site.	Broad spectrum, systemic with protective and curative action. Disrupts membrane function.
CAS number	15545-48-9	7287-19-6	79983-71-4
Chemical formula	C ₁₀ H ₁₃ ClN ₂ O	C ₁₀ H ₁₉ N ₅ S	C ₁₄ H ₁₇ Cl ₂ N ₃ O
Chemical structure			
Molecular mass (g mol ⁻¹)	212.68	241.36	314.21
Solubility in water (mg L ⁻¹) at 20°C	74	33	18
Environmental fate properties:			
Octanol-water partition coefficient (pH 7, 20°C)	P = 3.16 x 10 ² , Log P = 2.5	P = 2.19 x 10 ³ , Log P = 3.34	P = 7.94 x 10 ³ , Log P = 3.9
Dissociation constant at 25°C	N/A (Neutral)	4.1 (Weak base, pK _b = 9.95)	2.3 (Strong acid)

Vapour pressure at 25°C (mPa)	0.005	0.13	0.018
Henry's Law Constant at 25°C (Pa m ³ mol ⁻¹)	1.44 x 10 ⁻⁵	1.20 x 10 ⁻³	3.33 x 10 ⁻⁴
DT50 lab at 20°C (days)	59 (moderately persistent)	41 (moderately persistent)	122 (persistent) ^a
<i>K</i> _{oc}	196	400	1040
Key metabolites in soil	(i) 3-(3-chloro-p-tolyl)-1-methylurea	(i) 2-hydroxy-propazine; (ii) 2,4-bis(isopropylamino)-6-hydroxy-S-triazine; (iii) 2-methylthio-4-amino-6-isopropylamino-S-triazine; and, (iv) hydroxypropazine.	(i) 1,2,4-triazole; and, (ii) 1H-1,2,4-triazol-1-ylacetic acid.

* All data and diagrams were taken from the FOOTPRINT pesticide properties database (FOOTPRINT, 2006), collated by the University of Hertfordshire.

^aMeasured DT50 in laboratory at 20°C not available, typical DT50 only.

3.3. Soil properties

The three arable UK soils selected for study were chosen based on their differing physico-chemical properties. Study soils were collected, prepared and stored prior to use in accordance with OECD guideline 106 (2000). Fresh soils were collected from 0-15 cm depth, air-dried at ambient temperature (20-25°C) and then sieved to 2 mm. Soil moisture contents were determined by heating triplicate samples at 105°C for 24 hours and calculating the average difference in soil mass before and after heating. Soils were gamma irradiated at 35.40 kGy by Isotron Ltd (Bradford, UK) to sterilise and thus inhibit microbial degradation. Irradiated soils were subsequently stored under sterile conditions (at 4°C in darkness) until use. The three study soils were characterised by NRM Laboratories Ltd (Bracknell, UK). The results are given in Table 3.2. Although the Andover and Salop soils have a similar texture, they have a different pH; the Blackwood and Salop soils have a similar pH but the Blackwood soil has a greater sand content. Soils used in all experiments were taken from the same batch.

Table 3.2. Characterisation of the three study soils*.

Property	Blackwood	Andover	Salop
Sample location, OS grid reference	SE50917051	SE99105892	SP2660666114
Date collected	May 2009	May 2009	May 2009
Texture	Loamy sand	Clay loam	Loam
pH (in CaCl ₂)	5.6	6.9	5.8
Cation exchange capacity (meq/100 g)	29.0	20.2	25.5
Water holding capacity at 0.33 bar (% w/w)	19.2	27.6	21.7
Sand content (% w/w)	83	49	51
Silt content (% w/w)	8	32	26
Clay content (% w/w)	9	19	20
Organic matter content (% w/w)	5.6	5.1	5.2
Organic carbon content (% w/w) ^a	3.2	2.9	3.0

*Characterised by NRM Laboratories Ltd (Bracknell, UK).

^aCalculated using the van Bemmelen conversion factor of 1.724 (Pribyl, 2010).

3.4. Analytical methods

3.4.1. Analysis of ^{12}C -pesticide samples

3.4.1.1. High performance liquid chromatography (HPLC)

All ^{12}C -chlorotoluron, ^{12}C -prometryn and ^{12}C -hexaconazole samples generated by the experimental work were analysed by HPLC (Agilent 1100 Series, Agilent Technologies UK Ltd). HPLC method details are given in Table 3.3.

3.4.1.2. Liquid-chromatography time-of-flight mass-spectrometry (LC-TOF-MS)

LC-TOF-MS was only used to analyse ^{12}C -chlorotoluron supernatants and soil extracts from the samples used to test for degradation (see Chapter 4, section 4.2.4). LC-TOF-MS analysis was performed using an Agilent 1200 Series LC with G120 Time of Flight Mass Spectrometer (Santa Clara, CA, USA). LC was performed using a Waters Acquity BEH C_{18} (2.1 x 50 mm, 1.7 μm) column (at 35.0°C) with 0.2 μm in-line filter. Mobile phases were 5 mM ammonium acetate in water (Channel A) and methanol (Channel B). A gradient method and flow rate of 0.6 mL min^{-1} was used. The initial ratio of ammonium acetate/methanol was 98:2, changing to 2:98 over 5 minutes, held for 3.1 minutes before then returning to original conditions after 8.1 minutes (total run time 9 minutes). Retention time of parent chlorotoluron was 3.01 minutes. Injection volume was 3 μL in acetonitrile. TOF-MS analysis was carried out in positive mode electrospray with a nebulizer pressure of 45 psi, capillary of 4000 V, gas temperature of 450°C, drying gas flow at 15 L min^{-1} , skimmer of 60 V, fragmentor of 150 V and octopole RF voltage of 250 V. The mass range measured was 100-1100 m/z (mass to charge ratio). Total ion chromatographs were generated using Agilent Masshunter.

3.4.2. Analysis of ^{14}C -pesticide samples

3.4.2.1. Liquid scintillation counting (LSC)

All ^{14}C -pesticide samples generated during the thesis were analysed by LSC (LS 6500 Beckman Coulter Inc., Fullerton, USA). The LSC method counted each sample three times for a total of 15 minutes (5 minutes each). Duplicate blanks were used to account for the background radioactivity and results were corrected for quench and

Table 3.3. HPLC methods used to analyse ¹²C 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 ₃ PO 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)

* Acidified with ortho-phosphoric acid (H₃PO₄).

^a Acidified with formic acid (HCOOH).

luminescence. Limits of quantification were 0.00011, 0.00012 and 0.00022 $\mu\text{g mL}^{-1}$ for chlorotoluron, prometryn and hexaconazole, respectively.

3.4.2.2. Radio-HPLC

The ^{14}C -chlorotoluron, ^{14}C -prometryn and ^{14}C -hexaconazole samples used to test for degradation were analysed by Radio-HPLC (Hewlett Packard 1100 Series HPLC with Perkin-Elmer Radiomatic 625TR Flow Scintillation Analyser). Radio-HPLC analysis was used to identify the proportions of parent and metabolite that contributed to the radioactivity quantified by LSC. Radio-HPLC method details are given in Table 3.4.

3.4.2.3. Soil combustion

All ^{14}C -chlorotoluron, ^{14}C -prometryn and ^{14}C -hexaconazole soil samples used to calculate the mass balances were combusted (see Chapters 4 and 5). To prepare soil samples for combustion, soils were air-dried for 7 days and then ground using a pestle and mortar. Approximately 200 mg of dry, ground soil was weighed into a combustion cone sandwiched between two combustion caps. Soil samples were then oxidised in a Perkin-Elmer Oximate 80, Model 370 and finally analysed by LSC (Perkin-Elmer Tri-Carb 2810TR Liquid Scintillation Analyser). Limits of quantification were 0.00011, 0.00012 and 0.00022 $\mu\text{g mL}^{-1}$ for chlorotoluron, prometryn and hexaconazole, respectively (in both 0.01M CaCl_2 and MeOH).

3.5. Selection of optimal soil:solution ratios

Selection of optimal soil:solution ratios for the three pesticides (chlorotoluron, prometryn and hexaconazole) and three study soils (Blackwood, Andover and Salop) was carried out in accordance with OECD guideline 106 (2000) using ^{14}C -pesticide. Any deviation from the OECD guidelines has been acknowledged and justified.

3.5.1. Chlorotoluron

3.5.1.1. Method

Sorption of chlorotoluron to the Salop soil only (greatest sorption anticipated based on prior tests) was measured at four different soil:solution ratios (1:2, 1:3, 1:4 and 1:5). The 1:2, 1:3 and 1:5 soil:solution ratio tests were carried out in duplicate, and the 1:4 soil:solution ratio test was carried out in triplicate. Samples were prepared by

Table 3.4. Radio-HPLC methods used to analyse ¹⁴C 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

* Acidified with formic acid (HCOOH).

weighing out the desired amount of oven dry soil (see Table 3.3) into 50 mL Teflon® centrifuge tubes. For pre-equilibration, 19 mL of 0.01M CaCl₂ was added to each soil sample and then samples were shaken (HS 501 Digital IKA®-Werke reciprocal shaker) for at least 12 hours overnight (150 rpm) at room temperature. It should be noted here that the OECD guidelines recommend that the minimum volume of 0.01M CaCl₂ that should be used to pre-equilibrate the soils is 45 mL. Using such a volume for pre-equilibration was not possible however. The total volume of shaking solution that could be used was limited by the capacity of the Teflon® centrifuge tubes themselves. Although 50 mL nominal volume, the centrifuge tubes could not adequately hold 50 mL of 0.01M CaCl₂, even without soil. Therefore, the total amount of shaking solution used was 20 mL. ¹⁴C-chlorotoluron treatment solution was added to soil suspensions in 1 mL of 0.01M CaCl₂. The concentration of the treatment solution was quantified by LSC as 13.73 µg mL⁻¹ (see section 3.4.2.1 for LSC method). Samples were then shaken continuously under the same conditions as the pre-equilibration step for 24 hours. Samples were finally centrifuged (Hermle Z513K, LaborTechnik, Bench Top Centrifuge) at 3500 rpm for 10 minutes and a 200-µL aliquot was removed for quantification by LSC (LS 6500 Beckman Coulter Inc., Fullerton, USA).

3.5.1.2. Results

Sorption of chlorotoluron to the Salop soil measured at the four tested soil:solution ratios is given in Table 3.5.

Table 3.5. Sorption of chlorotoluron to Salop soil at varying soil:solution ratios.

Pesticide	Soil:solution ratio	Weight of soil (g)	Volume of solution (mL)	Soil type	% sorption (24 h)
Chlorotoluron	1:2	10	20	Salop	87.6 ^a , 88.0 ^b
	1:3	7	20	Salop	82.3 ^a , 82.5 ^b
	1:4	5	20	Salop	75.8 ^a , 76.8 ^b
	1:5	4	20	Salop	69.9 ^a , 70.9 ^b

^aReplicate A.

^bReplicate B.

Percentage sorption of chlorotoluron to the Salop soil was generally quite high for all soil:solution ratios tested (> 70%). According to the OECD guideline 106, sorption should be 20%, but preferably > 50%. After considering that sorption of

chlorotoluron to the Blackwood and Andover soils would be less than was observed here for the Salop soil, a 1:4 soil:solution ratio was selected as optimal.

3.5.2. Prometryn and hexaconazole

3.5.2.1. Method

Sorption of prometryn and hexaconazole was tested at three different soil:solution ratios and in all three study soils (Blackwood, Andover and Salop). For prometryn, sorption was measured at soil:solution ratios of 1:5, 1:10 and 1:20. Hexaconazole was anticipated to exhibit greater sorption to soil given its chemical properties, thus sorption was measured at the increased soil:solution ratios of 1:10, 1:20 and 1:50. One replicate per soil:solution ratio and soil type was used for both prometryn and hexaconazole. Samples were prepared by weighing out the desired amount of oven dry soil (see Table 3.4) into 50-mL Teflon® centrifuge tubes. It should be noted here that the OECD guidelines recommend that at least 1 g of soil should be used and preferably 2 g in order to obtain reliable results. However, for the same reason as described for chlorotoluron above, in order to achieve a 1:50 soil:solution ratio for hexaconazole in the test vessels, it was necessary to use 0.5 g of soil.

For pre-equilibration, 19 mL of 0.01M CaCl₂ was added to each soil sample (24 mL for the hexaconazole 1:50 ratio) and then suspensions were shaken for at least 12 hours overnight (150 rpm) at room temperature. ¹⁴C-prometryn and ¹⁴C-hexaconazole treatment solutions were added to soil suspensions in 1 mL of 0.01M CaCl₂; their concentrations were quantified by LSC as 4.73 and 5.21 µg mL⁻¹, respectively (see section 3.4.2.1 for LSC method). Samples were then shaken continuously under the same conditions as for the pre-equilibration step for 24 hours. After 24 hours, samples were centrifuged at 3500 rpm for 10 minutes and a 200-µL aliquot was removed for quantification by LSC. Samples were then re-suspended and shaken again (under the same conditions) for a further 96 hours to assess whether pesticide sorption to soil increased during this time.

3.5.2.2. Results

Sorption percentages of both pesticides to the three soil types at all soil:solution ratios are given in Table 3.6. As anticipated, sorption to the test soils after both 24 and 120 hours was considerably greater for hexaconazole than prometryn. At the lowest

comparable soil:solution ratio (1:10), sorption of hexaconazole was 87%, 73% and 70% for the Blackwood, Andover and Salop soils, respectively. For prometryn however, percentage sorption at this ratio was only 50%, 29% and 30% for the Blackwood, Andover and Salop soils, respectively. The greater sorption of hexaconazole to the three study soils was also apparent at the higher soil:solution ratio of 1:20.

Table 3.6. Sorption of prometryn and hexaconazole to the three test soils with varying soil:solution ratios.

Pesticide	Soil:solution ratio	Weight of soil (g)	Volume of solution (mL)	Soil type	% sorption (24 h)	% sorption (120 h)
Prometryn	1:5	4	20	Blackwood	65	70
	1:5	4	20	Andover	43	48
	1:5	4	20	Salop	44	53
	1:10	2	20	Blackwood	50	56
	1:10	2	20	Andover	29	35
	1:10	2	20	Salop	30	37
	1:20	1	20	Blackwood	37	44
	1:20	1	20	Andover	19	26
	1:20	1	20	Salop	22	29
Hexaconazole	1:10	2	20	Blackwood	87	90
	1:10	2	20	Andover	73	80
	1:10	2	20	Salop	70	77
	1:20	1	20	Blackwood	78	83
	1:20	1	20	Andover	61	68
	1:20	1	20	Salop	58	66
	1:50	0.5	25	Blackwood	62	68
	1:50	0.5	25	Andover	43	50
	1:50	0.5	25	Salop	41	50

Time-dependent sorption was observed for both prometryn and hexaconazole during 24 and 120 hours for all soil:solution ratios and soil types (Table 3.6). Taking this into account, a 1:10 soil:solution ratio and 1:50 soil:solution ratio were selected as optimal for prometryn and hexaconazole, respectively. Sorption of the two pesticides at these ratios were close to the least amount of sorption recommended by the OECD guidelines (20% preferably > 50%). However, the long adsorption periods for which the prometryn and hexaconazole laboratory studies were to be run, significantly increases the potential for time-dependent sorption. Thus, the selected ratios were anticipated to accommodate an increase in strength of sorption whilst still allowing accurate quantification of the aqueous phase after 168 days. As 24 hours is assumed to characterise equilibrium sorption, a 24-hour equilibration period was used in the

following Freundlich sorption studies to eliminate the effect of time-dependent sorption. The OECD guidelines also state that a 24-hour equilibration period is generally sufficient to measure equilibrium-sorbed pesticide.

3.6. Freundlich sorption studies

Freundlich sorption studies for the three pesticides (chlorotoluron, prometryn and hexaconazole) and three study soils (Blackwood, Andover and Salop) were also carried out in accordance with OECD guideline 106 (2000). Again, any deviation from the guidelines has been acknowledged and justified. The Freundlich sorption studies were carried out using ^{12}C -pesticide in order to minimise the amount of radiolabelled compound used; otherwise the high concentrations of pesticide required to do the tests would have equated to a substantial amount of radioactivity.

3.6.1. Method

Freundlich sorption studies for each pesticide and study soil were carried out in triplicate for a total of seven different concentrations (see Table 3.7). As the proposed experimental work (forced isotope exchange) involves application of high-concentration pesticide solution (up to $600\ \mu\text{g mL}^{-1}$ for chlorotoluron), concentrations C6 and C7 were included in order to incorporate sorption behaviour at very high concentrations.

As selected in the previous preliminary test, a 1:4 soil:solution ratio was used for chlorotoluron, a 1:10 soil:solution ratio was used for prometryn, and a 1:50 soil:solution ratio was used for hexaconazole. To prepare the soil samples, the desired amounts of oven dry soil were weighed into 50-mL Teflon® centrifuge tubes (5 g for chlorotoluron samples, 2 g for prometryn samples and 0.5 g for hexaconazole samples). Soils were then pre-equilibrated by adding either 10 mL (chlorotoluron and prometryn) or 5 mL (hexaconazole) of 0.01M CaCl_2 and then shaken for at least 12 hours overnight (150 rpm, room temperature). Pesticide treatment solutions were then applied to the pre-equilibrated soil suspensions in 10 mL 0.01M CaCl_2 for chlorotoluron and prometryn samples, and 20 mL 0.01M CaCl_2 for hexaconazole samples. Samples were then shaken continuously under the same conditions as the pre-equilibration step for 24 hours. After this period, samples were centrifuged at

3500 rpm for 10 minutes, and a 1-mL aliquot was removed for HPLC analysis (see section 3.4.1.1 for HPLC method) in order to measure percentage sorption with increasing pesticide concentration.

Table 3.7. Final concentrations of pesticide in solution before sorption.

Concentration	Chlorotoluron ($\mu\text{g mL}^{-1}$)	Prometryn ($\mu\text{g mL}^{-1}$)	Hexaconazole ($\mu\text{g mL}^{-1}$)
C1	0.5	0.3	0.2
C2	2.5	0.6	0.5
C3	5	1.25	1
C4	10	2.5	2
C5	20	5	4
C6	40	10	8
C7	50	15	12

It should be noted that as a result of difficulties dissolving all three pesticides at the higher concentrations, it was necessary to increase the volume of pesticide treatment solutions applied in order to achieve the required concentrations (10 mL for chlorotoluron and prometryn and 20 mL for hexaconazole). Hexaconazole treatment solutions were applied in 20 mL rather than 10 mL due to its lower solubility limit in water ($18 \mu\text{g mL}^{-1}$) than chlorotoluron ($74 \mu\text{g mL}^{-1}$) and prometryn ($33 \mu\text{g mL}^{-1}$). The addition of pesticide treatment solutions in such large volumes conflicts with the OECD guidelines, which state that the volume of pesticide treatment solution applied to the pre-equilibrated soil suspension, should not exceed 10% of the final volume of shaking solution. This is recommended in order to minimise changes to the pre-equilibration solution. However, although the addition of treatment solutions in 10 mL (chlorotoluron and prometryn) and 20 mL (hexaconazole) meant that their volume amounted to 50% and 80% of the final shaking solutions respectively, tests showed that a greater than 10% change to the pre-equilibration solution had a limited effect on total amounts of sorption.

3.6.3. Results

The sorption of the three pesticides to the three soils is shown in Figures 3.1 (chlorotoluron), 3.2 (prometryn) and 3.3 (hexaconazole). The Freundlich parameters

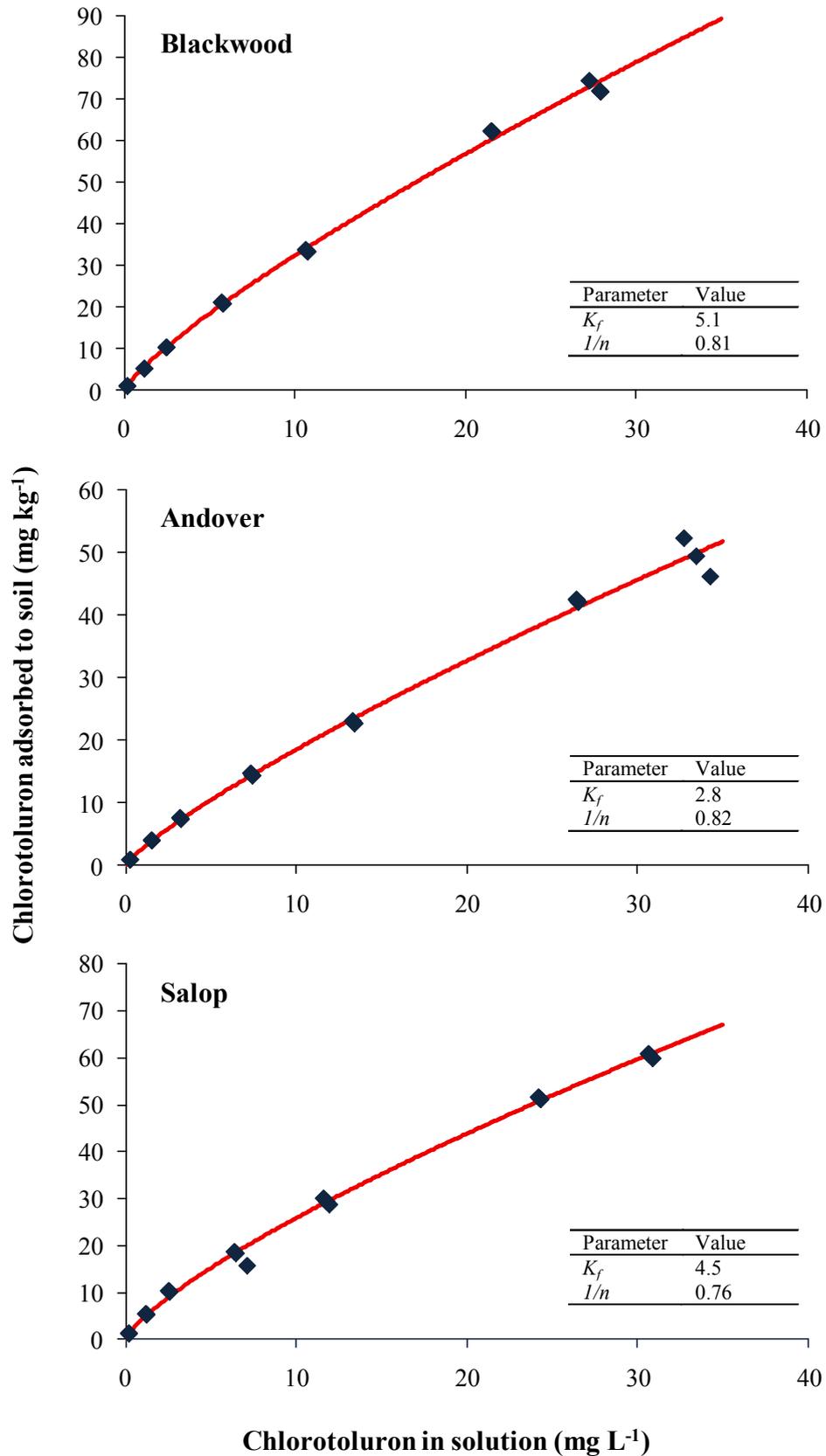


Figure 3.1. Sorption of chlorotoluron to Blackwood, Andover and Salop soils with increasing solute concentration.

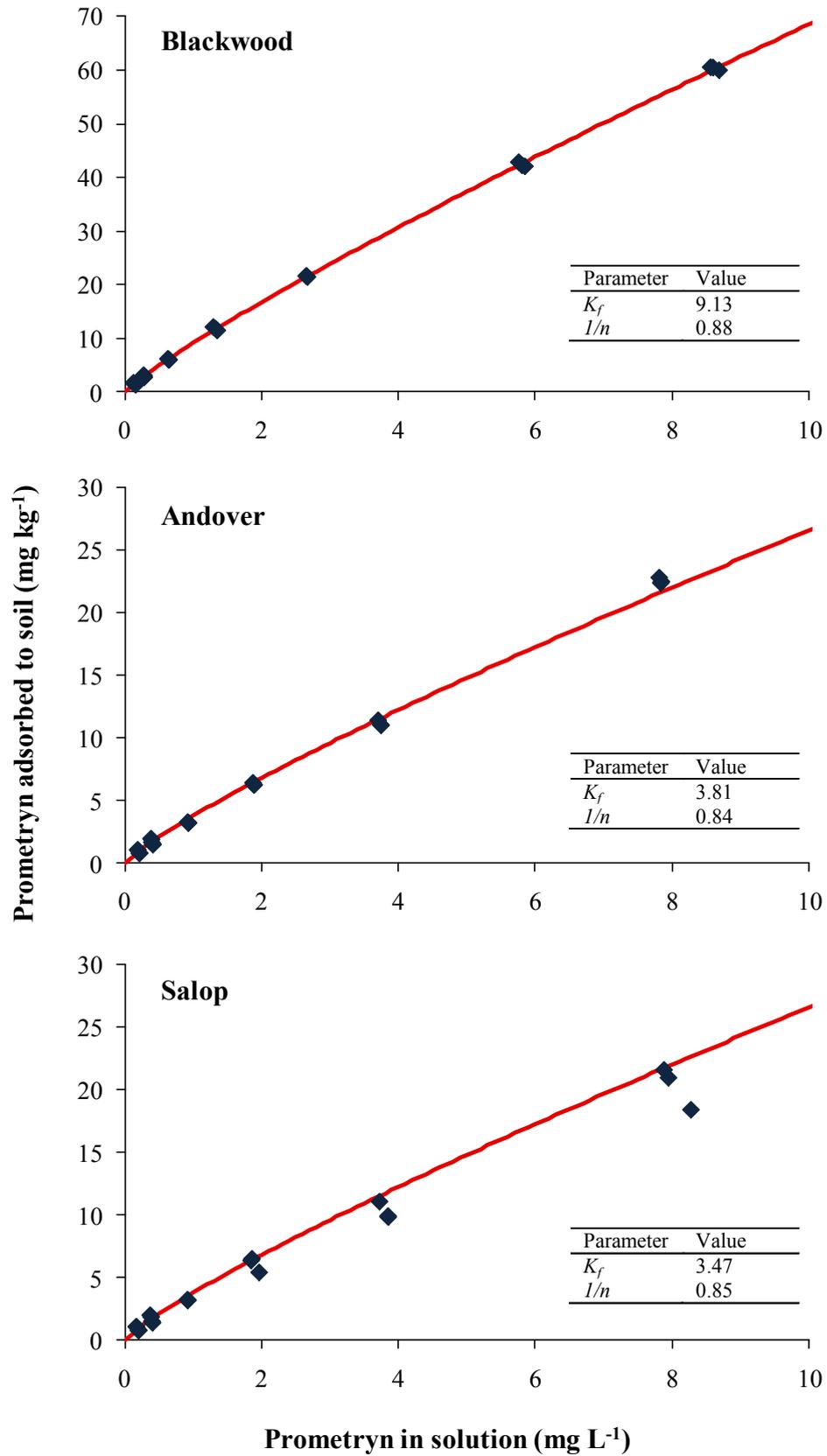


Figure 3.2. Sorption of prometryn to Blackwood, Andover and Salop soils with increasing solute concentration.

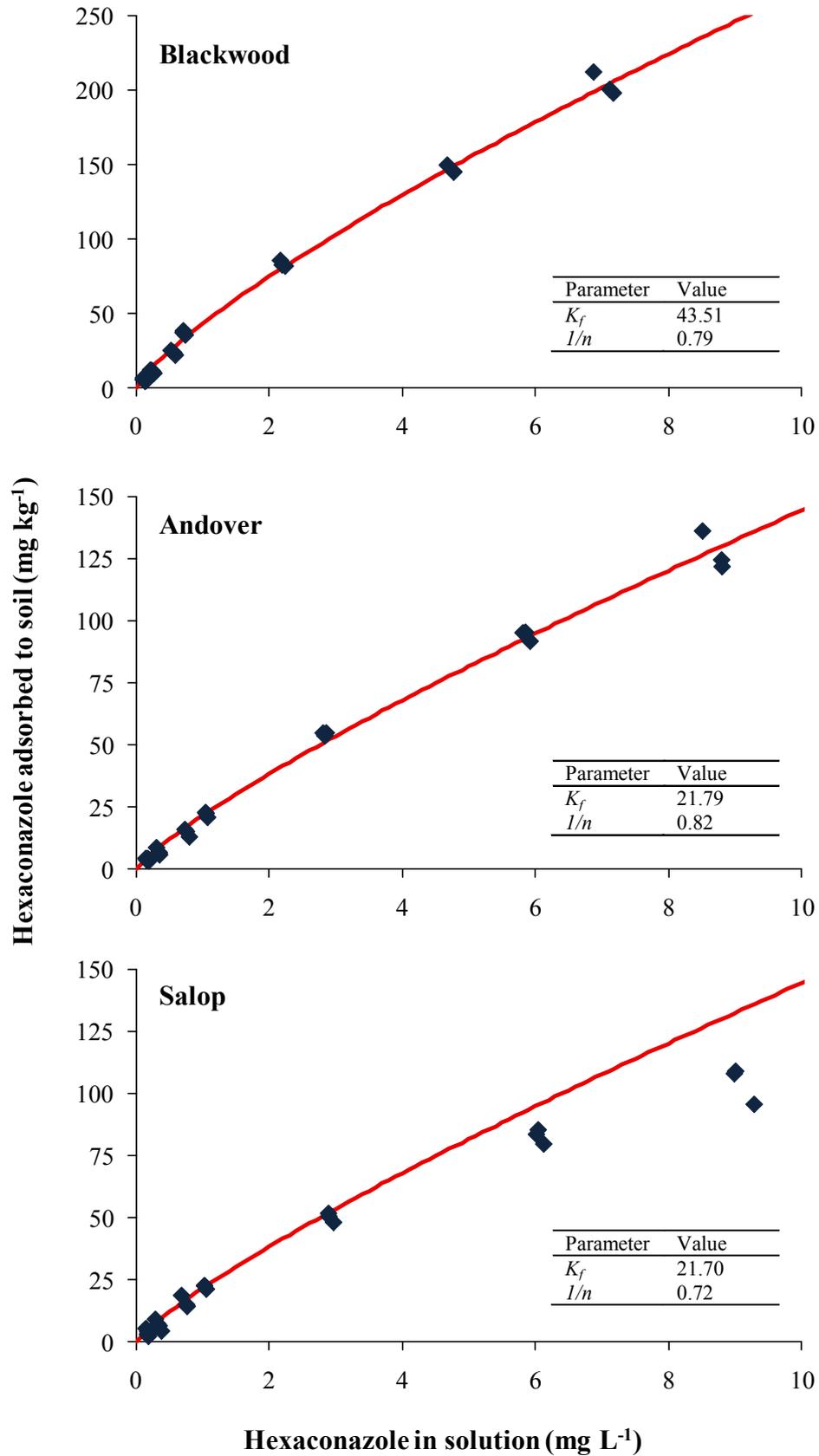


Figure 3.3. Sorption of hexaconazole to Blackwood, Andover and Salop soils with increasing solute concentration.

(K_f and $1/n$), which describe the distribution of pesticide between the soil and solution and non-linearity of sorption with increasing concentration are also included in each Figure. The two parameters were calculated using the Freundlich equation:

$$C_s = K_f \times C_{aq}^{1/n}$$

where C_s refers to the concentration of pesticide in the soil or solid phase ($\mu\text{g g}^{-1}$), C_{aq} refers to the concentration of pesticide in the aqueous phase ($\mu\text{g mL}^{-1}$) at equilibrium, K_f refers to the Freundlich distribution coefficient and $1/n$ refers to the Freundlich exponent. Initially, only estimates of the K_f and $1/n$ are used in the Freundlich equation to model the data. These estimates are then improved by using the Microsoft Excel Solver Add-On to reduce the residual sum of squares between the observed data and modelled values. Visually, the model fits to the data are generally very good with the exception of the model fit of hexaconazole sorption to the Salop soil data, which overestimates the sorption of the highest two concentrations. The model fit to the measured data for all pesticide and soil systems has been quantified statistically using the following model efficiency (ME) equation:

$$ME = 1 - \frac{\sum_{i=1}^n (C_i - O_i)^2}{\sum_{i=1}^n (O_i - \bar{O})^2}$$

where n is the total number of observations, O_i is the i^{th} observed value (with $i=1, 2, \dots, n$), C_i is the i^{th} value calculated by the model, and \bar{O} is the mean of all observed values. This equation may also be expressed as:

$$ME = 1 - \frac{RSS}{TSS}$$

where RSS is the residual sum of squares and TSS is the total sum of squares. The ME compares the sum of squared differences between the calculated and observed data (RSS) with the variability in the observed data. The calculated MEs for the model fits to the chlorotoluron, prometryn and hexaconazole Freundlich sorption data are given in Table 3.8.

As the ME may range from minus infinity to +1, with larger absolute values indicating better agreement between the measured data and modelled values, the

values given in Table 3.8 indicate that all model fits describe the measured data very well. The lowest ME was calculated for the hexaconazole/Salop soil test system, which deviates from the measured values at the highest concentrations as previously described.

Table 3.8. Calculated model efficiencies (ME) for the Freundlich isotherm data.

Soil type	Calculated model efficiency (ME) for each pesticide		
	Chlorotoluron	Prometryn	Hexaconazole
Blackwood	0.998	1.000	0.996
Andover	0.994	0.998	0.993
Salop	0.997	0.993	0.988

The K_f values obtained are significantly greater for hexaconazole than for both chlorotoluron and prometryn, indicating the greatest retention of that pesticide by the three study soils. The chlorotoluron and prometryn Freundlich distribution coefficients were similar, though with slightly greater adsorption of prometryn by the Blackwood and Andover soils, and slightly greater adsorption of chlorotoluron by the Salop soil. Sorption was the most linear for prometryn ($1/n$ values of 0.88, 0.84 and 0.85 for the Blackwood, Andover and Salop soils, respectively) and the least linear for hexaconazole ($1/n$ values of 0.79, 0.82 and 0.72 for the Blackwood, Andover and Salop soils, respectively).

3.7. Outlook

In the main experimental work, the final concentrations of chlorotoluron, prometryn and hexaconazole in the soil solution (before sorption) are 0.7, 0.8 and 0.8 $\mu\text{g mL}^{-1}$, respectively. These only correspond to the sorption described for concentrations C1-C3 (Table 3.7) of the Freundlich isotherms; thus - only the very lowest part of the Freundlich isotherms will describe the behaviour of these pesticides in the main experimental work. The fact that pesticide treatment solutions in the Freundlich sorption studies were added in large volumes and also high concentrations was necessary to incorporate the sorption behaviour that will occur during the forced isotope exchange experiment. The forced isotope exchange procedure (Chapters 4 and 5), involves repeated replacement of the supernatant (large volume) with a high concentration ^{12}C -pesticide solution. Thus, the parameters obtained by these Freundlich sorption studies are representative of sorption in the pesticide/soil systems

operating during the forced isotope exchange procedure. This justifies their use in the three-site model (Chapter 6) developed to describe the isotope exchange and forced isotope exchange procedures.

CHAPTER 4

LONG-TERM EXPERIMENTS TO INVESTIGATE IRREVERSIBILITY IN CHLOROTOLURON ADSORPTION TO SOIL

4.1. Introduction

This chapter presents the results of applying a sequential extraction procedure to three soil systems to which the herbicide chlorotoluron had been adsorbed. The sequential extraction procedure increased in harshness in the order: isotope exchange < forced isotope exchange < solvent extraction. Sequential extraction was used to define the relative amounts of sorbed chlorotoluron that may be mobilised when the strength of the extraction is increased, thus providing an estimate of the fraction of chlorotoluron taking part in irreversible sorption. The isotope exchange technique is based on the method used by Celis and Koskinen (1999a; 1999b). Isotope exchange or “self-exchange”, involves the use of ^{12}C and ^{14}C isotopes to characterise pesticide exchange kinetics *in-situ*, thus providing an estimate of the sorbed pesticide not taking part in exchange between the soil and solution.

The objectives of performing a sequential extraction based on the principles of isotope exchange, using chlorotoluron as the test compound, were to establish: (i) whether the isotope exchange technique is an effective measure of irreversibility in pesticide adsorption-desorption to soil; (ii) if there is an irreversibly bound fraction of parent compound not active in exchange between the soil and solution; (iii) whether this irreversible fraction changes (increases) with increasing adsorption time; and finally, (iv) if this irreversible fraction is influenced by soil type.

4.2. Materials and methods

4.2.1. Soils

The collection and preparation of the three study soils (Blackwood, Andover and Salop) for the experimental work are detailed in Chapter 3 (section 3.3).

4.2.2. Chemicals

Information concerning the purchase of ^{12}C - and ^{14}C -chlorotoluron, and its chemical and environmental fate properties are given in Chapter 3 (section 3.2).

4.2.3. Isotope exchange technique

An overview of the full methodology is shown in Figure 4.1. Long-term experiments commenced with the isotope exchange technique, implemented based on the method described by Celis and Koskinen (1999a; 1999b). The experimental design involved independent application of two isotopic forms of chlorotoluron (^{12}C and ^{14}C) to the three soils described above. The study was performed in triplicate and over four adsorption periods (7, 14, 28 and 56 days).

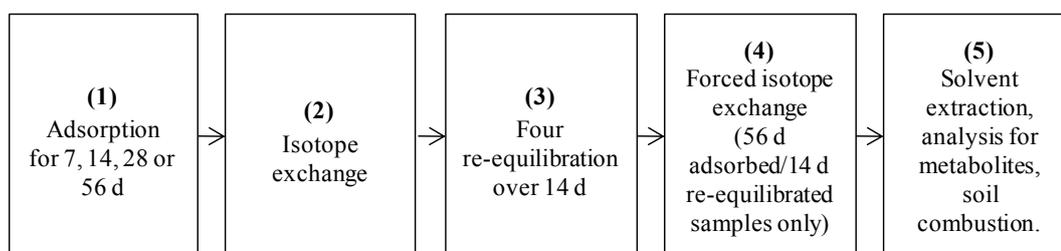


Figure 4.1. Overview of full methodology used.

All samples contained identical amounts of soil, solution and initial chlorotoluron concentration; however, half of the samples contained only ^{12}C -chlorotoluron and half contained only ^{14}C -chlorotoluron. Test vessels were 50-mL Teflon® centrifuge tubes, irradiated to sterilise for 20 minutes prior to use in a CL-1000 ultraviolet cross-linker (UVP Ltd, CA, USA). A 1:4 soil:solution ratio (5 g air-dry soil, 20 mL solution) was determined by preliminary study as optimal for all three soil types (see Chapter 3, section 3.5.1). Air-dried soils were then weighed into tubes and sterile 0.01M CaCl_2 was added. The experiment was performed in an incubator set to 4°C (Sanyo Fitotron RS232 incubator, with lights turned off) to minimise degradation, with continuous shaking at 150 rpm (HS 501 Digital IKA®-Werke reciprocal shaker). Soil suspensions were first pre-equilibrated by shaking overnight. Sterile ^{12}C - and ^{14}C -chlorotoluron treatment solutions were prepared to ensure equivalence in concentration. The ^{12}C - and ^{14}C -chlorotoluron treatment solutions (both 0.70 $\mu\text{g mL}^{-1}$) were then applied to the pre-equilibrated soils.

After their respective adsorption periods, samples were centrifuged at 3500 rpm for 10 minutes (Hermle Z513K, LaborTechnik, Bench Top Centrifuge). The supernatant from each sample was removed by weight (17.00 g) using a Pasteur pipette, taking care not to disturb the soil. A 200- μ L aliquot was taken from each ^{12}C supernatant to determine percentage sorption by HPLC analysis. The same sample volume was also removed from each ^{14}C supernatant but instead mixed with 10 mL of EcoScint A for 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 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- μ L aliquot of supernatant removed for LSC quantification) to measure exchange between ^{12}C - and ^{14}C -chlorotoluron isotopes over time.

4.2.4. Testing for degradation

Degradation was measured in parallel to the isotope exchange study. ^{12}C -chlorotoluron was applied to the three soils and again three replicates were used but this time with five adsorption periods (7, 14, 28, 56 and 70 days) to include the isotope exchange period after 56 days of adsorption. Soil suspensions were prepared and shaken exactly as in the main study using the same soil:solution ratio and initial mass of chlorotoluron. After each adsorption period samples were centrifuged, the supernatant was removed by weight (17.00 g) and a 1-mL aliquot was taken for analysis by HPLC. The soils were then extracted with solvent by adding 20.00 mL methanol (acidified with 0.1% ortho-phosphoric acid (H_3PO_4) to improve mass recovered) and shaking at 250 rpm for one hour (at room temperature). After centrifuging to separate the soil extract, a 1-mL aliquot was then taken for analysis by HPLC to derive the mass balance. Additionally, supernatants and soil extracts from 28, 56 and 70 days were analysed by LC-TOF-MS to validate mass balance results.

4.2.5. Forced isotope exchange

A forced isotope exchange procedure, involving the addition of a high-concentration ^{12}C -chlorotoluron solution in 0.01M CaCl_2 was carried out following the 14-day

isotope exchange phase to maximise the diffusion gradient for desorption of ^{14}C -chlorotoluron from the soil and provide an estimate of ^{14}C -chlorotoluron not taking part in exchange. The thoughts behind use of the forced isotope exchange procedure are described in Appendix A. This approach was found to be more effective in terms of time taken and amount desorbed than repeated shaking with pesticide-free solution e.g. 0.01M CaCl_2 only.

Only initially ^{14}C -chlorotoluron samples (three soils, three replicates) adsorbed for 56 days were selected for use in this part of the study as these samples had the greatest mass of ^{14}C -chlorotoluron sorbed to soil after the 14-day exchange phase. A total of 17 sampling points were used over a period of 204 days; sampling was initially once a week until 91 days, after which sampling continued once every five weeks. Samples were shaken between sampling points under the same conditions as for the isotope exchange method (150 rpm, 4°C). At each sampling point, samples were centrifuged and the supernatant was removed by weight (17.00 g). A 250- μL aliquot was taken for quantification by LSC (to measure desorption of ^{14}C from soil over time). The supernatant that was removed at each time-point was then replaced with fresh, high-concentration (40.01 $\mu\text{g mL}^{-1}$) ^{12}C -chlorotoluron solution.

After the full 204-day period, soils were extracted with solvent to identify whether the ^{14}C -chlorotoluron residue remaining sorbed to the soil after the forced isotope exchange procedure was available by destruction of the soil matrix. Solvent extraction was carried out until the additional recovery since the last sampling time was < 1% of the initially sorbed amount. The first three extractions were completed by removing the supernatant (taking a 250- μL aliquot for LSC quantification) and adding 20.00 mL of methanol (acidified with 0.1% H_3PO_4) before shaking at 250 rpm for 1 hour (at room temperature). The last four extractions were completed using the same method but with shaking for 24 hours instead. Supernatants and soil extracts were analysed by radio-HPLC for parent compound and metabolites, and soils were combusted to complete the mass balance.

4.2.6. Chemical analyses

The analytical methods used to carry out the chemical analyses are described in Chapter 3 (section 3.4). All ^{12}C -chlorotoluron isotope exchange and degradation

samples were analysed by the HPLC method described (Chapter 3, section 3.4.1.1). ^{12}C -chlorotoluron degradation samples (supernatants and soil extracts from 28, 56 and 70 days) were additionally analysed by LC-TOF-MS (Chapter 3, section 3.4.1.2). All ^{14}C -chlorotoluron isotope exchange and degradation samples were quantified by the LSC method described (Chapter 3, section 2.4.2.1). ^{14}C -chlorotoluron forced isotope exchange supernatants and soil extracts were analysed by radio-HPLC (Chapter 3, section 3.4.2.2). Radio-HPLC analysis calculated the percentage of radioactivity that could be allocated to the parent compound and metabolite as a proportion of the total radioactivity present (which represents 100%). Absolute amounts of radioactivity were not quantified by radio-HPLC (as this was achieved by LSC). After application of the sequential extraction procedure, all ^{14}C -chlorotoluron soil samples were finally combusted (Chapter 3, section 3.4.2.3).

4.3. Results and discussion

4.3.1. Isotope exchange

Sorption of chlorotoluron to the three test soils was observed in the order: Salop > Blackwood > Andover (Figure 4.2). There was good general agreement between percentage sorption values obtained for both ^{12}C - and ^{14}C -chlorotoluron, though the deviation between the two isotopes was observed to increase very slightly over time, particularly for the Blackwood and Andover soils (Figure 4.2).

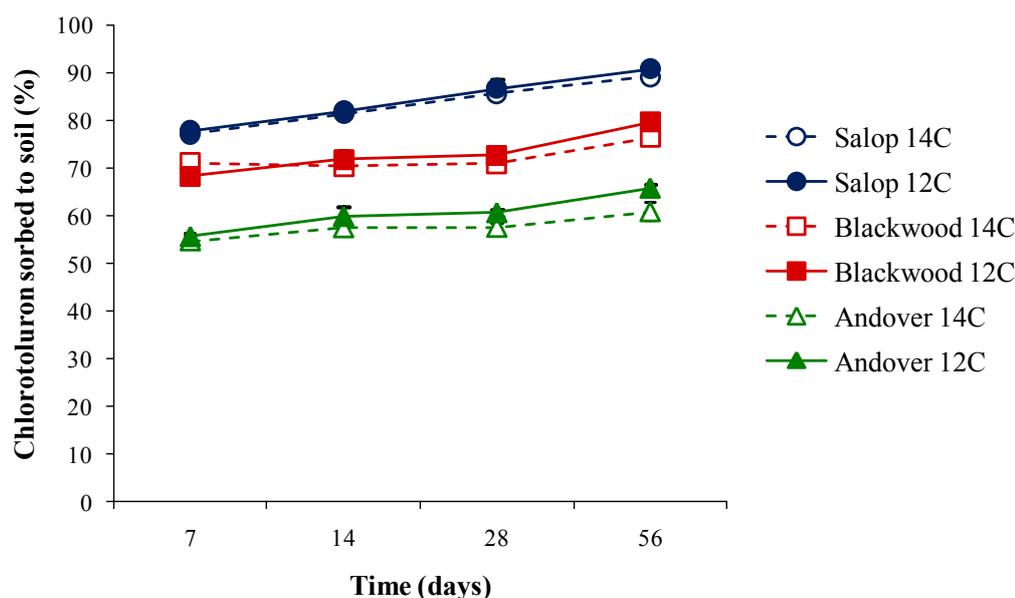


Figure 4.2. Sorption of ^{12}C - and ^{14}C -chlorotoluron to the three test soils over time.

Greater retention of chlorotoluron by the Salop soil than the Blackwood soil is likely to be explained by its larger clay content; stronger sorption of chlorotoluron to soil with a high clay content has been documented before (Hiller *et al.*, 2008). However, this makes it difficult to then explain why the least adsorption was observed to occur to the Andover soil, which has a similar silt and clay content to the Salop soil. The only apparent difference between the Andover and Salop soils is their pH, and as chlorotoluron is a non-ionic compound this would not affect its sorption behaviour. It is possible that the Andover and Salop soils may be constituted from different types of clay and it may be this difference that has affected the sorption behaviour of chlorotoluron. However, this can only be postulated since the nature of the clay fractions have not been assessed. The time-dependent sorption observed will be discussed further towards the end of this section.

The possibility of degradation was explored using LC-TOF-MS analysis. Total ion chromatographs did not identify chlorotoluron metabolites in any supernatants or soil extracts analysed from 28, 56 or 70 days of adsorption; ^{12}C -chlorotoluron samples did not differ (except for chlorotoluron peak) from the blank supernatants and soil extracts. Hence, it was assumed that negligible degradation of chlorotoluron occurred during the 70-day experiment. Over the same 70-day period, the proportion of total chlorotoluron that could not be extracted from the three soils increased to a maximum of 20 to 37% of that applied after 28 days, before decreasing slightly between 28 and 70 days to 10 to 35% of applied.

Figure 4.3 characterises isotope exchange in the study soils during the 14-day exchange phase. For initially ^{12}C samples, Figure 4.3 shows a significant reduction of ^{14}C -chlorotoluron in solution during 0 and 1 days after exchange. Meanwhile, for the initially ^{14}C samples, a significant increase of ^{14}C -chlorotoluron in solution is evident during the same period of time. The influx of ^{12}C - (initially ^{14}C sample) or ^{14}C - (initially ^{12}C sample) chlorotoluron at 0 days (supernatant exchange) resulted in considerable disparity between the ratios of the two isotopes in the soil and solution in both tubes. Thus, rapid adsorption (initially ^{12}C sample) and desorption (initially ^{14}C sample) of ^{14}C -chlorotoluron in one direction dominates as the system responds to accommodate for the new conditions.

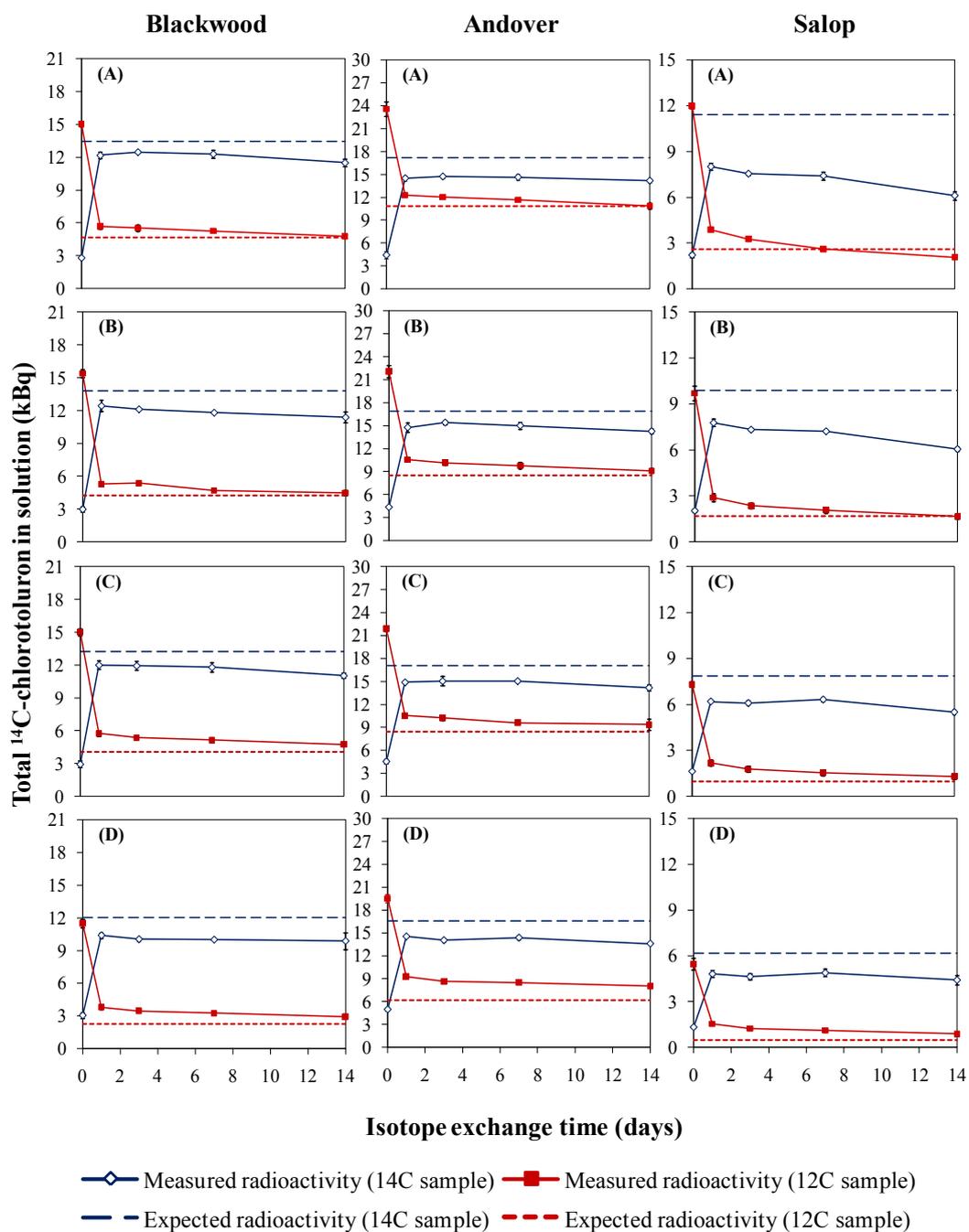


Figure 4.3. Chlorotoluron isotope exchange over time. The solid lines show the measured change in total ¹⁴C-chlorotoluron in solution over 14 days for the Blackwood, Andover and Salop soils after adsorption times of (A) 7 days; (B) 14 days; (C) 28 days; (D) 56 days. Diamonds represent the initially ¹⁴C-chlorotoluron samples, squares represent the initially ¹²C-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.

The remainder of the isotope exchange phase (between 1 and 14 days) sees further net exchange between ^{12}C - and ^{14}C -chlorotoluron isotopes become much slower. For both initially ^{12}C and ^{14}C samples, the disparity in isotope concentration has lessened as the majority of isotope exchange has already occurred. For initially ^{12}C samples, there is an overall decrease of ^{14}C -chlorotoluron in solution over time as this sorbs to the soil, displacing ^{12}C -chlorotoluron. For initially ^{14}C samples, the anticipated increase in ^{14}C -chlorotoluron in solution over time is short-lived. After the initial (0 to 1 day) increase in ^{14}C -chlorotoluron in solution (^{14}C -chlorotoluron release), there is a net decrease of ^{14}C -chlorotoluron in solution between 1 and 14 days which is particularly apparent for shorter adsorption times (7, 14, 28 days). After the longest adsorption time (56 days), the desorption and subsequent 're-adsorption' of ^{14}C -chlorotoluron back to the soil occurs to a lesser extent, with the measured ^{14}C -chlorotoluron in solution remaining more stable during the 14-day isotope exchange phase for all soil types (Figure 4.3). This phenomenon is the result of continued adsorption, demonstrating that the system had not reached equilibrium at the point of supernatant exchange, even after 56 days.

If adsorption equilibrium had been reached and all chlorotoluron was active in exchange between the soil and solution (meaning sorption was a fully reversible process) then Figure 4.3 would show the measured radioactivity in solution (solid lines) reach the expected radioactivity in solution (dashed or dotted lines) during the 14-day isotope exchange phase. Expected radioactivity lines were calculated based on the proportion of initial ^{14}C -chlorotoluron in solution after the respective adsorption period, so the same proportion of ^{14}C -chlorotoluron was expected to be in solution after the 14-day exchange phase if all sorbed chlorotoluron was participating in exchange. This is clearly not the case for the initially ^{14}C samples, as continued adsorption of ^{14}C -chlorotoluron that was desorbed into solution immediately after supernatant exchange means the measured radioactivity in solution actually deviates further from that expected over time. In a small number of cases however, for initially ^{12}C -chlorotoluron samples (e.g. Figure 4.3: Blackwood-B, Andover-A and Andover-B), the measured ^{14}C -chlorotoluron in solution appears to reach the expected radioactivity in solution after 14 days of isotope exchange suggesting complete reversibility. Although there is apparent full reversibility here, the trend is not observed in Figure 4.3 Blackwood-C and -D or Andover-C and -D (after 28 and 56 days of adsorption). Thus, as results between time-points are inconsistent, the

seemingly perfect exchange observed after 7 and 14 days of adsorption is not a robust conclusion. Additionally, Figure 4.3 Salop soil shows for the initially ^{12}C samples that after 7 and 14 days of adsorption (Figure 4.3: Salop-A and Salop-B) measured ^{14}C -chlorotoluron in solution actually falls beneath the expected radioactivity in solution. This demonstrates that continued adsorption is also evident in initially ^{12}C samples; however, the effect is mostly hidden by the anticipated decrease in ^{14}C -chlorotoluron over time due to exchange with sorbed ^{12}C -chlorotoluron.

Central to the explanation of Figure 4.3 is that the sorption of chlorotoluron to soil is time-dependent (Figure 4.2). For the Salop soil, sorption increases steadily with time during the 56-day adsorption phase of the isotope exchange experiment. For the Blackwood and Andover soils, the majority of the increase in sorption appears to occur between 7 and 14 days and also between 28 and 56 days, particularly for the ^{12}C -chlorotoluron samples. Reasons for this increase in sorption are likely to include diffusion into spatially remote areas, such as soil macro- and micropores (Ball and Roberts, 1991; Burgos *et al.*, 1996; Pignatello and Xing, 1996) and entrapment within soil organic matter (Brusseau *et al.*, 1991; Huang and Weber, 1997; Park *et al.*, 2004).

For the Blackwood and Andover soils, sorption increased from $70 \pm 1.7\%$ to $78 \pm 1.9\%$ and $55 \pm 3.3\%$ to $63 \pm 3.8\%$, respectively. The increase was greatest for the Salop soil though, for which sorption increased from $77 \pm 0.5\%$ to $90 \pm 1.0\%$ between 7 and 56 days. This means that for the longer adsorption times, less ^{14}C -chlorotoluron needed to be present in solution for perfect exchange to be observed. Thus, the position of the expected radioactivity lines shifts to become lower over time for both ^{12}C - and ^{14}C -chlorotoluron samples. Expected radioactivities in solution declined in the order Andover < Blackwood < Salop due to increasing strength of sorption. Since equilibrium was not reached, there is some uncertainty surrounding the true position of the expected radioactivity lines. An increase in sorption over time supports research by Gao *et al.* (2007) who also found chlorotoluron sorption to soil to be time-dependent, and suggested that the time-range to reach equilibrium was likely to be months or years. In contrast, Celis and Koskinen (1999a; 1999b) considered that triadimefon and imidacloprid-guanidine had reached sorption equilibrium during their 3-day isotope exchange tests.

Celis and Koskinen (1999a) developed the following equations to quantify the fraction of sorbed pesticide irreversibly bound to the soil:

$$\text{Initially } ^{12}\text{C sample: } \frac{R_s - R_{s,irr}}{R_e} = \frac{R_s'}{R_e'}$$

$$\text{Initially } ^{14}\text{C sample: } \frac{R_s - R_{s,irr}}{R_e} = \frac{R_s' - R_{s,irr}}{R_e'}$$

where R_e is the total ^{14}C -chlorotoluron in solution (Bq), R_s is the total ^{14}C -chlorotoluron sorbed to the soil (Bq) and $R_{s,irr}$ is the proportion of ^{14}C -chlorotoluron irreversibly sorbed to the soil (Bq) during the adsorption phase. R_e' and R_s' refer to the same parameters as above but at a given time after supernatant exchange. Using the above equations, Celis and Koskinen (1999a) observed asymmetry in their estimates for irreversible sorption in the Drummer soil. Estimates for the initially ^{12}C samples (^{14}C adsorption) were close to 0%, whereas estimates of the irreversible fraction for initially ^{14}C samples (^{14}C desorption) ranged from 14 to 34% for imidacloprid-guanidine and from 21 to 50% for triadimefon. Celis and Koskinen (1999a) attributed slow diffusion from restricted sorption sites, trapping in micropores of clay and organic matter and chemical transformation to more strongly sorbed species as contributing to the resistant component of sorption. However, the difference between the results for the initially ^{12}C and ^{14}C tubes suggests that this is more likely to result from continued adsorption following isotope exchange.

Sorption equilibrium was not attained during this chlorotoluron isotope exchange study so it was not appropriate to use their equations to quantify irreversible fractions (irreversible fractions that result range between -43 and 86% of the total sorption). Thus, the forced isotope exchange procedure was then implemented to improve the quantification of the fraction of sorbed ^{14}C -chlorotoluron not participating in the exchange process.

4.3.2. Forced isotope exchange

The result of the forced isotope exchange over time is given as the cumulative percentage recovery of initial chlorotoluron mass applied using this technique (Figure 4.4). The bulk of total ^{14}C -chlorotoluron was extracted with the first addition of ^{12}C -chlorotoluron with $17 \pm 1.3\%$ (Blackwood), $20 \pm 1.5\%$ (Andover) and $18 \pm 1.7\%$

(Salop) recovered after 1 day. This was followed by a gradual decline in ^{14}C -chlorotoluron recovery over time, which finally reached $< 1\%$ recovery of initial for the Blackwood and Andover soils and 2% for the Salop soil between 161 and 204 days.

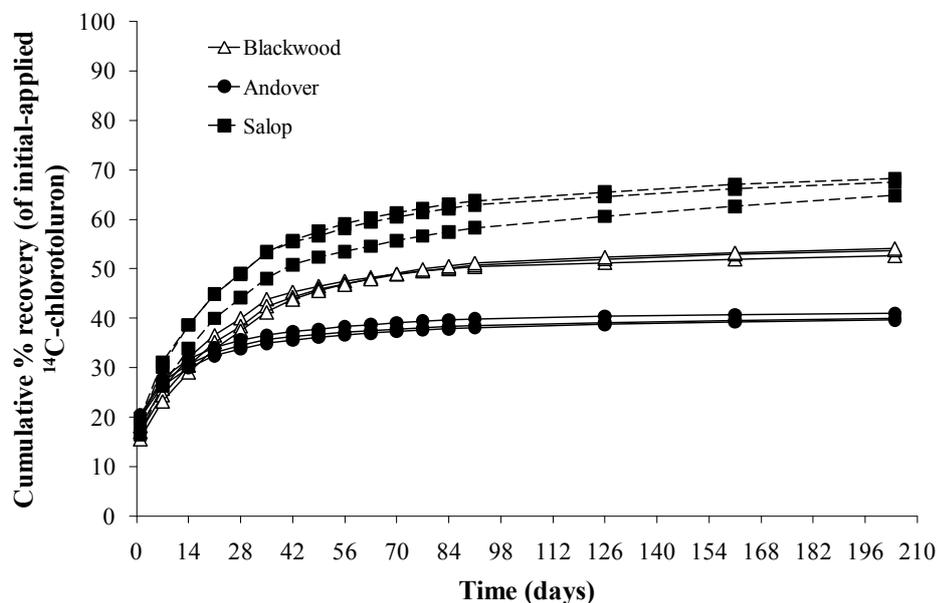


Figure 4.4. Cumulative percentage recovery of initial ^{14}C -chlorotoluron applied using the forced isotope exchange method. Samples are initially ^{14}C with 56-days adsorption and isotope exchange for 14-days (3 replicates per soil).

Although exposure to high concentrations of chlorotoluron in solution is far from normal field conditions, this approach is anticipated to leave the soil matrix essentially unchanged. This is in contrast with solvent extraction, where changes to the structure and activity of the soil matrix are inherent, limiting its use as an indication of availability of residues to be biodegraded or leached in natural soils (Mordaunt *et al.*, 2005).

The amount of ^{14}C -chlorotoluron recovered from the soil following the forced isotope exchange phase (as a percentage of initial ^{14}C -chlorotoluron applied) was $54 \pm 0.8\%$, $40 \pm 0.7\%$ and $67 \pm 1.8\%$ for the Blackwood, Andover and Salop soils, respectively. However, it should be noted that the forced exchange was still releasing small amounts of ^{14}C -chlorotoluron from soil at the end of the process, particularly for the Salop soil, albeit at very slow rates (Figure 4.4). Differences in recovery for the three soils reflect initial strength of sorption.

4.3.3. Mass balance

The complete mass balance of ^{14}C -chlorotoluron is given in Figure 4.5. Repeated solvent extraction after the forced isotope exchange released $5 \pm 0.7\%$, $2 \pm 0.2\%$ and $11 \pm 0.7\%$ of the initially applied ^{14}C -chlorotoluron from the Blackwood, Andover and Salop soils, respectively. Radio-HPLC analysis of forced exchange solutions identified that a small proportion of the initial ^{14}C -chlorotoluron applied to the soils had degraded during this phase of the study, though these were not identified. Degradation was quantified as $4 \pm 1.6\%$ (Blackwood), $4 \pm 2.3\%$ (Andover) and $2 \pm 1.5\%$ (Salop) of initial ^{14}C -chlorotoluron mass per sample. The half-life of chlorotoluron under laboratory conditions has been reported by Gao *et al.* (2007) to be 30 days, but soil sterilisation and experimental conditions of 4°C in the dark effectively inhibited degradation despite the long duration of the study (274 days). Combustion of the extracted soils released radioactivity equivalent to $2 \pm 0.4\%$, $1 \pm 0.1\%$ and $2 \pm 0.2\%$ of that applied for the Blackwood, Andover and Salop soils, respectively. These small fractions could not be identified, but fit with a classical definition of bound residues. It should be noted that these percentages would not necessarily reflect environmentally-relevant irreversible fractions, which are likely to be greater than those reported here; firstly, because these figures were obtained through destruction of the soil matrix via solvent-extraction and secondly, because the microbial community was eliminated. Finally, $3 \pm 0.9\%$ (Blackwood), $1 \pm 0.9\%$ (Andover) and $5 \pm 0.1\%$ (Salop) of initial-applied ^{14}C -chlorotoluron was unaccounted for by the chemical analyses. This may result from mineralisation to $^{14}\text{CO}_2$, volatilisation (as some degradation was observed) or cumulative errors during the length of the experiment due to the multiple solute exchanges undertaken.

4.4. Conclusions

The time-dependent sorption observed meant that it was not possible to estimate the proportion of ^{14}C -chlorotoluron taking part in irreversible sorption using the isotope exchange technique alone. The forced isotope exchange procedure improved the ability to estimate the fraction of sorbed pesticide that was not participating in exchange between the soil and solution after 204 days, however some ^{14}C -chlorotoluron was still desorbing from the soil at this time, albeit at very slow rates. This means that if the forced isotope exchange procedure had been continued for a

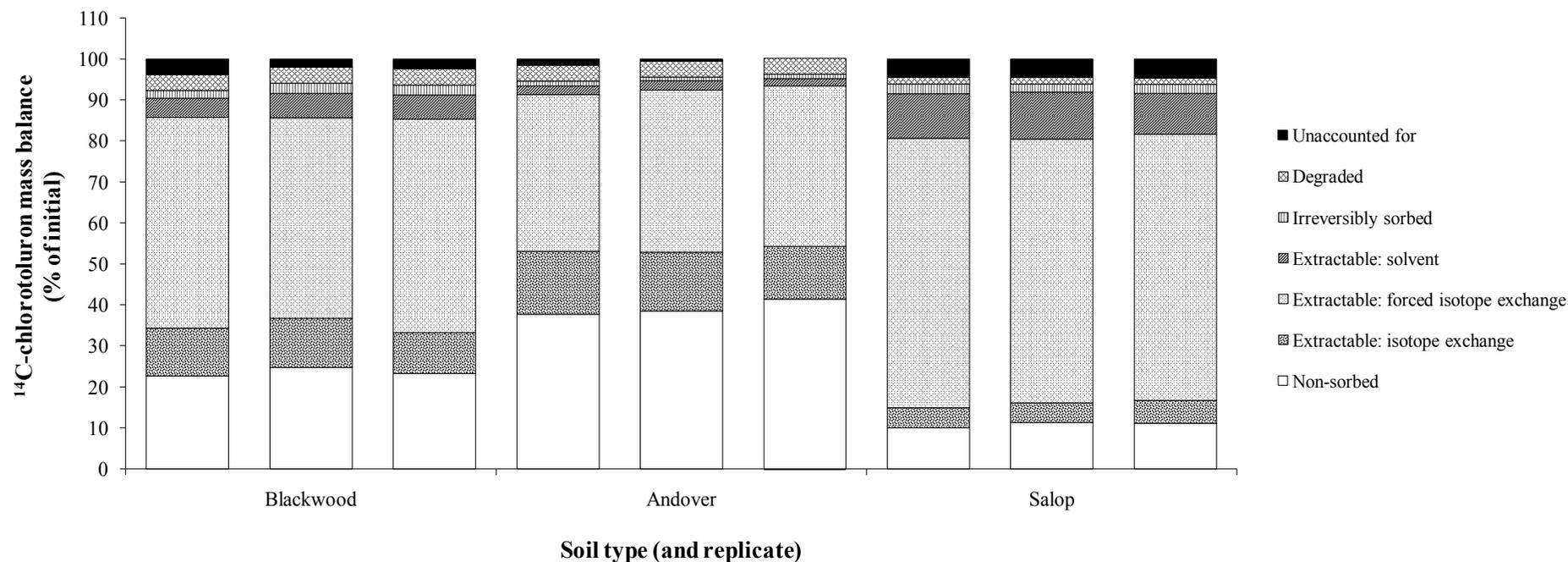


Figure 4.5. Mass balance of initially-applied ^{14}C -chlorotoluron to nine soil samples adsorbed for 56-days. Three replicate samples per soil type are shown. The non-sorbed fraction reflects ^{14}C -chlorotoluron present in the aqueous phase after the adsorption period, whilst that which partitioned to the soil is collectively represented by the three extractable fractions and irreversibly sorbed fraction. The three extractable fractions characterise the relative amounts of ^{14}C -chlorotoluron desorbed from the soil when the harshness of the extraction increased in the order: solvent > forced isotope exchange > isotope exchange. Further analysis revealed the irreversible fraction, proportion of metabolite(s) and a fraction unaccounted for by the chemical analyses.

longer period of time, it is likely that more sorbed ^{14}C -chlorotoluron would have been desorbed into solution. Observing such desorption end-points would however, require a prohibitive amount of experimental time. Extraction with organic solvent was able to recover some of the fraction of sorbed ^{14}C -chlorotoluron that could not be desorbed very readily using the forced isotope exchange procedure. However, the solvent-extractable fraction also includes a proportion of ^{14}C -chlorotoluron that would have been recovered by forced isotope exchange, given sufficient time. The final amounts of irreversible sorption measured were very small. This suggests that under abiotic conditions, irreversible sorption does not play a significant role in the fate of ^{14}C -chlorotoluron for the soil systems studied. The soil components specifically influencing the final irreversible fraction are difficult to identify, probably due to the small amounts of irreversibly sorbed ^{14}C -chlorotoluron observed overall.

In the following Chapter, the described sequential extraction procedure will be applied to the same soil systems for the pesticides prometryn and hexaconazole to assess whether the conclusions made for chlorotoluron are pesticide-specific or more general.

CHAPTER 5

LONG-TERM EXPERIMENTS TO INVESTIGATE IRREVERSIBILITY IN PROMETRYN AND HEXACONAZOLE ADSORPTION TO SOIL

5.1. Introduction

This study is a follow-up to the long-term chlorotoluron experiment described in Chapter 4 and had three major objectives: (i) to assess whether the conclusions reached for chlorotoluron were also true for pesticides prometryn and hexaconazole when adsorbed to the same three soils, under the same test system conditions; (ii) to test whether extending adsorption periods increased the likelihood of reaching sorption equilibrium; and, (iii) to determine whether irreversible fractions increased when the sequential extraction procedure was applied after increasing lengths of adsorption time (only applied after one adsorption period of 56-days for chlorotoluron). Although the methods applied here are essentially identical to those used in the preceding chlorotoluron study, some minor amendments were made to accommodate differences in pesticide properties; e.g. changes to the soil/solution ratio and concentrations of pesticide applied were required as a result of the differing sorption behaviours, solubility limits and specific activities of the pesticides.

5.2. Materials and methods

5.2.1. Soils

Details of the collection and preparation of the three study soils (Blackwood, Andover and Salop) for the experimental work are given in Chapter 3 (section 3.3).

5.2.2. Chemicals

Information concerning the purchase of ^{12}C and ^{14}C prometryn and hexaconazole, as well as their chemical and environmental fate properties, is given in Chapter 3 (section 3.2).

5.2.3. Isotope exchange technique

The four adsorption periods for both prometryn and hexaconazole were extended to 28, 56, 112 and 168 days as the longest adsorption period of 56-days for chlorotoluron was not sufficient to reach sorption equilibrium. To ensure minimal degradation, the prometryn and hexaconazole studies were performed under the same experimental conditions described in Chapter 4 (section 4.2). A 1:10 soil:solution ratio (2 g air-dry soil, 20 mL solution) was selected for use for all three prometryn-soil systems, and a 1:50 soil:solution ratio (0.5 g air-dry soil, 25 mL solution) was determined by preliminary study as optimal for hexaconazole (see Chapter 3, section 3.5.2). The studies were both performed in a dark constant environment room set to 4°C. Final concentration of both ^{12}C - and ^{14}C -prometryn treatment solutions applied to the pre-equilibrated soils were $0.78 \mu\text{g mL}^{-1}$ (in 0.01M CaCl_2). For hexaconazole, the final concentrations of ^{12}C - and ^{14}C -hexaconazole treatment solutions applied to the pre-equilibrated soils were 0.78 and $0.75 \mu\text{g mL}^{-1}$ (in 0.01M CaCl_2), respectively. Centrifugation speed was reduced from 3500 rpm (used for chlorotoluron and prometryn) to 3000 rpm for hexaconazole due to the lower mass of soil and greater volume of solution, though centrifugation time remained the same (10 minutes). At supernatant exchange, the supernatant removed from the prometryn and hexaconazole samples was 18.00 g and 24.00 g, respectively.

5.2.4. Testing for degradation

Separate ^{12}C and ^{14}C prometryn and hexaconazole samples were used to test for degradation. For ^{12}C -prometryn and ^{12}C -hexaconazole samples, five adsorption periods were used (28, 56, 112, 168 and 182 days), with the longest time point necessary to include the 14-day isotope exchange period following the 168-day adsorption period. ^{12}C -pesticide soil suspensions were prepared and shaken exactly as in the isotope exchange study using the same soil/solution ratio and initial masses of prometryn and hexaconazole. After each adsorption period samples were centrifuged, the supernatant was removed by weight (18.00 g for prometryn, 24.00 g for hexaconazole) and a 1-mL aliquot was taken for HPLC analysis. Soils were then extracted with solvent by adding 20.00 mL methanol (acidified with 0.1% HCOOH to improve recovery) and shaking at 250 rpm for 24 hours (at room temperature). After centrifuging to separate the soil extract, another 1-mL aliquot was taken for HPLC

analysis. The solvent-extraction procedure was repeated twice to ensure maximum recovery.

For ^{14}C -prometryn and ^{14}C -hexaconazole samples, only two adsorption periods were used (56 and 182 days). ^{14}C -pesticide soil suspensions were prepared and shaken exactly as in the isotope exchange study using the same soil/solution ratio and initial masses of prometryn and hexaconazole. After each adsorption period samples were centrifuged, the supernatant was removed by weight (18.00 g for prometryn, 24.00 g for hexaconazole) and two 250- μL aliquots were taken for analysis by LSC and radio-HPLC. Soils were then also extracted with solvent by adding 20.00 mL methanol (acidified with 0.1% HCOOH to improve recovery) and shaking at 250 rpm for 24 hours (at room temperature). After centrifuging to separate the soil extract, two 250- μL aliquots were removed for analysis by LSC and radio-HPLC. The solvent-extraction procedure was again repeated twice to ensure maximum recovery. Soil pellets were then finally combusted to derive the mass balance.

5.2.5. Forced isotope exchange

^{14}C -prometryn and ^{14}C -hexaconazole samples adsorbed for 56, 112 and 168 days were subjected to the forced isotope exchange procedure to observe its effect after different periods of adsorption time. A total of 14 sampling points were used over a period of 175 days for prometryn, and 12 sampling points over a period of 145 days for hexaconazole. For both pesticides, sampling was initially once a week until 49 days, after which sampling continued once every two weeks until 91 days, with sampling then once every four weeks until the end of their respective experimental time-frame (145 days for hexaconazole and 175 days for prometryn). At each sampling point, samples were centrifuged and the supernatant was removed by weight (18.00 g for prometryn, 24.00 g for hexaconazole); a 200- μL aliquot was taken for quantification by LSC. The supernatants were then replaced with fresh, high-concentration ^{12}C -pesticide solutions. The average concentrations added were 29.85 $\mu\text{g mL}^{-1}$ and 14.91 $\mu\text{g mL}^{-1}$ for prometryn and hexaconazole, respectively.

After the full forced isotope exchange period, soils were extracted with solvent to identify whether the ^{14}C -prometryn and ^{14}C -hexaconazole residue remaining sorbed to the soil was solvent-extractable. The solvent extraction procedure was again

repeated twice. The extractions were performed by removing the supernatant (taking a 250- μ L aliquot for LSC quantification) and adding 20.00 mL of methanol (acidified with 0.1% HCOOH) before shaking at 250 rpm for 24 hours (at room temperature). Supernatants and soil extracts were further analysed by radio-HPLC to assess the proportions of parent compound and metabolite(s), and soils were finally combusted to complete the mass balance.

5.2.6. Chemical analyses

The analytical methods used to carry out the chemical analyses are described in Chapter 3 (section 3.4). All ^{12}C -prometryn and ^{12}C -hexaconazole isotope exchange and degradation samples were analysed by the HPLC. All ^{14}C -pesticide isotope exchange and degradation samples were quantified by LSC. ^{14}C -pesticide samples used to test for degradation were additionally analysed by radio-HPLC. Radio-HPLC analysis calculated the percentage of radioactivity that could be allocated to the parent compound and metabolite as a proportion of the total radioactivity present (which represents 100%). Absolute amounts of radioactivity were not quantified by radio-HPLC (as this was achieved by LSC). After application of the sequential extraction procedure, all ^{14}C -prometryn and ^{14}C -hexaconazole soil samples were combusted.

5.3. Results and discussion

5.3.1. Sorption

Total amounts of sorption to the three test soils over the four adsorption periods were relatively similar for both pesticides (Figure 5.1); this was dependent on the isotope however, and is discussed further towards the end of this section. Percentage sorption was calculated by subtracting the mass of pesticide measured in the liquid phase from the mass of pesticide initially-applied, with the assumption that the remaining pesticide was sorbed to the soil. Percentage sorption was observed in the order Blackwood > Salop > Andover for both prometryn and hexaconazole.

The Blackwood soil has the lowest silt and clay contents (see Chapter 3, Table 3.2). As clay and silt are known to have an important influence on pesticide sorption (Chapter 2, section 2.4.2.4), this suggests that retention of prometryn and

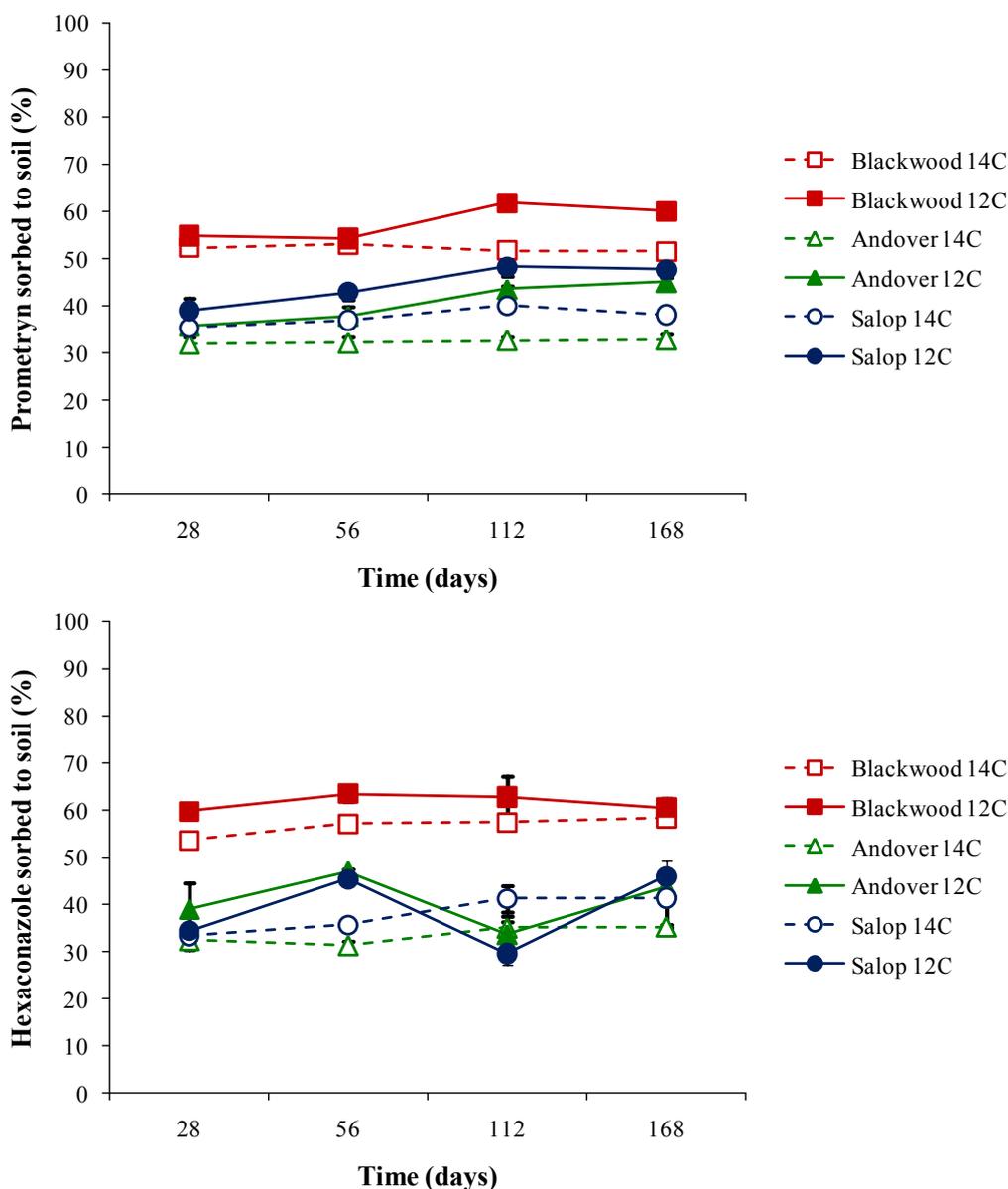


Figure 5.1. Sorption of ^{12}C and ^{14}C prometryn and hexaconazole to the three test soils after 28, 56, 112 and 168 days.

hexaconazole is predominantly determined by another component of the soil. The sorption behaviour of triazole fungicides (including hexaconazole) in soil has been studied by Singh (2002; 2005), who reported that the organic carbon content is the single largest parameter affecting sorption of the triazole fungicides studied. As the Blackwood soil did marginally have the highest organic carbon content (Chapter 3, Table 3.2), it is likely that this is the reason for the greatest retention of hexaconazole by that soil. Singh (2002) also reported that hydrogen bonding and charge-transfers were likely to be the mechanisms binding hexaconazole to humic acids. The importance of the organic matter fraction in pesticide retention has also been

demonstrated for prometryn. Baskaran and Kennedy (1999) observed greatest sorption of prometryn to the soil with the highest organic carbon content; additionally, Khan (1982) found that after one year of incubation in soil, prometryn was found to be mainly associated with the organic matter fractions (humin, humic acid and fulvic acid), with more than half of the total prometryn residue in the humic fraction.

Figure 5.1 shows that for ^{14}C -prometryn samples there was very little variance in amounts of pesticide sorbing to soil between 28 and 168 days; however, an increase in sorption of ^{12}C -prometryn was observed to occur for all soils during the same period of time. For ^{12}C -prometryn sorption to the Blackwood and Andover soils, the majority of the increase occurred between 56 and 112 days, whilst for the Salop soil a steady increase in ^{12}C -prometryn sorption occurred between 28 and 112 days, after which sorption then plateaued between 112 and 168 days. For ^{12}C - and ^{14}C -hexaconazole, sorption to the Blackwood soil was relatively consistent during 28 and 168 days. For ^{12}C - and ^{14}C -hexaconazole sorption to the Andover and Salop soils however, increases in sorption were observed between 28 and 168 days. For ^{14}C -hexaconazole samples, the majority of the increase in sorption occurred between 56 and 112 days. The sorption of ^{12}C -hexaconazole to the Andover and Salop soils at 112 days is anomalous; if these values are disregarded, then although there is an increase in sorption ^{12}C -hexaconazole between 28 and 56 days, sorption is similar at 56 and 168 days.

These differences in sorption depending on the isotope and soil type make it difficult to establish whether equilibrium was reached, particularly for the ^{12}C samples. However, as results mostly rely on ^{14}C data, it is possible to make some conclusions. For ^{14}C -prometryn samples, sorption equilibrium appears to have been reached after 28 days as the increase in amounts of ^{14}C -prometryn sorbing to soil when the adsorption period was increased is negligible; this is also the case for ^{14}C -hexaconazole sorption to the Blackwood soil. Increases in amounts of ^{14}C -hexaconazole sorbed to the Andover and Salop soils were observed between 56 and 112 days; sorption appeared to plateau between 112 and 168 days. This may be reflected in the forced isotope exchange and mass balance results if an increase in the strength of sorption occurred, preventing remobilisation of a fraction of the sorbed pesticide. This is discussed further in section 5.3.4.

Two clear patterns can be identified from Figure 5.1: (i) consistently greater sorption to soil was observed for the ^{12}C samples than for the ^{14}C samples, for both prometryn and hexaconazole and all soil types; and, (ii) with the exception of hexaconazole sorption to the Blackwood soil, the increase in sorption observed between 28 and 168 days was also consistently greater for the ^{12}C samples. There is thus, a discrepancy between the ^{12}C and ^{14}C sorption data obtained for prometryn and hexaconazole. This phenomenon was not observed for the chlorotoluron samples, with similar amounts of sorption observed for both ^{12}C - and ^{14}C -chlorotoluron isotopes (Chapter 4, Figure 4.2).

Both ^{12}C and ^{14}C isotopes should behave identically. The fact that sorption was consistently greater for the ^{12}C samples suggests that the difference in sorption observed may be due to an experimental artefact. The concentrations of ^{12}C - and ^{14}C -hexaconazole solution in contact with the soils did differ slightly ($0.75\ \mu\text{g mL}^{-1}$ applied to ^{12}C -hexaconazole samples, and $0.78\ \mu\text{g mL}^{-1}$ applied to ^{14}C -hexaconazole samples). However, it is unlikely that adding more ^{12}C -hexaconazole would have resulted in greater relative sorption to soil given the results obtained from the Freundlich sorption studies (Chapter 3, section 3.6); these indicate that as the concentration of hexaconazole added increases, percentage sorption should decrease. Furthermore, equivalent amounts of prometryn were added to both ^{12}C ($0.78\ \mu\text{g mL}^{-1}$) and ^{14}C samples ($0.78\ \mu\text{g mL}^{-1}$), and the effect was even more pronounced for this chemical.

Another possible cause could have been that the ^{14}C -prometryn and ^{14}C -hexaconazole treatment solutions contained a slightly greater proportion of methanol than the ^{12}C -prometryn and ^{12}C -hexaconazole treatment solutions. It is stipulated by OECD guideline 106 that the solvent used to help dissolve the test substance (methanol in this case) should not exceed 0.1% of the solution coming into contact with the soil. However, as none of the treatment solutions prepared surpassed this level, and were at least a factor of ten lower than this limit, it is also unlikely that this could have been the cause of the discrepancy between the data.

The only other factor which could be taken into consideration is the different methods of analysis used to measure the two isotopes. The ^{12}C samples were analysed by HPLC and the ^{14}C samples were analysed by LSC. The limit of quantification (LOQ)

for LSC is significantly lower (and thus more sensitive) than that for HPLC so it is possible to quantify much lower concentrations. However, all of the prometryn and hexaconazole samples analysed by HPLC exceeded the calculated LOQs for their respective methods. Another difference between the two methods is that LSC is non-specific, therefore it is not able to differentiate between parent compound and metabolite in the way that HPLC can. If the parent forms of prometryn and hexaconazole had degraded into metabolites in solution, the HPLC method would only quantify the parent compound, whereas LSC would quantify both parent and metabolite. This would affect the relative quantities of ^{12}C - and ^{14}C -pesticide measured in solution; less ^{12}C -pesticide would be quantified in the aqueous phase, giving the impression of greater sorption to soil. However, as hexaconazole did not degrade during in the experiment (section 5.3.2) this is not a plausible explanation. Therefore, it has to be concluded that the cause of the stronger sorption, which was observed consistently for both ^{12}C -pesticides and all soil types is an unknown experimental artefact.

It is difficult to assess the implication of these findings. The discrepancy between sorption of the two isotopes would only have an effect on the isotope exchange results. These data may be compromised because the principle of self-exchange relies on the assumption that ^{12}C - and ^{14}C -pesticide isotopes behave in exactly the same way. Thus, the sorption equilibrium reached after the adsorption period may be disturbed at the supernatant exchange step, with the following implications. For initially ^{12}C samples, the ^{14}C supernatant replacing the original ^{12}C supernatant will contain a higher concentration of pesticide than the original, thus a greater reduction in ^{14}C -pesticide in solution may take place during isotope exchange as more ^{14}C -pesticide is available in solution to exchange with the ^{12}C -pesticide sorbed to the soil. The opposite effect would therefore occur for initially ^{14}C samples with the replacement ^{12}C supernatant containing a lower concentration of pesticide than the original ^{14}C supernatant, limiting the desorption of ^{14}C -pesticide into solution as less ^{12}C -pesticide is available to take part in the exchange. However, the isotope exchange graphs plotted for prometryn and hexaconazole (see section 5.3.3) do not appear to have been affected by the difference in sorption of ^{12}C - and ^{14}C -pesticide, with isotope exchange during the 14-day period occurring as anticipated. As it is difficult to quantify the true extent of this effect, no corrections to the data have been made.

5.3.2. Degradation

The results of the degradation analysis showed that the parent hexaconazole molecule did not degrade over the duration of the experiment as no metabolites were found during the length of the study. Some degradation of prometryn did take place however (Figure 5.2).

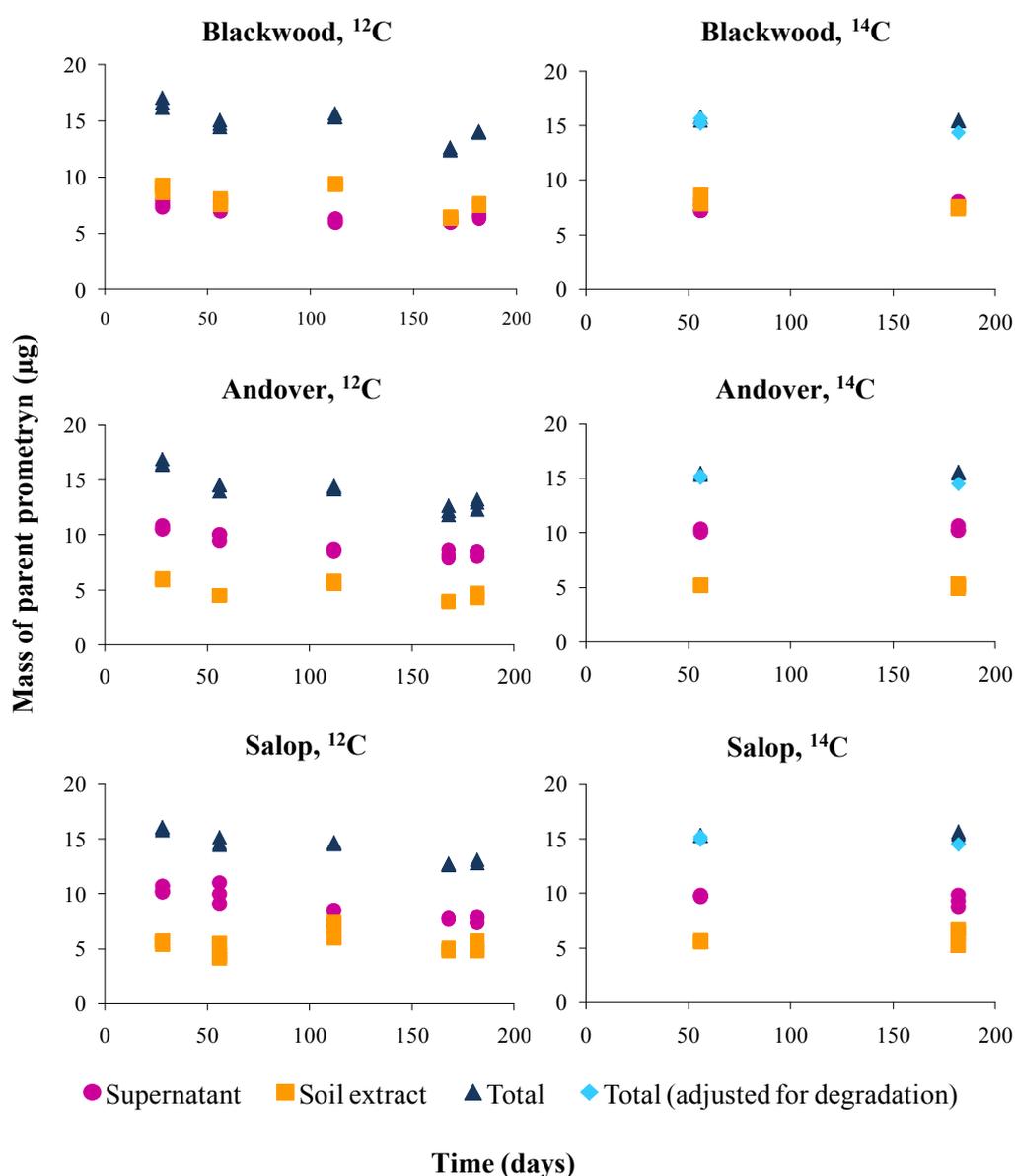


Figure 5.2. Total mass of parent prometryn recovered over time. The masses of ^{12}C -prometryn recovered are shown down the left-hand side, whilst the masses of ^{14}C -prometryn recovered are shown on the right-hand side. All three replicates per time point are plotted. The mass of prometryn in solution, extracted from the soil and total mass of prometryn recovered are plotted. For ^{14}C -prometryn samples, the total mass recovered is adjusted after radio-HPLC analysis had quantified the proportion of ^{14}C -prometryn metabolite.

The masses recovered for ^{12}C -prometryn will be discussed first. The mass of ^{12}C -prometryn recovered was observed to decrease over time (between 28 and 182 days) for all soil types. After 28 days the masses of ^{12}C -prometryn recovered were $107 \pm 2.9\%$, $107 \pm 1.8\%$ and $103 \pm 1.0\%$ of initial-applied for the Blackwood, Andover and Salop soils, respectively. However, after 182 days the mass recovered had decreased to $90 \pm 0.4\%$, $82 \pm 3.1\%$ and $83 \pm 1.2\%$ of initial-applied for the Blackwood, Andover and Salop soils, respectively. The masses of ^{12}C -prometryn measured in both the solution and soil extracts declined over time for all soil types.

For the ^{14}C samples however, the mass of ^{14}C -prometryn recovered was still approximately 100%, even after 182 days. The total mass of ^{14}C -prometryn recovered after 56 days was $101 \pm 1.2\%$, $99 \pm 0.8\%$ and $99 \pm 0.1\%$ for the Blackwood, Andover and Salop soils, respectively. After 182 days, the mass of ^{14}C -prometryn recovered was still $100 \pm 0.25\%$, $100 \pm 0.49\%$ and $99 \pm 1.74\%$ for the Blackwood, Andover and Salop soils, respectively. These data would be plausible if degradation of the parent prometryn molecule resulted in generation of ^{14}C -labelled metabolite(s). Thus, the initial mass of radioactivity applied can still be recovered after 182 days but the non-specific nature of LSC quantification means that a differentiation cannot be made between the parent molecule and any ^{14}C -labelled metabolite(s) that may be present. For this reason, samples were also analysed by radio-HPLC in order to quantify the proportion of radioactivity attributable to the parent molecule.

The radio-HPLC analysis revealed that after 56 days the fraction of radioactivity attributable to parent ^{14}C -prometryn was $97 \pm 0.3\%$, $97 \pm 0.1\%$ and $98 \pm 1.9\%$ for the Blackwood, Andover and Salop soils, respectively. After 182 days, the proportion of parent prometryn was 92%, 94%, 97% for the Blackwood, Andover and Salop soils, respectively (no replicates as samples were combined and concentrated). Therefore, although confirming the presence of metabolites, the mass of parent ^{14}C -prometryn quantified by the radio-HPLC analysis was greater than that measured for the ^{12}C -prometryn samples for all soil types.

A plausible explanation for this discrepancy may be the difference in the way ^{12}C - and ^{14}C -prometryn samples were prepared for their respective HPLC and radio-HPLC analyses. Whilst the ^{12}C -prometryn samples were sampled directly into vials for HPLC analysis, the ^{14}C -prometryn samples were first concentrated under a flow of

nitrogen at 40°C for approximately 24 hours. The samples were subsequently re-suspended and then sampled into the HPLC vials in preparation for radio-HPLC analysis. Depending on the properties of the metabolite(s), it may be possible that they either volatilised or adhered to the glass of the concentrating vial during the concentration process. As the radioactivity was not quantified by the radio-HPLC analysis, this would mean that the metabolite(s) would not have been detected by the radio-HPLC analysis, and proportionally, the parent prometryn molecule would have artificially appeared to form a greater amount of the total radioactivity.

Overall, the discrepancy between amounts of degradation observed for ^{12}C - and ^{14}C -prometryn samples has little impact on the results. As the results mostly rely on the ^{14}C -prometryn data, and little evidence of degradation was observed for those samples (Figure 5.2), the data were not corrected for minimal losses of parent compound. Although it is likely that the chemistry, properties and sorptive behaviour of the transformation products will differ from that of parent prometryn, it is not possible to correct the prometryn measurements for this without first identifying the metabolite(s). Although this is a simplification and partial limitation of the study, the metabolite is assumed to sorb to the same extent as the parent compound.

5.3.3. Isotope exchange

Figures 5.3 and 5.4 characterise isotope exchange over 14 days in the three study soils for prometryn and hexaconazole, respectively. The isotope exchange behaviour is similar for the two pesticides. Immediately after supernatant exchange (between 0 and 1 day), there is a simultaneous rapid increase and decrease in both ^{14}C -prometryn and ^{14}C -hexaconazole in solution in the initially ^{14}C and ^{12}C samples, respectively (as was also observed for chlorotoluron). This effect is mirrored quite well between initially ^{12}C and ^{14}C samples, with similar amounts of ^{14}C -prometryn and ^{14}C -hexaconazole adsorbed and desorbed during the same period of time. The symmetry of this effect relies on the fact that the same sorption equilibrium was reached in both ^{12}C and ^{14}C samples; although this was not the case here (see section 5.3.1), the anticipated effect was still observed.

When comparing the isotope exchange behaviour observed for chlorotoluron and that observed for prometryn and hexaconazole it clear that for initially ^{14}C samples, the

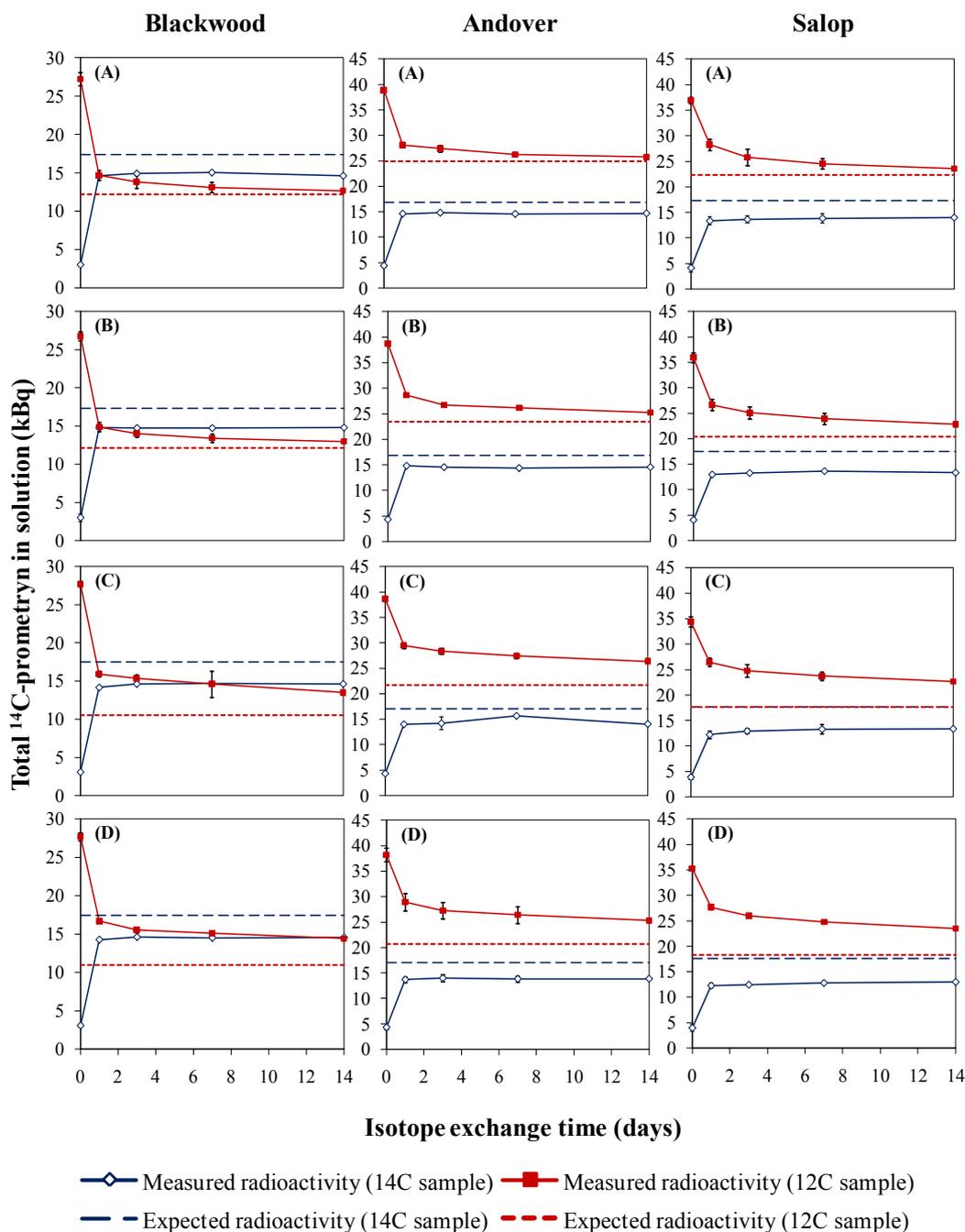


Figure 5.3. Prometryn isotope exchange over time. The solid lines show the change in total ^{14}C -prometryn in solution over 14 days for the Blackwood, Andover and Salop soils after adsorption times of (A) 28 d; (B) 56 d; (C) 112 d; (D) 168 d. Diamonds represent the initially ^{14}C -prometryn samples, 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.

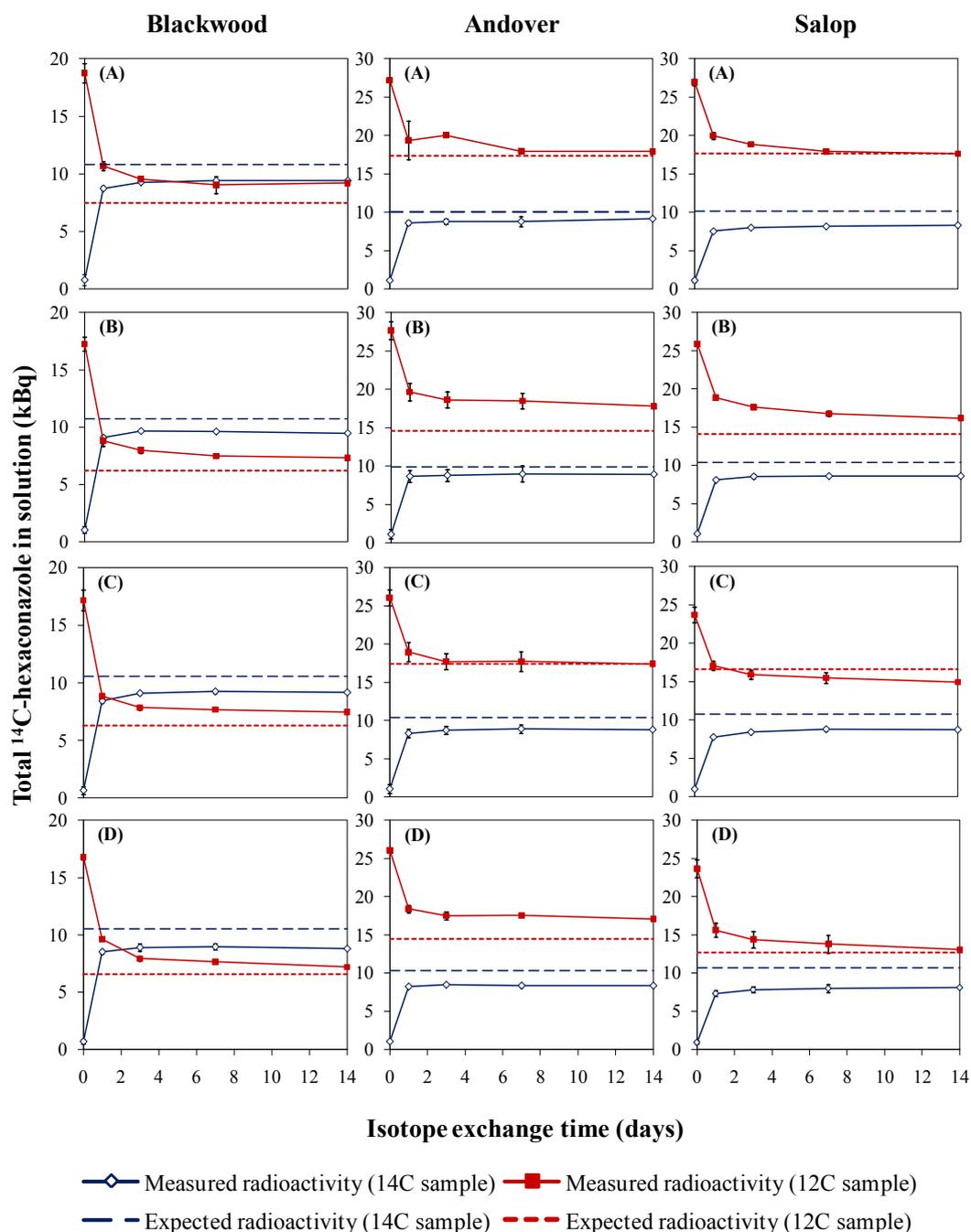


Figure 5.4. Hexaconazole isotope exchange over time. The solid lines show the change in total ¹⁴C-hexaconazole in solution over 14 days for the Blackwood, Andover and Salop soils after adsorption times of (A) 28 d; (B) 56 d; (C) 112 d; (D) 168 d. Diamonds represent the initially ¹⁴C-hexaconazole samples, squares represent the initially ¹²C-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.

^{14}C -pesticide measured in solution is observed to be constant or slightly increase during the isotope exchange phase for both prometryn and hexaconazole and all soil types, whereas this was observed to consistently decrease for chlorotoluron. This may be explained by the minimal amounts of time-dependent sorption observed for prometryn and hexaconazole over 168 days, compared to that observed for chlorotoluron over 56 days. Thus, as the processes of adsorption and desorption were closer to reaching a dynamic equilibrium for prometryn and hexaconazole, adsorption was no longer the dominant process (as was the case for chlorotoluron).

For prometryn, the measured ^{14}C -prometryn in solution for both initially ^{12}C and ^{14}C samples never reaches the expected radioactivity in solution over time. This gives the impression that irreversible sorption is taking place, when in reality it is more likely that 14 days is not long enough to observe any further exchange over time. This explanation may also be applied to the majority of the hexaconazole isotope exchange data, though there are some exceptions. In Figure 5.4, the isotope exchange behaviour plotted for three of the initially ^{12}C -hexaconazole samples (Andover-C, Salop-A and Salop-C), ^{14}C -hexaconazole measured in solution appears to either meet the expected concentration in solution or exceed it. However, the Andover and Salop ^{12}C sorption data at 112 days were anomalous, which may have resulted in the incorrect positioning of the expected radioactivity lines and explain the results observed. As it is still not clear, even after minimising the effect of time-dependent sorption, whether irreversible sorption is taking place, the forced isotope exchange procedure was applied to initially ^{14}C -prometryn and ^{14}C -hexaconazole samples. This time the procedure was applied after three increasing periods of adsorption (56, 112 and 168 days).

5.3.4. Forced isotope exchange

Figures 5.5 and 5.6 show the percentage of initially-applied chemical released after each successive exchange step for the Blackwood, Andover and Salop soils after increasing lengths of adsorption time (56, 112 and 168 days). For both ^{14}C -prometryn and ^{14}C -hexaconazole, the extent of desorption was observed in the order Blackwood > Salop > Andover. This is likely to reflect the amounts of initial ^{14}C -prometryn and ^{14}C -hexaconazole sorption to soil, which were observed in the same order for both pesticides. Approximately 50% of initially-applied ^{14}C -prometryn and ^{14}C -

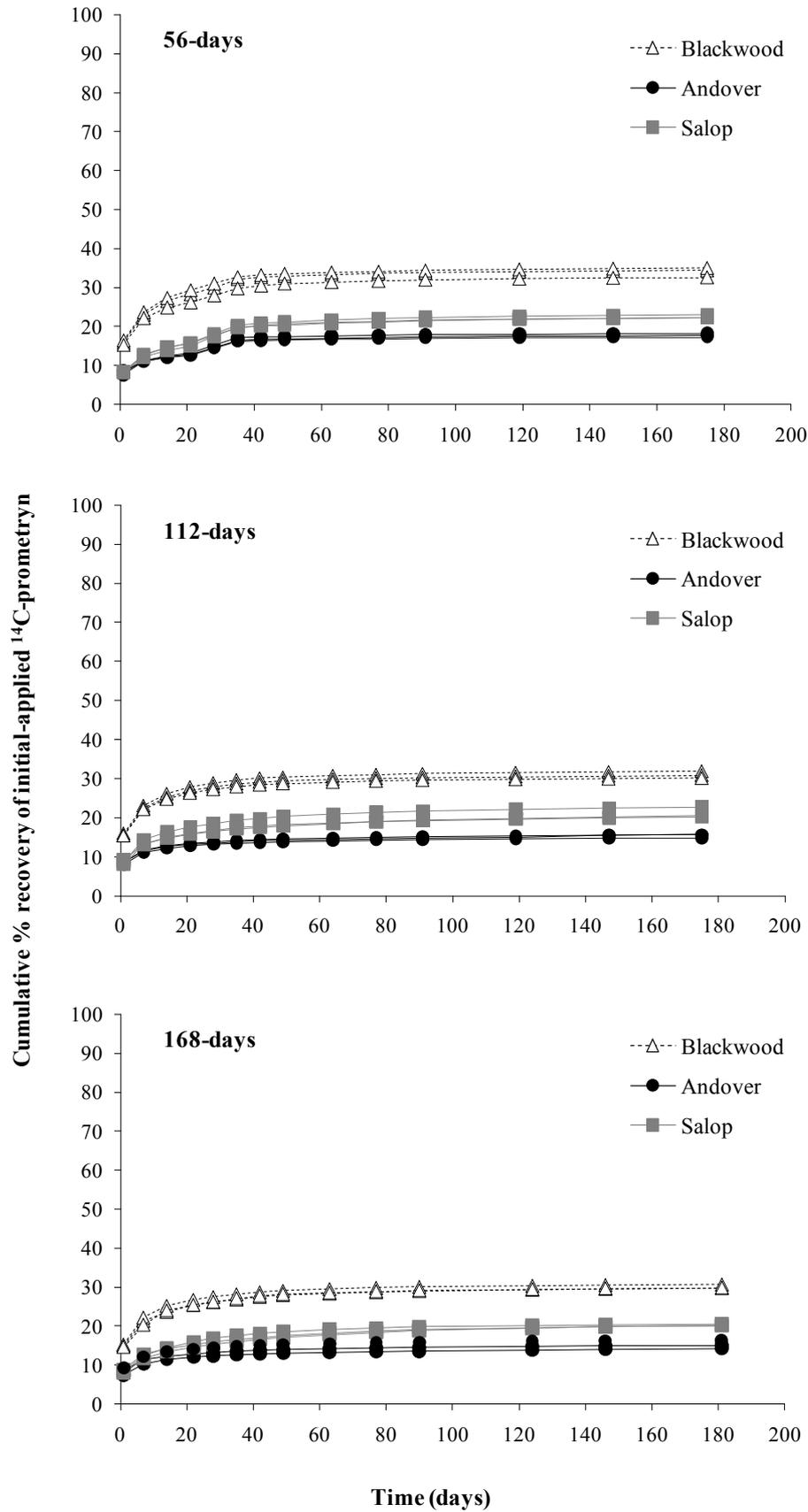


Figure 5.5. Cumulative percentage recovery of initially-applied ^{14}C -prometryn using forced isotope exchange.

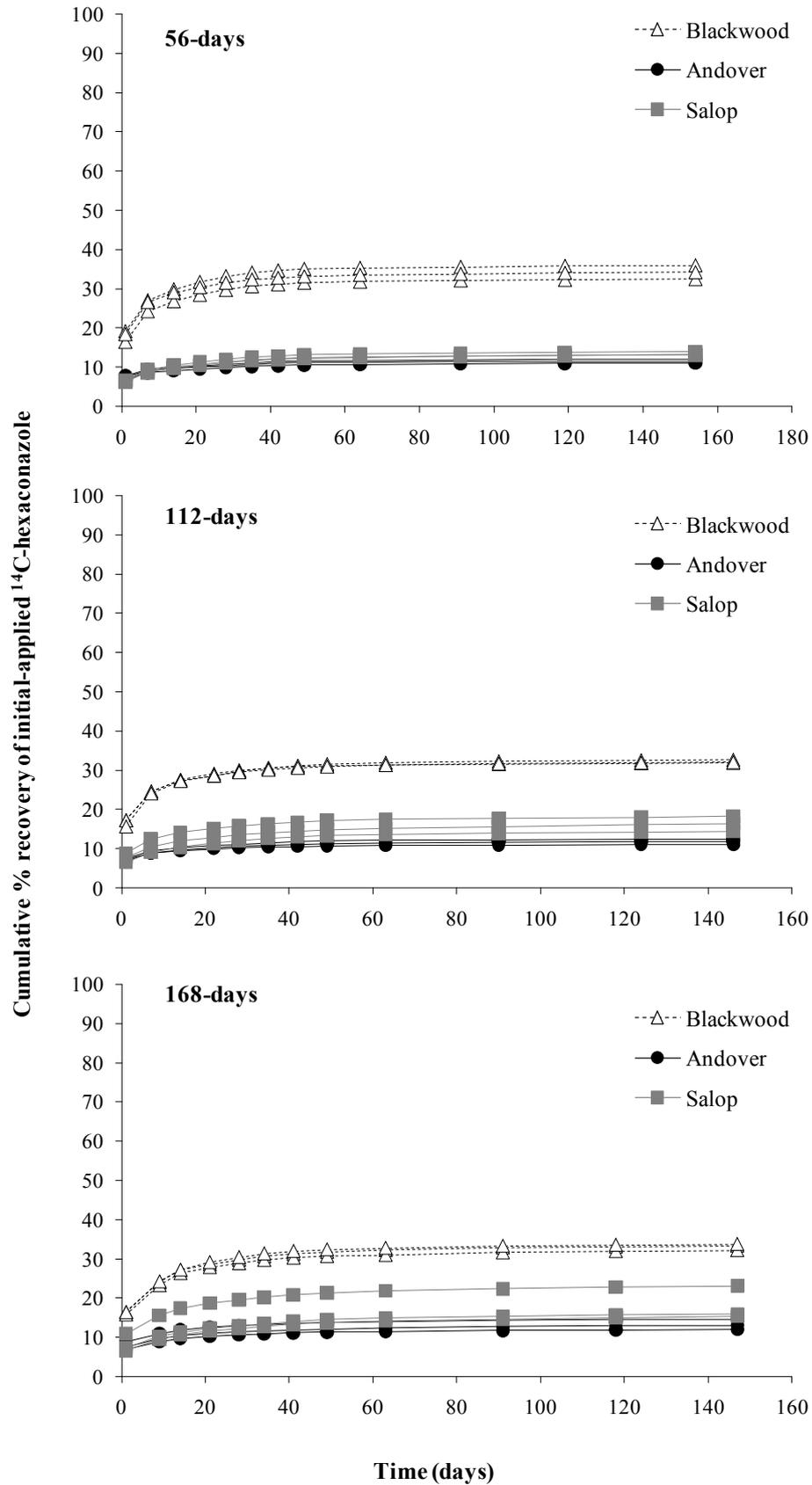


Figure 5.6. Cumulative percentage recovery of initially-applied ^{14}C -hexaconazole using forced isotope exchange.

hexaconazole was recovered with the first additions of ^{12}C -prometryn and ^{12}C -hexaconazole across all time-points (see Table 5.1). At the final sampling point (147 days hexaconazole, 175 days prometryn) the amounts of ^{14}C -prometryn and ^{14}C -hexaconazole released had gradually declined to between 0.1 - 0.3% recovery of the mass initially-applied. Longer adsorption times appeared to have a negligible effect on initial desorption (after 1 day) of ^{14}C -prometryn and ^{14}C -hexaconazole.

Table 5.1. ^{14}C -prometryn and ^{14}C -hexaconazole forced isotope exchange data, including end-points of initially-applied ^{14}C -prometryn and ^{14}C -hexaconazole masses recovered using the forced isotope exchange technique.

Pesticide	Adsorption time (days)	Soil type	Recovery of initial after 1 day (%)	Recovery at last sampling point (%)	Total recovery of initial (%)	
Prometryn	56	Blackwood	16 ± 0.5	0.2 ± 0.01^a	34 ± 1.3	
	56	Andover	8 ± 0.5	0.1 ± 0.01^a	18 ± 0.5	
	56	Salop	8 ± 0.1	0.2 ± 0.02^a	22 ± 0.5	
	112	Blackwood	16 ± 0.3	0.2 ± 0.01^a	31 ± 0.9	
	112	Andover	8 ± 0.3	0.1 ± 0.03^a	15 ± 0.5	
	112	Salop	9 ± 0.6	0.3 ± 0.01^a	21 ± 1.4	
	168	Blackwood	15 ± 0.4	0.2 ± 0.01^a	30 ± 0.6	
	168	Andover	8 ± 1.0	0.2 ± 0.05^a	15 ± 1.1	
	168	Salop	8 ± 0.4	0.3 ± 0.06^a	20 ± 0.3	
	Hexaconazole	56	Blackwood	18 ± 1.4	0.2 ± 0.03^b	34 ± 1.7
		56	Andover	7 ± 0.5	0.1 ± 0.01^b	11 ± 0.5
		56	Salop	6 ± 0.3	0.2 ± 0.03^b	13 ± 0.5
112		Blackwood	17 ± 0.9	0.1 ± 0.03^b	32 ± 0.4	
112		Andover	7 ± 0.3	0.1 ± 0.01^b	12 ± 0.7	
112		Salop	8 ± 1.1	0.2 ± 0.04^b	16 ± 1.9	
168		Blackwood	16 ± 0.4	0.2 ± 0.2^b	33 ± 0.8	
168		Andover	8 ± 1.0	0.1 ± 0.003^b	13 ± 1.3	
168		Salop	8 ± 2.3	0.3 ± 0.4^b	18 ± 4.3	

^aPrometryn forced isotope exchange period was carried out over 175 days.

^bHexaconazole forced isotope exchange period was carried out over 147 days.

A reduction in pesticide desorption was expected to occur as a function of contact time with the soil. Longer adsorption periods have been reported to allow time for the solute to further diffuse into remote regions of the soil matrix (Lesan and Bhandari, 2003), increasing sorption non-linearity. For hexaconazole however, longer adsorption times had little effect on the total mass of initially-applied ^{14}C -hexaconazole recovered, particularly for the Salop soil, for which the amount of

initially-applied pesticide recovered actually increased with increasing adsorption time. For prometryn, the total mass of ^{14}C -prometryn recovered was observed to decline very slightly over time; this effect was more significant for the Blackwood and Andover soils and mainly occurred between 56 and 112 days. As no considerable increases in sorption were observed between 28 and 168 days (section 5.3.1), sorption equilibrium may have been reached prior to the first time-point (56-days); therefore, it follows that no significant reductions in amounts of initially-applied ^{14}C -hexaconazole and ^{14}C -prometryn recovered should be expected.

Total masses recovered were very similar for ^{14}C -prometryn and ^{14}C -hexaconazole, suggesting that the sorption behaviour of both pesticides at the selected soil:solution ratios was similar. It should also be noted, as for chlorotoluron, that the forced isotope exchange procedure was still releasing very small amounts of sorbed ^{14}C -prometryn and ^{14}C -hexaconazole at the end of the process, albeit at very slow rates. Thus, even though total sorption to soil of ^{14}C -prometryn and ^{14}C -hexaconazole was significantly less than for ^{14}C -chlorotoluron, the desorption process still requires a significant amount of time in order for desorption end-points to be reached.

5.3.5. Prometryn and hexaconazole mass balances

The complete mass balances for ^{14}C -prometryn over time are given in Figures 5.7, 5.8 and 5.9. The complete mass balances for ^{14}C -hexaconazole over time are subsequently presented in Figures 5.10, 5.11 and 5.12. As total sorption was relatively lower for prometryn and hexaconazole compared to chlorotoluron, the majority of ^{14}C -prometryn and ^{14}C -hexaconazole was non-sorbed. After the isotope exchange and forced isotope exchange procedures, repeated solvent extraction further released only very small amounts of initially-applied ^{14}C -prometryn and ^{14}C -hexaconazole (see Table 5.2). Radio-HPLC analysis of the forced isotope exchange solutions identified that no degradation of ^{14}C -hexaconazole occurred during the study but that a small amount of ^{14}C -prometryn degradation did take place, as discussed in section 5.3.2.

Combustion of the ^{14}C -prometryn and ^{14}C -hexaconazole extracted soils only released very small amounts of radioactivity, though absolute values were fairly small, these

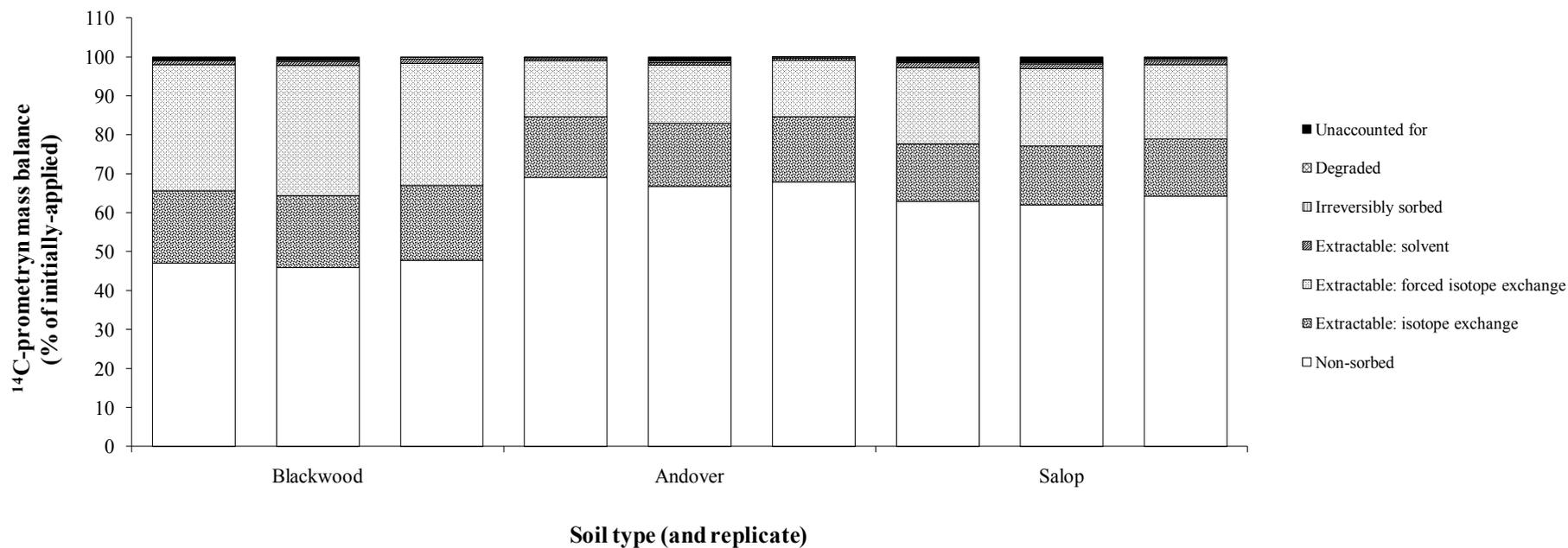


Figure 5.7. Mass balance of initially-applied ^{14}C -prometryn to nine soil samples adsorbed for 56-days. Three replicate samples per soil type are shown. The non-sorbed fraction reflects ^{14}C -prometryn present in the aqueous phase after the adsorption period, whilst that which partitioned to the soil is collectively represented by the three extractable fractions and irreversibly sorbed fraction. The three extractable fractions characterise the relative amounts of ^{14}C -prometryn desorbed from the soil when the harshness of the extraction increased in the order: isotope exchange < forced isotope exchange < solvent. Further analysis revealed the irreversible fraction, degradation and a fraction unaccounted for by the chemical analyses.

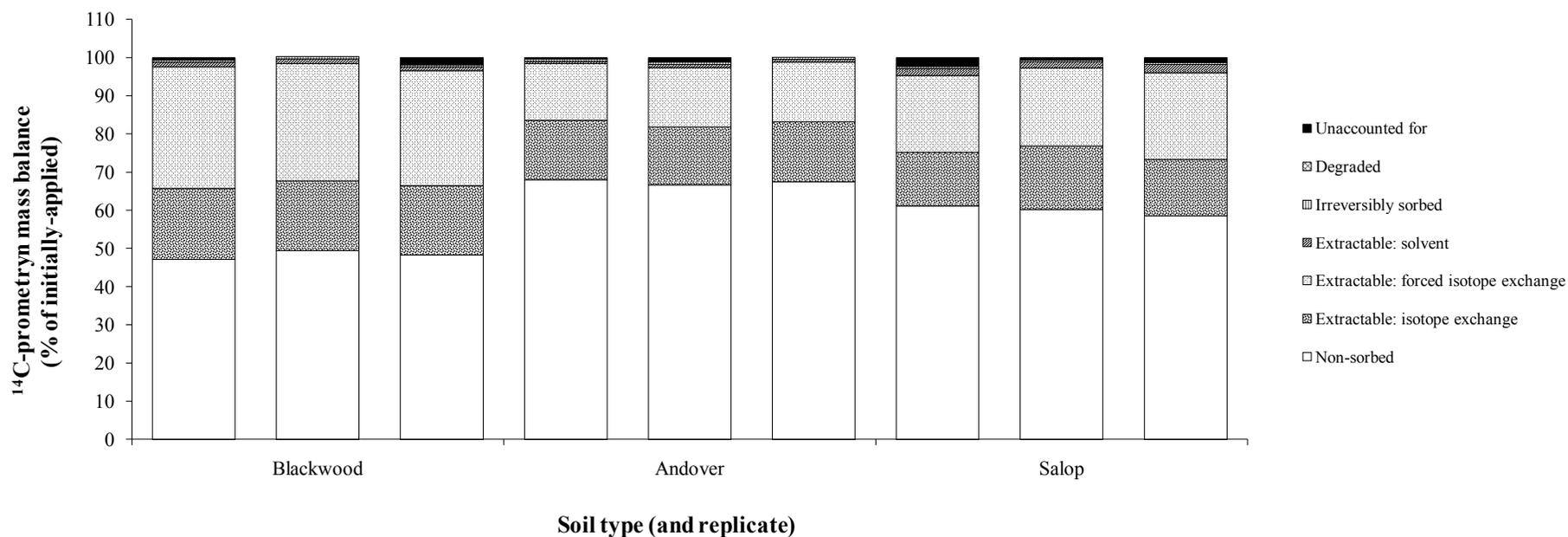


Figure 5.8. Mass balance of initially-applied ¹⁴C-prometryn to nine soil samples adsorbed for 112-days. Three replicate samples per soil type are shown. The non-sorbed fraction reflects ¹⁴C-prometryn present in the aqueous phase after the adsorption period, whilst that which partitioned to the soil is collectively represented by the three extractable fractions and irreversibly sorbed fraction. The three extractable fractions characterise the relative amounts of ¹⁴C-prometryn desorbed from the soil when the harshness of the extraction increased in the order: isotope exchange < forced isotope exchange < solvent. Further analysis revealed the irreversible fraction, degradation and a fraction unaccounted for by the chemical analyses.

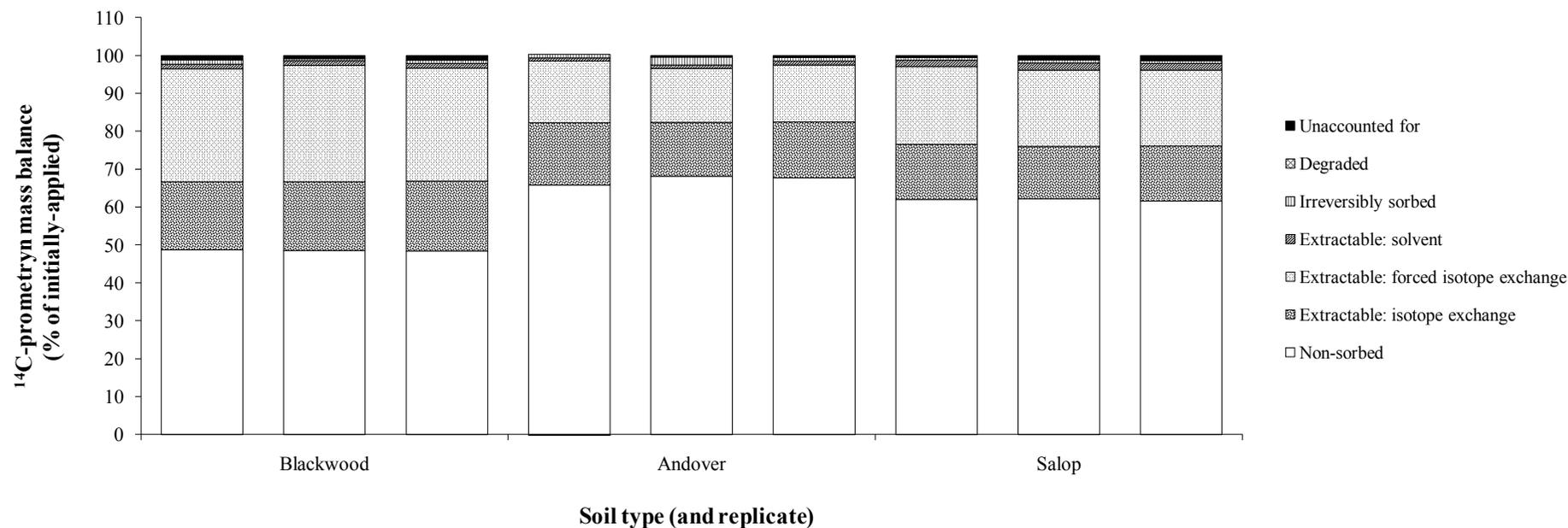


Figure 5.9. Mass balance of initially-applied ^{14}C -prometryn to nine soil samples adsorbed for 168-days. Three replicate samples per soil type are shown. The non-sorbed fraction reflects ^{14}C -prometryn present in the aqueous phase after the adsorption period, whilst that which partitioned to the soil is collectively represented by the three extractable fractions and irreversibly sorbed fraction. The three extractable fractions characterise the relative amounts of ^{14}C -prometryn desorbed from the soil when the harshness of the extraction increased in the order: isotope exchange < forced isotope exchange < solvent. Further analysis revealed the irreversible fraction, degradation and a fraction unaccounted for by the chemical analyses.

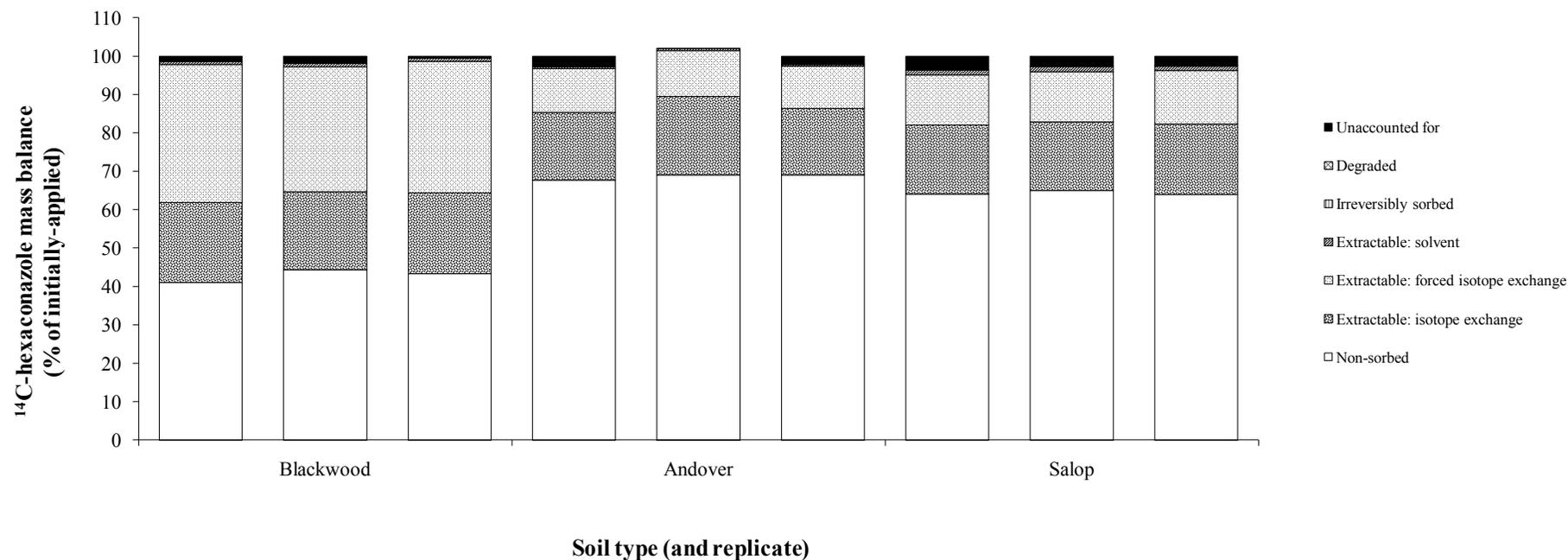


Figure 5.10. Mass balance of initially-applied ^{14}C -hexaconazole to nine soil samples adsorbed for 56-days. Three replicate samples per soil type are shown. The non-sorbed fraction reflects ^{14}C -hexaconazole present in the aqueous phase after the adsorption period, whilst that which partitioned to the soil is collectively represented by the three extractable fractions and irreversibly sorbed fraction. The three extractable fractions characterise the relative amounts of ^{14}C -hexaconazole desorbed from the soil when the harshness of the extraction increased in the order: isotope exchange < forced isotope exchange < solvent. Further analysis revealed the irreversible fraction, degradation and a fraction unaccounted for by the chemical analyses.

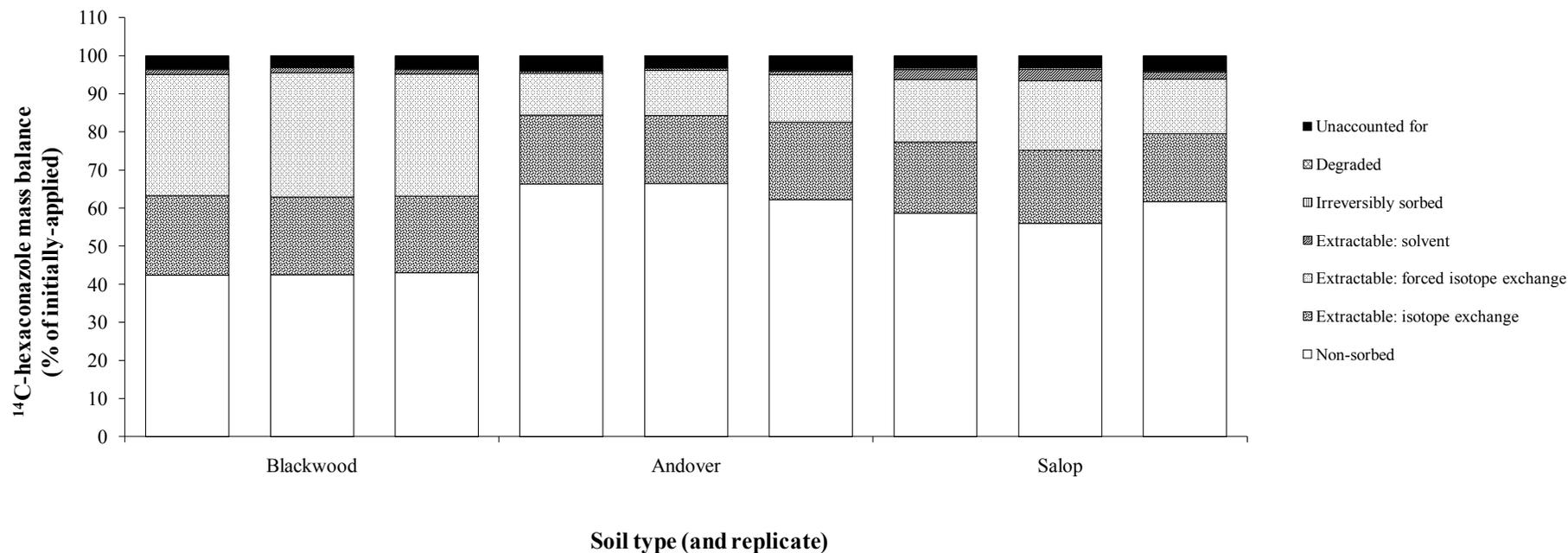


Figure 5.11. Mass balance of initially-applied ^{14}C -hexaconazole to nine soil samples adsorbed for 112-days. Three replicate samples per soil type are shown. The non-sorbed fraction reflects ^{14}C -hexaconazole present in the aqueous phase after the adsorption period, whilst that which partitioned to the soil is collectively represented by the three extractable fractions and irreversibly sorbed fraction. The three extractable fractions characterise the relative amounts of ^{14}C -hexaconazole desorbed from the soil when the harshness of the extraction increased in the order: isotope exchange < forced isotope exchange < solvent. Further analysis revealed the irreversible fraction, degradation and a fraction unaccounted for by the chemical analyses.

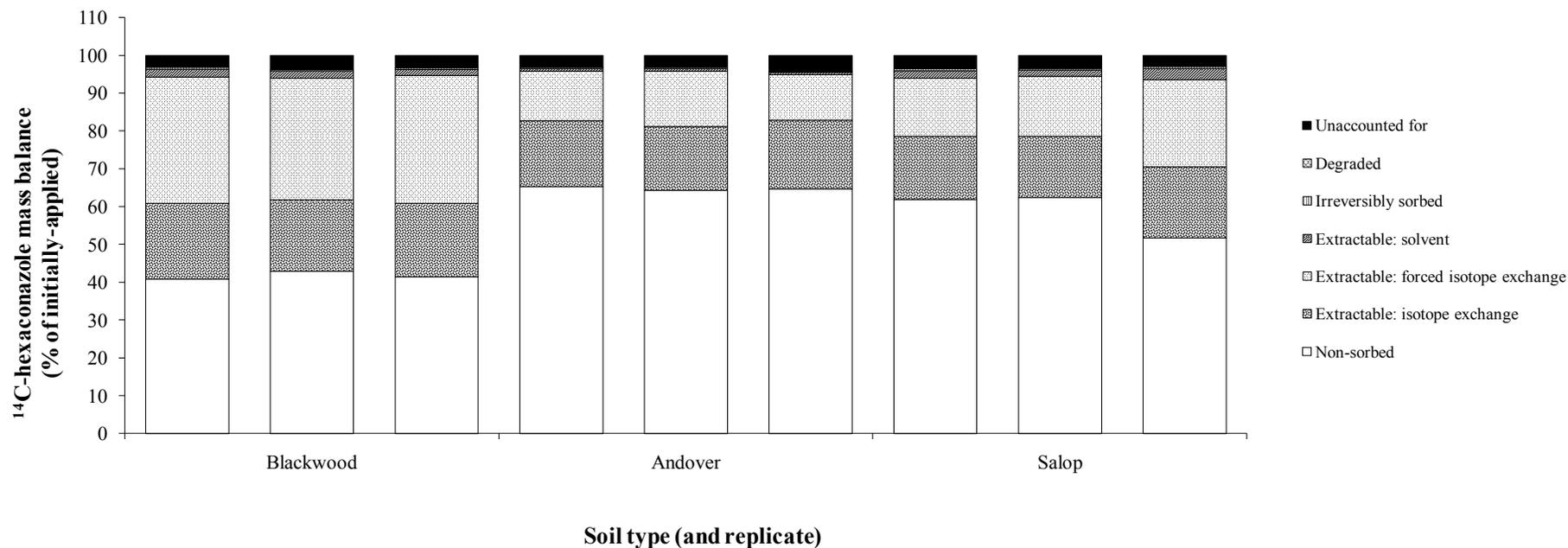


Figure 5.12. Mass balance of initially-applied ^{14}C -hexaconazole to nine soil samples adsorbed for 168-days. Three replicate samples per soil type are shown. The non-sorbed fraction reflects ^{14}C -hexaconazole present in the aqueous phase after the adsorption period, whilst that which partitioned to the soil is collectively represented by the three extractable fractions and irreversibly sorbed fraction. The three extractable fractions characterise the relative amounts of ^{14}C -hexaconazole desorbed from the soil when the harshness of the extraction increased in the order: isotope exchange < forced isotope exchange < solvent. Further analysis revealed the irreversible fraction, degradation and a fraction unaccounted for by the chemical analyses.

Table 5.2. Recoveries (with standard deviation) of initial-applied ^{14}C -prometryn and ^{14}C -hexaconazole by solvent extraction and soil combustion.

Pesticide	Adsorption time (days)	Soil type	Recovery of initial by solvent extraction (%)	Recovery of initial by soil combustion (%)	Unaccounted for (%)	
Prometryn	56	Blackwood	0.9 ± 0.06	0.3 ± 0.10	0.6 ± 0.39	
	56	Andover	0.5 ± 0.05	0.3 ± 0.06	0.4 ± 0.57	
	56	Salop	1.0 ± 0.09	0.3 ± 0.01	1.1 ± 0.44	
	112	Blackwood	1.0 ± 0.07	0.5 ± 0.11	0.7 ± 1.10	
	112	Andover	0.6 ± 0.06	0.6 ± 0.07	1.5 ± 0.61	
	112	Salop	1.6 ± 0.21	0.5 ± 0.06	0.4 ± 0.86	
	168	Blackwood	1.1 ± 0.03	0.8 ± 0.27	1.1 ± 0.22	
	168	Andover	0.7 ± 0.11	1.3 ± 0.60	0.2 ± 0.44	
	168	Salop	1.5 ± 0.08	0.8 ± 0.04	1.0 ± 0.44	
	Hexaconazole	56	Blackwood	0.7 ± 0.03	0.2 ± 0.03	1.1 ± 0.72
		56	Andover	0.4 ± 0.08	0.1 ± 0.01	0.9 ± 2.58
		56	Salop	1.1 ± 0.13	0.2 ± 0.02	2.8 ± 0.58
112		Blackwood	1.1 ± 0.06	0.3 ± 0.005	3.2 ± 0.25	
112		Andover	0.6 ± 0.22	0.2 ± 0.03	3.5 ± 0.49	
112		Salop	2.2 ± 0.58	0.4 ± 0.03	3.5 ± 0.46	
168		Blackwood	1.7 ± 0.25	0.4 ± 0.11	3.4 ± 0.35	
168		Andover	0.6 ± 0.06	0.3 ± 0.01	3.5 ± 0.63	
168		Salop	0.9 ± 0.65	0.5 ± 0.06	3.3 ± 0.35	

fractions did increase slightly for both pesticides after longer adsorption periods (see Table 5.2). This suggests that a small increase in strength of binding did occur when ^{14}C -prometryn and ^{14}C -hexaconazole were in contact with the soil for longer. The mechanisms responsible for this are likely to include diffusion into spatially remote areas, such as soil macro- and micropores (Ball and Roberts, 1991; Burgos *et al.*, 1996; Pignatello and Xing, 1996) and entrapment within soil organic matter (Brusseau *et al.*, 1991; Huang and Weber, 1997; Park *et al.*, 2004). As for chlorotoluron, these small fractions could not be identified, but fit with a classical definition of bound residues. Again, it should be noted that these percentages would not completely reflect irreversible fractions measured in the field, which are likely to be greater than those reported here; firstly, because under field conditions rainwater is the main solvent as opposed to organic solvent, and secondly, because the microbial community was eliminated. These factors would both affect pesticide fate in the environment and are discussed further in Chapter 7 (section 7.1).

Finally, a proportion of the initially-applied ^{14}C -prometryn and ^{14}C -hexaconazole was unaccounted for. For ^{14}C -prometryn these values varied from an average of 0.2-1.5%, though standard deviations were large between replicates. For ^{14}C -hexaconazole, the fraction that was unaccounted for was significantly larger than for ^{14}C -prometryn samples, amounting to $3.4 \pm 0.4\%$, $3.5 \pm 0.6\%$ and $3.3 \pm 0.4\%$ after 168 days for the Blackwood, Andover and Salop soils, respectively. It is postulated for prometryn that these minor 'losses' may be the result of mineralisation to $^{14}\text{CO}_2$, volatilisation or cumulative errors due to the multiple solute exchanges undertaken during the length of the experiment. Due to the strong sorption of hexaconazole, it is unlikely that any mineralisation or volatilisation occurred though it may be possible that some adsorption of this fungicide to the test vessels occurred. Although no degradation was observed for hexaconazole (section 5.3.2), recovery of initial-applied ^{14}C -hexaconazole (^{14}C degradation samples) after 182 days averaged 98, 94 and 93% for the Blackwood, Andover and Salop soils respectively. Recovery of initial-applied after 56 days was very similar. No evidence has been found in the literature however, to suggest that hexaconazole may sorb to PTFE. Celis and Koskinen (1999a) studied another triazole fungicide (triadimefon) in glass centrifuge tubes with a PTFE lid and reported no losses during their study. Thus, it is not clear why the largest proportions of initially-applied hexaconazole remain unaccounted for. Further assessments are required in order to draw robust conclusions on the subject.

5.4. Conclusions

The majority of initially-applied ^{14}C -prometryn and ^{14}C -hexaconazole was remobilised using the forced isotope exchange procedure; only minimal amounts of sorbed ^{14}C -pesticide were additionally released by solvent-extraction and soil combustion. As the forced isotope exchange procedure was still releasing small amounts of sorbed pesticide at the end of the experimental time-frame, it is likely that had the procedure been continued, all of the initially sorbed ^{14}C -prometryn and ^{14}C -hexaconazole would have been eventually released. This suggests that under abiotic conditions, irreversible sorption does not play a significant role in the fate of ^{14}C -prometryn and ^{14}C -hexaconazole for the soil systems studied, also confirming the results obtained for ^{14}C -chlorotoluron, despite the longer periods of adsorption. The factors influencing the formation of bound residues for the pesticide/soil systems studied are still uncertain therefore, and require further investigation.

It has been reported that microbial activity has a significant effect on the formation of bound residues (Burauel and Führ, 2000; Rice *et al.*, 2002). As the soil microbial community was eliminated from the study soils in this work, and only minimal amounts of irreversible sorption was observed, this raises three key questions: (i) is it possible to assess from the literature whether irreversible sorption is most likely microbially-mediated? (ii) if irreversible sorption is microbially-mediated then it probably results from biodegradation of the parent molecule. Is it appropriate to use a definition of bound residues that includes both parent and non-parent forms in the definition? (iii) if bound residues are composed of non-parent material, what is the implication of this for concerns about the bioavailability of those residues? These matters are discussed in detail in the general discussion (Chapter 7, section 7.1).

In the following Chapter, mathematical modelling is used to further explore the sorption processes operating in the pesticide/soil systems studied, and attempts to establish the point at which the release of sorbed ^{14}C -prometryn or ^{14}C -hexaconazole from soil comes to an end.

CHAPTER 6

MODELLING PESTICIDE SORPTION BEHAVIOUR

6.1. Introduction

The results of the isotope exchange and forced isotope exchange of chlorotoluron, prometryn and hexaconazole to the three study soils have been presented in Chapters 4 and 5. As desorption end-points were not reached during the experimental time-frame, there is still uncertainty over whether the remaining sorbed pesticide was participating in non-equilibrium (with potential for release) or irreversible sorption. In order to interpret the data in greater detail, a three-site mathematical model was developed to further explore the sorption processes responsible for the measurements obtained. Differential equations were used to simulate the sorption behaviour of the three pesticides over time, enabling prediction of their behaviour beyond the time-scale of the measured data. The three-site model was first applied to the pesticide/soil systems studied by Celis and Koskinen (1999a; 1999b) in order to test the model. The three-site model was then applied to the pesticide/soil systems studied in this thesis, with the addition of the forced isotope exchange procedure.

6.2. Methods

6.2.1. The three-site model

A three-site model was developed to conceptualise three different types of sorption sites. Its purpose was to simulate: (i) instantaneous exchange between solution and soil in the equilibrium phase; (ii) slow but reversible binding on non-equilibrium sorption sites, characterised by a first-order adsorption and desorption reaction with identical rate constant k_{des} ; and, (iii) slow movement from non-equilibrium sites to irreversible sites, using the rate constant k_{irr} (Figure 6.1). Degradation was disregarded due to difficulty in accounting for sorption behaviour of metabolites. This was appropriate since degradation was shown to be limited (Chapters 4 and 5).

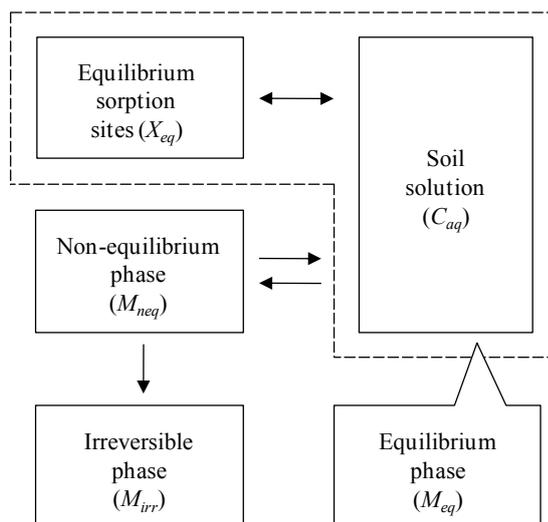


Figure 6.1. Schematic representation of the three-site model. The equilibrium phase (M_{eq}) includes both the concentration ($\mu\text{g mL}^{-1}$) of pesticide in the soil solution (C_{aq}) and the pesticide sorbed ($\mu\text{g g}^{-1}$) at equilibrium sites (X_{eq}).

The quantity of pesticide sorbed at equilibrium sites (X_{eq}) was derived using the following equation:

$$X_{eq} = K_{feq} \times C_{aq}^{1/n}$$

where K_{feq} is the equilibrium Freundlich sorption distribution coefficient and $1/n$ is the Freundlich exponent describing non-linearity. The Taylor expansion is used to estimate C_{aq} ($\mu\text{g mL}^{-1}$), thus the Freundlich equation uses an iteration procedure in order to solve the mass balance.

X_{eq} is then used to determine the total mass of pesticide in the equilibrium phase (M_{eq}), as demonstrated by the following equation:

$$M_{eq} = (V_{sol} \times C_{aq}) + (M_{soil} \times X_{eq})$$

where the parameters V_{sol} and M_{soil} , respectively, refer to the volume of solution (mL) and mass of soil (g) used in the experimental system. In order to derive the mass of pesticide in the non-equilibrium (M_{neq}) and irreversible phases (M_{irr}) of sorption, the following differential equations were used:

$$\frac{dM_{neq}}{dt} = (k_{des} \times F_{ne} \times K_{feq} \times C_{aq}^{1/n}) - (k_{des} \times M_{neq}) - (k_{irr} \times M_{neq})$$

$$\frac{dM_{irr}}{dt} = k_{irr} \times M_{neq}$$

where k_{des} is the desorption or exchange rate coefficient (d^{-1}), F_{ne} is the ratio of sorption in the non-equilibrium phase to sorption in the equilibrium phase, and k_{irr} is the rate coefficient controlling irreversible sorption (d^{-1}).

6.2.2. Adsorption and isotope exchange

The experimental set-up, based on Celis and Koskinen's isotope exchange technique (1999a; 1999b), involved preparation of samples that initially contained either ^{12}C - or ^{14}C -pesticide (see Chapters 4 and 5, sections 4.2 and 5.2 for detailed methodology). After specific periods of adsorption to soil (continuous shaking for 7, 14, 28 or 56 days for chlorotoluron and 28, 56, 112 or 168 days for prometryn and hexaconazole), a supernatant exchange was carried out between the corresponding initially ^{12}C and ^{14}C samples. As a consequence, all samples eventually contained both isotopes, however, with either ^{12}C - or ^{14}C -pesticide sorbed to soil. Samples were then shaken continuously for a further 14 days with samples of the supernatant taken at specific time intervals (1, 3, 7 and 14 days) to measure isotope exchange over time. The three-site model described above was therefore applied four times per pesticide in order to estimate the behaviour of ^{12}C and ^{14}C isotopes individually during the adsorption phase and then simultaneously after supernatant exchange, for both initially ^{12}C samples (Tube A) and initially ^{14}C samples (Tube B) (Figure 6.2).

In order to calculate the isotope masses transferred between the two corresponding tubes at supernatant exchange, the following equations were used:

In Tube A:

$$M_{eq}^{12C} = M_{eq}^{in\ tube\ A} - (V_{rem} \times C_{aq}^{in\ tube\ A})$$

$$M_{eq}^{14C} = V_{add} \times C_{aq}^{in\ tube\ B}$$

In Tube B:

$$M_{eq}^{12C} = V_{add} \times C_{aq}^{in\ tube\ A}$$

$$M_{eq}^{14C} = M_{eq}^{in\ tube\ B} - (V_{rem} \times C_{aq}^{in\ tube\ B})$$

where V_{rem} is the original volume of supernatant removed from each tube at supernatant exchange. V_{add} is the added volume of supernatant from the other tube, replacing the original supernatant.

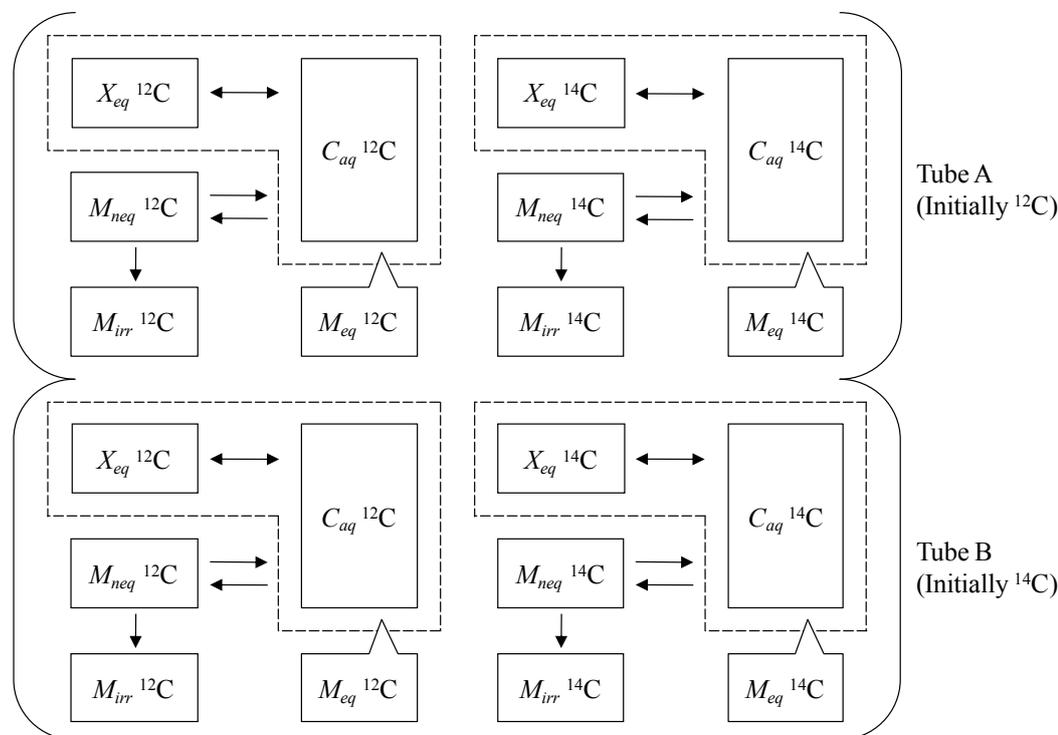


Figure 6.2. Expanded schematic representation of the three-compartment model to account for the fact that: (i) Tubes A were initially ^{12}C and Tubes B were initially ^{14}C ; and, (ii) after supernatant exchange Tubes A and B contained both ^{12}C and ^{14}C isotopes.

In the model, the equilibrium sorption reaction is assumed to be instantaneous. As this reaction does not differentiate between ^{12}C - and ^{14}C -pesticide, the Freundlich equation is performed for both tubes simultaneously. This necessitates calculating the fraction of total pesticide isotopes that ^{14}C -pesticide represents in Tube A and Tube B (F_{14C}):

$$F_{14C} = M_{eq}^{14C} \div M_{eq}$$

where M_{eq}^{14C} refers only to the mass of ^{14}C -pesticide in the equilibrium phase. F_{14C} is calculated by the model at each time step. The fraction of ^{14}C -pesticide is considered in the flow from the equilibrium to the non-equilibrium phases. In order to account for this in the model, the following equations are used to calculate the concentrations of ^{12}C - and ^{14}C -pesticide in solution for Tubes A and B, respectively:

In Tube A:

$$C_{aq}^{12C} = (1 - F_{14C \text{ in tube A}}) \times C_{aq \text{ in tube A}}$$

$$C_{aq}^{14C} = F_{14C \text{ in tube A}} \times C_{aq \text{ in tube A}}$$

In Tube B:

$$C_{aq}^{12C} = (1 - F_{14C \text{ in tube B}}) \times C_{aq \text{ in tube B}}$$

$$C_{aq}^{14C} = F_{14C \text{ in tube B}} \times C_{aq \text{ in tube B}}$$

Furthermore, for output purposes the specific activity ($\text{Bq } \mu\text{g}^{-1}$) was also included as a parameter in the model, and is used to convert the concentration in $\mu\text{g mL}^{-1}$ in the aqueous phase into radioactivity (Bq mL^{-1}). This is necessary because although the model calculates the concentrations in $\mu\text{g mL}^{-1}$, the original measurements were in Bq mL^{-1} .

6.2.3. Forced isotope exchange

Finally, the forced isotope exchange procedure was applied to initially ^{14}C samples after 56, 112 or 168 days of adsorption and 14 days of isotope exchange only. This involved the removal of the supernatant and its subsequent replacement with a high concentration ^{12}C -pesticide solution over a period of time. This procedure was anticipated to increase the rate at which ^{14}C -pesticide desorbed from the soil. The following equation thus describes the removal of the supernatant and subsequent addition of the high-concentration ^{12}C -pesticide solution in Tube B:

$$M_{eq}^{12C} = M_{eq}^{12C} - (V_{rem1} \times C_{aq}^{12C}) + M_{12C \text{ added}}$$

$$M_{eq}^{14C} = M_{eq}^{14C} - (V_{rem1} \times C_{aq}^{14C})$$

where V_{rem1} is the volume of supernatant removed during the forced isotope exchange procedure (to differentiate it from the volume removed by isotope exchange) and M_{12C} is the total mass of ^{12}C -pesticide added in the solution.

6.2.4. Measured parameters, optimised parameters and optimisation method

The model has three unknown parameters that describe the sorption behaviour of the pesticide: (i) the ratio of non-equilibrium sorption to equilibrium sorption (F_{ne}); (ii) the desorption rate (k_{des}); and, (iii) the rate of irreversible sorption (k_{irr}). The

Freundlich distribution coefficient (K_{feq}) and the Freundlich exponent ($1/n$) were measured independently by Freundlich sorption studies (see Chapter 3, section 3.6) for the pesticide/soil systems studied in this thesis. Celis and Koskinen (1999a; 1999b) also provided K_{feq} and $1/n$ values after carrying out Freundlich sorption studies in their papers for the pesticide/soil systems studied. The three unknown parameters were optimised using the Marquardt method and weighted least squares. The optimisation was carried out using the default settings of ModelMaker© software, version 4.0, using a convergence change of 0.1 and 50 convergence steps. The default Marquardt settings were: initial lambda of 0.01; minimum change of 1×10^{-5} ; and fractional change of 0.01. Individual weighting was used with a data error fraction of 1.

For adsorption and isotope exchange, the model was optimised against measurements obtained from Tube A (initially ^{12}C) and Tube B (initially ^{14}C). For the full isotope exchange model (which includes the full data-set: adsorption, isotope exchange and forced isotope exchange) the model was only optimised against Tube B as only initially ^{14}C samples were used in the forced isotope exchange part of the study. The model fit was implemented step-wise, by first fitting the parameters to the adsorption and isotope exchange data and then using those values as the initial starting values to fit the forced isotope exchange data. Parameters were optimised to the data for all three time-points (56, 112 and 168 days) for prometryn and hexaconazole (only one time-point, 56 days, was used for chlorotoluron). Different starting values were also tested.

6.3. Results and discussion

6.3.1. Adsorption and isotope exchange model

6.3.1.1. Modelling Celis and Koskinen's isotope exchange data

As previously described, the methodology implemented to measure irreversible sorption in this thesis was based on the isotope exchange technique developed by Celis and Koskinen (1999a; 1999b). The three-site model was applied in order to describe the data obtained from the five pesticide/soil systems studied in their two isotope exchange papers (Celis and Koskinen, 1999a; Celis and Koskinen, 1999b): (i) imidacloprid-guanidine/Hanford soil; (ii) imidacloprid-guanidine/Drummer soil; (iii)

imidacloprid/SiCL (silty clay loam) soil; (iv) imidacloprid-urea/SiCL soil; and, (v) imidacloprid-guanidine/LS (loamy sand) soil. The raw data were provided by Celis and Koskinen.

The measured and optimised constants used to parameterise the model are given in Table 6.1. The Freundlich distribution coefficients (K_{feq}) and Freundlich exponents ($1/n$), were measured by the authors in both of their papers, thus these values were used in the three-site model. Attempts to include the k_{irr} in the optimisation with the F_{ne} and k_{des} meant that the model could not generate robust F_{ne} , k_{des} and k_{irr} values; optimisation errors exceeded the parameter estimates calculated by the model. Thus, in order for the three-site model to accurately describe Celis and Koskinen's isotope exchange data, it was necessary to set the parameter for irreversible sorption (k_{irr}) to zero.

The model fit is plotted against the isotope exchange data measured by Celis and Koskinen for all pesticide/soil systems in Figures 6.3 and 6.4. Visually, the model describes the measured isotope exchange data well. The model efficiency (ME) equation described in Chapter 3 (section 3.6.3) was used to quantify how well the two-site model was able to describe the five pesticide/soil systems studied (Table 6.2). Plots of residuals are also given in Appendix B (Figures B.1 and B.2). As optimisation was carried out using weighted least squares, all residuals are plotted as a fraction of the observed values in order to illustrate their relative deviation from the experimental data. Despite the fact that the optimise errors generated by ModelMaker© were generally quite high, the calculated model efficiencies are close to one for all models tested (≥ 0.984). This indicates that although there is some uncertainty concerning the accuracy of the optimised parameter values, the two-site model was able to describe Celis and Koskinen's isotope exchange data reasonably well.

The model also confirms the difference between measured data from initially ^{12}C and ^{14}C samples and relative deviation from their respective expected radioactivity lines. The measured data for initially ^{12}C samples is always closer to the expected radioactivity than for initially ^{14}C samples (Figures 6.3 and 6.4). This is due to the fact that equilibrium was not reached during the 24-hour adsorption phase. Without attaining equilibrium, adsorption is still the dominant process, thus comparatively,

Table 6.1. Measured and optimised parameter values of the model used to describe Celis and Koskinen's (1999a; 1999b) isotope exchange data for the five pesticide/soil systems studied.

Parameter	Parameter values per pesticide/soil system				
	Imidacloprid-guanidine/Hanford	Imidacloprid-guanidine/Drummer	Imidacloprid/SiCL ^a	Imidacloprid-urea/SiCL ^a	Imidacloprid-guanidine/LS ^b
<i>Measured values:</i>					
K_{feq} (Freundlich distribution coefficient)	6.75	42.2	6.70	3.10	3.57
$1/n$ (Freundlich exponent)	0.92	0.83	0.87	0.88	1.05
Initial mass Tube A, initially ¹² C (µg)	3.15	3.15	3.15	3.15	3.15
Initial mass Tube B, initially ¹⁴ C (µg)	3.15	3.15	3.15	3.15	3.15
Mass of soil (g)	0.5	0.5	2.0	2.0	2.0
Specific activity (Bq µg ⁻¹)	246.03	246.03	239.68	239.68	239.68
Volume of supernatant removed (mL)	8.0	8.0	7.0	7.0	7.0
Volume of supernatant replaced (mL)	8.0	8.0	7.0	7.0	7.0
Total volume of shaking solution (mL)	10.0	10.0	10.0	10.0	10.0
<i>Optimised values:</i>					
F_{ne}^*	0.16 (0.10)	0.049 (0.010)	1.07 (0.20)	0.81 (0.072)	2.80 (0.23)
k_{des}^*	0.016 (0.017)	0.090 (0.11)	0.034 (0.013)	0.037 (0.0070)	0.027 (0.0040)
<i>Fixed values:</i>					
k_{irr}	0.0	0.0	0.0	0.0	0.0

^aSiCL refers to silty clay loam.

^bLS refers to loamy sand.

*Optimise error of parameter generated by ModelMaker© given in brackets.

desorption of ^{14}C -pesticide from the initially ^{14}C sample occurs more slowly.

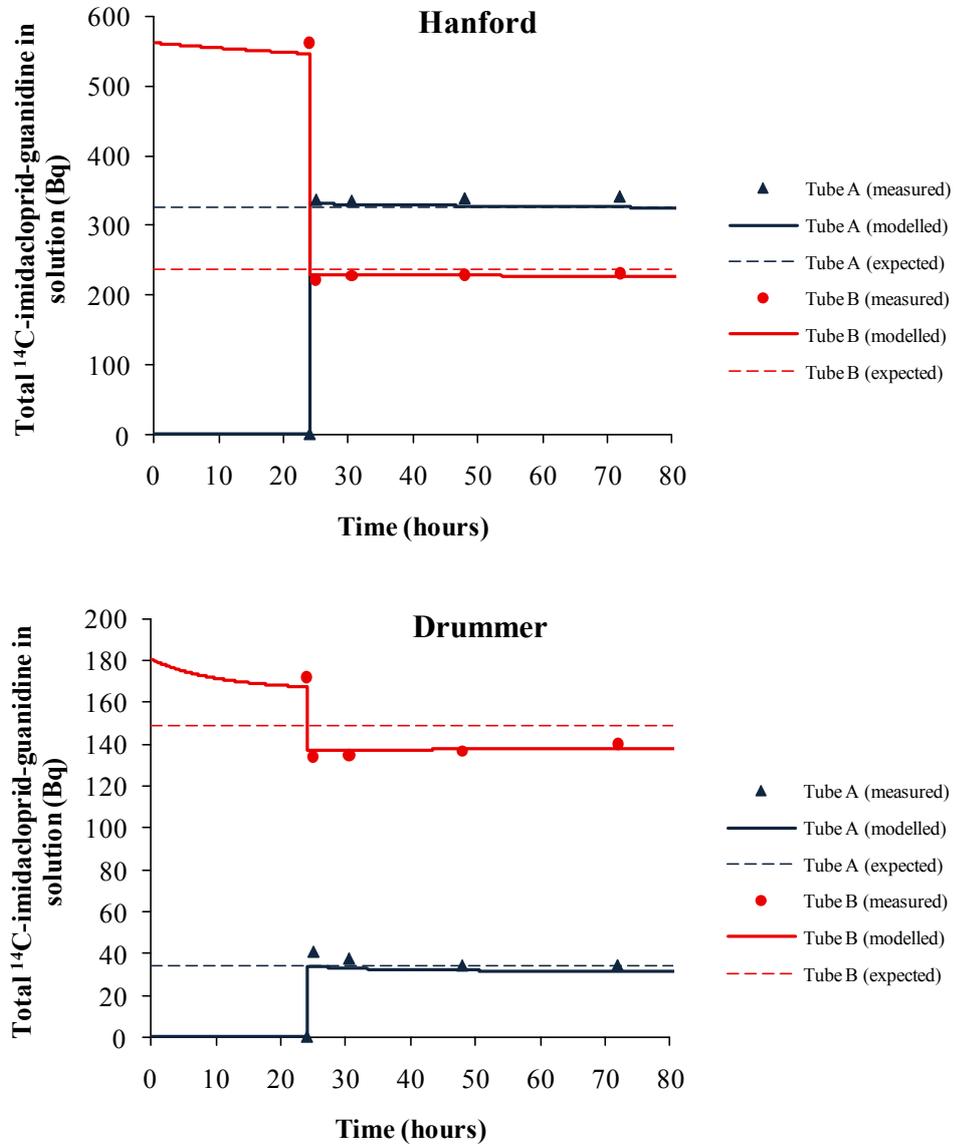


Figure 6.3. Isotope exchange of imidacloprid-guanidine in the Hanford and Drummer soils (Figure 6, Celis and Koskinen 1999a).

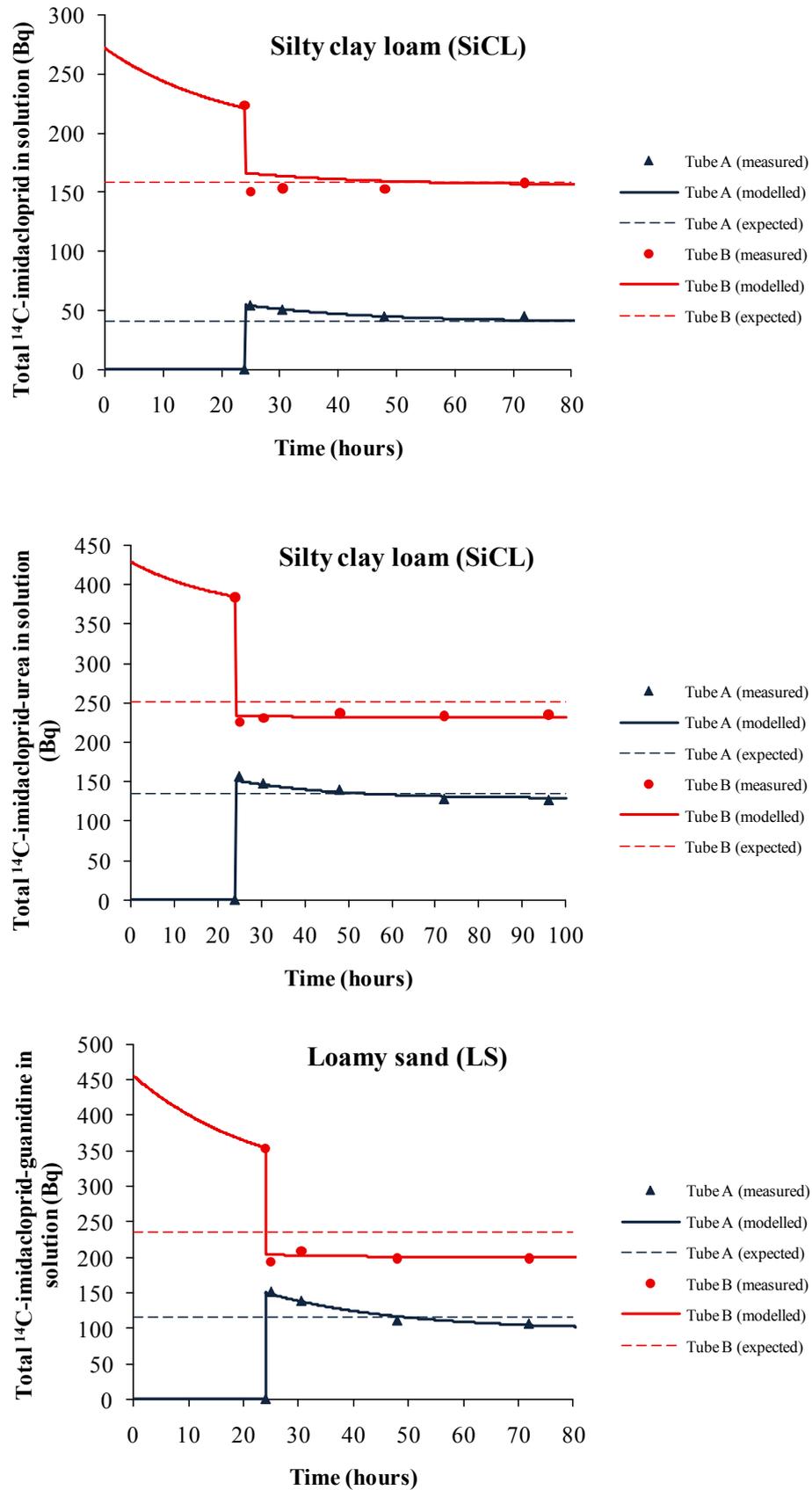


Figure 6.4. Isotope exchange in the three soil systems studied (Figure 2, Celis and Koskinen 1999b).

Table 6.2. Calculated model efficiencies (ME) for Celis and Koskinen's data.

Pesticide/soil system	Calculated model efficiency (ME)
Imidacloprid-guanidine/Hanford soil ^a	0.996
Imidacloprid-guanidine/Drummer soil ^a	0.997
Imidacloprid/SiCL soil ^b	0.984
Imidacloprid-urea/SiCL soil ^b	0.997
Imidacloprid-guanidine LS soil ^b	0.996

^aData from Celis and Koskinen (1999a), Figure 6.

^bData from Celis and Koskinen (1999b), Figure 2.

The ability of three-site model to describe Celis and Koskinen's data well, despite the fact the model parameter describing irreversible sorption was set to zero, conflicts with the results Celis and Koskinen obtained using their two-site model. Celis and Koskinen (1999a) reported that whilst the majority of sorption (90%) occurred on reversible, easily desorbable sites, 10% of sorption occurred on irreversible sites. The application of this three-site model to Celis and Koskinen's data has shown that the sorption behaviour they observed can be explained by slow but reversible sorption. The importance of slow sorption kinetics has been documented by a number of authors (Ball and Roberts, 1991; Pignatello and Xing, 1996; Altfelder and Streck, 2006); thus, this process cannot be ignored in the interpretation of isotope exchange studies.

The experimental design and the short time-scales involved in the adsorption and isotope exchange phases restricts the ability to discriminate between slow, reversible and irreversible sorption. It is difficult to identify whether the sorbed pesticide that did not take part in isotope exchange during the experimental time-frame is slowly reversible or irreversibly sorbed. Thus, the fact that the three-site model describes the isotope exchange data with the k_{irr} set to zero does not prove that irreversible sorption did not occur. Abundant evidence has shown that attaining sorption equilibrium for many compounds may take days, months or even years (Ball and Roberts, 1991; Beulke *et al.*, 2004; Renaud *et al.*, 2004). Thus, carrying out isotope exchange studies using longer adsorption and isotope exchange time-scales may augment the ability of isotope exchange studies to differentiate between slow, reversible and irreversible sorption.

6.3.1.2. Modelling chlorotoluron adsorption and isotope exchange data

The three-site model was initially applied to describe only the adsorption and isotope exchange data obtained for chlorotoluron in the Blackwood, Andover and Salop soils. The data values plotted represent total ^{14}C -chlorotoluron in solution during the adsorption and isotope exchange phases. The data in the adsorption phase included total ^{14}C -chlorotoluron measured in solution after all four periods of adsorption (7, 14, 28 and 56), although isotope exchange data were obtained after 56 days of adsorption only.

The measured and optimised constants used to parameterise the model are given in Table 6.3. The optimised parameters (F_{ne} and k_{des}) were fitted to the data obtained from Tube A (initially ^{12}C) and Tube B (initially ^{14}C). The optimise error for F_{ne} and k_{des} were better than those observed for Celis and Koskinen's data, particularly for the F_{ne} . This suggests that there is less uncertainty in the parameter values estimated by the model for the chlorotoluron data. The decision was again taken to set the parameter for irreversible sorption (k_{irr}) to zero to describe the adsorption and isotope exchange data. Attempts to include the k_{irr} in the optimisation with the F_{ne} and k_{des} for chlorotoluron also meant that robust F_{ne} , k_{des} and k_{irr} values could not be obtained; optimisation errors obtained again significantly exceeded the parameter estimates calculated by the model. This suggests that at this stage in the experiments the model was not sensitive enough to detect changes in irreversible sorption.

The model fit is plotted against the measured data for all soil types in Figure 6.5. On a visual basis, the model describes the measured data well for the Blackwood, Andover and Salop soils. The calculated MEs are given in Table 6.4 (residuals are plotted in Appendix B, Figure B.3). The MEs are slightly lower than those calculated for Celis and Koskinen's data. The worst model fit was for the Blackwood data, likely to be a result of the poorer fitting to the adsorption data (Figure 6.5). However, all ME values are still close to 1, indicating good model fit to the data overall. The three-site model is therefore, also able to explain the chlorotoluron isotope exchange data using only slow, reversible sorption.

The three-site model also confirms the difference between measured data from initially ^{12}C and ^{14}C samples and deviation from their respective expected radioactivity lines. As also observed during Celis and Koskinen's isotope exchange

study, the measured data for initially ^{12}C samples is always closer to the expected radioactivity lines than for initially ^{14}C samples (Figure 6.5). This confirms that sorption equilibrium was not reached for chlorotoluron after the 56-day adsorption phase. The model shows that this is the expected behaviour, if the model assumptions apply.

Table 6.3. Measured and optimised parameter values of the chlorotoluron isotope exchange model.

Parameters	Parameter values per soil type		
	Blackwood	Andover	Salop
<i>Measured values:</i>			
K_{feq} (Freundlich distribution coefficient)	5.1	2.8	4.5
$1/n$ (Freundlich exponent)	0.81	0.82	0.76
Initial mass Tube A, initially ^{12}C (μg)	13.9	13.9	13.9
Initial mass Tube B, initially ^{14}C (μg)	13.8	13.8	13.8
Mass of soil (g)	5.0	5.0	5.0
Specific activity ($\text{Bq } \mu\text{g}^{-1}$)	4569.5	4569.5	4569.5
Volume of supernatant removed (mL)	16.3	16.3	16.3
Volume of supernatant replaced (mL)	16.7	16.7	16.6
Total volume of shaking solution (mL)	20.0	20.0	20.0
<i>Optimised values:</i>			
F_{ne}^*	4.75 (0.21)	4.34 (0.18)	16.79 (0.48)
k_{des}^*	0.040 (0.0058)	0.059 (0.0092)	0.025 (0.0021)
<i>Fixed values:</i>			
k_{irr}	0.0	0.0	0.0

* Optimise error of parameter generated by ModelMaker© given in brackets.

Table 6.4. Calculated model efficiencies (ME) of the modelled and measured values for chlorotoluron adsorption and isotope exchange data.

Soil type	Calculated model efficiency (ME)
Blackwood	0.873
Andover	0.961
Salop	0.914

As estimations of the irreversible fraction could not be calculated by the model at this stage, the forced isotope exchange procedure was carried out. It was anticipated that this procedure would improve the sensitivity of the model and its ability to provide estimates of the irreversibly sorbed fraction.

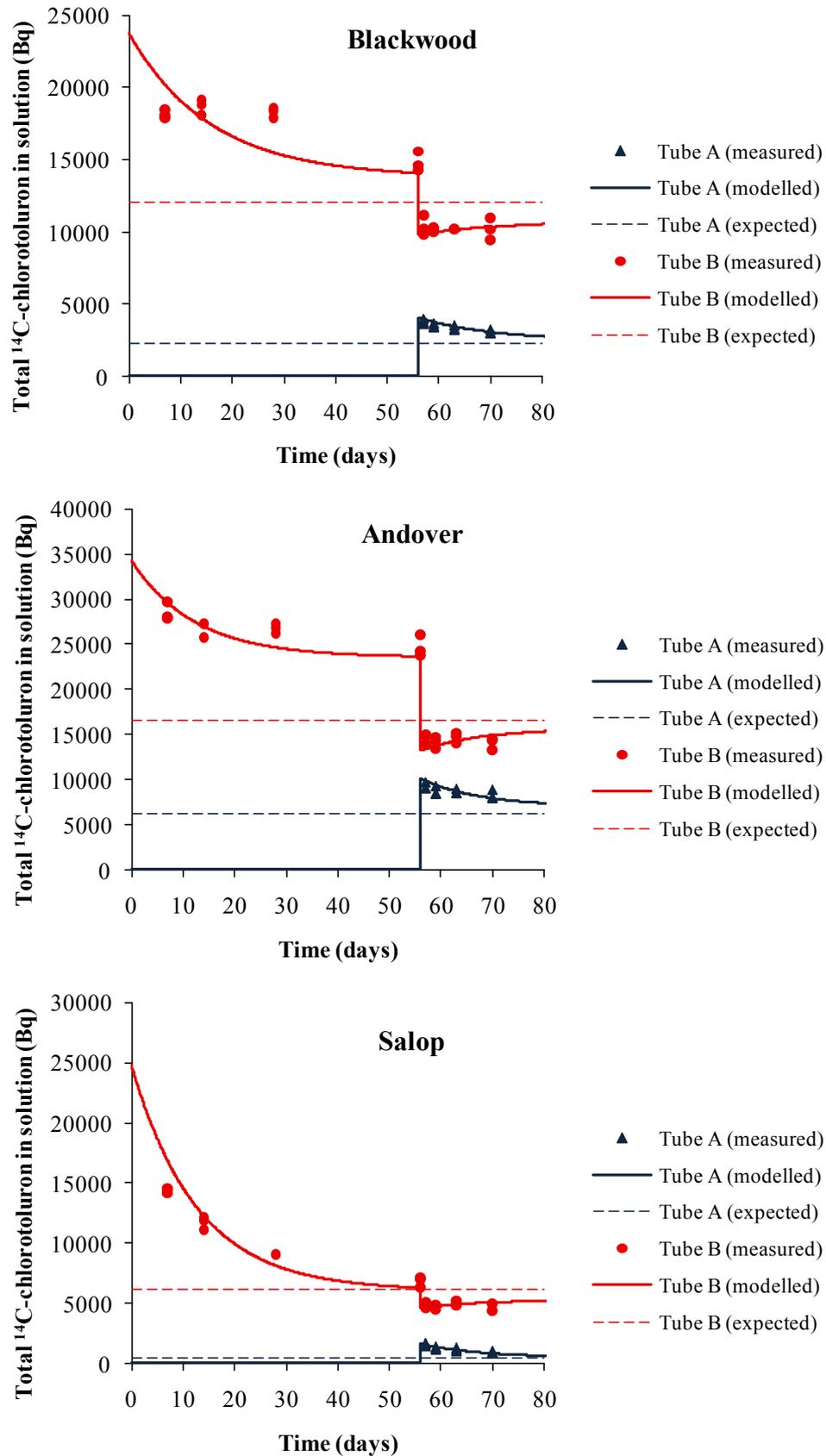


Figure 6.5. Model fit to the chlorotoluron adsorption and isotope exchange data for the Blackwood, Andover and Salop soils, after 56 days adsorption.

6.3.2. Adsorption, isotope exchange and forced isotope exchange model

6.3.2.1. Chlorotoluron data

The three-site model was then applied to the complete chlorotoluron data-set, which includes the adsorption, isotope exchange data and forced isotope exchange data. The model was only optimised to the data obtained for Tube B here, as only the initially ^{14}C -chlorotoluron samples were subject to the forced isotope exchange procedure. The F_{ne} and k_{des} parameter values from the model fit to the adsorption and isotope exchange data above were used as the initial starting values for optimisation of the complete data-set. As the k_{irr} was zero in the model fit to the adsorption and isotope exchange data, the initial value of the k_{irr} was set to 0.005 d^{-1} to begin with, and then optimised from there. Different starting values were also tested to assess whether the same values for k_{irr} were attained after optimisation.

The final optimised parameter values are given in Table 6.5. When optimising the three unknown parameters to the full data-set in order to include the forced isotope exchange data, the model fit was visually better when the k_{irr} was included in the optimisation. Thus, it was necessary to include an irreversible component in order to describe the data. This suggests that the forced isotope exchange data improved the power of the model for providing estimates of the proportion of chlorotoluron that was taking part in irreversible sorption.

The model fits are plotted against the measured data in Figure 6.6. Generally, when compared to the adsorption and isotope exchange data (Figure 6.5) the model fits for the data-sets are as a whole, poorer. This is confirmed by the calculated MEs given in Table 6.6 (with the exception of the Blackwood soil, for which the model efficiency improved). Optimise errors for the k_{des} were similar to those generated for the chlorotoluron isotope exchange model, however the optimise error increased for the F_{ne} . Optimise error for the k_{irr} varied depending on the soil type. Though the uncertainty in k_{irr} was fairly low for the Salop soil, it was very high for the Andover soil. Differences between the data-sets and parameters for each soil type affect the uncertainty of the optimised parameters.

For all soil types it appears that the initial starting value of the modelled ^{14}C -pesticide concentration in solution is too high. The K_{feq} and $1/n$ values were determined by

Table 6.5. Additional measured and optimised parameter values of the chlorotoluron isotope exchange model when including the forced isotope exchange data.

Parameters	Parameter values per soil type		
	Blackwood	Andover	Salop
<i>Measured values^a:</i>			
Mass of ¹² C chlorotoluron added (µg)	520	600	600
Volume of supernatant removed (mL) ^b	13.0	15.0	15.0
Volume of ¹² C solution added (mL) ^b	13.0	15.0	15.0
<i>Optimised values:</i>			
F_{ne}^*	3.89 (0.62)	1.91 (0.75)	13.59 (1.02)
k_{des}^*	0.019 (0.0023)	0.019 (0.0034)	0.022 (0.0027)
k_{irr}	0.0042 (0.0011)	0.0021 (0.0033)	0.0069 (0.00053)

^aIn addition to those already described for the adsorption and isotope exchange data.

^bVolumes of supernatant removed and added during forced isotope exchange.

*Optimise error of parameter generated by ModelMaker© given in brackets.

Table 6.6. Calculated model efficiencies (ME) for the chlorotoluron adsorption, isotope exchange and forced isotope exchange model.

Soil type	Calculated model efficiency (ME)
Blackwood	0.965
Andover	0.939
Salop	0.803

independent Freundlich isotherm experiments (Chapter 3, section 3.6) using ¹²C. As there was little difference in sorption behaviour of ¹²C- and ¹⁴C-labelled compounds observed for chlorotoluron (Chapter 4, Figure 4.2), the overestimation of the adsorption data is likely to be the result of the optimisation method, which used weighted least squares and thus places more emphasis on the latter time-points. Plots of the residuals further support this and show that the model systematically overestimates the adsorption phase (Appendix B, Figure B.4). The isotope exchange data are underestimated; the forced isotope exchange data are initially overestimated and then increasingly underestimated towards the latter time-points (Appendix B, Figure B.4).

Though the model fit to the adsorption and isotope exchange data can be visually improved by optimising the parameters using ordinary least squares, the model fit to

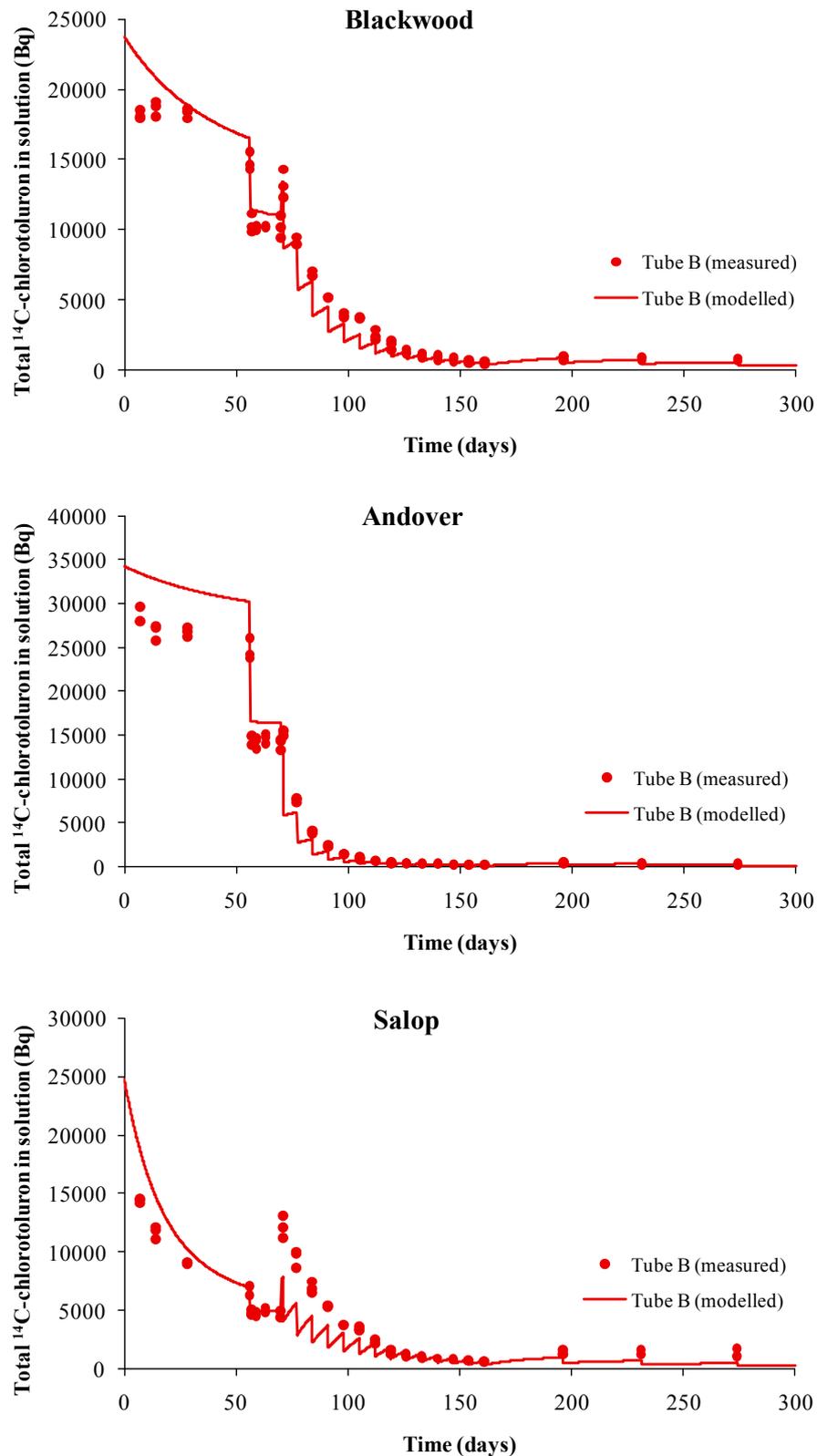


Figure 6.6. Model fit to the chlorotoluron adsorption, isotope exchange and forced isotope exchange data for the Blackwood, Andover and Salop soils, after 56 days adsorption, 14 days isotope exchange and 204 days for forced isotope exchange. Data are plotted in triplicate.

the latter time-points of the forced isotope exchange data deviated further from the observed data. Thus, the decision to use weighted least squares was taken as it was considered more important that the end-points of the data-set were described better in order to use the model to make robust estimations of the irreversible fraction at the end of the experimental time-frame (see section 6.3.3 for model estimations). More ^{14}C -chlorotoluron was released by the forced isotope exchange procedure in reality than the model predicts. This is a weakness of the model.

6.3.2.2. *Prometryn and hexaconazole data*

The three-site model was also applied in order to describe the adsorption, isotope exchange and forced isotope exchange data obtained for the pesticides prometryn and hexaconazole. For these two pesticides, the forced isotope exchange procedure was implemented after three different periods of adsorption (rather than after only 56-days for chlorotoluron). The schematic displayed in Figure 6.2 was therefore applied a total of three times in the models for each pesticide in order to simultaneously describe the measurements obtained for isotope exchange and forced isotope exchange after 56, 112 and 168 days of adsorption. Model fitting was also carried out step-wise with the parameters used to describe the adsorption and isotope exchange phases used as starting values for the complete data-sets.

The measured and optimised values used to parameterise the prometryn and hexaconazole models are given in Table 6.7. The models for both pesticides were only optimised to the data measurements obtained for Tube B, as only the initially ^{14}C -prometryn and ^{14}C -hexaconazole samples were subject to the forced isotope exchange procedure. The same parameters were used to describe the data-sets obtained after all three periods of adsorption for both pesticides. When compared to the optimise errors obtained for the chlorotoluron model, similar uncertainties were also observed in estimation of F_{ne} , k_{des} and k_{irr} for prometryn and hexaconazole. Based on this, it is possible to make some comparisons between the F_{ne} , k_{des} and k_{irr} values obtained for chlorotoluron, prometryn and hexaconazole (see section 6.6.3).

For prometryn, the model fits are plotted against the measured data values for the Blackwood, Andover and Salop soils in Figures 6.7, 6.8 and 6.9, respectively. The three graphs for each soil type display the model fit to the adsorption, isotope exchange and forced isotope exchange data obtained after 56, 112 and 168 days of

Table 6.7. Measured and optimised parameter values of the prometryn and hexaconazole isotope exchange model when including the forced isotope exchange data.

Parameters	Prometryn Blackwood	Andover	Salop	Hexaconazole Blackwood	Andover	Salop
<i>Measured values:</i>						
K_{feq} (Freundlich distribution coefficient)	9.13	3.81	3.47	43.51	21.79	21.70
$1/n$ (Freundlich exponent)	0.88	0.84	0.85	0.79	0.82	0.72
Initial mass Tube A, initially ^{12}C (μg)	15.55	15.55	15.55	19.53	19.53	19.53
Initial mass Tube B, initially ^{14}C (μg)	15.53	15.53	15.53	18.85	18.85	18.85
Mass of soil (g)	2.0	2.0	2.0	0.5	0.5	0.5
Specific activity ($\text{Bq } \mu\text{g}^{-1}$)	4158.8	4158.8	4158.8	2261.0	2261.0	2261.0
Volume of supernatant removed (mL)	18.03	18.03	18.03	23.99	24.05	24.05
Volume of supernatant replaced (mL)	17.70	17.69	17.69	23.73	23.78	23.78
Mass of ^{12}C hexaconazole (μg)	508.57	509.17	508.57	343.53	343.53	343.68
Volume of supernatant removed (mL) ^a	17.03	17.05	17.09	23.04	23.04	23.05
Volume of ^{12}C solution added (mL) ^a	17.04	17.06	17.04	23.04	23.04	23.05
Total volume of shaking solution (mL)	20.0	20.0	20.0	25.0	25.0	25.0
<i>Optimised values:</i>						
F_{ne}^*	0.57 (0.077)	0.46 (0.14)	1.08 (0.14)	0.10 (0.012)	0.18 (0.050)	0.21 (0.031)
k_{des}^*	0.014 (0.0010)	0.016 (0.0025)	0.021 (0.0014)	0.029 (0.0019)	0.015 (0.0022)	0.025 (0.0021)
k_{irr}^*	0.0079 (0.00081)	0.0045 (0.0025)	0.0033 (0.0012)	0.0063 (0.0014)	0.013 (0.0020)	0.0085 (0.0017)

^aAverage volumes of supernatant removed and added during the forced isotope exchange procedure.

*Optimise error of parameter generated by ModelMaker© given in brackets.

adsorption, using the same parameters. For hexaconazole, the model fits to the data obtained for the adsorption, isotope exchange and forced isotope exchange procedures after 56, 112 and 168 days are plotted in Figures 6.10, 6.11 and 6.12 for the Blackwood, Andover and Salop soils, respectively. The plots of the residuals are given in Appendix B (Figures B.5 to B.10). MEs are given in Table 6.8. The MEs show that the model fit to the prometryn data was very good overall, though the plots of residuals showed that the model generally overestimates the isotope exchange data and the beginning of the forced isotope exchange data, and then begins to slightly underestimate the forced isotope exchange data towards the latter time-points.

Table 6.8. Model efficiencies (ME) for prometryn and hexaconazole for all soil types and time-points.

Pesticide	Soil type	Time-point	Calculated model efficiency (ME)
Prometryn	Blackwood	56	0.996
		112	0.993
		168	0.986
	Andover	56	0.998
		112	0.998
		168	0.998
	Salop	56	0.995
		112	0.995
		168	0.996
Hexaconazole	Blackwood	56	0.996
		112	0.996
		168	0.997
	Andover	56	0.992
		112	0.991
		168	0.989
	Salop	56	0.984
		112	0.987
		168	0.981

For hexaconazole, the model was visually able to describe the Blackwood data the most accurately, though there was some overestimation of the isotope exchange data and underestimation of the forced isotope exchange data (Appendix B, Figure B.8). For both the Andover and Salop data however, the model systematically underestimated the adsorption data and most of the forced isotope exchange data (Appendix B, Figures B.9 and B.10). The worst model fit was to the Salop soil (Table

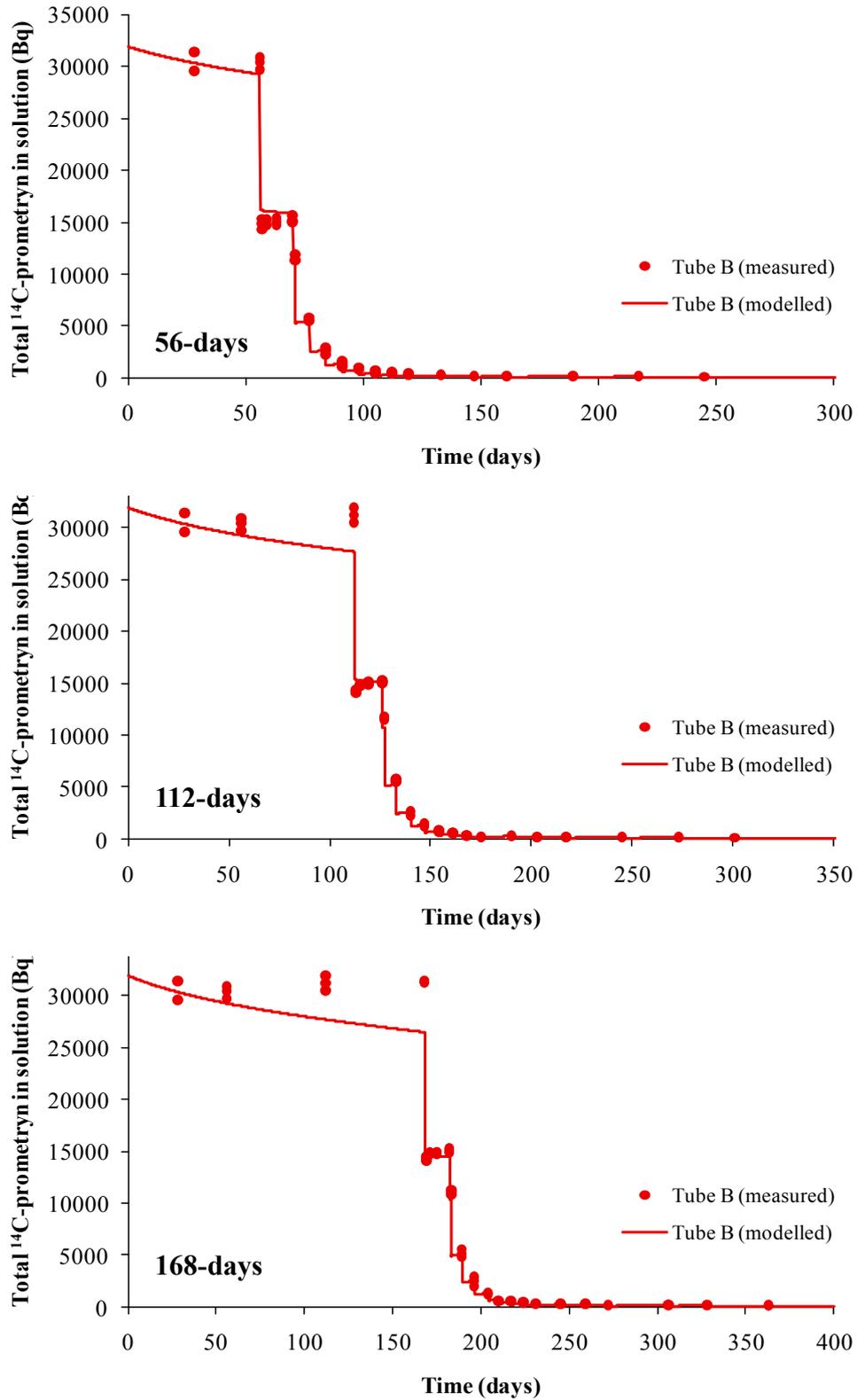


Figure 6.7. Model fit to the prometryn adsorption, isotope exchange and forced isotope exchange data for the Blackwood soil, after 56, 112 and 168 days of adsorption, 14 days isotope exchange and 175 days for forced isotope exchange. Data are plotted in triplicate.

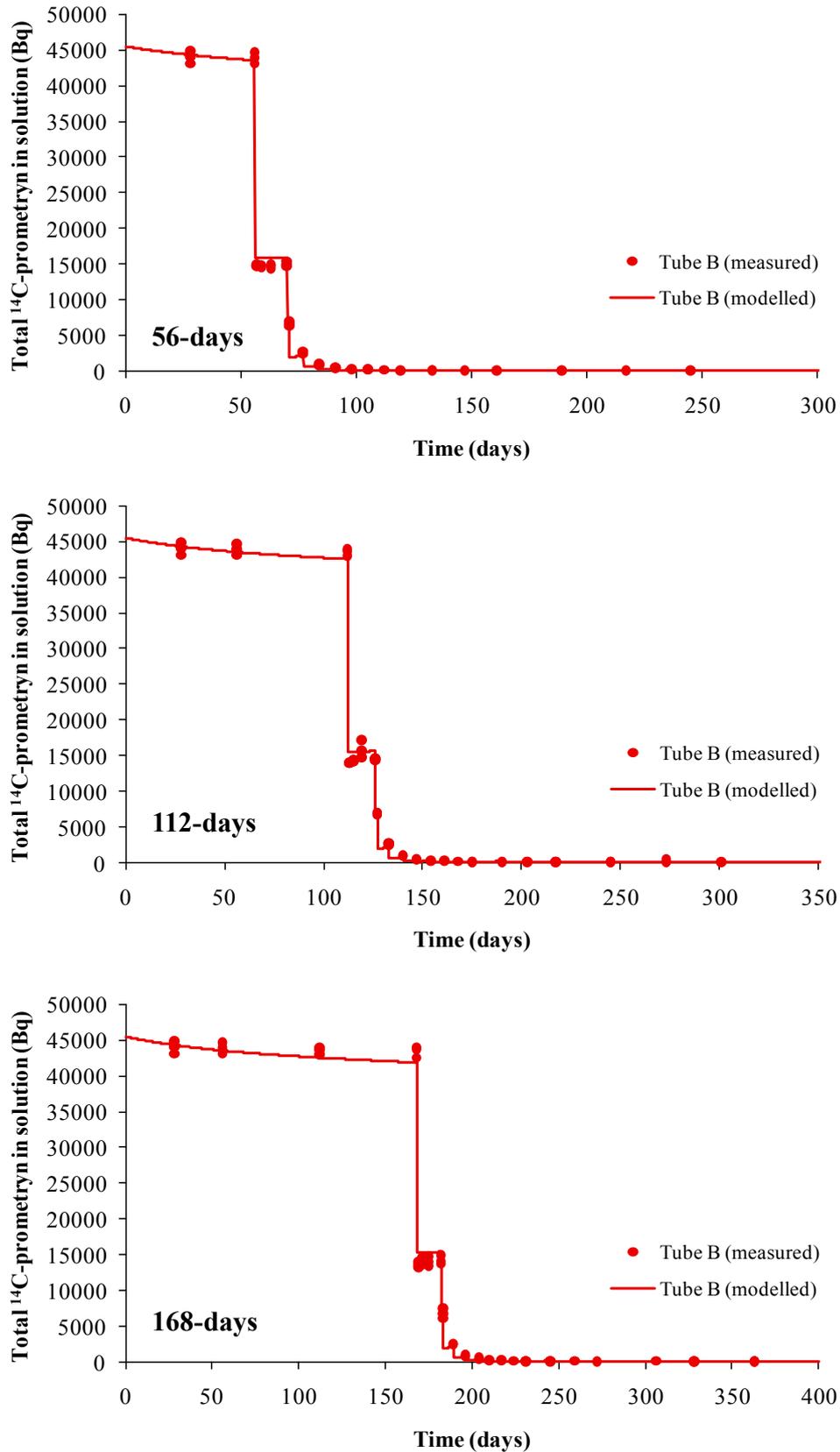


Figure 6.8. Model fit to the prometryn adsorption, isotope exchange and forced isotope exchange data for the Andover soil, after 56, 112 and 168 days of adsorption, 14 days isotope exchange and 175 days for forced isotope exchange. Data are plotted in triplicate.

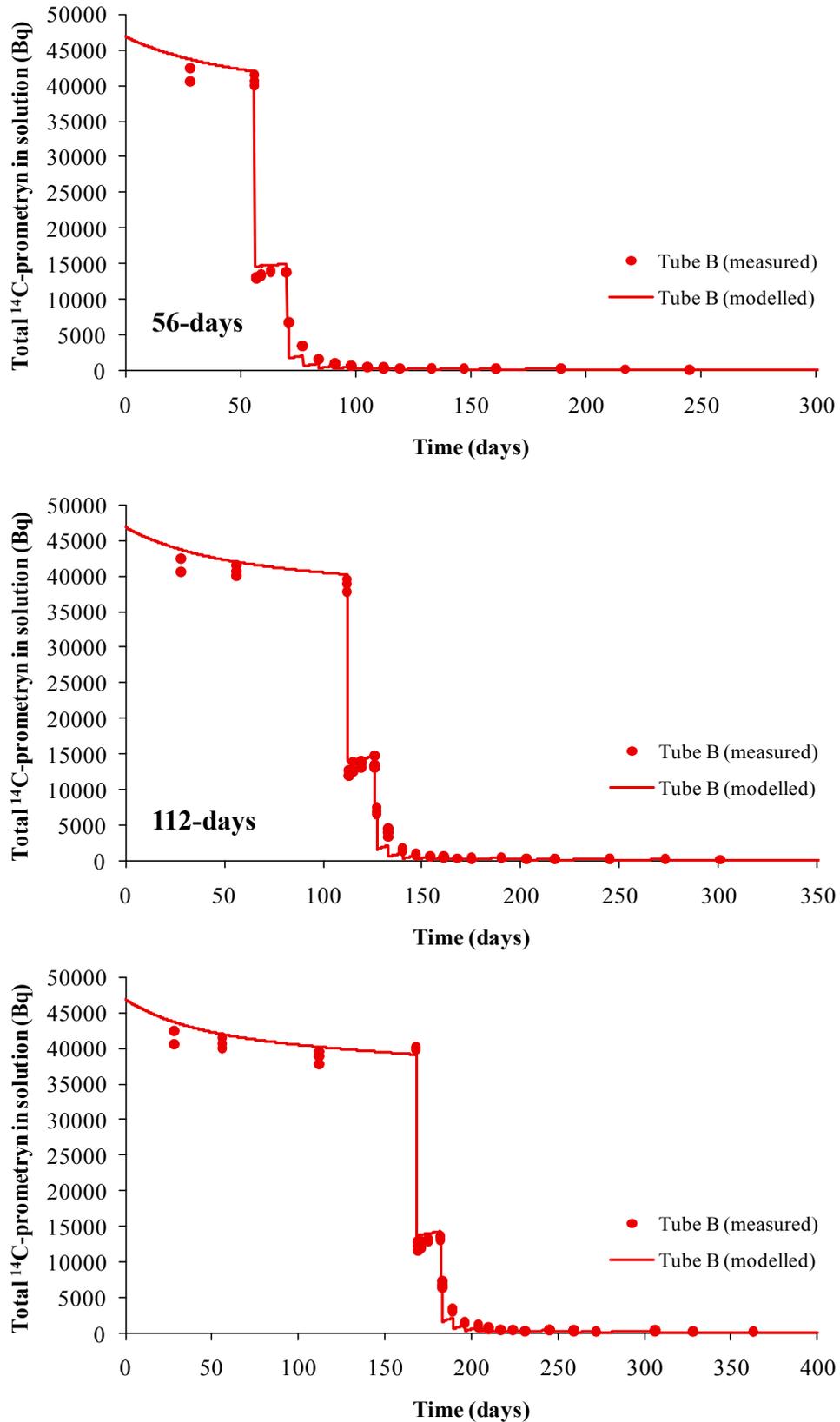


Figure 6.9. Model fit to the prometryn adsorption, isotope exchange and forced isotope exchange data for the Salop soil, after 56, 112 and 168 days of adsorption, 14 days isotope exchange and 175 days for forced isotope exchange. Data are plotted in triplicate.

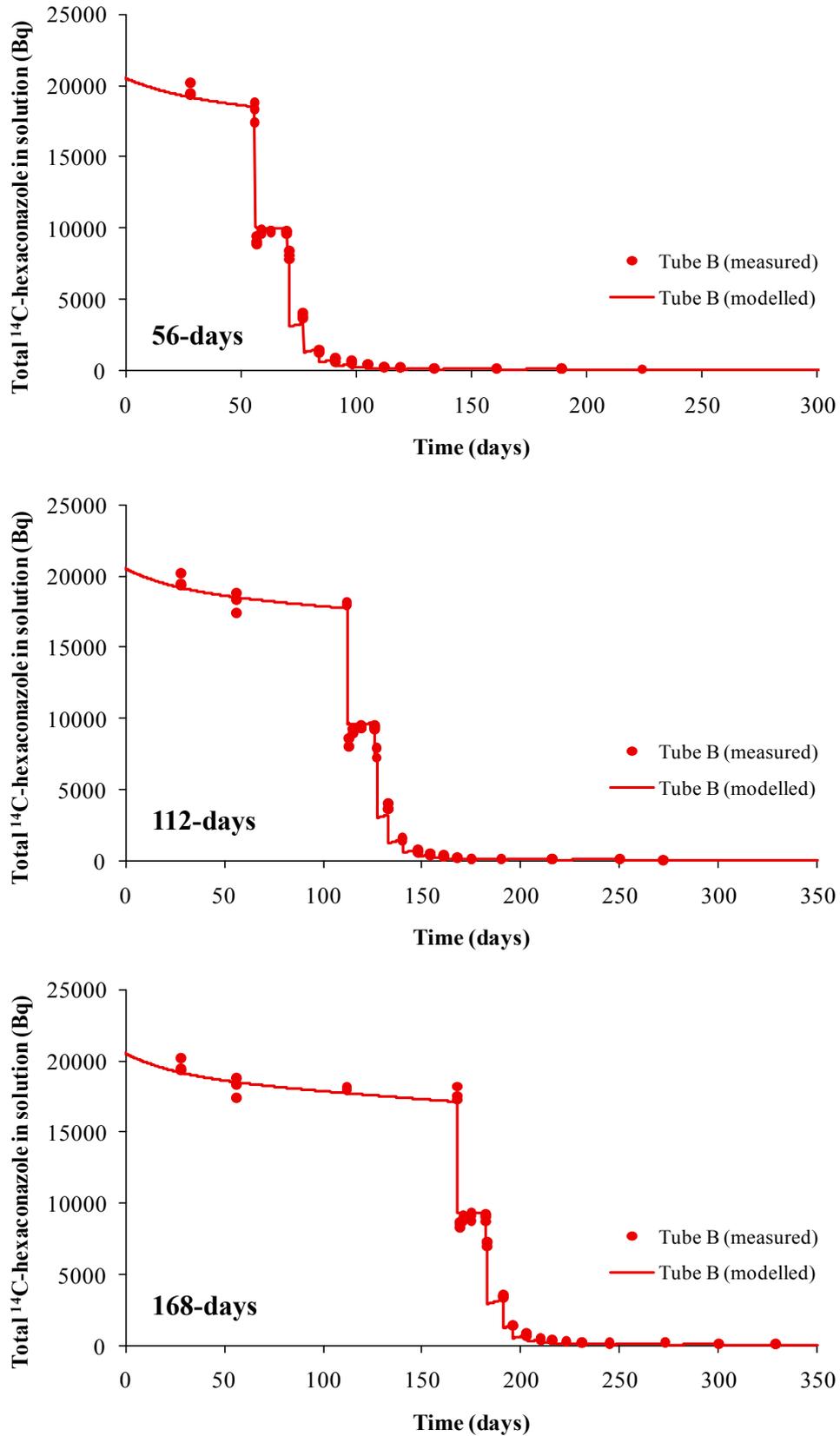


Figure 6.10. Model fit to the hexaconazole adsorption, isotope exchange and forced isotope exchange data for the Blackwood soil, after 56, 112 and 168 days of adsorption, 14 days isotope exchange and 147 days for forced isotope exchange. Data are plotted in triplicate.

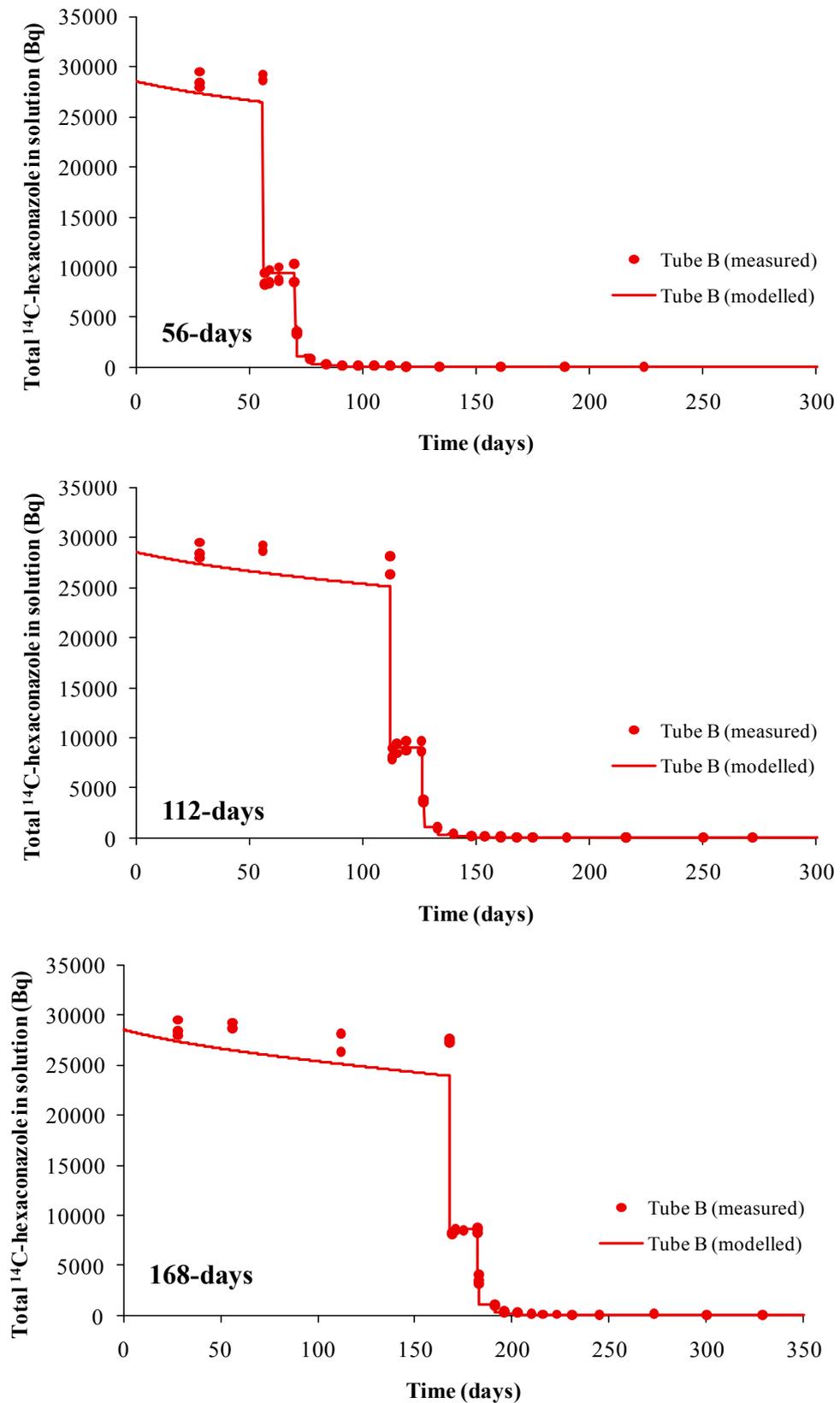


Figure 6.11. Model fit to the hexaconazole adsorption, isotope exchange and forced isotope exchange data for the Andover soil, after 56, 112 and 168 days of adsorption, 14 days isotope exchange and 147 days for forced isotope exchange. Data are plotted in triplicate.

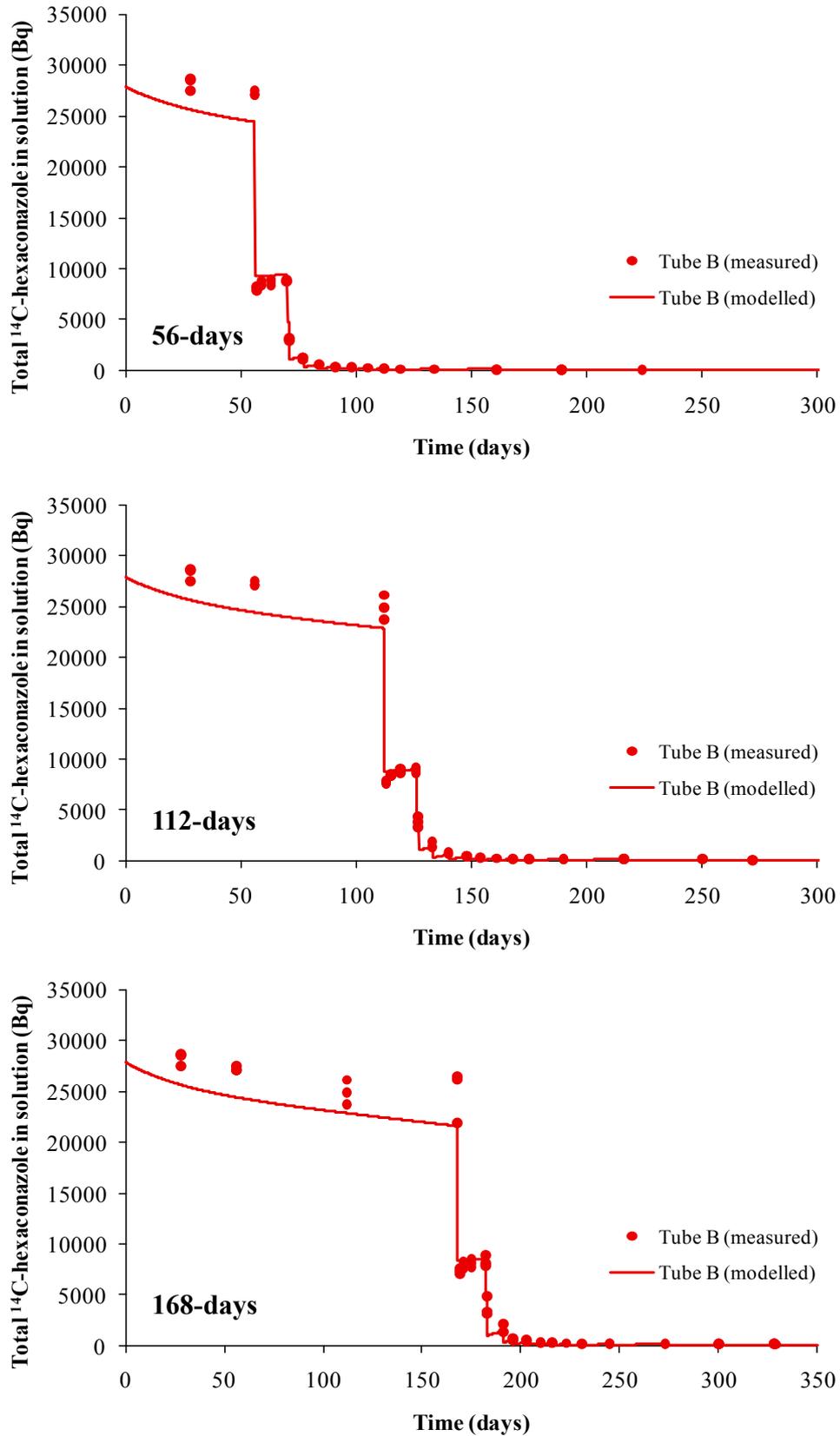


Figure 6.12. Model fit to the hexaconazole adsorption, isotope exchange and forced isotope exchange data for the Salop soil, after 56, 112 and 168 days of adsorption, 14 days isotope exchange and 147 days for forced isotope exchange. Data are plotted in triplicate.

6.8). As the Freundlich isotherm for that particular pesticide/soil system did not describe the observed data very well at the higher concentrations, it is likely that this contributed to the poorer model fit (Chapter 3, Figure 3.3).

As explained for chlorotoluron, the poor model fit to the adsorption data is likely to be the result of using the weighted least squares method to estimate the unknown parameters. However, for prometryn and hexaconazole a difference in sorption behaviour was observed for ^{12}C - and ^{14}C -pesticide. As the Freundlich sorption studies were carried out using ^{12}C -pesticide and the model uses K_{feq} and $1/n$ values derived from experiments using ^{12}C -pesticide to predict ^{14}C -pesticide sorption behaviour, this may contribute to the poor description of the adsorption data. However, this effect is not likely to be significant as poor fitting to the chlorotoluron adsorption data was also observed for ^{12}C - and ^{14}C -chlorotoluron (Chapter 4, Figure 4.2), despite the two isotopes exhibiting similar sorption behaviour.

6.3.3. Irreversible fractions and comparison of optimised parameter values

The optimised parameter values for the adsorption and isotope exchange models, and subsequent models including forced isotope exchange data, are given in Table 6.9. Comparing the ratios of pesticide taking part in non-equilibrium to equilibrium sorption (F_{ne}), rates of desorption (k_{des}) and rates of irreversible sorption (k_{irr}) between the pesticide/soil systems studied allows some insight into differences between the sorption processes operating. As there is an element of uncertainty surrounding the absolute values of the optimised parameters, this must be considered when assessing the differences between the F_{ne} , k_{des} and k_{irr} values estimated by the model for each pesticide/soil system.

Optimised F_{ne} values vary considerably depending on the pesticide and soil type. The highest F_{ne} values were observed for the chlorotoluron isotope exchange data, though these values decreased slightly when the forced isotope exchange data and k_{irr} were included in the model. This is likely to be the result of the irreversible component reducing the amount of chlorotoluron participating in non-equilibrium sorption. The highest F_{ne} overall was observed for chlorotoluron sorption to the Salop soil. This was also true of the prometryn data; F_{ne} values were similar for hexaconazole sorption to all soil types. k_{des} values were relatively similar across all pesticide/soil systems. The

Table 6.9. Optimised parameter values and optimise error for all models.

Pesticide	Soil type	F_{ne}	F_{ne} optimise error	k_{des}	k_{des} optimise error	k_{irr}	K_{irr} optimise error
<i>Isotope exchange model:</i>							
Imidacloprid-guanidine	Hanford	0.16	0.10	0.016	0.017	N/A	N/A
Imidacloprid-guanidine	Drummer	0.049	0.010	0.090	0.11	N/A	N/A
Imidacloprid	SiCL	1.07	0.20	0.034	0.013	N/A	N/A
Imidacloprid-urea	SiCL	0.81	0.072	0.037	0.0070	N/A	N/A
Imidacloprid-guanidine	LS	2.80	0.23	0.027	0.0040	N/A	N/A
Chlorotoluron	Blackwood	4.75	0.21	0.040	0.0058	N/A	N/A
	Andover	4.34	0.18	0.059	0.0092	N/A	N/A
	Salop	16.79	0.48	0.025	0.0021	N/A	N/A
<i>Forced isotope exchange model:</i>							
Chlorotoluron	Blackwood	3.89	0.62	0.019	0.0023	0.0042	0.0011
	Andover	1.91	0.75	0.019	0.0034	0.0021	0.0033
	Salop	13.59	1.02	0.022	0.0027	0.0069	0.00053
Prometryn	Blackwood	0.57	0.077	0.014	0.0010	0.0079	0.00081
	Andover	0.46	0.14	0.016	0.0025	0.0045	0.0025
	Salop	1.08	0.14	0.021	0.0014	0.0033	0.0012
Hexaconazole	Blackwood	0.10	0.012	0.029	0.0019	0.0063	0.0014
	Andover	0.18	0.050	0.015	0.0022	0.013	0.0020
	Salop	0.21	0.031	0.025	0.0021	0.0085	0.0017

highest values were obtained in the isotope exchange models, suggesting that desorption rates were slower for the forced isotope exchange models. This is again likely to be the result of including an irreversible sorption parameter. k_{irr} values were also similar across all pesticide/soil systems. For prometryn, the greatest k_{irr} value was obtained for the Blackwood soil but for chlorotoluron and hexaconazole the greatest k_{irr} values were obtained for the Salop soil. However, values of k_{irr} were small in general.

The model estimates of initially-applied pesticide taking part in irreversible sorption at the end of the experimental time-frame have been compared with the measured values, and are shown in Table 6.10. Though there is a large deviation between amounts of irreversible sorption predicted by the model and amounts of irreversible sorption measured by soil combustion, this is partly due to the fact that some of the modelled irreversibly sorbed fraction was shown to be solvent-extractable (Table 6.10). However, when the measured irreversibly-sorbed pesticide and solvent-extractable pesticide are added together, modelled values are still greater than the measured values (with the exception of the chlorotoluron/Andover soil system). The measured data shows that there is some increase in the amount of pesticide in the irreversible phase over time, which is also confirmed by the model. Irreversible binding has a more significant role in the model than the measured data. This highlights the difficulty in including irreversible sorption in pesticide sorption models, as it is difficult to obtain detailed experimental data accurate enough to support the necessary additional model parameters.

6.4. Conclusions

There were two aims of using the three-site model to describe the isotope exchange data: (i) to interpret the sorption processes operating in pesticide/soil systems studied; and, (ii) quantification of the irreversible fraction. Application of the three-site model to Celis and Koskinen's (1999a; 1999b) isotope exchange data and the chlorotoluron isotope exchange data showed that the measurements obtained could be described using two sorption sites (instantaneous exchange and slow but reversible binding) with no need to include sites for irreversible sorption. It was necessary however, to include all three sorption sites in order to adequately describe the chlorotoluron, prometryn and hexaconazole forced isotope exchange data.

Table 6.10. Modelled vs. measured irreversible sorption values as a percent of initially-applied pesticide.

Pesticide	Soil type	Adsorption period (days)	Modelled irreversible sorption (μg) [*]	Mass of initially-applied ¹⁴ C-pesticide (μg)	Modelled irreversible sorption (%)	Measured irreversible sorption (%) ^a	Solvent-extractable (%)	Measured irreversible sorption plus solvent-extractable (%)
Chlorotoluron	Blackwood	56	1.37	13.76	9.96	2.27 ± 0.36	5.48 ± 0.66	7.75
	Andover	56	0.27	13.76	1.96	1.07 ± 0.12	2.03 ± 0.23	3.10
	Salop	56	4.65	13.76	33.79	2.19 ± 0.22	10.77 ± 0.73	12.96
Prometryn	Blackwood	56	0.70	15.53	4.51	0.29 ± 0.10	1.09 ± 0.06	1.38
	Andover	56	0.22	15.53	1.42	0.33 ± 0.06	0.56 ± 0.05	0.89
	Salop	56	0.38	15.53	2.45	0.34 ± 0.01	1.21 ± 0.11	1.55
	Blackwood	112	1.27	15.53	8.18	0.54 ± 0.11	1.14 ± 0.07	1.68
	Andover	112	0.42	15.53	2.70	0.58 ± 0.07	0.72 ± 0.07	1.30
	Salop	112	0.70	15.53	4.51	0.48 ± 0.06	1.89 ± 0.19	2.37
	Blackwood	168	1.81	15.53	11.65	0.83 ± 0.27	1.27 ± 0.03	2.10
	Andover	168	0.61	15.53	3.93	1.35 ± 0.60	0.89 ± 0.13	2.24
	Salop	168	1.01	15.53	6.50	0.77 ± 0.04	1.78 ± 0.14	2.55
Hexaconazole	Blackwood	56	0.65	18.85	3.45	0.18 ± 0.03	0.89 ± 0.04	1.07
	Andover	56	0.90	18.85	4.77	0.14 ± 0.01	0.45 ± 0.09	0.59
	Salop	56	1.00	18.85	5.31	0.19 ± 0.02	1.23 ± 0.11	1.42
	Blackwood	112	1.18	18.85	6.26	0.29 ± 0.00	1.28 ± 0.04	1.57
	Andover	112	1.68	18.85	8.91	0.25 ± 0.03	0.68 ± 0.23	0.93
	Salop	112	1.85	18.85	9.81	0.36 ± 0.03	2.47 ± 0.62	2.83
	Blackwood	168	1.70	18.85	9.02	0.39 ± 0.11	1.89 ± 0.26	2.28
	Andover	168	2.43	18.85	12.89	0.28 ± 0.01	0.70 ± 0.06	0.98
	Salop	168	2.67	18.85	14.16	0.50 ± 0.06	2.18 ± 0.67	2.68

*Mass of pesticide in irreversible compartment at the end of the experimental time-frame.

^aBy soil combustion.

In most existing sorption models, irreversible binding is not included. Selecting an appropriate model to describe desorption profiles has been reported to be system-specific however. For example, Saffron *et al.* (2006) divided the desorption profile into three regimes: (i) a fast or instantaneous regime, where desorption occurs at rates not captured by the first few sampling points; (ii) a dynamic regime in which rates are well measured by the sampling scheme; and, (iii) a slow regime where rates are slower than can be measured given the combination of data uncertainty and duration of sampling. They found that although naphthalene desorption was best described by two regimes, all three regimes were required to adequately describe the desorption behaviour of atrazine. Furthermore, Johnson *et al.* (2001) compared six modelling approaches in their ability to describe phenanthrene desorption data: (i) three-parameter kinetic model; (ii) five-parameter kinetic model; (iii) gamma-distribution model; (iv) one-parameter pore-diffusion model; (v) two-parameter pore-diffusion model; and, (vi) three-parameter biphasic polymer diffusion model. They concluded that all desorption profiles were at least biphasic and that models composed of two-regimes provide a good basis for describing desorption profiles.

The fact that it was not necessary to include sites for irreversible binding in order to describe the isotope exchange data is likely to be the result of the experimental design. The data generated meant that it was not possible to identify whether the sorbed pesticide was taking part in slow, reversible sorption or irreversible sorption. After analysing the experimental data it was concluded that irreversible sorption does not play any significant role in the fate of sorbed chlorotoluron, prometryn and hexaconazole, under abiotic conditions, for the soil systems studied. However, it was necessary to incorporate sites for irreversible binding in order to adequately describe the forced isotope exchange data, though modelled and measured amounts of irreversible sorption did not match very closely, and overall amounts of irreversible sorption were small in general. Contaminant transport models (e.g. PEARL, MACRO) are therefore likely to provide adequate estimates of the quantities of pesticide which may potentially reach surface and groundwaters. Since most sorption models do not include irreversible sorption, this may only lead to a possible overestimation of the risk of pesticide transport to surface and groundwaters. It is however, always better to provide conservative estimates rather than underestimate the risk that sorbed pesticide residues may pose to the quality of surface and groundwaters.

If measured amounts of irreversible sorption had been larger then it would have been possible to extrapolate the model beyond the experimental time-frame. However, because amounts of irreversible sorption were so small this was not necessary. Finally, the three-site model shows that experiments were definitely long enough to conclude that little irreversible sorption occurred. Some interpretation of the sorption processes operating and an estimate of the irreversible fraction was possible, though further work is required to develop shorter experiments that are able to sufficiently differentiate between slowly reversible sorption and irreversible sorption.

CHAPTER 7

GENERAL DISCUSSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

7.1. General discussion

Sorption phenomena have been researched extensively in recent decades, e.g. Pignatello and Xing (1996), Reid *et al.* (2000), Barriuso *et al.* (2008). The common purpose of such research is to assess how pesticide retention by soil may limit pesticide mobility and bioavailability in the field. Although this aim is broad, working toward its achievement requires a detailed understanding of specific pesticide/soil interactions and processes occurring over varied spatial and temporal scales. A combination of the diverse range of synthetic pesticide products in existence, the heterogeneous nature of the different soil types they come into contact with and inadequate experimental procedures means many of these interactions are still not fully understood.

The current understanding of sorption phenomena was reviewed in Chapter 2. Although it has been demonstrated that sorption may be described in terms of three empirically-distinct kinetic fractions – a fast desorbing fraction, a slowly desorbing fraction and a very slowly desorbing fraction (van den Heuvel and van Noort, 2006; Chai *et al.*, 2007; Sormunen *et al.*, 2008; Yang *et al.*, 2010) – a proportion of sorbed pesticide has also been shown to irreversibly bind to the soil matrix (Mordaunt *et al.*, 2005; Barriuso *et al.*, 2008). Quantifying this irreversible fraction is important in order to assess the amount of sorbed pesticide potentially available for leaching. At present, there is no scientific consensus that recognises a standard laboratory method which adequately discriminates between slowly reversible and irreversible sorption. An approach with the ability to differentiate between the two sorption processes would be a valuable tool for improving the accuracy of contaminant transport models.

The general aim of the work carried out in this thesis was to quantify the amounts of pesticide taking part in irreversible sorption under abiotic conditions, and to thus determine its significance for assessing pesticide fate in the environment. This also

involved developing an experimental method with the ability to discriminate between pesticide taking part in slow, reversible sorption and irreversible sorption. This was achieved via the use of isotope or “self-exchange” which enables *in-situ* quantification of the sorbed pesticide not taking part in continuous adsorption-desorption between the soil and aqueous phase. A sequential extraction procedure (isotope exchange < forced isotope exchange < solvent extraction) was carried out to determine the significance of irreversible sorption for three pesticides (chlorotoluron, prometryn and hexaconazole), under abiotic conditions. The aforementioned pesticides were adsorbed to three soils (Blackwood, Andover and Salop) that differed in their physico-chemical properties in order to identify the soil components with the greatest influence over irreversible sorption. The effect of increasing the adsorption period on the magnitude of irreversible sorption was also tested for all pesticide/soil systems concerned.

The key findings of the research were:

- (i) The experimental time-frame was long enough to conclude that minimal or no irreversible sorption was observed for the pesticide/soil systems studied. The vast majority of initial-applied ^{14}C -chlorotoluron, ^{14}C -prometryn and ^{14}C -hexaconazole had been measured in the aqueous phase by the end of the forced isotope exchange phase (Table 7.1). This was particularly apparent for ^{14}C -prometryn (96.46 to 98.72% accounted for) and ^{14}C -hexaconazole (93.71 to 98.54% accounted for). Furthermore, as desorption end-points had still not been reached by the end of the forced isotope exchange phase (small amounts of ^{14}C -pesticide were still being released, albeit at very slow rates), this suggests that if the procedure had been continued, greater amounts of sorbed pesticide would have been remobilised. Thus, under abiotic conditions, no significant amount of sorbed pesticide was taking part in irreversible binding for the pesticide/soil systems studied;
- (ii) The minimal amounts of irreversible sorption observed (Chapters 4 and 5, Figures 4.5 and 5.7 to 5.12) suggest that the parent forms of ^{14}C -chlorotoluron, ^{14}C -prometryn and ^{14}C -hexaconazole participate in very slow but reversible sorption under abiotic conditions; this carries the

implication that all of the initially sorbed ^{14}C -chlorotoluron, ^{14}C -prometryn and ^{14}C -hexaconazole in the parent form is potentially available for transport and leaching, given sufficient time. This conclusion should be limited to the pesticide/soil systems studied and laboratory conditions as the same conclusion is not likely to apply under biotic conditions;

Table 7.1. Total ^{14}C -pesticide quantified by the end of the forced isotope exchange procedure (expressed as the percent of initial-applied).

Pesticide	Soil type	Adsorption period (days)	Total ^{14}C -pesticide (% of initial-applied) after forced isotope exchange*	
Chlorotoluron	Blackwood	56	85.58 ± 0.18	
	Andover	56	92.39 ± 1.02	
	Salop	56	80.93 ± 0.63	
Prometryn	Blackwood	56	98.05 ± 0.28	
	Andover	56	98.72 ± 0.68	
	Salop	56	97.39 ± 0.54	
	Blackwood	112	97.57 ± 0.94	
	Andover	112	98.22 ± 0.73	
	Salop	112	96.24 ± 0.96	
	Blackwood	168	96.79 ± 0.49	
	Andover	168	97.56 ± 1.00	
	Salop	168	96.46 ± 0.56	
	Hexaconazole	Blackwood	56	97.86 ± 0.69
		Andover	56	98.54 ± 2.51
		Salop	56	95.77 ± 0.58
Blackwood		112	95.25 ± 0.23	
Andover		112	95.53 ± 0.58	
Salop		112	93.71 ± 0.21	
Blackwood		168	94.29 ± 0.33	
Andover		168	95.51 ± 0.56	
Salop		168	94.01 ± 0.45	

*204 days for chlorotoluron, 175 days for prometryn and 145 days for hexaconazole.

- (iii) Increasing the length of the adsorption period had a small positive effect on the final amounts of irreversibly sorbed ^{14}C -prometryn and ^{14}C -hexaconazole measured (Chapter 5, Table 5.2). The effect of increasing strength of sorption with time is also revealed by Table 7.1; total amounts of initial-applied ^{14}C -prometryn and ^{14}C -hexaconazole accounted for after the forced isotope exchange phase decreased as a function of increasing

adsorption time. This is likely to be the result of the increasing length of adsorption time allowing diffusion into spatially remote areas, such as soil macro- and micropores (Ball and Roberts, 1991; Burgos *et al.*, 1996; Pignatello and Xing, 1996) and entrapment within soil organic matter (Brusseau *et al.*, 1991; Huang and Weber, 1997; Park *et al.*, 2004);

- (iv) Varying the composition of the soil had little effect on final amounts of irreversibly sorbed ^{14}C -chlorotoluron, ^{14}C -prometryn and ^{14}C -hexaconazole (Chapters 4 and 5, Figures 4.5 and 5.7 to 5.12). This is probably an effect of the minimal amounts of irreversible sorption observed overall; it is therefore unlikely that differences between the soil types had the capacity to influence irreversible binding interactions. Furthermore, all three soils were of a temperate and arable origin; no soils with extremely different properties were tested which may also have influenced the final amounts of irreversible sorption observed.
- (v) The experimental design of the isotope exchange technique means that it is not possible to identify whether pesticide still sorbed at the end of the 14-day isotope exchange phase is participating in slowly reversible or irreversible sorption (Chapters 4 and 5, sections 4.3.1 and 5.3.3). Although measuring the amount of ^{14}C -pesticide in solution may be used to assess the quantity of ^{14}C -pesticide not taking part in continuous adsorption-desorption between the soil and solution, it is clear that this technique is not powerful enough to conclude that the ^{14}C -pesticide that did not desorb from the soil during the 14-day isotope exchange period is irreversibly sorbed;
- (vi) The forced isotope exchange procedure is able to provide an indication of the amounts of ^{14}C -pesticide which remain sorbed to the soil after multiple supernatant exchanges (replacement with a high-concentration ^{12}C -pesticide solution in order to maintain a high diffusion gradient over a protracted time period). The technique also highlights the exceptionally long periods of time over which desorption occurs and provides some insight into desorption kinetics. For chlorotoluron, prometryn and hexaconazole, the bulk of sorbed ^{14}C -pesticide was found to desorb with

the first addition of high-concentration ^{12}C -pesticide solution, after which the amount of ^{14}C -pesticide desorbing from the soil markedly declined over time (Chapters 4 and 5, sections 4.3.2 and 5.3.4). Desorption endpoints had still not been reached at the end of the experimental time-frame however (204 days chlorotoluron, 175 days prometryn and 145 days hexaconazole);

- (vii) The three-site model developed to further elucidate the non-equilibrium and irreversible sorption processes observed also confirmed that sorption behaviour could be mostly explained by non-equilibrium sorption, particularly for the shorter experiments (Chapter 6). No irreversible component was required in order to explain the data obtained from the isotope exchange studies. This is likely to be the result of the experimental design, as previously discussed, which meant that it was not possible to identify whether the sorbed pesticide was participating in irreversible sorption; data could be adequately explained by non-equilibrium sorption. An irreversible component was required however, to explain the forced isotope exchange data, suggesting an improvement in the experimental design in its ability to measure irreversible sorption.

Although only minimal amounts of irreversible sorption were observed, the results obtained have raised additional questions. The following areas, outlined at the end of Chapter 5, necessitate some further discussion: (i) considering whether irreversible sorption is most likely microbially-mediated since little irreversible sorption was observed to occur under abiotic conditions for the pesticide/soil systems studied; (ii) assessing whether it is therefore appropriate to use a definition of bound residues that includes both parent and non-parent forms; and, (iii) if bound residues are comprised of non-parent material, determining the implication of this for concerns about the bioavailability of those residues. Although there is some discussion of these points in the following passages, it is clear that despite their importance, a lack of conclusive evidence restricts the ability to reach definitive answers.

To address the first point, there is strong evidence in the literature to suggest that microbial activity plays a significant role (via biodegradation) in bound residue formation. The majority of this information comes from studies that have tried to

identify the parent compound or metabolite(s) by applying different degradation techniques (Northcott and Jones, 2000). For example: (i) Reuter *et al.* (1999) found that the formation of bound residues for the herbicide ^{14}C -isoproturon was largely dependent on the prior degradation of ^{14}C -isoproturon to the metabolite 4-isopropylaniline; (ii) Wang *et al.* (2009) observed that degradation of the parent compound (new herbicide ZJ0273) was accompanied by formation of bound residues and mineralisation to CO_2 ; and, (iii) Chilom *et al.* (2004) found that bound residue formation of naphthalene was low, but formation was 5-20 times higher for its primary metabolite *cis*-naphthalene-1,2-dihydrodiol.

It is well known that the presence of hydroxyl and carboxyl groups greatly enhances the chemical reactivity of metabolites, and thus their ability to form bound residues (Richnow *et al.*, 1997; Barriuso and Benoit, 2006). In addition to this effect, it is also possible for the soil microbial community to influence soil bound residue formation via another route. Microorganisms degrading parent material are also able to assimilate the derived carbon into their cellular components (e.g. fatty acids and amino acids), which in turn become stabilised within the soil organic matter (Nowak *et al.*, 2010). Thus, a proportion of soil bound residue may also be biogenic in nature. A recent publication by Nowak *et al.* (2010) showed that after 64 days of incubation, 44% of initially-applied 2,4-D had been converted to microbial biomass and finally to biogenic residues. As such residues would not pose any ecotoxicological hazard this clearly has important implications for risk assessment. Biogenic residues are not considered in the IUPAC definition of bound residues (Chapter 2, section 2.6.1) and their exclusion may lead to overestimation of the risk bound residues pose to the environment (Kästner *et al.*, 1999; Nowak *et al.*, 2010).

The effects of microbial activity may also depend on the pesticide in question. Irreversible sorption has been reported to occur in a number of studies in which sodium azide (NaN_3) was applied to minimise bacterial growth and biodegradation (Chen *et al.*, 2004; Sander and Pignatello, 2009; Yu *et al.*, 2010). As no degradation was found to occur during the experimental time-frames of these studies, this suggests that the fractions of irreversible sorption reported refer only to parent material. All three studies attributed the occurrence of irreversible sorption to some form of physical entrapment or matrix deformation, such that adsorption and desorption were occurring to and from different physical environments. However,

Chen *et al.* (2004) and Yu *et al.* (2010) used only very short adsorption and desorption times (24 and ≤ 96 hours, respectively), suggesting that the irreversible fractions observed are unlikely to be indefinitely irreversible. Sander and Pignatello (2009) used considerably longer adsorption (140 days) and desorption (87 days) periods, however they concluded that although a small fraction of pesticide desorbs extremely slowly, it is too early to conclude that it is permanently trapped. Therefore, to also address point two, this evidence suggests that at this stage it is necessary to include both parent and non-parent materials in the definition of bound residues. The chemical identity of bound residues is still uncertain as is their irreversible status; however it is possible to recognise that in addition to the parent material, non-parent material may be even more likely to take part in similar irreversible binding reactions, though this is expected to be largely dependent on the chemical structure of the pesticide in question.

Furthermore, the positioning of the radio-label has also been shown to affect both mineralisation and the measurement of bound residues originating from metabolised compounds (Kästner *et al.*, 1999; Barriuso *et al.*, 2008; Nowak *et al.*, 2010). If the ^{14}C -labelling in the pesticide chemical structure is positioned on a labile molecular fragment (i.e. one that can be easily mineralised), the measured fraction of bound residues will tend to be low (Barriuso *et al.*, 2008). In contrast, if a stable moiety of the compound is ^{14}C -labelled, the measured fraction of bound residues will appear higher in comparison (Kästner *et al.*, 1999; Barriuso *et al.*, 2008; Nowak *et al.*, 2010). Therefore, though the true amounts of bound residue are the same independent of the label position, the quantity of bound residue measured differs.

Finally, there is still considerable uncertainty concerning the chemical identity of soil bound residues. As bound residues can only be quantified using ^{14}C -labelled compounds, little knowledge of their chemical structure is ever revealed (Kästner *et al.*, 1999; Barraclough *et al.*, 2005). Residues may consist of the parent compound, chemical metabolites or biogenic metabolites (Kästner *et al.*, 1999). Metabolites are also difficult to study since they are usually present in low concentrations (Chilom *et al.*, 2004). Most metabolites however, tend to be more hydrophilic and less toxic than the parent compound (Selim *et al.*, 1999; Kolpin *et al.*, 2000), though there are some exceptions to this rule (Tixier *et al.*, 2001). Such evidence suggests that if bound residues are mostly comprised of non-parent material, assessing their potential

mobility and bioavailability would strongly depend on the pesticide and metabolite in question. Thus, to address point three, it is still too early to define the implication of metabolite or biogenic bound residues for pesticide/metabolite mobility and bioavailability.

The three areas identified for discussion are clearly still subject to debate. Both the situations in which microorganisms play a significant role and the specific chemical identity of measured soil bound residues remain largely unknown; this lack of conclusive evidence limits the ability to make appropriate judgements. Thus, further experimental work is required, of which the first step would be to gain a greater insight into the chemical identity of pesticide bound residues, including the role of microorganisms, before making any further assessments (see section 7.3).

7.2. Conclusion

The work carried out in this thesis has demonstrated that under abiotic conditions the majority of sorbed pesticide participates in very slow but reversible binding; this phenomenon was found to be largely independent of the pesticide properties, soil type or length of adsorption time. Under abiotic conditions, and for the pesticide/soil systems studied, irreversible sorption does not play any significant role in limiting the quantities of pesticide available for leaching into surface and groundwaters. However, though this work has shown that all of the initially-sorbed pesticide was eventually remobilised, desorption occurred over exceptionally long time-scales, despite using forced isotope exchange and relatively large volumes of soil solution.

The use of isotopes is thought to overcome ambiguities inherent to experimental techniques previously employed to establish sorbate entrapment as the cause of hysteresis (Sander and Pignatello, 2005). However, it still relies on specifying a period of time over which isotope- or “self-exchange” may occur. As this work showed that protracted experimental time-frames are required to reach desorption end-points, it follows that for the pesticide/soil systems studied, the greater the length of the desorption period, the greater the amount of pesticide desorbed. Thus, there are still difficulties in using this approach to differentiate between slowly reversible and irreversible sorption, as irreversible fractions are often a function of time and still remain operationally-defined.

The minimal amounts of irreversible sorption measured in this thesis suggest that irreversible sorption phenomena are of little significance when assessing the environmental fate of the studied pesticides and soils under abiotic conditions. However, a soil microbial community would be present in the field situation, as well as other abiotic transfer and degradation processes. It may be possible therefore, that biological and/or chemical degradation play an important role in the formation of irreversibly sorbed pesticide residues in the pesticide/soil systems studied. There are still many questions to be answered on the subject, and these are considered in the following section, which provides some recommendations for future research.

7.3. Recommendations for future research

Recommendations specific to this thesis:

- (i) The adsorption of the three study pesticides (chlorotoluron, prometryn and hexaconazole) to the three test soils under abiotic conditions was found to be predominantly reversible, given sufficient time. Studying pesticide sorption kinetics under non-sterile soil conditions would provide a valuable comparison. The presence of an active soil microbial community may positively influence the fractions of irreversibly sorbed pesticide measured in the study pesticide/soil systems and should be explored in greater detail. However, this must be addressed with care as degradation would confound interpretation of the results.

- (ii) It is important to recognise that degradation of the parent compound will occur in the field, although for the purpose of this work both abiotic and microbial degradation were controlled. The contribution of transformation products in forming irreversibly sorbed pesticide residues is considered significant, though still poorly understood. Metabolites were not explored in any detail in this thesis. The study of non-parent material is important as it is included in the definition of bound residues and would thus contribute to a more complete understanding of pesticide fate in the environment. Metabolites are often less toxic than the parent and thus subject to lesser levels of control.

- (iii) Further work should be carried out to identify whether soils with more extreme properties affect the occurrence of irreversible sorption; it was not possible to make any robust conclusions from the soils tested in this thesis.
- (iv) Further research into assessing the most effective “natural” methods or approaches to differentiate between readily available, potentially available and non-available pesticide would greatly improve the robustness of input parameters used in contaminant transport models for predicting pesticide leaching. Methods with the ability to reach desorption end-points under more practical time-frames would also be a valuable asset.
- (v) The work carried out in this thesis concentrated on the availability of sorbed pesticide for leaching. However, it is also essential to assess the implication of results in terms of their bioavailability.

More general recommendations:

- (i) The studies carried out in this thesis were implemented in the laboratory. It would be valuable to investigate more realistic methods of assessing readily available, potentially available and non-available pesticide under environmentally-relevant conditions e.g. lysimeter studies. This would enable a more accurate assessment of potential pesticide behaviour in field situations.
- (ii) Under abiotic conditions and using the selected approaches, irreversible sorption was found to be insignificant. This suggests that current contaminant transport models are adequate in their ability to provide reasonable estimates of amounts of pesticide likely to reach surface and groundwaters. However, it is clear that irreversible sorption may be more significant in some situations, which may lead to an overestimation of their potential risk. Where it is relevant, aiming to account for irreversible sorption in contaminant transport models would be beneficial to improve their accuracy as a tool for predicting pesticide fate. This would however, first require development of an appropriate method to generate detailed experimental data concerning the significance of irreversible sorption for a range of pesticides and scenarios.

- (iii) Working towards standardising definitions of non-extractable residue, bound residue and irreversible sorption and the laboratory procedures used to measure them would greatly improve consistency and comparability between studies.
- (iv) Assessing the chemical identity of bound residues is important to accurately predict their potential for leaching and bioavailability. For instance, whether the majority of soil bound residue is comprised of non-parent or biogenic material will have different implications for environmental fate than if it were parent material.
- (v) The isotopic compound exchange experiments described in this thesis used mass per unit volume to prepare identical ^{12}C - and ^{14}C -pesticide solutions; however, using mass per unit volume does not account for the slightly greater molecular weight carried by the ^{14}C -labelled pesticide molecules. Therefore, in future isotope exchange experiments it would be more appropriate to use molarity in order to ensure equivalence in concentration between ^{12}C - and ^{14}C -pesticide solutions. This may help to minimise the differences that were observed between ^{12}C - and ^{14}C -pesticide in this thesis.

APPENDIX A

METHOD DEVELOPMENT: ASSESSING PESTICIDE DESORPTION KINETICS

A.1. Introduction

While adsorption is considered to control the persistence and irreversible binding of pesticides, desorption determines the release rate and potential mobility of pesticides in soils (Boivin *et al.*, 2005). Thus, a pesticide's potential for remobilisation over time has important implications for future solute transfer to surface and groundwaters, and bioavailability. The ability to predict long-term pesticide adsorption and desorption behaviour is limited by the multifaceted interactions occurring between the pesticide, soil system and ecological receptors. Complexity arises from the fact that as well as soils being heterogeneous environmental matrices with varying spatial and temporal gradients of organic carbon, pH, and particle size distribution, the ecological receptors themselves also differ significantly in terms of physiology and behaviour; the chemical structure of the pesticide in question must also be considered (Lanno *et al.*, 2004).

The complexity of these interactions causes ambiguity in prediction of both the pesticide available for transport or leaching to surface and groundwaters and the level of exposure ecological receptors will receive, necessitating further research to explore and document the manner in which pesticides interact with soils and soil biota (Ehlers and Loibner, 2006). This uncertainty has led to a conservative approach being adopted in exposure assessment, which assumes that the total concentration of a contaminant present in a given soil or sediment is available for uptake by potential receptors (Ehlers and Loibner, 2006). However, numerous laboratory and field studies have demonstrated that this is not the case (Ehlers and Loibner, 2006). The assumption that the biological effects of a contaminant are related to its total soil concentration results in overestimation of their potential risk; organisms will only respond to the bioavailable fraction (Gomez-Eyles *et al.*, 2010; Yang *et al.*, 2010). There is also increasing recognition that elevated contaminant levels in soil and sediment are not necessarily indicative of their availability for transport or leaching

(Peijnenburg and Jager, 2003; Chai *et al.*, 2007; Sormunen *et al.*, 2008). Furthermore, both bioavailability and potential for transport or leaching are often observed to decline as a function of increasing residence time in soil (Beulke *et al.*, 2004).

The International Standardisation Organisation (ISO) committee for soil and site assessment has specified that a simple approach would be to define an actually available fraction, a potentially available fraction and a non-available fraction (ISO, 2004). In order to produce site-specific risk assessments of contaminants, techniques must be utilised that measure the bioavailable fraction of chemicals in soil and/or the fraction available for transport and leaching (the quantities of which will be related), and not total chemical concentrations (Lanno *et al.*, 2004). However, environmental assessment of these fractions is typically a time-consuming process as a result of the protracted time-scales required to reach desorption end-points, which is frequently in the range of weeks, months or even years (Kan *et al.*, 1994; Altfelder and Streck, 2006). It is also often difficult to identify a distinct boundary between each of the three fractions, which typically appear continuous and may only become discernible when expressed as a function of time. Improving the practicality and environmental relevance of such studies necessitates the development of methodology that accelerates the rate of desorption, whilst simultaneously generating results comparable to the fraction realistically available for transport and leaching and/or the bioavailable fraction. A considerable amount of research has already focussed on developing non-exhaustive techniques to assess contaminant bioaccessibility to soil and sediment biota and some good correlations have been found between the rapidly desorbing fraction and bioavailability (Rhodes *et al.*, 2010; Yang *et al.*, 2010).

This appendix documents the assessment of three selected sorbent materials (Tenax TA®, Gerstel Twister® stir bars, Empore™ SPE disks) and a technique (forced isotope exchange) anticipated to increase the efficiency of determining the actually available, potentially available and non-available fractions. The theory behind the use of the three sorbent materials is the same: to act as an infinite sink for organic contaminants desorbing from geosorbents and thus, keep the aqueous phase solute-free. The establishment of a strong diffusion gradient between soil and solution is expected to accelerate desorption of pesticide into the aqueous phase. Over time, the rate of desorption is anticipated to slow, as the diffusion gradient lessens, and eventually reach an end-point when no more compound is desorbed into the aqueous

phase (indicating the potentially available fraction); subsequent analysis of the soil would reveal the non-available fraction (Figure A.1). Although this schematic is also likely to reflect the desorption behaviour of a natural soil system, the introduction of sorbent material is likely to considerably shorten the overall time-scale. The higher the affinity between the sorbent and sorbate, the more efficient this process would become; therefore, the three aforementioned sorbent materials were tested for sorption efficacy as a function of both their adsorption rate and capacity.

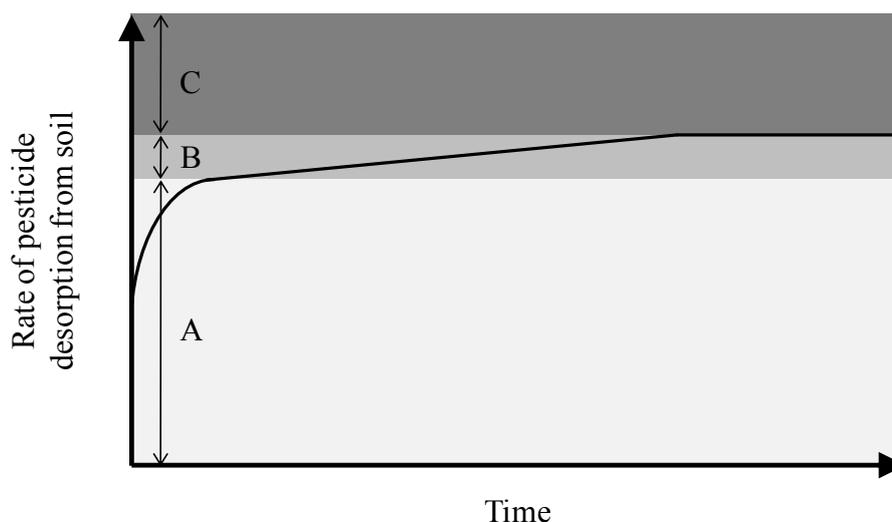


Figure A.1. Anticipated rate of pesticide desorption over time, indicating (A) the rapidly desorbing (actually available) fraction; (B) the potentially available fraction, slow pesticide release as the rate of desorption lessens over time; and, (C) the non-available fraction as the rate of desorption is 0.

Tenax TA® is recognised as one of the most promising materials to measure the rapidly desorbing fraction (Yang *et al.*, 2010). It is a porous polymer based on 2,6-diphenyl-*p*-phenylene oxide (Cornelissen *et al.*, 1997). Although Tenax TA® was originally designed to trap volatiles and semi-volatiles from air, its ability to act as an unlimited sink for organic contaminants desorbing from geosorbents has been extensively demonstrated in the literature (Cornelissen *et al.*, 2000; van den Heuvel and van Noort, 2006; Yang *et al.*, 2010). Its advantages include having a large adsorption capacity, floating on water (low density) and easy separation from the aqueous phase. Sequential desorption experiments using Tenax TA® has facilitated the study of desorption kinetics. In recent years, Tenax TA® extraction has been used extensively to assess the bioavailability of contaminants in soil systems despite the fact it has not been standardised by ISO (Yang *et al.*, 2010).

The Gerstel Twister® is used in stir-bar sorptive extraction (SBSE) procedures, and is an alternative to liquid extraction. The Gerstel Twister® (stir-bar) has three essential components: (i) a magnetic stirring rod to transfer the rotating movement of a stirring plate to the sample liquid; (ii) a layer of polydimethylsiloxane (PDMS) to act as the sorptive extraction phase, adsorbing analytes from solution; and, (iii) a glass layer that sits in between the aforementioned layers to cover the magnetic stirring rod, preventing decomposition of the PDMS layer catalysed by the metal of the magnetic rod (David *et al.*, 2003). Extraction time is controlled kinetically, determined by sample volume, stirring speed and stir bar dimensions (David *et al.*, 2003). The sorbed chemical may be desorbed from the Gerstel Twister® by either thermal or liquid means, depending on the suitability of the compound to each method.

Empore™ SPE disks encompass a variety of adsorbent chemistries, though C₈, C₁₈ and styrenedivinylbenzene (SDB-XC) are the most common (Green and Abraham, 2000). The C₁₈ and SDB-XC disks were tested here as their use in the extraction of pesticides from water has previously been demonstrated by Barceló *et al.* (1994). Empore™ SPE disks are composed of an inert material, usually polytetrafluoroethylene (PTFE), into which the specified sorptive material is embedded (Horne and Holt-Larkin, 1997; Green and Abraham, 2000; Tollbäck *et al.*, 2006). Their uniform and densely packed particle distribution in combination with their high surface area facilitates their effective extraction of organic compounds from aqueous matrices (Horne and Holt-Larkin, 1997). Other sorbent materials with the potential to keep the aqueous phase solute-free are available e.g. Amberlite™ XAD4, Hypersol-Macronet™ sorbent resins, lignin, activated carbon and clays, however these have not been tested here.

Finally, the forced isotope exchange procedure uses a different approach but maintains the same aim (to increase the desorption rate). The procedure is based on the principles of isotope exchange described by Celis and Koskinen (1999a; 1999b). Both the isotope exchange and forced isotope exchange methods use ¹²C and ¹⁴C isotopes to characterise the irreversible fraction *in-situ*. However, instead of the sorption equilibrium being undisturbed as in isotope exchange (see Chapters 4 and 5), the forced isotope exchange procedure involves a repeated influx of excess ¹²C-pesticide added in solution, thus essentially ‘forcing’ desorption of sorbed ¹⁴C-pesticide from the soil. This method is based on the assumption that not all sorption

sites are readily accessible. Thus the high-concentration of ^{12}C -pesticide added over time means that adsorption to readily-accessible sites is increasingly biased towards ^{12}C -pesticide as the supply of ^{12}C -pesticide to the soil surface to take part in adsorption would be essentially instantaneous, with desorption the rate-limiting step. It would therefore be possible to identify both the proportion of ^{14}C -pesticide sorbed to readily-accessible sites and the ^{14}C -pesticide sorbed to non-accessible sites (i.e. the non-available fraction) through measurement of ^{14}C -pesticide in solution. Each of the aforementioned sorbents and forced isotope exchange technique are described as separate experiments in the following sections.

A.2. Materials and chemical analyses

A.2.1. Soils

The collection and preparation details for the three study soils (Blackwood, Andover and Salop) used in the experimental work are given in Chapter 3 (section 3.3).

A.2.2. Chemicals

Information concerning the purchase of ^{12}C - and ^{14}C -chlorotoluron and its chemical and environmental fate properties are given in Chapter 3 (section 3.2).

A.2.3. Sorbents and other materials

The polydimethylsiloxane (PDMS) coated stir bar or Twister® (length 20 mm, PDMS thickness 1.0 mm) was obtained from Gerstel (Mülheim an der Ruhr, Germany). Tenax TA® adsorbent resin (60-80 mesh) and Empore™ SPE disks (C_{18} and SDB-XC, both 47 mm) were purchased from Sigma-Aldrich Ltd (Dorset, UK). The nylon mesh (120 and 250 μm) was purchased from ZMSystems (Winchester, UK). A cloth (similar to lining fabric) was used, obtained from the laboratory.

A.2.4. Chemical analyses

The analytical methods used to carry out the chemical analyses mentioned are described in Chapter 3 (section 3.4). All ^{12}C -chlorotoluron samples were analysed by

the HPLC method described (Chapter 3, section 3.4.1.1). All ^{14}C -chlorotoluron samples were quantified by the LSC method described (Chapter 3, section 3.4.2.1).

A.3. Experiment 1: Stir-bar sorptive extraction (SBSE)

A.3.1. Methodology

Two chlorotoluron test samples were prepared; the first a solution and the second a soil suspension. The solution was prepared in a 60 mL glass amber jar containing 19.00 mL of 0.01M CaCl_2 and 1 mL of chlorotoluron treatment solution (quantified by HPLC at $49.66 \mu\text{g mL}^{-1}$). The stir-bar was added to the solution and rotated rapidly on the magnetic stirrer for one hour at room temperature. The stir-bar was then transferred (by first tapping lightly against the side of the amber jar to remove excess liquid) from the ‘adsorption’ solution into an ‘extraction’ solution, which consisted of 20 mL of methanol acidified with 0.1% H_3PO_4 . The stir-bar was then rotated in the extraction solution at the same speed as during the adsorption step for one hour to allow desorption into solution. A 1-mL aliquot was taken from both the adsorption and extraction solutions and analysed by HPLC.

The soil suspension was prepared by weighing 5.0 g of dry Salop soil into a 50 mL Teflon® centrifuge tube. To obtain the desired 1:4 soil:solution ratio (previously determined optimal for this soil type, Chapter 3, section 3.5.1) 20 mL of shaking solution was added, consisting of 18.80 mL 0.01M CaCl_2 (to account for 0.20 mL moisture in soil) and 1 mL of chlorotoluron treatment solution (quantified by HPLC at $49.70 \mu\text{g mL}^{-1}$). The soil suspension was continuously shaken (HS 501 Digital IKA®-Werke reciprocal shaker) at 150 rpm for 24 hours at room temperature for adsorption to take place. The sample was then centrifuged (Hermle Z513K, LaborTechnik, Bench Top Centrifuge) at 3500 rpm for 10 minutes and a 1-mL aliquot of the supernatant was removed for HPLC analysis. The stir-bar was introduced into the soil suspension and shaken under the same conditions for a further 24 hours. The stir-bar was then removed from the soil suspension using tweezers, submerged in 20 mL of the same extraction solution as used for the previous test (solution only) and magnetically-stirred rapidly for one hour. A 1-mL aliquot was taken from the extraction solution, also for analysis by HPLC.

A.3.2. Results and discussion

The results showing the effectiveness of the stir-bar and the other sorbents are given in Table A.1. For both the solution and soil suspension, the results of the HPLC analysis showed that both 'extraction' solutions contained no chlorotoluron (or $< 0.01 \mu\text{g mL}^{-1}$ as this is the limit of quantification). However, a small reduction (2.13%) of chlorotoluron in the aqueous phase was observed for the sample containing solution only. Furthermore, HPLC analysis confirmed that most, if not all, of the initially-applied chlorotoluron was present in the 'adsorption' solutions. This indicates that very little, if any, chlorotoluron adsorbed to the stir-bar in the first place and also means that a poor extraction procedure was not the cause of the absence of chlorotoluron in the extraction solution. It may be possible that the poor adsorption of chlorotoluron was a result of the stir-bars being insufficiently pre-conditioned. Some research (Popp *et al.*, 2001; Kole *et al.*, 2011; Tölgyessy *et al.*, 2011) describes how the stir-bars used were pre-conditioned by heating at 200-300°C for a number of hours under a flow of helium or nitrogen of 50-100 mL min⁻¹. Other research neglects to mention this procedure as 'pre-conditioning' however, instead referring to it as 're-conditioning' (Tan *et al.*, 2008); whilst other research does not mention it at all (Blasco *et al.*, 2004). It is unclear therefore, whether 'pre-conditioning' is necessary to 'clean' (by thermal desorption) stir-bars that have already been used (before it is appropriate to use them again), or whether it is a necessary procedure in order for the stir-bar to work efficiently. Research using such conditioning techniques had used thermal desorption by means of extracting the chemical from the stir-bar, whereas research that did not mention it, had used liquid desorption instead. Thus, perhaps conditioning the stir-bar is not a necessary procedure in all cases. Chlorotoluron is thermally sensitive, therefore it would have been inappropriate to use thermal desorption in this instance. Furthermore, stir-bar sorptive extraction (SBSE) has been proved reasonably effective for chlorotoluron in water samples before (83% recovery with 116 μL PDMS) (Sandra *et al.*, 2003), thus perhaps it is really an additional unknown factor, which had caused this ineffectiveness. Due to these problems, efforts were concentrated upon the other adsorbents to assess their potential.

Table A.1. Relative reduction of chlorotoluron mass in solution after 24 hours contact with specified sorbent.

Experiment number	Method of adsorption	Treatment solution concentration ($\mu\text{g mL}^{-1}$)	Total mass in solution after pesticide adsorption to soil (μg)	Total mass in solution after contact with adsorbent (μg)	Relative reduction of mass in solution (%)
1	Gerstel Twister®, solution only	49.66	-	48.60	2.13
	Gerstel Twister®, soil suspension	49.70	16.79	16.80	0.00
2	Tenax TA® free, solution only	3.88	-	0.00	100.00
	Tenax TA® free, Andover soil suspension	3.88	2.79	0.00	100.00
	Andover soil suspension control*	3.88	2.76	(2.08)	(24.64)
	Tenax TA® bag (fine mesh), Salop soil suspension ^a	3.94	0.47 ± 0.003	0.35 ± 0.008	25.53 ± 2.15
	Tenax TA® bag (coarse mesh), Salop soil suspension	3.71	1.48	0.82	44.63
	Tenax TA® bag (cloth), Salop soil suspension	3.94	0.43	0.40	6.98
3	Empore™ SPE disk, C ₁₈ (half disk)	3.71	1.22	0.15	87.70
	Empore™ SPE disk, SDB-XC (half disk) ^a	3.71	1.11 ± 0.03	0.06 ± 0.01	94.76 ± 1.07
	Empore™ SPE disk, C ₁₈ (full disk)	3.71	1.02	0.12	88.24
	Empore™ SPE disk, SDB-XC (full disk)	3.71	0.99	0.04	95.96

- Sample did not contain soil, thus quantification was not applicable.

*No Tenax TA® was added to the control sample but data were included (in brackets) for comparability with soil suspension plus Tenax TA®.

^aTests were carried out in duplicate, thus the standard deviation has been given.

A.4. Experiment 2: Tenax TA®

A.4.1. Methodology

The Tenax TA® was conditioned prior to use in the following tests, using the method described by Cornelissen *et al.* (1997). The Tenax TA® was rinsed three times with acetone and three times with hexane (10 mL per 1 g of Tenax TA®) using filtering equipment attached to a vacuum line in a fumehood. The vacuum was left flowing for a further 20 minutes after the solvents had passed through in order to allow the Tenax TA® to dry completely. The Tenax TA® was subsequently transferred using a clean spatula into a glass LSC vial and stored at room temperature until required for use.

A.4.1.1. Tenax TA® adsorption test

Three separate chlorotoluron samples were prepared in 50 mL Teflon® centrifuge tubes: (i) a solution with Tenax TA® added after 24 hours; (ii) a soil suspension with Tenax TA® added after 24 hours adsorption; and, (iii) a second soil suspension *without* the addition of Tenax TA® after 24 hours adsorption (to act as a control). For the two soil suspensions, 2.00 g of dry Andover soil was weighed into separate 50 mL Teflon® centrifuge tubes. The Andover soil was selected for use because previous work (Chapter 3, Figure 3.1) had shown that chlorotoluron had the least affinity for the Andover soil. As the literature has shown Tenax TA® to have a large adsorption capacity it was anticipated that it would be capable of adsorbing large chlorotoluron concentrations so this was tested. For pre-equilibration, 19.00 mL of 0.01M CaCl₂ was added to all three samples, which were then shaken (HS 501 Digital IKA®-Werke reciprocal shaker) at 150 rpm for four hours at room temperature. The soil:solution ratio was also increased from 1:4 to 1:10 to further facilitate a higher solute concentration in the aqueous phase.

The chlorotoluron treatment solution was then applied to all three samples in 1-mL aliquots with HPLC analysis confirming that the mass added was 3.88 µg mL⁻¹. Samples were returned to the shaker for 24 hours for adsorption to soil to take place after which the samples containing soil were centrifuged (Hermle Z513K, LaborTechnik, Bench Top Centrifuge) at 3000 rpm for 10 minutes. A 200-µL aliquot from each sample was removed for quantification by HPLC. The Tenax TA® adsorbent resin (0.20 g) was then introduced into the relevant samples (i and ii). The

samples containing soil (ii and iii) were re-suspended, and all samples were then returned to the shaker for a further 24 hours. The samples containing soil were then centrifuged again and a 200- μ L aliquot was then removed from each sample for analysis by HPLC.

A.4.1.2. Making Tenax TA® ‘beads’

The sodium alginate beads were made following the method described by van Beinum *et al.* (2000). To prepare the sodium alginate solution, 0.50 g of sodium alginate was added to 100 mL of water and left to stir magnetically at a moderate speed overnight to form a gel. A 0.2M CaCl₂ solution was also prepared, of which approximately 500 mL was poured into a glass beaker. The 0.2M CaCl₂ solution was then also magnetically stirred at a low to moderate speed. A small amount of Tenax TA® (> 0.10 g) was added to some sodium alginate gel (approx. 20 mL) in a beaker and stirred together. Drops of the sodium alginate gel containing Tenax TA® were then dropped into the 0.2M CaCl₂ solution to form beads.

A.4.1.3. Tenax TA® ‘bag’ experiment

The following four samples were prepared: (i) fine nylon mesh, 120 μ m, two replicates; (ii) coarse nylon mesh, 250 μ m, one replicate; and, (iii) cloth, one replicate. Tenax TA® ‘bags’ were made by cutting out a circle of material (fine nylon mesh, coarse nylon mesh or cloth) and placing it in a beaker to form a well at the bottom in which to hold the Tenax TA®. Tenax TA® was then weighed (0.20 g) into the mesh (or cloth); the mesh (or cloth) was then twisted around at the top and secured tightly with a cable tie, excess mesh (or cloth) and cable tie were then trimmed off. Soil suspensions were prepared by weighing 5.00 g of dry Salop soil into four Teflon® centrifuge tubes. To obtain the desired 1:4 soil:solution ratio 18.80 mL of 0.01M CaCl₂ was added to the soils. Suspensions were shaken at 150 rpm to pre-equilibrate overnight in an incubator set to 4°C (Sanyo Fitotron RS232 incubator, with lights turned off). ¹⁴C-chlorotoluron treatment solution was added in 1 mL of 0.01M CaCl₂ to the samples (7.51 kBq or 3.94 μ g for the fine mesh and cloth samples, and 16.94 kBq or 3.71 μ g for the coarse mesh sample); all samples were then returned to the shaker at 150 rpm in the incubator for 24 hours. The soil suspensions were removed from the shaker and centrifuged at 3000 rpm for 10 minutes. A 200- μ L aliquot was removed from each sample and dispensed into an LSC vial and mixed with 10 mL EcoScint A for analysis by LSC in order to

determine the radioactivity in solution before addition of the Tenax TA® ‘bags’. Soil suspensions were re-suspended, the Tenax TA® ‘bags’ were added to the samples and they were returned to the shaker at 150 rpm for 24 hours. Another 200- μ L aliquot was removed from each sample at this stage for quantification by LSC.

A.4.2. Results and discussion

When Tenax TA® was in direct contact with the soil solution (A.4.1.1. Tenax TA® adsorption test), it was found to be remarkably effective at adsorbing chlorotoluron from solution. After 24 hours shaking with Tenax TA®, HPLC analysis confirmed that no chlorotoluron (or $< 0.01 \mu\text{g mL}^{-1}$) was present in solution; hence it was 100% effective at keeping the aqueous phase solute-free (Table A.1). This is also highlighted by the fact that in the soil suspension where no Tenax TA® was added, 75% of the chlorotoluron present in solution (after the 24-hour adsorption phase) was still present in solution after an additional 24 hours. Although there was still a reduction (25%), this is likely to be an effect of continued sorption of chlorotoluron to soil, resulting from non-attainment of sorption equilibrium during the first 24 hours.

Extraction of Tenax TA® to determine the mass balance necessitates its removal from the centrifuge tube. This proved near unattainable and quite time-consuming due to its small size and non-polarity causing it to adhere to both the spatula and shoulders of the centrifuge tube. This difficulty has often been avoided in the literature by conducting Tenax TA® solid-phase extraction experiments in separation funnels as opposed to centrifuge tubes (Cornelissen *et al.*, 1997; Cornelissen *et al.*, 2000; van Noort *et al.*, 2003; Leppänen and Kukkonen, 2006; van den Heuvel and van Noort, 2006; Baczynski *et al.*, 2010; Yang *et al.*, 2010). The use of separation funnels allowed simple separation of the Tenax TA® as it floats on top of the aqueous phase, thus during separation it adheres to the glass wall of the separation funnel. However, it was desirable to carry out the Tenax TA® experiment in centrifuge tubes due to the anticipated scale of the experiment and thus the large number of samples required.

Some Tenax TA® experiments have already been carried out using vials, test tubes, glass bottles and glass centrifuge tubes as test vessels (Kan *et al.*, 2000; Reeves *et al.*, 2004; Chai *et al.*, 2007; Trimble *et al.*, 2008; Liang *et al.*, 2010). The removal of

Tenax TA® from these vessels has been described by: (i) using a stainless steel spatula or scoop to transfer the Tenax TA® into a pre-weighed vial (Kan *et al.*, 2000; Liang *et al.*, 2010); (ii) after centrifugation pouring the Tenax TA® and supernatant through a hexane-rinsed sieve to separate, then pouring the original supernatant back into the vial to prevent losses of fine suspended sediment or organic carbon (Reeves *et al.*, 2004); or, (iii) by stating that the Tenax TA® was ‘removed’ but not giving the means by which this was achieved (Chai *et al.*, 2007; Trimble *et al.*, 2008). The difficulties encountered in removing the Tenax TA® from the centrifuge tube in this test may result from the structure of centrifuge tube used, which had a narrowed neck for the lid that widened into the body of the tube itself, and thus prevented access to the Tenax TA® remaining beneath the shoulders of the tube. The use of a vessel with straight sides therefore, may ameliorate this effect. Using Tenax TA® in its ‘free’ manner means that some loss of solution is likely to be inevitable if the Tenax TA® has to be removed by the means described above, particularly if sequential Tenax TA® extractions are involved. Thus, it was decided to evaluate containment of the Tenax TA®, thus making it easier to introduce, and more importantly to remove, from the centrifuge tubes whilst also minimising the loss of solution.

One thought was to incorporate the Tenax TA® into sodium alginate gel to make Tenax TA® ‘beads’; however, the non-polarity of the Tenax TA® meant that it did not combine particularly well with the polar sodium alginate gel to form beads. Hence, further method development involved the idea of enclosing the Tenax TA® in a porous nylon mesh. It was anticipated that using such a material would allow the aqueous phase to infiltrate the nylon mesh and come into contact with the Tenax TA®, while simultaneously maintaining the efficacy of the Tenax TA®. However, containing the Tenax TA® in this way introduced two further complications, the first of which was a reduction in extraction efficiency. The finer nylon mesh (120 µm) reduced the efficacy of the Tenax TA® to a greater extent than the coarse nylon mesh (250 µm), with reduction of the concentration of chlorotoluron in solution by $26 \pm 2.15\%$ and 45% , respectively (Table A.1). This is plausible since it would be more difficult for the aqueous phase to infiltrate nylon mesh with a smaller mesh size. The cloth material, although appearing to be less water-repellent than the nylon mesh, clearly prevented the free flow of chlorotoluron to the Tenax TA® as the chlorotoluron in solution was only reduced by 7% during 24 hours (Table A.1). It is therefore clear that attempting to encapsulate the Tenax TA® to improve its

practicality considerably reduced its extraction efficiency. The small size of the Tenax TA® means it is difficult to enclose within a material with a mesh size small enough to keep the Tenax TA® contained whilst also allowing the uninhibited flow of aqueous phase.

The second complication arose from the fact that addition of the mesh and cable tie increased the density of the Tenax TA®, resulting in some cases, in the Tenax TA® ‘bags’ attaching to the soil pellet during centrifugation. This problem could perhaps be overcome by centrifuging at a slower speed, though the balance between centrifuging at sufficient speed for full liquid/solid separation without the Tenax TA® ‘bag’ becoming attached to the soil would require some testing to ensure that results are consistent. This is because some Tenax TA® ‘bags’ floated on top of the supernatant, even though they were centrifuged under the same conditions as those that became associated with the soil pellet. Without overcoming this problem it is difficult to remove the Tenax TA® ‘bag’ without also transferring soil into the extraction fluid. The use of Empore™ SPE disks was anticipated to be a suitable alternative to the free Tenax TA®, provided their adsorption capacity was sufficient, as the sorbent material is compacted in a convenient disk form.

A.5. Experiment 3: Empore™ SPE disks

A.5.1. Methodology

A.5.1.1. C₁₈ and SDB-XC Empore™ SPE half disk test

Three soil suspensions were prepared by weighing 5.00 g of dry Blackwood soil into three separate 50 mL Teflon® centrifuge tubes. To obtain the desired 1:4 soil:solution ratio, 19.00 mL of 0.01M CaCl₂ was added to the soils and shaken overnight to pre-equilibrate. Chlorotoluron treatment solution was applied in 1 mL and was quantified by LSC as 16.94 kBq (or 3.71 µg mL⁻¹). The soil suspensions were then returned to the shaker for 28 days to allow adsorption to take place. Samples were then removed and centrifuged at 3000 rpm for 10 minutes. A 250-µL aliquot was taken from the supernatant, dispensed into an LSC vial and mixed with 10 mL of EcoScint A for analysis by LSC. Each Empore™ disk (one C₁₈ and one SDB-XC) was conditioned using the following method: (i) disk placed onto vacuum manifold and clipped in; (ii) added 10 mL acetone (HPLC grade); (iii) applied vacuum to dry the disk; (iv) added

10 mL of isopropanol (HPLC grade); (v) applied vacuum to the dry disk; (vi) added 10 mL of methanol (HPLC grade); (vii) applied vacuum and made sure that the disk did not run dry; (viii) added 10 mL water (HPLC grade); (ix) applied vacuum and made sure that the disk did not run dry. Half of a C₁₈ Empore™ SPE disk and the two halves of an SDB-XC Empore™ SPE disk were then placed into each of the three separate centrifuge tubes. The soil suspensions were then re-suspended and returned to the shaker at 4°C at 150 rpm for 24 hours. Samples were then centrifuged again and another 250-μL aliquot was taken from the supernatant and analysed by LSC.

A.5.1.2. C₁₈ and SDB-XC Empore™ full disk test over time

Two more soil suspensions were prepared by weighing 5.00 g of dry Blackwood soil into two 50 mL Teflon® centrifuge tubes. To obtain a 1:4 soil:solution ratio, 19.00 mL of 0.01M CaCl₂ was added to the soil and shaken overnight to pre-equilibrate. Chlorotoluron treatment solution was applied in 1 mL and was quantified by LSC as 16.94 kBq (or 3.71 μg mL⁻¹). A control using the same volumes and concentrations was prepared without any soil. The three samples were then returned to the shaker for 66 days. The samples were then removed and those containing soil were centrifuged at 3000 rpm for 10 minutes. A 250-μL aliquot was taken from all three sample supernatants, dispensed into an LSC vial and mixed with 10 mL of EcoScint A for analysis by LSC. Both Empore™ disks were conditioned using the same method as described in the previous test. The Empore™ disks were added to the relevant samples. The soil suspensions were then re-suspended and returned to the shaker at 4°C at 150 rpm for 24 hours. After 24 hours the samples were then removed from the shaker and those containing soil were again centrifuged at 3000 rpm for 10 minutes. Another 250-μL aliquot was then taken from the supernatants and analysed by LSC. The centrifuge tubes were then re-suspended (without removing the Empore™ disks) and returned to the shaker at 4°C at 150 rpm for 24 hours. This was repeated once more to gauge the capacity of the Empore™ disk after 24, 48 and 72 hours (i.e. to see if the chlorotoluron in solution remained constant over time or was depleted further).

A.5.2. Results and discussion

In the first test, using the half C₁₈ and SDB-XC Empore™ disks, the concentration of chlorotoluron in solution was reduced by 88% and 95 ± 1.1%, respectively (Table A.1); these appear to be the best results obtained so far with the exception of the free

Tenax TA®. However, the results between sorbents cannot directly be compared because the total mass of chlorotoluron in solution before adding the sorbent was different and only the concentration of chlorotoluron in solution has been measured. The difference in affinity for chlorotoluron adsorption between the two Empore™ disks is likely to be the result of their differing chemistry; SDB-XC was observed to be marginally more effective at adsorbing chlorotoluron from solution than C₁₈.

As using the half disks did not completely deplete the solution of chlorotoluron, full C₁₈ and SDB-XC Empore™ disks were tested to assess whether depletion of chlorotoluron from solution could be improved. However, there was little further reduction of chlorotoluron in solution when using the full C₁₈ and SDB-XC Empore™ disks instead of the half disks. Chlorotoluron adsorbed from solution was only slightly greater (~0.5%) for the full disks, increasing to 88% and 96% for the full C₁₈ and SDB-XC disks, respectively. Shaking with the full Empore™ disks over 48 and 72 hours also had negligible effect on further adsorption of chlorotoluron from solution. Between 24 and 72 hours, the reduction in solution concentration increased from 88 to 91% and 96 to 97% for the C₁₈ and SDB-XC Empore™ disks, respectively. Though these results appear to indicate that adsorption capacity was essentially reached after the first 24 hours, without further analysis involving extraction of both the Empore™ disk and soil, only partial conclusions can be drawn. This is because it is unclear whether further desorption from the soil was taking place during this time as well as continued adsorption of chlorotoluron onto the Empore™ disk. Therefore, the only conclusion that can be made is that the solution concentration had not changed significantly.

As no literature examples have been found specifically describing the efficacy of Empore™ SPE disks for chlorotoluron adsorption, the results are difficult to assess in terms of what would normally be expected. However, Barceló *et al.* (1994) have reported the average percentage recovery of 32 pesticides and pesticide transformation products from river water spiked at 1-5 µg L⁻¹ (considerably lower concentrations than were tested here). They found that recovery ranged from 30-120% though only four of the recoveries were less than 60%. The authors also found that recoveries increased with the use of two Empore™ SPE disks as opposed to one, but this would be a significantly additional cost given the number of samples to be run. The rationale for testing half Empore™ disks to begin with was to minimise

costs due to their fairly considerable expense. Furthermore, as full mass balances have not been determined the extent of their adsorption capacity still remains unknown. Therefore, it could be possible that sequential Empore™ disk ‘extractions’ would be necessary in order to reach the non-available fraction. The nature of using such a method would also require optimising extraction procedures for both the soil and Empore™ disks. As the results of this Empore™ disk test were inconclusive, and the efficacy of the Empore™ disks was not immediately apparent, the forced isotope exchange procedure was tested.

A.6. Experiment 4: Forced isotope exchange

A.6.1. Methodology

The nine initially ^{14}C samples (three soils: Blackwood, Andover and Salop, and three replicates) used previously in the chlorotoluron isotope exchange study (see Chapter 4 for experimental set-up and isotope exchange procedure) were selected to test this method. The samples chosen had been adsorbed for 56 days, and so would have had the greatest mass of ^{14}C -chlorotoluron sorbed to the soil after the 14-day isotope exchange phase. The supernatant removed at each time-point was replaced with fresh, high-concentration ($40.01 \mu\text{g mL}^{-1}$) ^{12}C -chlorotoluron solution. Samples were shaken under the same conditions (150 rpm, 4°C) as the isotope exchange method in between sampling points. Sampling was carried out at first after one day and then once a week, for four weeks in this preliminary test. At each forced isotope exchange sampling point, the soil suspensions were centrifuged (3500 rpm, 10 minutes) and the supernatant was removed by weight (17.00 g). A 250- μL aliquot from each supernatant was then taken for quantification by LSC (to measure the change of ^{14}C -chlorotoluron in solution over time).

A.6.2. Results and discussion

During the four-week preliminary test, good recoveries of sorbed ^{14}C -chlorotoluron were obtained using this method. The bulk of sorbed ^{14}C -chlorotoluron was extracted with the first addition of ^{12}C -chlorotoluron with $25 \pm 2.2\%$ (Blackwood), $42 \pm 0.8\%$ (Andover) and $21 \pm 1.8\%$ (Salop) recovered after one day. The amount of sorbed ^{14}C -chlorotoluron extracted increased steadily during 7, 14 and 21 days, and cumulatively

reached $57 \pm 2.6\%$ (Blackwood), $72 \pm 2.0\%$ (Andover) and $55 \pm 2.5\%$ (Salop) after 28 days. Although this test was initially intended as preliminary work, the simplicity and effectiveness of its use meant that the procedure was continued for an extended time-scale. Thus, the complete forced isotope exchange data are shown and discussed in Chapter 4 (Figure 4.4), together with the other chlorotoluron results.

A.7. Conclusions

Although the free Tenax TA® appeared to be the most effective adsorbent of chlorotoluron in solution and thus the optimal adsorbent material to use in such a study, its incompatibility with the available Teflon® centrifuge tubes restricted its feasibility. Clearly something which was not tested here was the subsequent extraction of the adsorbents to assess the quantities of chlorotoluron adsorbed. This is very important because a complete mass balance would necessitate quantification of the pesticide in solution, pesticide sorbed to the soil and the pesticide that had been sorbed onto the adsorbent. The forced isotope exchange procedure was therefore selected as the most suitable method to accelerate the desorption of ^{14}C -chlorotoluron from soil and characterise the non-available fraction *in-situ*. In addition to the reasons described above for its choice, this method also did not require development of an optimal method for extraction, which may have introduced further complications into the methodology. The forced isotope exchange technique is anticipated to be a powerful indicator of the extent to which remobilisation of previously adsorbed pesticide occurs, revealing the non-available or irreversible fraction and providing a quantitative estimate of the fraction of sorbed pesticide available for transport and leaching.

APPENDIX B

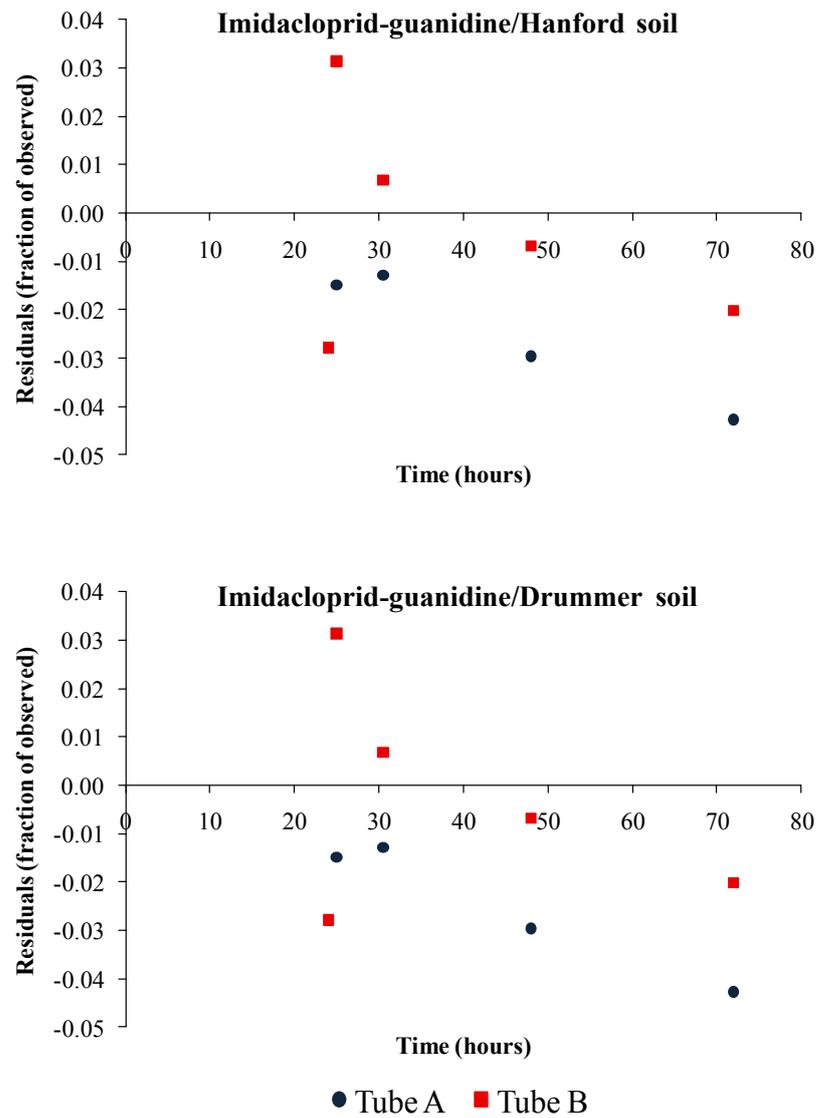


Figure B.1. Relative deviation of the model from the observed values for Celis and Koskinen's (1999a) isotope exchange data, Figure 6.

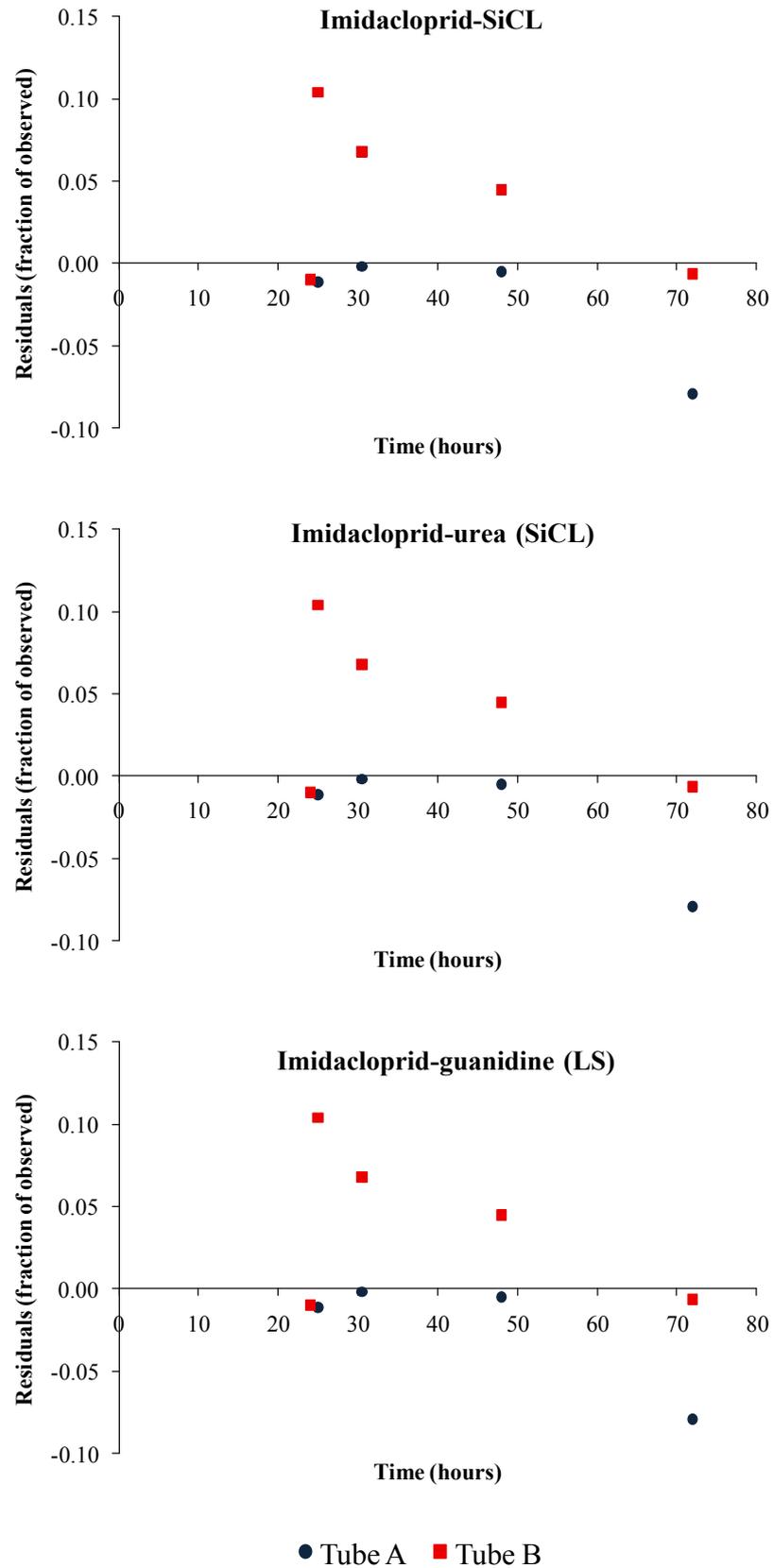


Figure B.2. Relative deviation of the model from the observed values for Celis and Koskinen's (1999b) isotope exchange data, Figure 2.

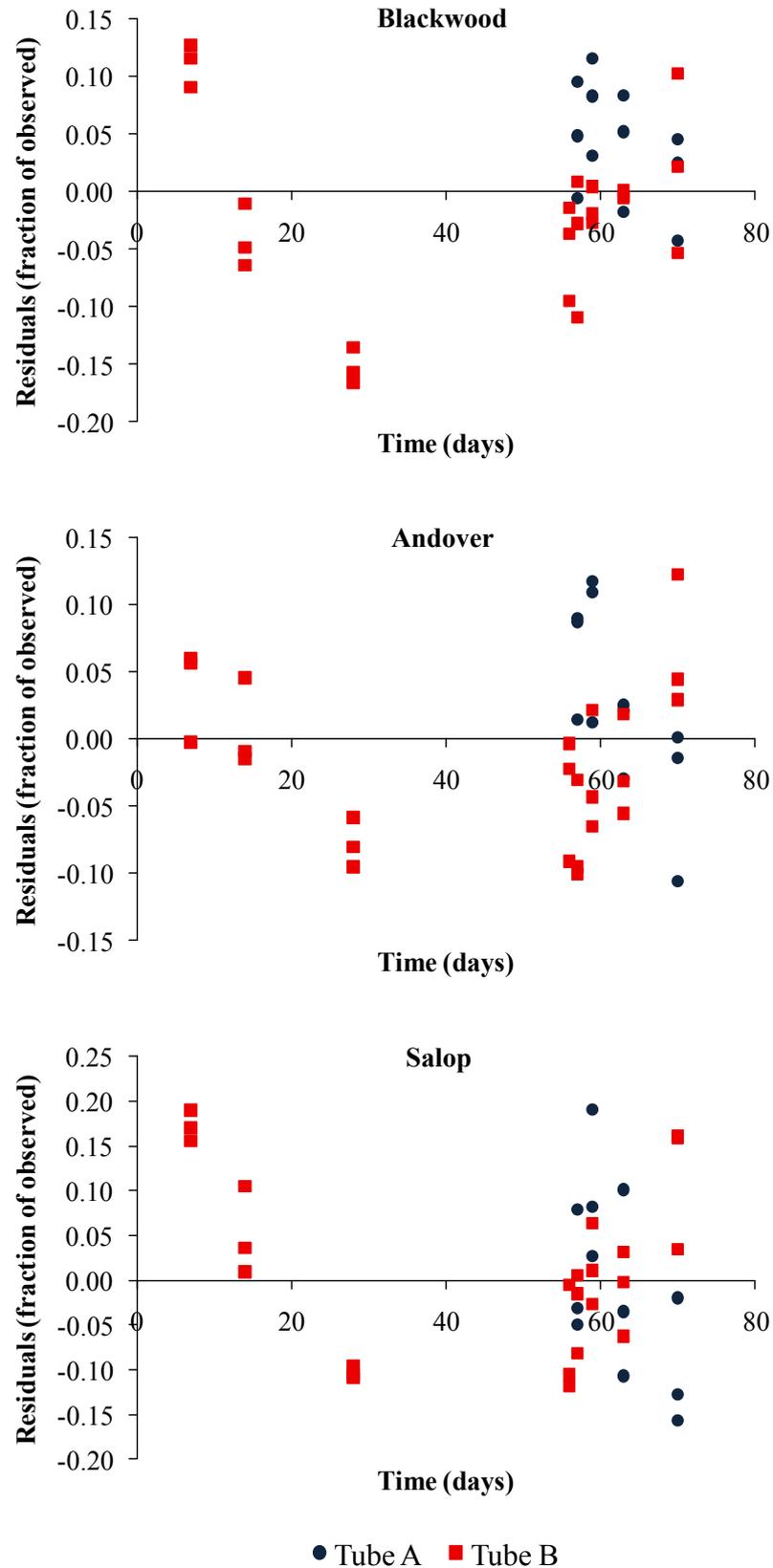


Figure B.3. Relative deviation of the model from the observed values for the chlorotoluron isotope exchange data for the three studied soils.

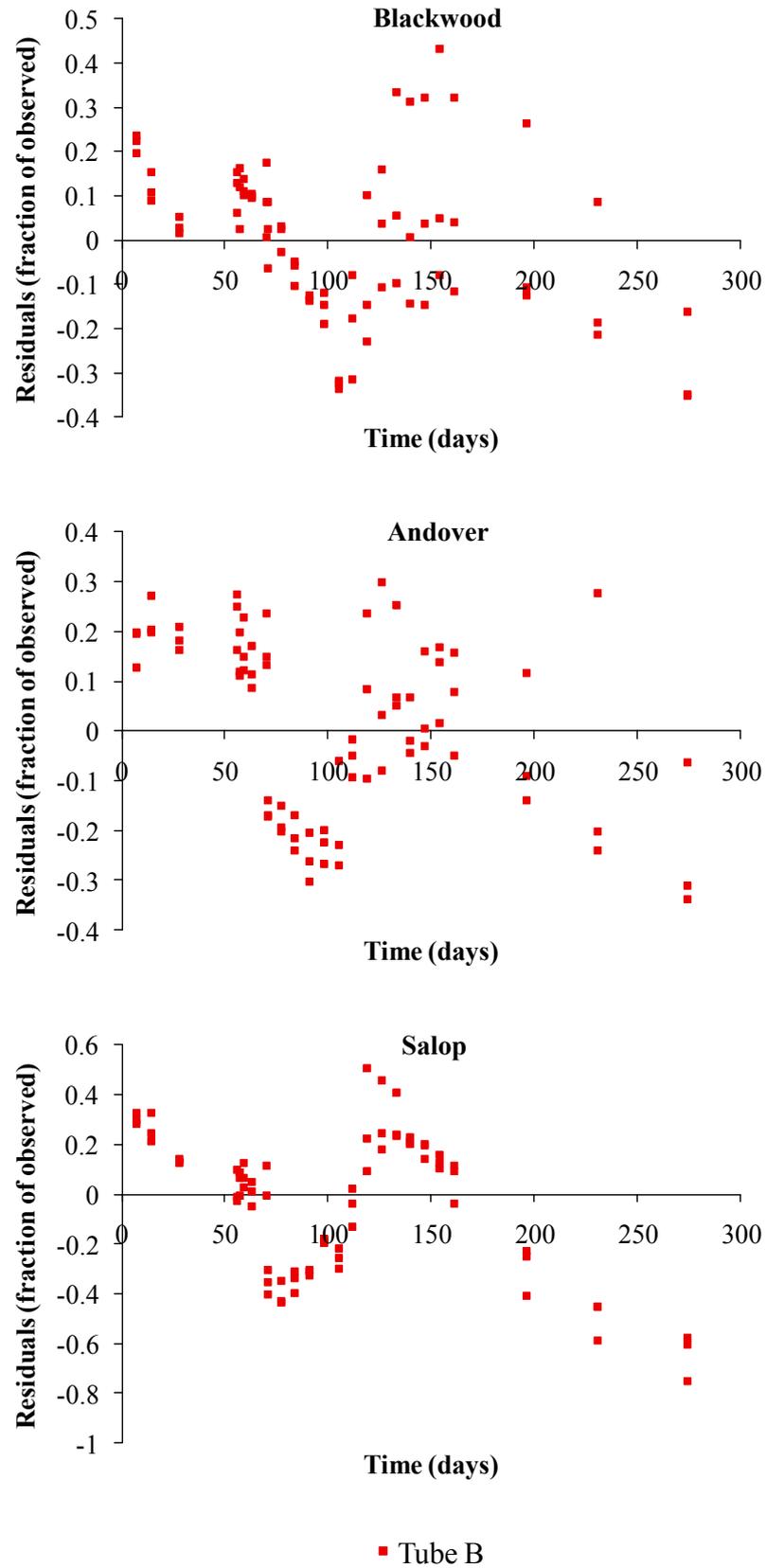


Figure B.4. Relative deviation of the model from the observed values for the chlorotoluron adsorption, isotope exchange and forced isotope exchange data after 56 days of adsorption, and the three studied soils.

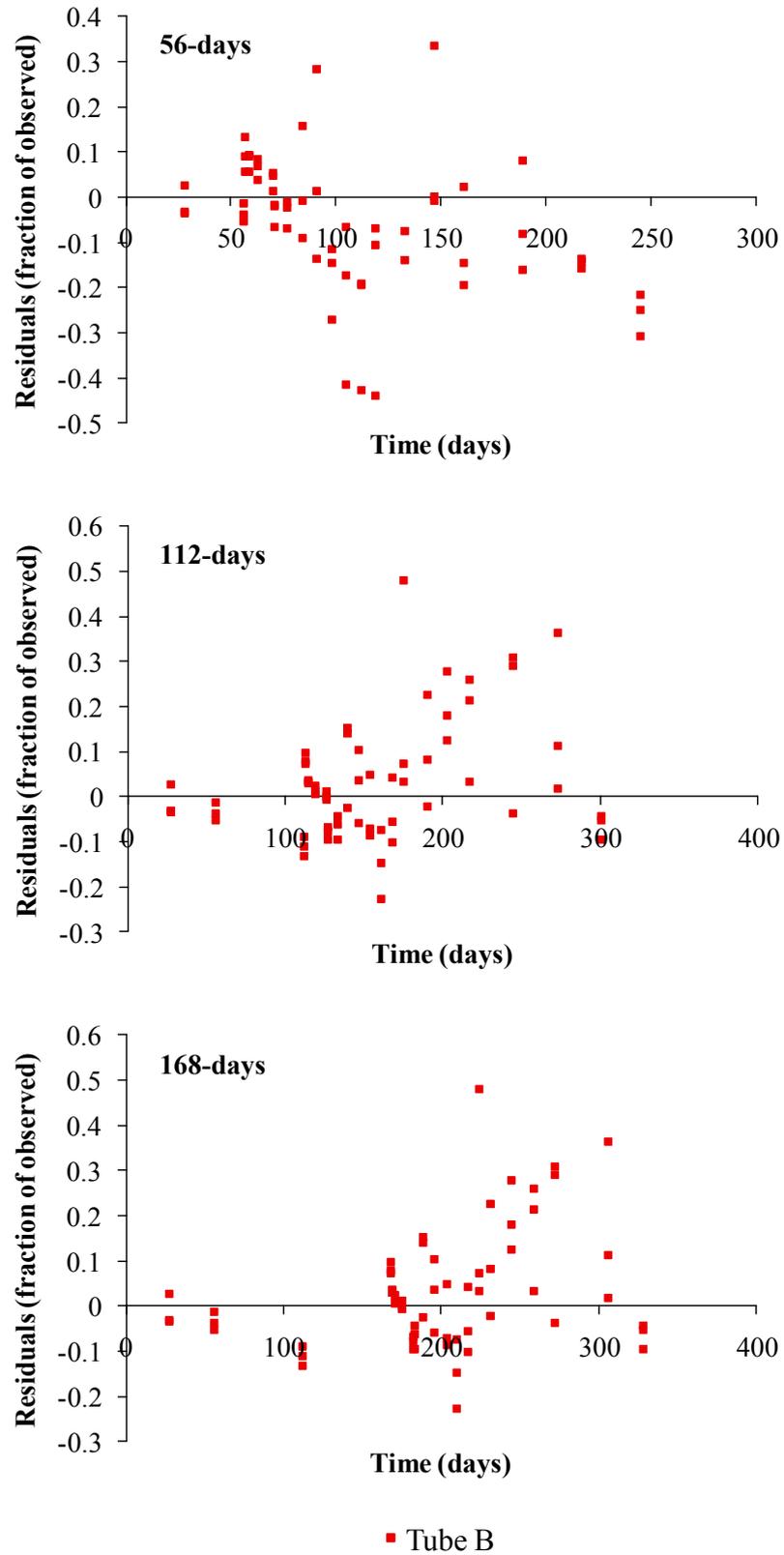


Figure B.5. Relative deviation of the model from the observed values for the prometryn adsorption, isotope exchange and forced isotope exchange data after 56, 112 and 168 days of adsorption, for the Blackwood soil.

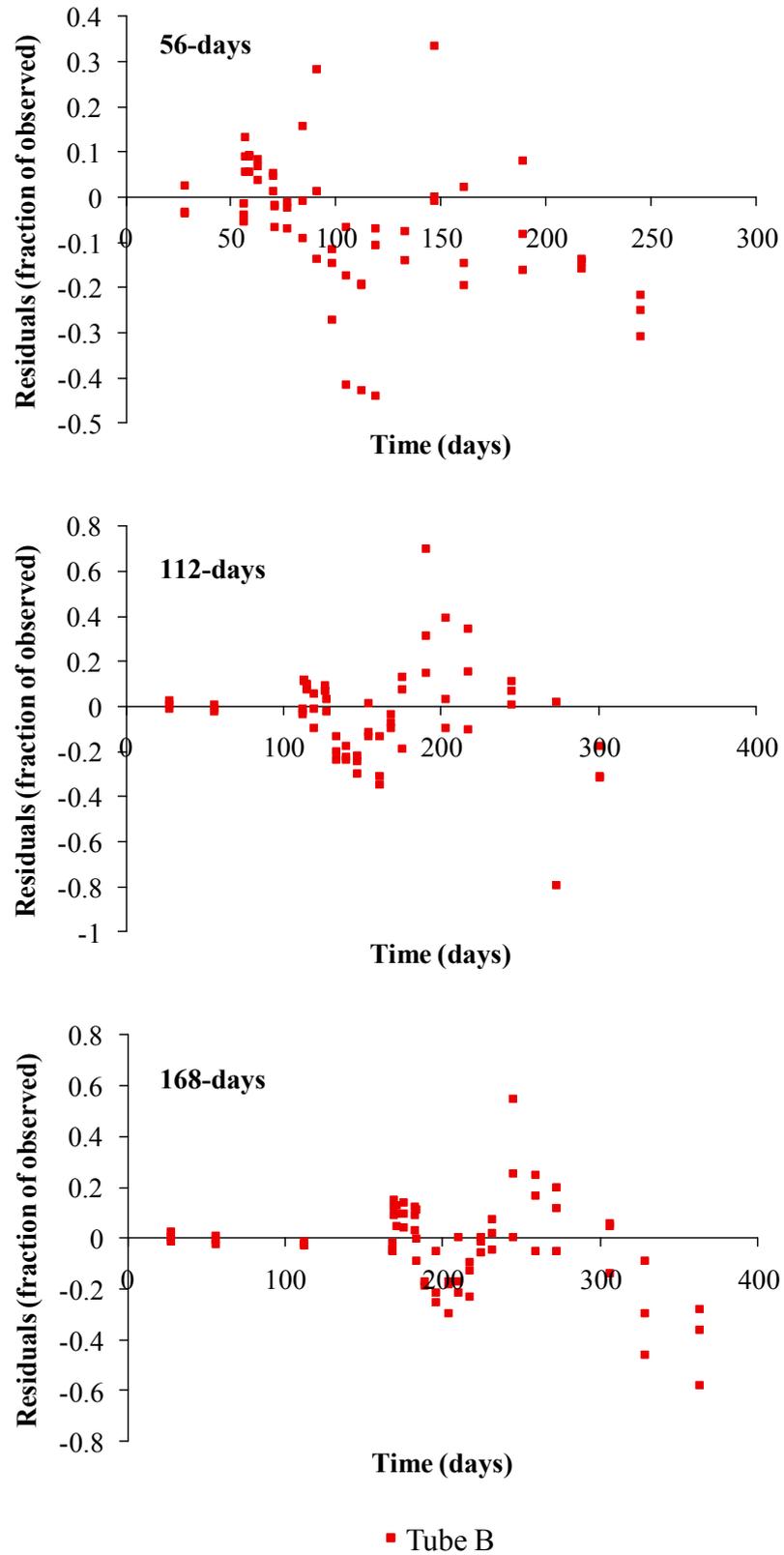


Figure B.6. Relative deviation of the model from the observed values for the prometryn adsorption, isotope exchange and forced isotope exchange data after 56, 112 and 168 days of adsorption, for the Andover soil.

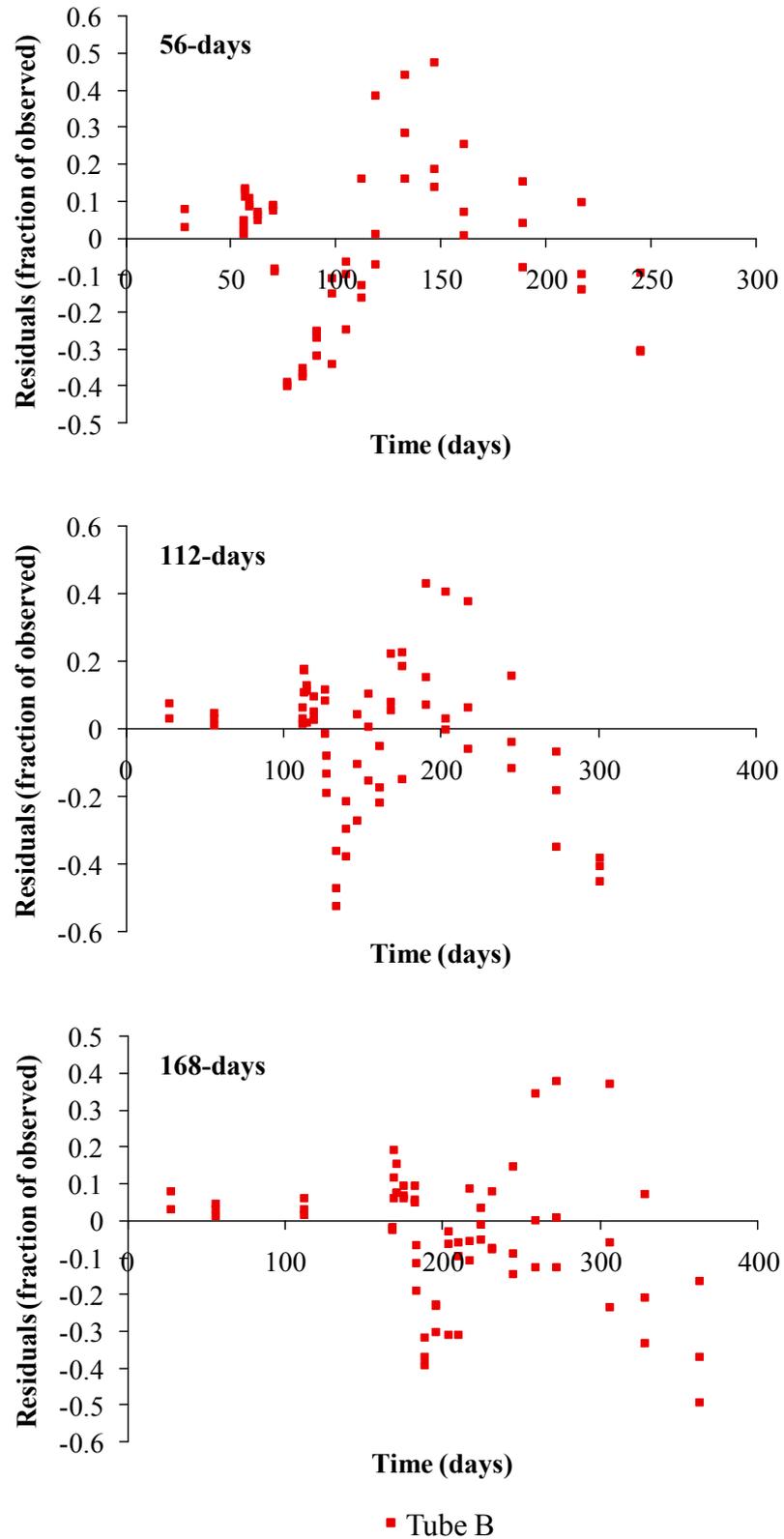


Figure B.7. Relative deviation of the model from the observed values for the prometryn adsorption, isotope exchange and forced isotope exchange data after 56, 112 and 168 days of adsorption, for the Salop soil.

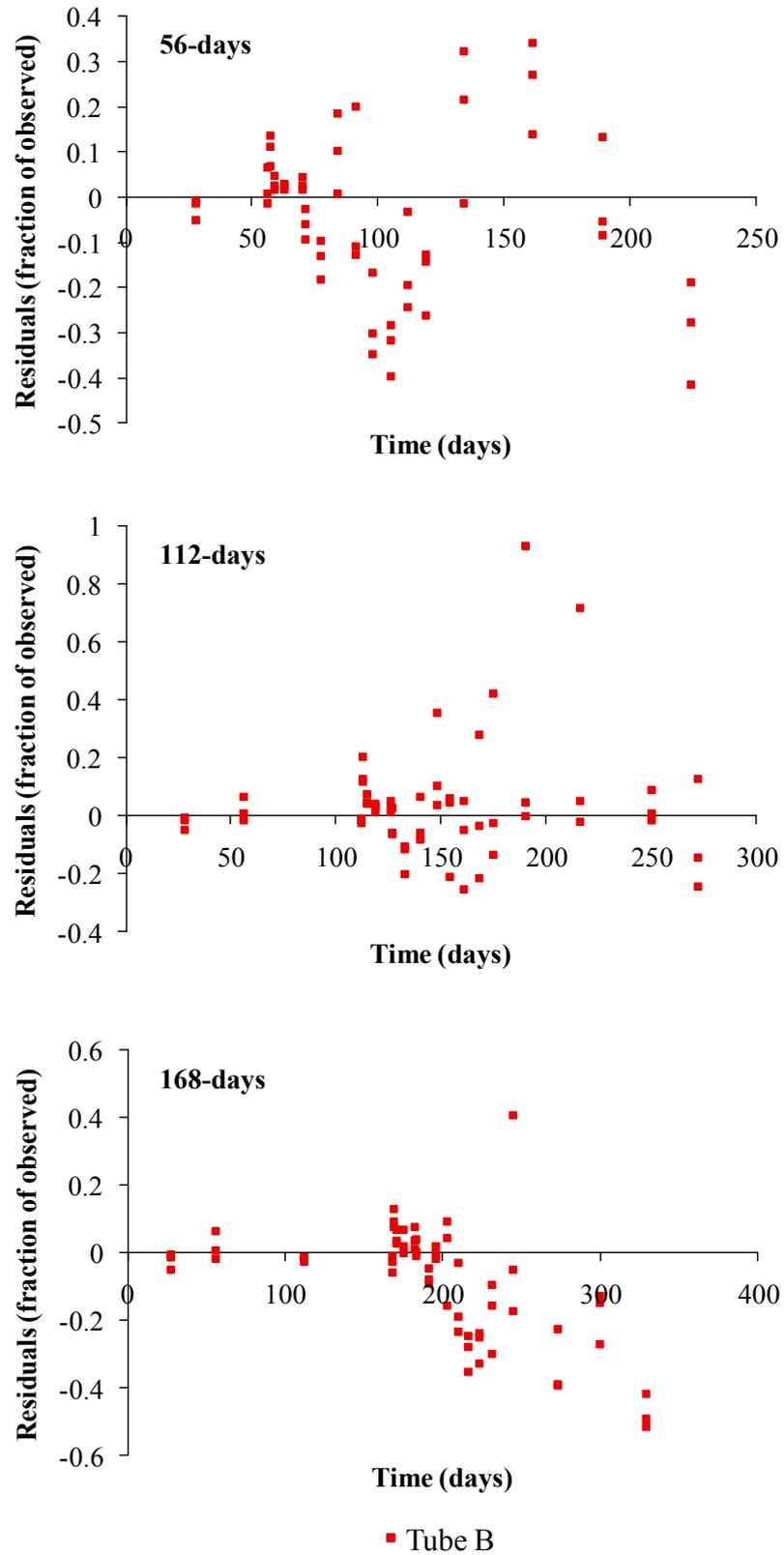


Figure B.8. Relative deviation of the model from the observed values for the hexaconazole adsorption, isotope exchange and forced isotope exchange data after 56, 112 and 168 days of adsorption, for the Blackwood soil.

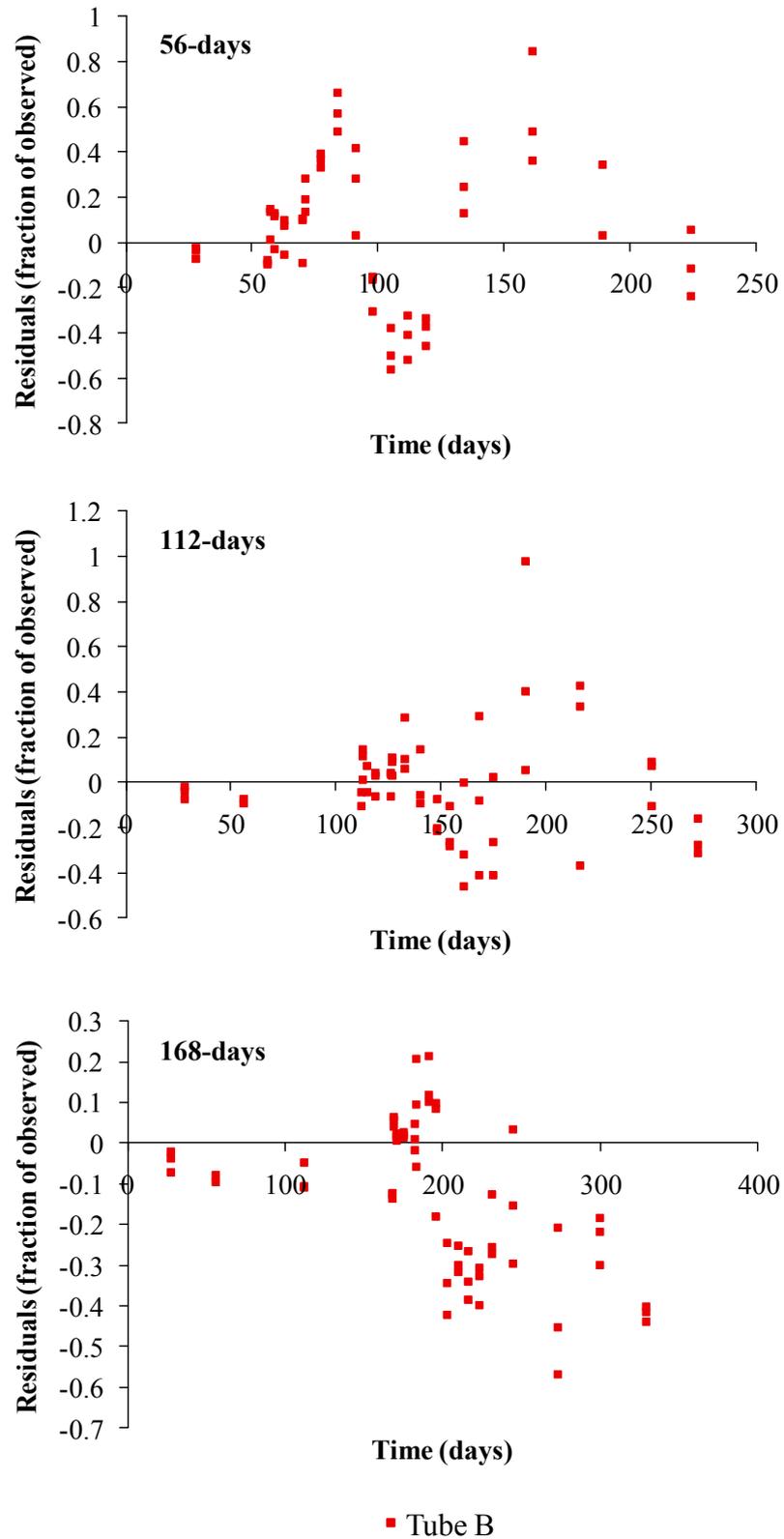


Figure B.9. Relative deviation of the model from the observed values for the hexaconazole adsorption, isotope exchange and forced isotope exchange data after 56, 112 and 168 days of adsorption, for the Andover soil.

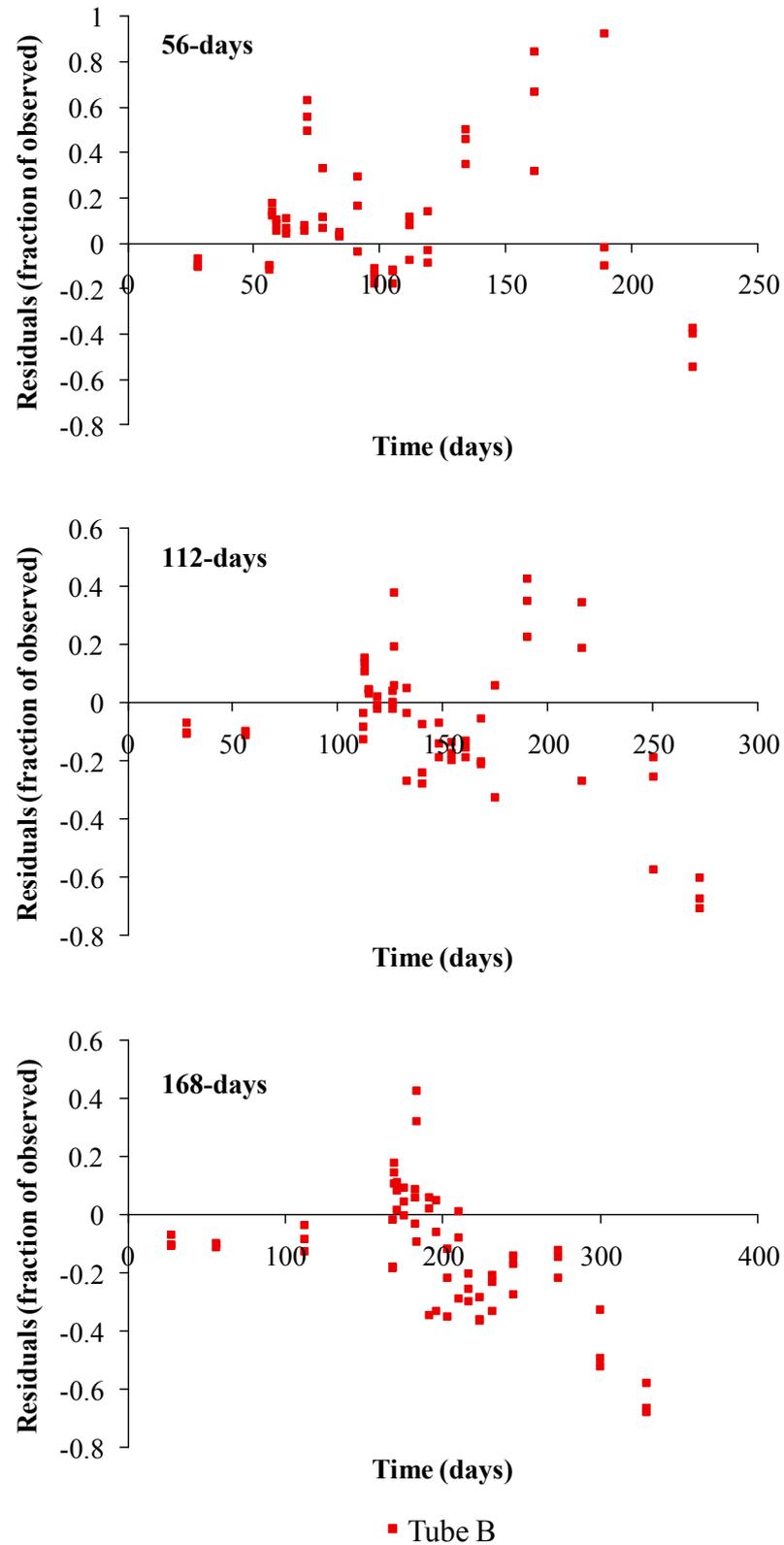


Figure B.10. Relative deviation of the model from the observed values for the hexaconazole adsorption, isotope exchange and forced isotope exchange data after 56, 112 and 168 days of adsorption, for the Salop soil.

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