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New Sampling and Analytical Methods: Pesticides and Transformation Products in Chalk Aquifers

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New Sampling and Analytical Methods: Pesticides and Transformation Products in Chalk Aquifers

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This report summarises the findings of the research undertaken with regard to pesticides and their transformation products in chalk aquifers. The work formed part of a European study, funded by the EC, into this topic area. The information is for use by Agency staff and others involved in the management of the quality of water contained in ground water aquifers.

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EXECUTIVE SUMMARY

Although there are numerous instances of pesticides being detected in groundwater, little is understood about the mechanisms by which they enter groundwater after application for agricultural and non-agricultural weed control. The first step to improve current understanding of pesticide transport mechanisms, together with the fate and persistence of target pesticides following application, lies in the development of suitable methodology for detecting these compounds and their degradation products in solid aquifer material. The project described in this report was co-funded by the Environment Agency and the European Commission DG-XII to develop, to validate and to use sampling and analytical methods for this purpose.

A review of the existing literature was carried out of the environmental effects of transformation products (TPs) of the active ingredients of pesticides and the non-pesticidal substances, known as formulation chemicals (FCs), used in pesticides. Environmental TPs were identified of many high-usage pesticides. Several have been reported in water sources in the UK at significant levels (close to or exceeding 0.1 $\mu g\ l^{-1}$), for example, deethylatrazine and other TPs of atrazine and simazine. Little information is available on the physico-chemical properties of TPs, and it was not possible to assess their potential to contaminate drinking water sources. General information on the chemicals used in FCs was obtained, as well as usage data for adjuvant active ingredients. A detailed review of the FCs used in pesticide products was not possible because the information required is held in confidence by regulatory authorities and the pesticide manufacturers.

Work previously undertaken by WRc has demonstrated the difficulties and uncertainties in detecting pesticides at very low concentration in solid aquifer material. The laboratory development of appropriate analytical methodology for TPs has therefore, formed a major part of the work programme. To validate the new analytical methods for TPs in aquifer material, there was a requirement to test the methods on aquifer material known or suspected of containing the compounds under investigation. This validation has been undertaken on both the saturated and unsaturated zones of the aquifer. WRc have also undertaken as part of their objectives, a demonstration and comparison of the efficiency and suitability of two contrasting monitoring borehole designs.

Prior to the commencement of this project there had been very little published work in the UK which dealt with the fate and persistence of pesticides, in particular in the unsaturated zones of the aquifer. This study of pesticides and their transformation products would have been impossible without the development and validation of new analytical methodology as part of the project. The development of analytical methods for triazine herbicides in chalk aquifer material complements previous methods for other classes of pesticides in chalk and sandstone aquifers. The analytical methodology will allow further detailed profiling work to be completed in other aquifers, and the data so produced will have important consequences to land management and aquifer protection, since the accuracy of any modelling of pesticide transport depend heavily on these measurements of pesticide behaviour in the subsurface.

Although monitoring of the unsaturated zone of aquifers for pesticides is in its infancy the recent profiling work has improved current understanding of the fate of agriculturally applied pesticides and their impact on water quality. Profiling has served to show that agricultural herbicides have the potential to migrate through significant thickness of unsaturated material

and infiltrate groundwater where there is karstic flow (fissure flow), or high flux rates. The work has specifically identified that the major TPs of atrazine can, in the presence of favourable hydrogeological conditions, present a real risk to groundwater quality, this has important implications to current recommendation for pesticide use in the agricultural environment. The current project could be used as a basis of repeat studies to study the rate of decay of pesticides and their TPs in aquifers and further improve pesticide transport models. The profiling data when used in conjunction with off the shelf modelling tools now make it possible to quantify herbicide fluxes moving toward the water table.

KEY WORDS

Pesticides, Transformation Products, Analytical Methodology, Saturated and Unsaturated Zone Profiling.

1. INTRODUCTION

1.1 Background

Although there are numerous instances of pesticides being detected in groundwater, little is understood about the mechanisms by which they enter groundwater after application for agricultural and non-agricultural weed control. The first step to improve current understanding of pesticide transport mechanisms, together with the fate and persistence of target pesticides following application, lies in the development of suitable methodology for detecting these compounds and their degradation products in solid aquifer material.

The title for this Environment Agency/European Commission project was "The Development of Analytical and Sampling Methods for Priority Pesticides and Relevant Transformation Products in Aquifers". It was agreed that validation of this new methodology would be undertaken on both the saturated and unsaturated zones of the aquifer.

The field demonstration segment of the project has served to provide real material to validate the analytical methodology. WRc have also undertaken as part of their objectives, a demonstration and comparison of the efficiency and suitability of two contrasting monitoring borehole designs.

1.2 Aims of the Project

Work previously undertaken by WRc has demonstrated the difficulties and uncertainties in detecting pesticides at very low concentration in solid aquifer material. The laboratory development of appropriate analytical methodology for Transformation Products (TPs) has therefore, by necessity, formed a major part of the work programme of this Agency Study.

To validate the new analytical methods for TPs in aquifer material, there was a requirement to test the methods on aquifer material known or suspected of containing the compounds under investigation. Where possible the compounds should also be present at depth in the profile. The former was obviously an essential condition of the selection of a study site, the latter was likely to be accommodated at a site where repeat applications of the same pesticide have been made over a number of years. Ideally a period of time should have elapsed between the first application of the selected pesticide and the time chosen for the sampling survey. This will help to ensure degradation processes are well advanced.

The scenarios described above represent the best possible combination of conditions for both validation purposes and the production of profiles of pesticide movement in the unsaturated and saturated zones of the aquifer. For this reason a site should not be selected for which the history of the site is unknown or where the only known pesticide application of interest is in the study year. It should be noted that it is impossible to critically validate analytical methods by the use of artificially spiked samples alone, although spiked and blank 'real' samples represent an important step in the validation process. The additional information regarding pesticide movement in the unsaturated zone gained from the validation work will obviously be invaluable in understanding the processes of pesticide migration in the unsaturated zone. The

quantification of loading to groundwater for the parent compound and its TPs may also be a step nearer. However, it should be born in mind that with respect to the objectives of this contract, this aspect of the work is of secondary importance to the validation of the new methodology. The number of samples collected is not the important issue, rather, it is the quality of the samples obtained. The aim is not to establish the rate of pesticide movement down the profile (by long term monitoring) but provide a set of good quality test samples on which to validate the analytical methodology.

WRc has previously developed multi-residue methods for acid herbicides and uron and carbamate pesticides in chalk and sandstone aquifer materials. A method for triazine herbicides in sandstone aquifer materials was also developed, but technical difficulties prevented its application to chalk aquifer materials. There was therefore a need to develop and validate a method for these important herbicides, to ensure that methods are available for monitoring the major classes of pesticides in the most important aquifer materials.

2. PESTICIDE TRANSFORMATION PRODUCTS AND FORMULATION CHEMICALS

2.1 Introduction

The potential of pesticides to migrate from their point of application and to reach water resources is well known. The environmental effects of pesticides include effects on aquatic life and also increases in the pesticide concentration in sources of drinking water above the legally prescribed maximum permissible concentration. The approval for use of a pesticide is based on an assessment of the potential environmental effects that it may have.

There is a growing realisation that the application of pesticides may pose threats to water sources other than those posed by the active ingredients. There is also the potential threat from the products formed by the degradation of the active ingredient and from the non-pesticidal chemicals which are included as ingredients in the pesticide formulation. In this section the environmental effects of these two categories of substances are considered. Appendix A contains the detailed results of the review.

2.2 Transformation products of pesticides

Pesticide transformation products (TPs) are chemicals formed in the environment from the degradation of pesticides. TPs can be formed by photolysis, microbial degradation, chemical degradation and plant metabolism. The main sources of TPs in groundwater and aquifer materials are likely to be processes that occur in the soil, due to soil having usually relatively high quantities of pesticide, high microbial populations and surfaces and chemistry which aid chemical degradation. The most important degradation process in the soil is probably microbial degradation.

Many TPs are likely to be relatively harmless substances, such as acetic acid, urea, and ultimately carbon dioxide. However, some are similar in their mobility and ecotoxicity to the parent pesticides and merit further study. The TPs that are of most concern are those that are likely to leach into groundwater in significant quantities and that may have ecotoxic effects. This implies that they do not sorb to soil to any great extent and are resistant to further degradation. A TP of concern could be formed even from a pesticide that is itself non-leaching.

The study of TPs and their potential environmental effects is complicated because each pesticide active ingredient may form several different TPs as it degrades, and in principle each TP must be considered for a thorough assessment of the potential effects of the TPs.

The regulatory position on TPs is unclear. For protection of waters used as sources of drinking water, it may be considered that TPs should be treated similarly to pesticides. The EC Directive on water intended for human consumption (80/778/EEC) defines parameter 55 as 'Pesticides and related products', for which a maximum admissible concentration (MAC) of $0.1\ \mu g\ l^{-1}$ is set. 'Related products' has been interpreted by the Department of the Environment as polychlorinated biphenyls (PCBs) and polychlorinated terphenyls (PCTs). However, some

EU Member states have included TPs in their legislation covering pesticides in drinking water. There is no specific legislation covering the acceptable environmental concentrations of TPs and no Environmental Quality Standards (EQS) have been set for TPs.

There have been reports of the occurrence of pesticide TPs in groundwater in the UK, Europe and the USA. It is therefore important that the significance of pesticide TPs is assessed.

2.2.1 Methodology

For a thorough assessment of the potential impact of pesticide TPs on groundwater quality, the following information would be required:

(a) identity of the principal TPs of the major usage pesticides,

(b) data on whether the principal TPs are likely to leach into groundwater, by reference to their physico-chemical properties,

(c) data on whether the TPs likely to leach into groundwater are of concern toxicologically.

Progress with the first aim was made in a review of TPs carried out by WRc for the Department of the Environment in 1992 (Cable *et al.* 1994). The approach taken then was to attempt to identify the TPs of a list of pesticides with high usage. A list of 68 priority pesticides was drawn up based on the criteria of either high agricultural usage (>50 tonnes per annum in England and Wales, >25 tonnes per annum in Scotland or >5 tonnes per annum in Northern Ireland) or of high non-agricultural usage (>50 tonnes per annum) in England and Wales. The list, in order of decreasing usage, is as follows:

Isoproturon, chloromequat, mecoprop, mancozeb, fenpropimorph, maneb, chlorotoluron, chlorothalonil, MCPA, tri-allate, trifluralin, pendimethalin, prochloraz, metamitron, carbendazim, tridemorph, fenpropidin, glyphosate, methyl bromide, propachlor, dimethoate, propiconazole, mecoprop-p, TCA (sodium trichloroacetate), terbutryn, chloridazon, simazine, phenmedipham, methabenzthiazuron, captan, 2,4-DB, bromoxynil, propyzamide, 1,3-dichloropropene, paraquat, flutriafol, ioxynil, diflufenican, triadimenol, linuron, aldicarb, ethofumesate, flusilazole, dithianon, diclofop-methyl, fluroxypyr, pirimicarb, bentazone, MCPB, lindane, difenzoquat, cyanazine, diquat, flamprop-S-isopropyl, demeton-S-methyl, ethirimol, triazophos, iprodione, amitrole, trietazine, metazachlor, imazamethabenz-methyl, dichlorprop, dicamba, fentin hydroxide, atrazine, diuron, 2,4-D.

The review undertaken for the DoE identified the main TPs for 46 of these pesticides; however, there were many significant gaps, such as 22 pesticides for which no information on TPs was found. The approach taken in the current review was to use the earlier review as a starting point and to up-date it with recent findings and to attempt to fill in the gaps in knowledge. The search for information to fill the gaps in knowledge included carrying out literature searches using the CAS and BIOSIS computer databases and contacting relevant pesticide manufacturers. The full list of pesticide TPs was then compiled using the DoE review and the information from the literature review and manufacturers.

2.2.2 Results and discussion

The review of TPs of the widely used pesticides is presented in Appendix A, which gives the identity of the principal TPs of the pesticides (Table A.1).

It proved possible to identify TPs for 13 of the 22 pesticides for which information on TPs had not been found previously. Additional information (in most cases, the names of more TPs) was obtained for eight of the 46 pesticides for which some TPs had been identified previously (Cable *et al.* 1994).

In most cases, it is not possible to ascertain which TPs are likely to leach into groundwater, since there is insufficient information available. It is likely that it would not be possible to obtain this information for most pesticides without experimental work to establish their physico-chemical properties. Use of simple predictive models such as the GUS index (Gustafson 1989) could help to identify TPs likely to leach. In this system, a GUS index is assigned, based on the octanol-water partition (log Kow) and half-life (t½) of the TP, and it allows categorisation of the TP as 'probable leacher', 'transitional leacher' and 'improbable leacher'. Obviously, the minimum data requirements for applying this model include the log Kow and t½ for the TP.

It is known that some of the TPs listed in Table A.1 have been reported in drinking water sources in the UK at levels above 0.1 $\mu g\,l^{-1}$. Some (notably, deethylatrazine) have been detected in drinking water supplies. Many TPs have been reported at significant concentrations in ground or surface waters in Europe, North America, South Africa, and elsewhere. These TPs include aldicarb sulphoxide, aldicarb sulphone, deethylatrazine, deisopropylatrazine, dealkylatrazine, hydroxyatrazine, *N*-isopropylanthranilamide (AIPA), 3-chloroallyl alcohol, DCPMU, DCPU, α-HCH, ETU, 2,6-dichlorobenzamide (BAM), DCPA di-acid and MITC. Although, in many cases, local or national agricultural or geological pecularities can explain the presence of a particular TP (e.g. high usage, shallow groundwaters), it is possible that some of these TPs will be detected in UK drinking water sources in the future.

To assess the environmental impact of the TPs, it would also be required to identify the ecotoxicological properties of the substances. For the great majority of the TPs in the table, this information is not available. No EQSs have been set for any pesticide TPs. Therefore to establish the ecotoxicological properties of all the TPs identified in the table, which itself only refers to the most highly-used pesticides, would be a very considerable task.

A 'threshold concentration' may be defined, an environmental concentration above which, if a parent pesticide occurs, or is predicted to occur, its TPs are studied more intensively. It may be that 0.1 $\mu g\,l^{-1}$ should be, arbitrarily, taken as a practicable threshold concentration. The purpose of this simplification is to reduce the magnitude of the task to manageable levels. The limitation is that some TPs are more mobile than the parent pesticide and may leach significantly even though the parent is known not to leach.

Alternative, simpler methods could be used to evaluate the environmental effects of TPs and to prioritise those pesticides and TPs for which more detailed study is required. It may be possible, for example, to simulate in laboratory experiments the degradation of a pesticide and then to test the ecotoxic properties of the mixture of TPs formed. Such an experiment would not yield individual values for ecotoxicological parameters of TPs, but rather the combined

values of a mixture of different TPs. Nevertheless, the method could be used to prioritise future research needs. The use of theoretical computer models that relate molecular structure to physico-chemical or ecotoxicological properties could also be investigated. Structure-activity relationships (SARs) could draw on the larger database of properties of the parent pesticides to predict the properties of TPs of similar structure. The feasibility of this would need to be demonstrated, by comparing predictions with known properties for example.

2.3 Formulation chemicals

Formulation chemicals (FCs) are non-pesticidal chemicals used in pesticide formulations. These include solvents, surfactants, safeners (i.e. additives that limit the phytotoxic effects of the active ingredient) and inert co-formulants. Those FCs that enhance the pesticide's desired effect are termed adjuvants.

The actual role of FCs can range from altering surface properties so that the pesticide can easily enter plants, to making the product easier to prepare or apply.

The impact of FCs on the quality of environmental waters is largely unknown at present. As FCs can be present in large quantities in the pesticide formulation, the impact is worth investigating, even though FCs have been selected partly on the grounds of their comparatively low toxicity.

Although some FCs allow the pesticidally active ingredients to be applied at a much lower rate than would otherwise be the case, hence reducing the environmental impact, the possibility of water contamination by FCs still needs to be evaluated.

2.3.1 Methodology

The potential impact of FCs (as well as TPs) was also considered in the review undertaken for the DoE (Cable *et al.* 1994). However, no information on usage was found then and it was impossible to identify the chemicals used in the pesticides used in the UK.

Although there is a legal requirement for the pesticides in commercial products to be disclosed upon registration, this requirement does not extend to the non-pesticide formulants. Consequently, the non-pesticide formulations tend to be regarded as confidential.

For this study, contact was made with the regulatory authorities (Pesticides Safety Directorate and Pesticides Usage Survey Group within MAFF) and with suppliers of pesticides, to see whether information could be provided on the usage or even the identify of the pesticide FCs used in the UK. Little progress was made in this area, due to the confidential nature of the information.

Literature searches were carried out using the CAS and BIOSIS computer databases, and a number of papers were found on the topic of FCs and related topics. Most published papers on FCs concern their effect on improving the performance of the active pesticidal active ingredient. Usage data for adjuvants for 1994 in Great Britain was obtained from Central Science Laboratories, Harpenden.

2.3.2 Results and discussion

It did not prove possible to obtain detailed information on the composition of individual FCs, as this is held in confidence by PSD. Usage data were also generally unavailable. Types of solvents and surfactants that may be used in pesticide formulations were identified and these are listed in Appendix A.

For one type of FC, adjuvants, it proved possible to obtain information on the usage of the active ingredients of the adjuvant. The information is provided in detail in Appendix A.

The total usage of adjuvant active ingredients during 1994 was 1719 tonnes. The data indicate that adjuvant active ingredients include alkyl and nonyl phenol ethoxylates (total usage of these substances for 1994 was 162 tonnes). Other surfactants were also used. Usage of the adjuvants generally peaks during the summer months, mirroring the usage of the co-applied pesticides.

Attention has focused recently on alkyl and nonyl phenol ethoxylates and their environmental degradation products, alkyl and nonyl phenols, as they are considered by some to be potential oestrogens. It has been suggested that their use in pesticide formulation be withdrawn (Anon 1996). It may be that these usages are relatively low compared with the known usage in other products. It is therefore important that the impact of such FCs through the application of pesticides is kept in perspective. However, it is not known whether the direct entry to the environment that is associated with spray application of pesticides increases the environmental impact, particularly for groundwaters. WRc is engaged in a separate desk-based study for the European Commission DG-III on the environmental effects of surfactants generally.

A review of the solvents used in pesticide formulations is underway by a MAFF Committee, but information on the identity of the solvents to be reviewed is not publicly available.

It is possible that FCs (and their TPs, and co-products with active ingredients) may have a significant impact on the environment. It is already established that non-pesticides can have a synergistic effect on the fate of the active ingredients. For example, solvents may increase pesticide leaching, or the surfactant used in the formulation of a pesticide may enhance the degradation of the pesticide in soils, etc. (this has been reported in the case of glyphosate).

2.4 Selection of pesticides and transformation products for experimental studies

2.4.1 General

For the purposes of this experimental study of pesticide TP, it was necessary to select a small number of pesticides for whose TPs analytical and sampling methodologies were to be developed. The information from the review of pesticide TPs was used in this selection.

The pesticides selected for the study were atrazine and isoproturon. Both herbicides have been detected in surface and groundwaters in the UK (and other parts of Europe), and in drinking water supplies above the MAC of 0.1 $\mu g\ l^{-1}$. Isoproturon has the largest usage of agricultural herbicides in the UK. WRc has also carried out previous studies at field sites in the UK where historical records of pesticide usage indicated that both of these pesticides had been used. Immunoassay kits are also available for the determination of both pesticides in water.

The important TPs of the selected pesticides were found by reference to the literature. A literature review was carried out using references obtained from a computer literature search of the CAS and BIOSIS previews databases. The UK manufacturers responsible for the pesticides were also contacted to obtain their views on which were likely to be the most important TPs in aquifer material.

The main route for the formation of TPs is likely to be through degradation in the soil, as the pesticides are in the soil zone for a reasonable time and the conditions for degradation (temperature, microbial population) are suitable. However, although the temperature and microbial population are not favourable in the aquifer material, the residence time of the chemical is long and there may be further potential for degradation.

2.4.2 Transformation products of atrazine

A pathway for the degradation of atrazine in soil has been proposed by Kruger *et al.* (1993). Although hydroxyatrazine (HYA) is the major TP formed in soil (Jones *et al.* 1982, Winkelmann and Klaine 1991a), it is strongly sorbed by the soil and not as available for leaching as some other TPs (Winkelmann and Klaine 1991a, Schiavon 1988). Deethylatrazine (DEA) and deisopropylatrazine (DIA) are the next most abundant TPs in soil (Winkelmann and Klaine 1991b). The leachability of these TPs is reported to be DEA > DIA > HYA (Schiavon 1988). Schiavon (1988) also showed atrazine plus metabolites in soil leachate to be mostly DEA (30-50%), then totally dealkylated atrazine or 2-chloro-4,6-diamino-1,3,5-triazine (DAA, 10-28%), atrazine (11-24%) and DIA (4-7.5%). Other studies have also shown that DEA is the major TP in soil leachate and that DIA is present at lower concentrations (Adams and Thurman 1991, Felding 1992). HYA has been shown to be a minor component of soil leachate (Muir and Baker 1976).

Agertved *et al.* (1992) reported no degradation of atrazine over a period of 96 days in a Canadian sand aquifer. Klint *et al.* (1990) also reported no degradation of atrazine after 539 days under simulated groundwater conditions. However, Wehtje *et al.* (1983) reported

that limited degradation of atrazine to HYA did occur under aquifer conditions, with about 3% of the atrazine being degraded after 70 days. McMahon *et al.* (1992) also report deethylation of atrazine by alluvial-aquifer sediment although the rate was slow.

It therefore appears that the major TP most likely to occur in groundwater and aquifer materials is DEA. Lower concentrations of DIA, DAA and HYA may also occur. Indeed DEA has been detected a number of times in groundwater (DeLuca 1990, Pionke and Glotfelty 1990, Isensee *et al.* 1990). DIA was also detected in the former two of these studies. A personal communication with staff at Ciba Agriculture, Cambridge also confirmed that these are likely to be the most important TPs.

By analogy with atrazine, the TPs of simazine are likely to be deethylsimazine, dealkylated simazine and hydroxysimazine (HYS). The former two of these TPs are identical to DIA and DAA respectively. It was therefore important to develop an analytical method for DEA and DIA in aquifer material. If possible the analytical method should also be able to analyse DAA, HYA and HYS.

2.4.3 Transformation products of isoproturon

Comparatively few references were found on the degradation of isoproturon. This means information on which TPs are likely to be most common in aquifers is limited.

A pathway for the degradation of isoproturon in soil has been proposed by Mudd *et al.* (1983). The singly-demethylated isoproturon is the major TP in soil (Berger and Heitefuss 1990; Fournier *et al.* 1975). It is reported that the isopropylaniline (IPA) formed is bound to the soil (Bollag *et al.* 1978). Information on the occurrence of isoproturon TPs in soil leachate, groundwater or aquifer material could not be found. As it is likely that demethylisoproturon (DMI) is relatively water-soluble, it was assumed that it would also be the major TP in soil leachate and groundwater. A personal communication with staff of Rhône-Poulenc Agriculture Ltd, Ongar, also confirms that DMI is likely to be the most important TP. It was therefore important to be able to analyse for this compound in aquifer material. If possible the analytical method should also be able to determine some of the other TPs of isoproturon such as didemethylisoproturon (DDMI) and IPA.

2.5 Conclusions

The TPs of many high-usage pesticides have been identified. Several have been reported in water sources in the UK at significant levels (close to or exceeding 0.1 $\mu g\ l^{-1}$), for example, deethylatrazine and other TPs of atrazine and simazine. Many others have been detected in surface and ground waters in other countries.

Little information is available on the physico-chemical properties of TPs, and it has not been possible to assess their potential to contaminate drinking water sources. More information on the physico-chemical properties of these compounds is needed. Information on the ecotoxicological properties of the TPs is also generally unavailable. It is likely that, if a

systematic assessment of the environmental effects of pesticide TPs is to be undertaken, the task will need to be reduced in complexity and magnitude through the use of simplifying assumptions or prioritisation procedures to identify the most significant TPs.

General information on the chemicals used in FCs was obtained, as well as usage data for adjuvant active ingredients. A detailed review of the FCs used in pesticide products was not possible because the information required is held in confidence by regulatory authorities and the pesticide manufacturers. It is suggested that the Environment Agency should access and evaluate this information through involvement in the regulatory approval mechanisms. FCs include some widely used chemicals, so the environmental impact associated with pesticide use may not be significant. However, it is not possible to confirm this without detailed information on the composition and usage of the products.

For the purposes of this study, atrazine and isoproturon TPs have been selected for the development and use of analytical methods.

3. DEVELOPMENT AND VALIDATION OF LC-MS ANALYTICAL METHODS

The analytical methods for atrazine, simazine and their TPs, and isoproturon and its TPs, in chalk aquifer materials were developed based on a literature review and previous work carried out at WRc on the development of analytical methods for pesticides in aquifer materials (Forbes *et al.* 1993, 1994a, b). Reference samples of the TPs of isoproturon were kindly provided by Rhone Poulenc, UK. Methods based on solvent extraction of the chalk and subsequent gas- or liquid chromatography mass spectrometry (GC- or LC-MS) have been used successfully for some pesticides, so these methods were the main focus of the work. The methods that were developed for atrazine TPs and isoproturon TPs are described in detail in Appendices E and F respectively. The development work has been described in detail in previous reports to the Agency (Moore *et al.* 1995, 1996).

3.1 Analytical method for atrazine, simazine and transformation products

3.1.1 Development of analytical method

The following HPLC conditions were tested and found to separate the compounds of interest:

Column:	Spherisorb 5 C8 25 cm x 4.6 mm ID.
Mobile phase:	45% 0.1 M ammonium acetate.
Flow rate:	1 ml min-1.
Detector:	UV absorbance at 220 nm.

Four extraction solvents (acetone, methanol, 90:10 methanol/water, or 70:18:12 acetonitrile (ACN)/water/ammonia) identified in the review as being good extraction solvents for atrazine and its TPs were tested.

Eight 250 g samples of chalk (known to be free of pesticides) were spiked at 2 $\mu g\ kg^{-1}$ with simazine, atrazine, DEA, DIA, DAA and HYA. Duplicate samples were extracted using one of the four solvents. The filtered extracts and associated washings were concentrated by Turbovap to 1 ml. The concentrated extracts and washings were transferred to a 1 ml vial and blown down to about 0.2 ml with a gentle stream of nitrogen before filtration through a Whatman Anotop 10 0.2 µm filter. The extracts were blown down to dryness with a gentle stream of nitrogen and heating. The dry extracts were reconstituted in 0.1 ml of LC eluent containing 5 $mg\ l^{-1}$ of internal standard (d5-atrazine). The extracts and some standards containing 5 $mg\ l^{-1}$ of the compounds of interest and internal standard were then run on LC-MS using the optimised conditions. The results of the experiment are given in Table 3.1.

Table 3.1 Testing various extraction solvents for atrazine, simazine and TPs

		Recoveries at 2 µg kg^{-1} (%)					
Extraction solvent	Sample	DAA	DIA	DEA	HYA	Simazine	Atrazine
Acetone	1	56	74	84	0	84	75
	2	49	57	81	23	81	85
Methanol	1	37	40	47	15	48	62
	2	22	27	30	1	33	60
Methanol/water	1	41	41	49	50	48	46
	2	87	89	127	209	134	60
ACN/water/ammonia	1	27	50	56	97	68	60
	2	32	31	44	83	45	38

The extraction solvent which achieved the most consistently reasonable recoveries is acetone, so it was chosen for the method. The LC-MS response for HYA was very variable even for the standard, hence the poor and variable recoveries for this compound. The available evidence suggests this is a less abundant TP, so the lack of an analytical method for it was not judged critical.

3.1.2 Validation of the LC-MS analytical method

The performance of the analytical methods were tested in two parts: a linearity test, and a precision test. The linearity test determines the linear range of the analytical method over a wide concentration range (0-10 µg kg^{-1}) and the precision test determines the precision, bias and limit of detection of the analytical method. The method is described in detail in Appendix E.

Linearity test

Duplicate 250 g samples of chalk (powdered in a knife mill) were left blank. Further duplicate 250 g samples were then spiked with 0.05, 0.1, 0.2, 0.5, 1, 2, 5 and 10 µg kg^{-1} of simazine, atrazine, DAA, DIA, DEA, HYA and d5-atrazine (internal standard). The samples were left overnight to equilibrate and then analysed by the analytical method described above. Figure 3.1 shows the LCMS chromatogram obtained for the 0.2 µg kg^{-1} spike.

The calibration curves for atrazine and DEA are illustrated in Figure 3.2 and Figure 3.3. The calibration curves for all the compounds appear to be linear over the range from 0.05 to 10 µg l^{-1}. DAA appears to be non-linear below 0.5 µg kg^{-1} so the working range of DAA is likely to be from 0.5 to 10 µg kg^{-1}. As expected, the HYA response is small and variable.

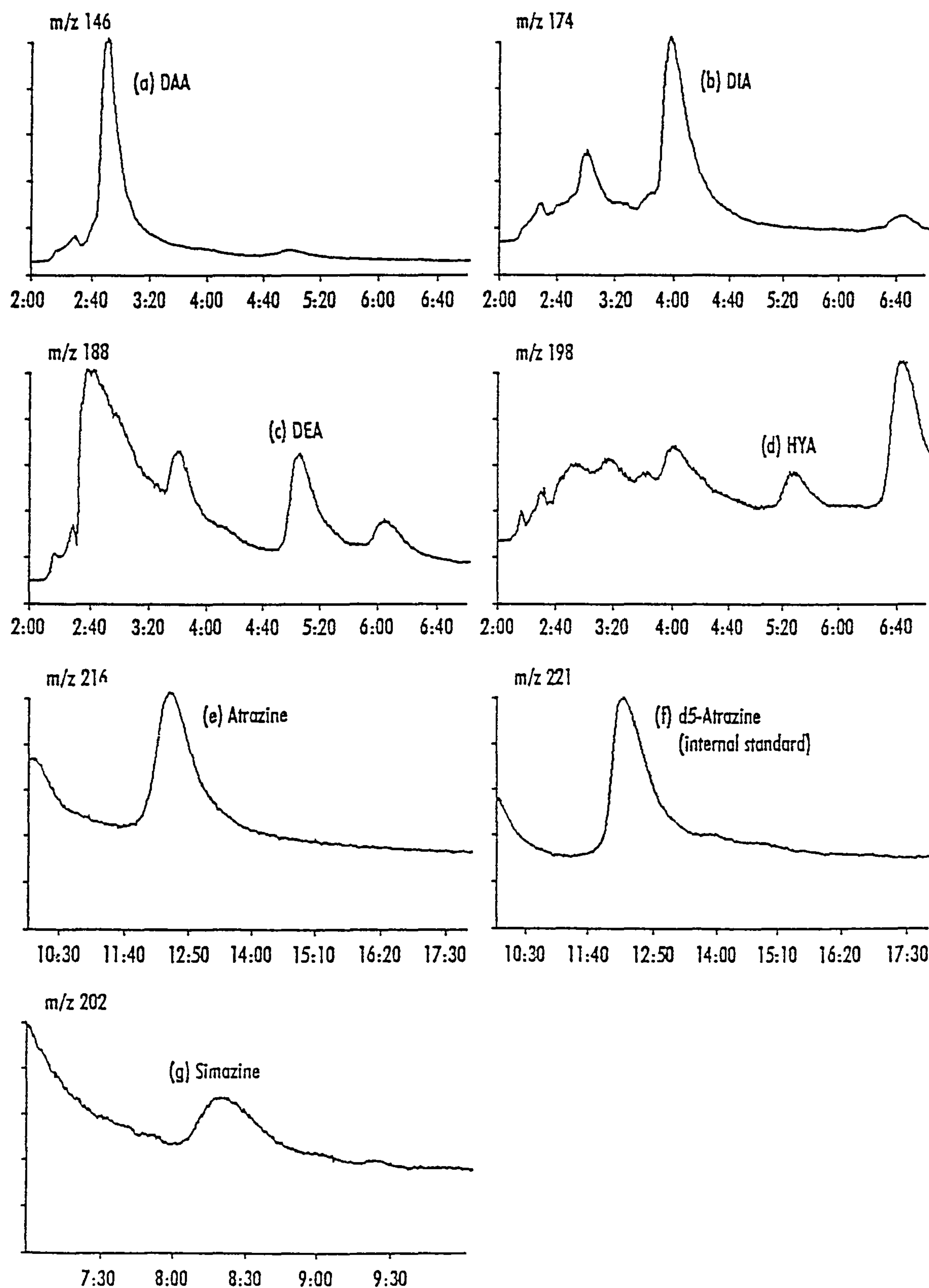
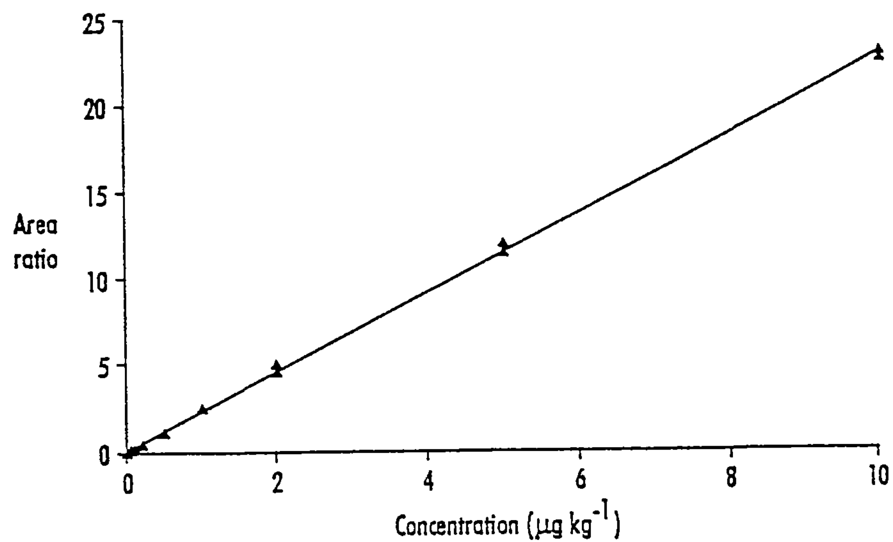


Figure 3.1 LC-MS mass chromatograms for extract of chalk sample spiked at 0.2 µg kg^{-1} with atrazine, simazine and TPs (DAA at 1 µg kg^{-1})



Expansion of low concentrations

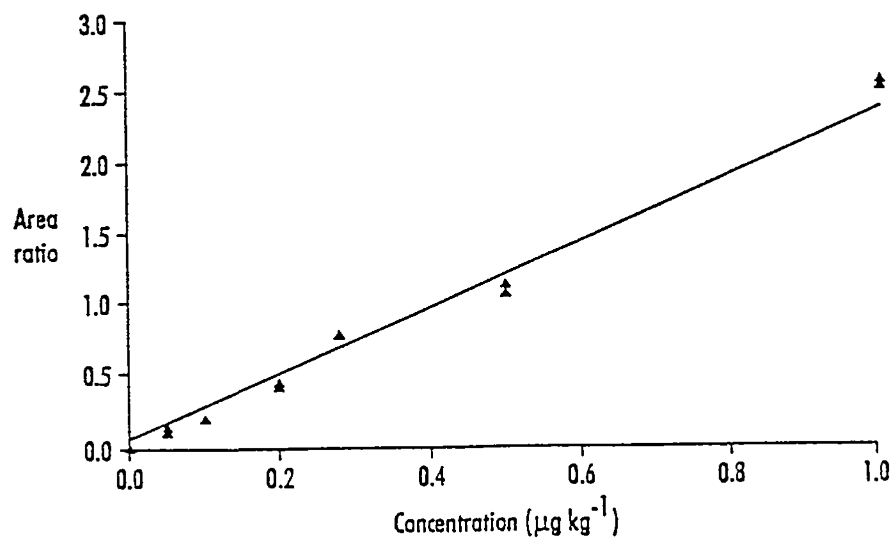
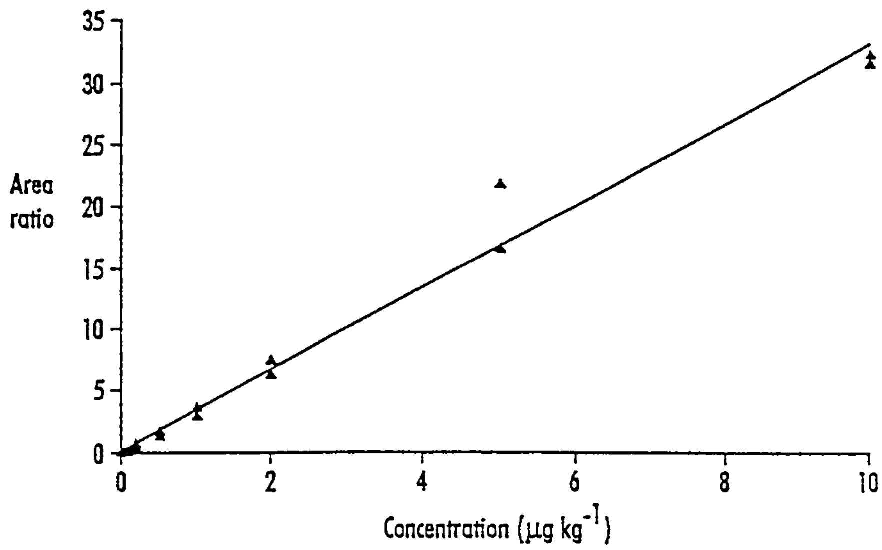


Figure 3.2 Linearity test of analytical method for atrazine in chalk



Expansion of low concentrations

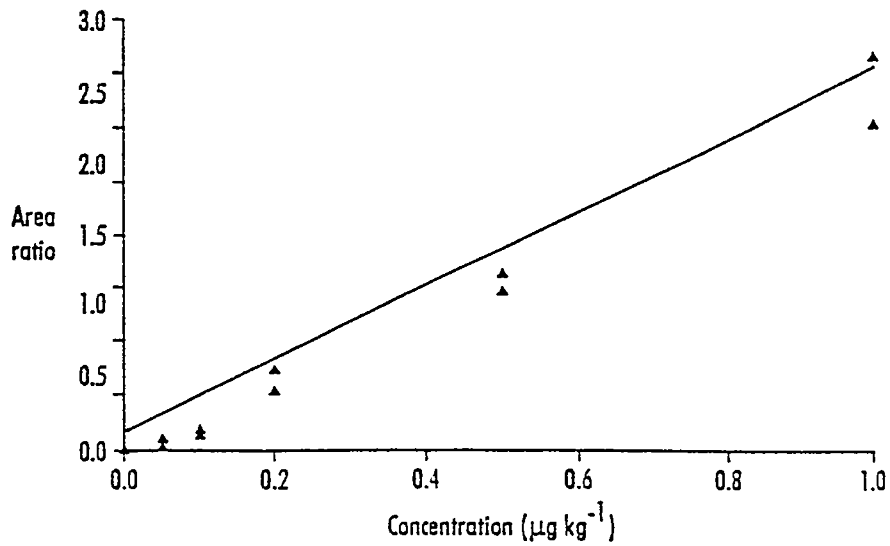


Figure 3.3 Linearity test of analytical method for deethylatrazine in chalk

Precision test

The precision test consisted of the analysis of three batches of duplicate samples of blank, low concentration spiked and high concentration spiked chalk samples. The blank was spiked at 0.05 $\mu g\ kg^{-1}$ (for all compounds except DAA which was spiked at 0.5 $\mu g\ kg^{-1}$) in order to provide an LC-MS response that would allow calculation of the limits of detection. A low level spike of 0.2 $\mu g\ kg^{-1}$ (all compounds except DAA, which was at 1 $\mu g\ kg^{-1}$) and a high level spike of 2 $\mu g\ kg^{-1}$ (all compounds except DAA, which was at 5 $\mu g\ kg^{-1}$) were chosen so as to cover the main concentration range of interest, based on previous analyses of pesticides in chalk samples from the study sites (Clark *et al.* 1992). The samples were spiked the same way as for the linearity test and then analysed by the analytical method described above.

The results are summarised in Table 3.2. The precision test has shown that the analytical method achieves good detection limits and precision, considering the difficult nature of the analysis. No significant bias could be detected for any of the determinands.

3.2 Analytical method for isoproturon and its transformation products

3.2.1 Development of analytical method

The HPLC conditions used by (Forbes *et al.* 1994a) for the analysis of isoproturon were tested and found to provide adequate separation of isoproturon and the TPs of interest (DMI, DDMI and IPA) as follows:

Column: Spherisorb S5 ODS-1 25 cm x 4.6 mm ID.

Mobile phase: 63% methanol 37% 0.1 M ammonium acetate.

Flow rate: 1 ml min^{-1}.

Detector UV absorbance at 240 nm.

Acetone was tested as a solvent for the extraction of isoproturon and its TPs. Three 250 g samples of pesticide-free chalk were spiked at 2 $\mu g\ kg^{-1}$ with isoproturon, DMI, DDMI and IPA, and extracted with acetone as for the atrazine method. The dry extracts were reconstituted in 0.1 ml of 63% methanol, 37% 0.1 M ammonium acetate containing 5 mg l^{-1} of an internal standard (benzanilide). For the performance testing, d_3-isoproturon was custom-synthesised and was used as an internal standard. The extracts and some standards containing 5 mg l^{-1} of the compounds of interest and internal standard were then run on LCMS using the optimised conditions. The results of the experiment are given in Table 3.3. Figure 3.4 shows the thermospray mass spectra of isoproturon and DMI. Figure 3.5 shows the LC-MS mass chromatograms of isoproturon and TPs.

Table 3.2 Performance characteristics of the analytical method for atrazine, simazine and TPs in chalk

Performance characteristic	Spiking level ($\mu g\ kg^{-1}$)	Value of performance characteristic ($\mu g\ kg^{-1}$) for Atrazine	Simazine	DEA	DIA	DAA
LOD		0.040	0.023	0.158	0.063	0.341
Mean	0.05	0.051	0.041	0.034	0.048	0.422
S_w	(DAA 0.5)	0.006	0.004	0.024	0.009	0.051
Mean	0.2	0.190	0.196	0.207	0.168	0.994
S_w	(DAA 1)	0.007	0.015	0.016	0.022	0.102
S_b		0.003	0.020	0.027	0.051	0.271
S_t		0.008	0.025	0.031	0.056	0.290
RSD_t (%)		4	13	15	33	29
Degs F		5	3	3	2	2
Mean	2	1.989	2.219	2.173	2.092	5.200
S_w	(DAA 5)	0.089	0.544	0.550	0.529	1.652
S_b		0.125	0.291	0.142	0.103	1.670
S_t		0.154	0.617	0.568	0.539	2.349
RSD_t		8	28	26	26	45
Degs F		3	4	5	5	3

LOD - Limit of detection as defined by Cheeseman and Wilson (1989)
Mean - Mean concentration of analytical results
S_w - Within batch standard deviation
S_b - Between batch standard deviation
S_t - Total standard deviation
RSD_t - Relative total standard deviation
Degs F - Degrees of freedom

Table 3.3 Testing acetone for extraction of isoproturon and TPs

	Recoveries at 2 µg kg^{-1} (%)			
Sample	Isoproturon	DMI	DDMI	IPA
1	74	74	56	0.5
2	69	68	50	1
3	81	80	*126	0.5
Mean	75	74	53	0.7
RSD(%)	8	8	8	43

* Outlier not included in calculation of mean.

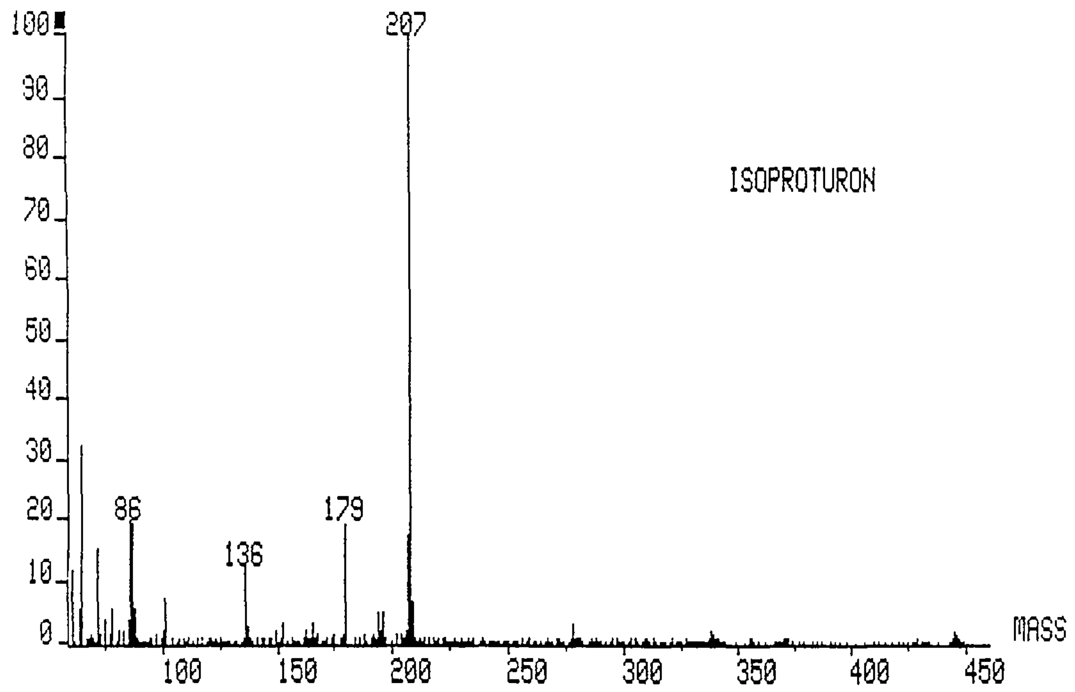
The recoveries for isoproturon and the most important TP (DMI) are reasonable at around 75%. The recovery for DDMI is poorer (50%), but acceptable considering the matrix. It appears that IPA is not recovered to any significant extent. It therefore seems that the analytical method will not be able to analyse for IPA. The available evidence suggests this is a less abundant TP, so the lack of an analytical method for it was not judged critical.

3.2.2 Analytical method for isoproturon and transformation products

A 250 g sample of powdered chalk was spiked with 125 ng of internal standard (d_3 isoproturon) in 10 ml of water and left overnight in the dark to equilibrate. This was then extracted by shaking with 500 ml of acetone for 4 hours at 180 revolutions per minute on an orbital shaker. The chalk was then filtered off using a GF/F glass fibre filter with a GF/D prefilter under vacuum. The acetone extract and associated glassware washings was then concentrated to about 1 ml using a Zymark Turbo-Vap 500 evaporator at a water bath temperature of 40 °C and fan speed C. The extract was evaporated to about 0.2 ml using a gentle stream of nitrogen and warming (45 °C). The extract was passed through a 0.2 µm syringe filter. The extract and syringe filter washings were evaporated to dryness with a gentle stream of nitrogen and warming (45 °C). The extract was reconstituted in 100 µl of 63:37 methanol / 0.1 M ammonium acetate solution. The extract was analysed by LC-MS using the following conditions:

Column:	Spherisorb 5 µm ODS-1 250 x 4.6 mm.
Eluent:	63:37 Methanol / 0.1 M ammonium acetate solution.
Flow rate:	1 ml min^{-1}.
Injection loop:	20 µl.
Ion source:	Plasmaspray operated at 200 °C with 600 µA discharge current.

Typical retention times and ions monitored are shown in Table 3.4.



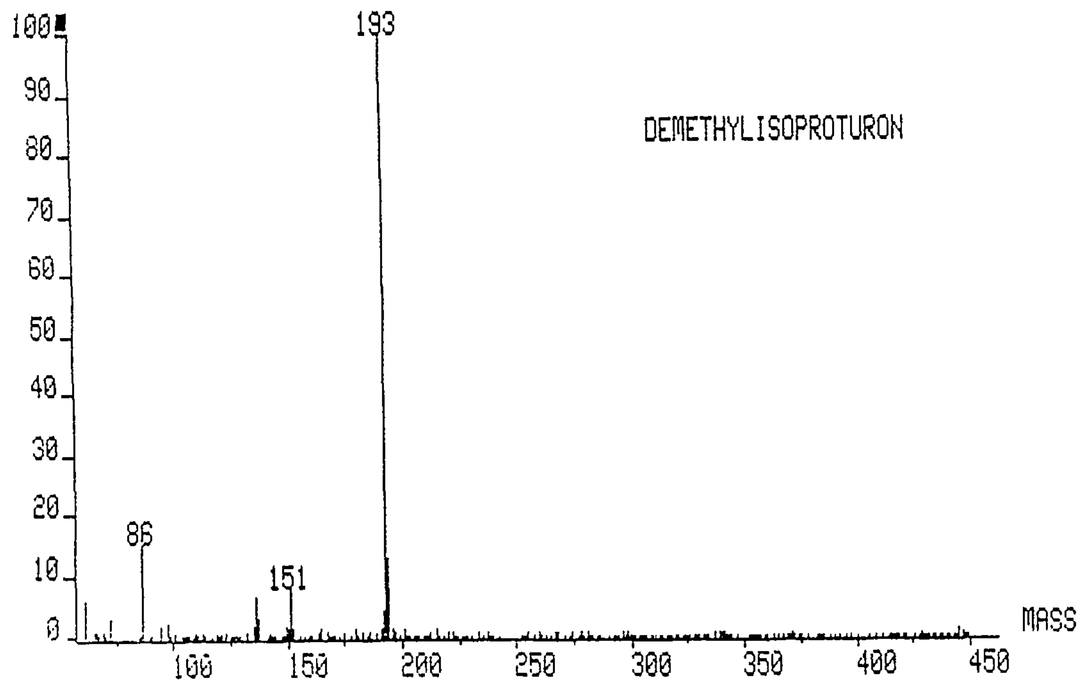


Figure 3.4 Discharge-assisted thermospray mass spectrum of isoproturon and DMI

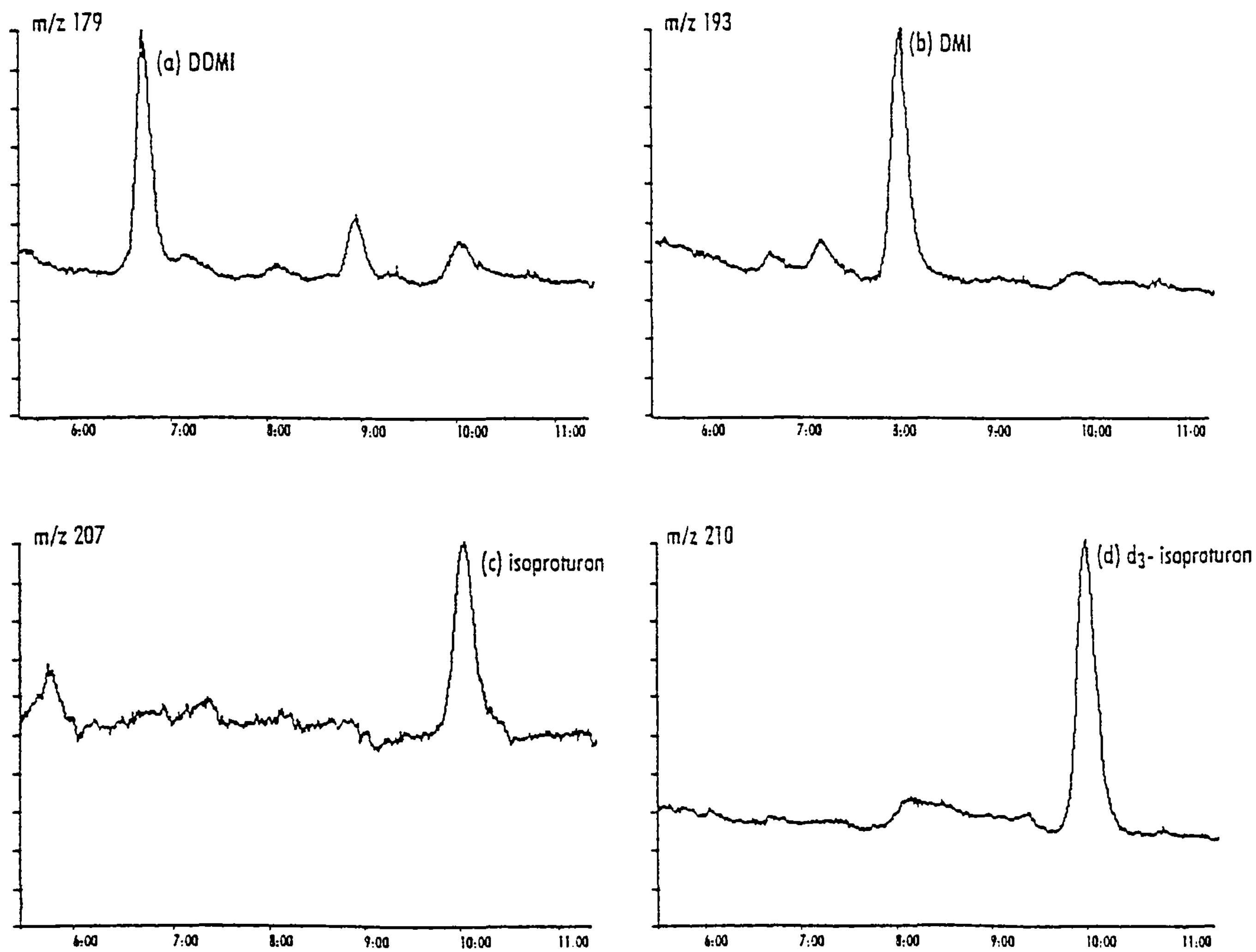


Figure 3.5 LC-MS chromatograms for extract of chalk sample spiked at 0.2 µg kg^{-1} with isoproturon and TPs

Table 3.4 Retention times and ions monitored for isoproturon and its transformation products

Compound	Retention time (min:sec)	m/z monitored
DDMI	6:50	179
DMI	8:10	193
d_3 isoproturon	10:08	210
isoproturon	10:12	207

3.2.3 Validation of the analytical method for isoproturon and transformation products

The performance of the analytical methods were tested in two parts: a linearity test, and a precision test. The linearity test determines the linear range of the analytical method over a wide concentration range (0-10 µg kg^{-1}) and the precision test determines the precision, bias and limit of detection of the analytical method. The method is described in detail in Appendix F.

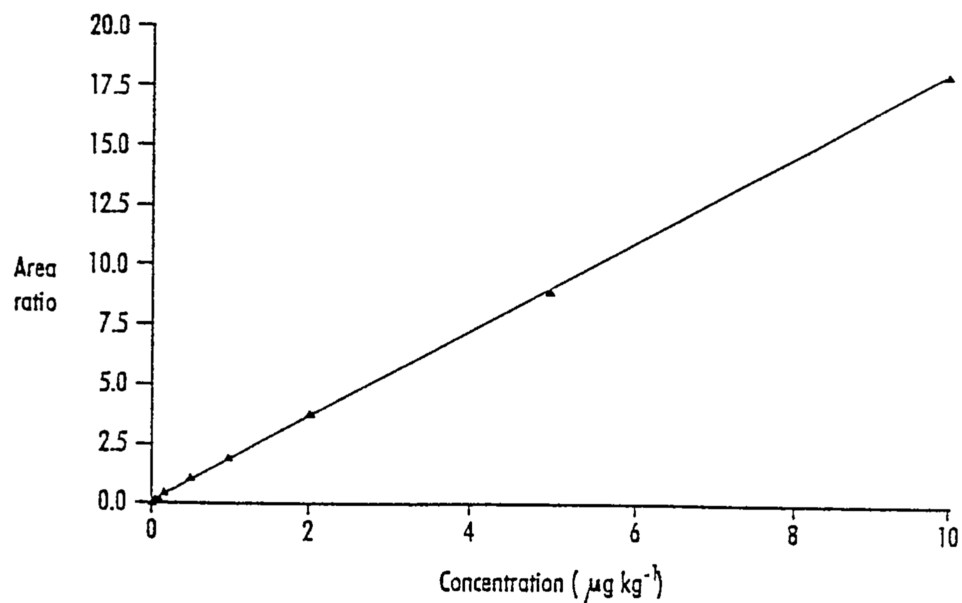
Linearity test

Duplicate 250 g samples of chalk (powdered in a knife mill) were left blank. Further duplicate 250 g samples were then spiked with 0.05, 0.1, 0.2, 0.5, 1, 2, 5 and 10 µg kg^{-1} of isoproturon, DMI, DDMI. The samples were left overnight to equilibrate and then analysed by the analytical method described above.

The calibration curves for isoproturon, DMI and DDMI obtained during this work are shown in Figures 3.6, 3.7 and 3.8 respectively. The calibration curves for all the compounds appeared to be linear over the range from 0.05 to 10 µg kg^{-1}.

Precision test

The precision test consisted of the analysis of three batches of duplicate samples of blank, low concentration spiked and high concentration spiked chalk samples. The blank was spiked at 0.02 µg kg^{-1} in order to provide an LC-MS response that would allow calculation of the limits of detection. A low level spike of 0.2 µg kg^{-1} and a high level spike of 2 µg kg^{-1} were chosen so as to cover the main concentration range of interest, based on previous analyses of pesticides in chalk samples from the study sites (Clark *et al.* 1992). The samples were spiked the same way as for the linearity test and then analysed by the analytical method described above.



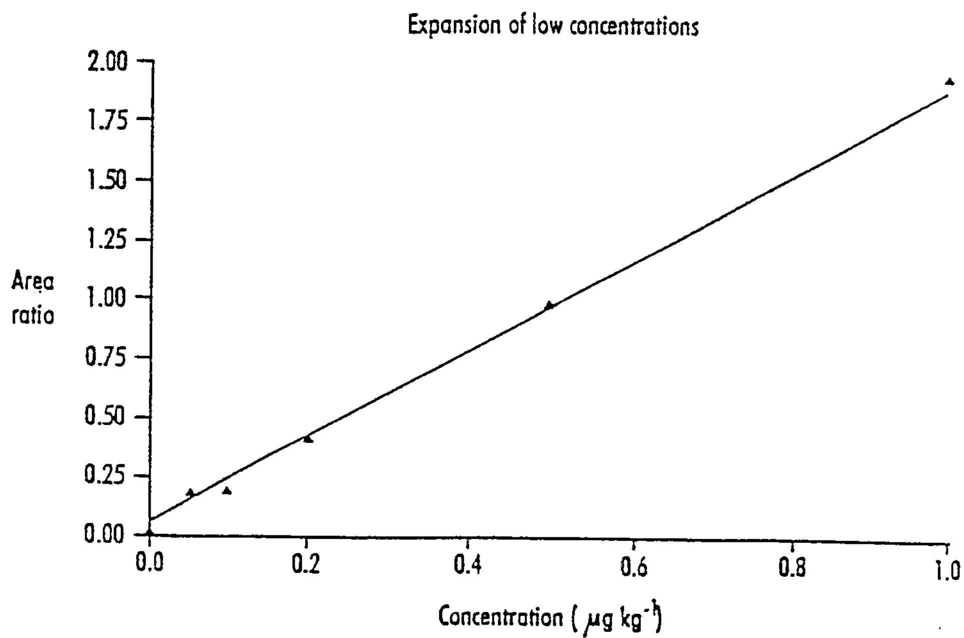
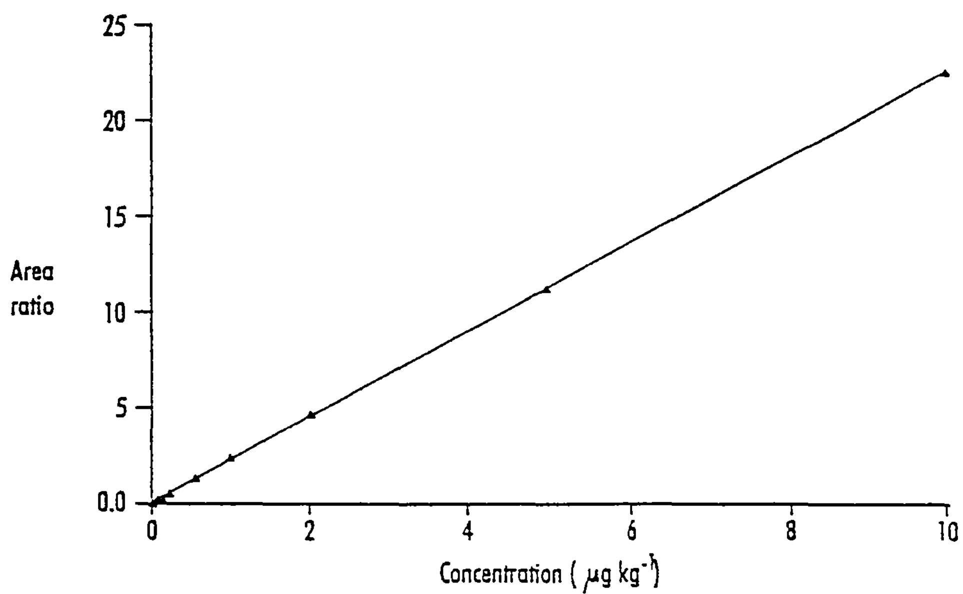


Figure 3.6 Linearity test of analytical method for isoproturon in chalk



Expansion of low concentrations

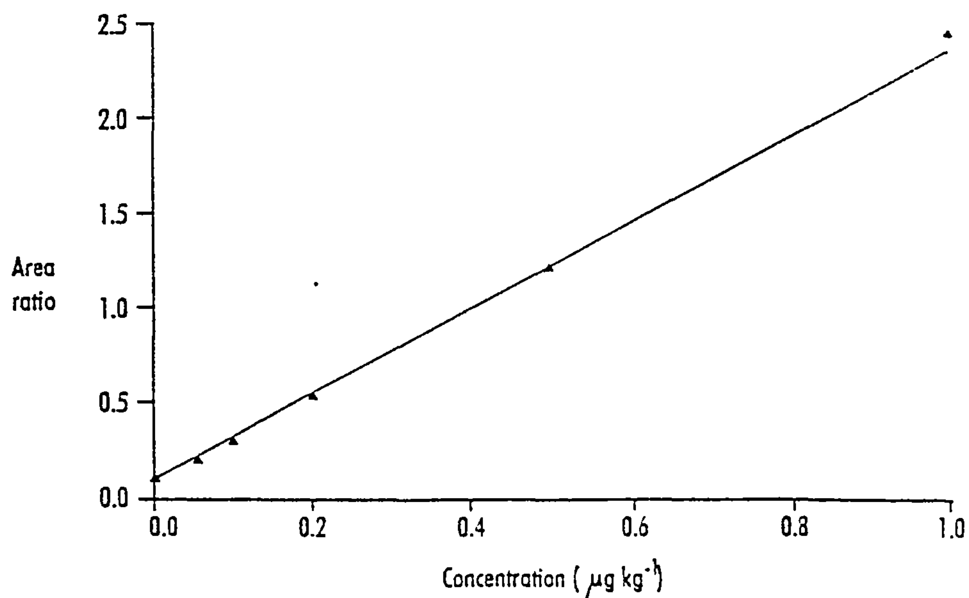
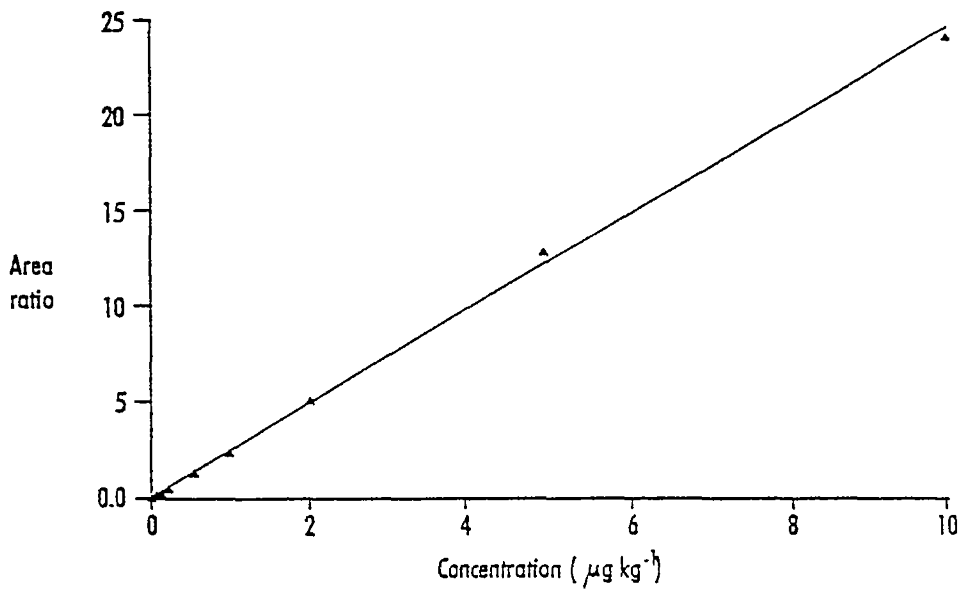


Figure 3.7 Linearity test of analytical method for DMI in chalk



Expansion of low concentrations

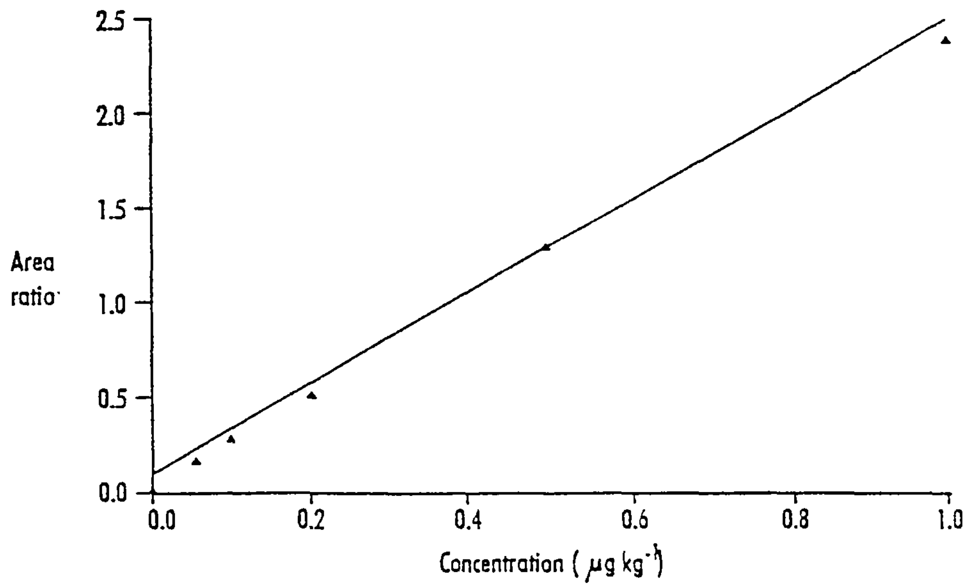


Figure 3.8 Linearity test of analytical method for DDMI in chalk

The results of the ANOVA analysis of the precision test data are summarised in Table 3.5. The precision test has shown that the analytical method achieves good detection limits and precision, considering the difficult nature of the analysis. No significant bias could be detected for any of the determinands at the low and high spiking levels.

Table 3.5 Performance characteristics of the analytical method for isoproturon and TPs in chalk

Performance characteristics	Spiking level ($\mu g\ kg^{-1}$)	Value of performance characteristic ($\mu g\ kg^{-1}$) for		
		Isoproturon	DMI	DDMI
LOD		0.094	0.198	0.090
Results from 'Blank' samples				
Mean	0.02	0.017	0.045	0.045
S_w		0.014	0.030	0.014
Results from low level spiked samples				
Mean	0.2	0.208	0.205	0.212
S_w		0.012	0.004	0.024
S_b		0.014	0.013	0.000
S_t		0.018	0.014	0.024
RSD_t (%)		9	7	11
Degs F		3	2	5
Results from high level spiked samples				
Mean	2	2.063	1.932	1.900
S_w		0.060	0.252	0.352
S_b		0.069	0.000	0.194
S_t		0.092	0.252	0.402
RSD_t		4	13	21
Degs F		3	5	4

LOD - Limit of detection as defined by Cheeseman and Wilson (1989)
Mean - Mean concentration of analytical results
S_w - Within batch standard deviation
S_b - Between batch standard deviation
S_t - Total standard deviation
RSD_t - Relative total standard deviation
Degs F - Degrees of freedom

4. ASSESSMENT OF IMMUNOASSAY KITS

Immunoassays have been widely used for environmental analysis including analysis for pesticides (Watts and Hegarty 1995). A particular advantage of the technique is that only small volumes of water are required for the analysis, so porewater spun from cores can be analysed. The kits tested were the Aquascreen immunoassay kits (IAs) for atrazine and isoproturon supplied by Guildhay, UK. These IAs were tested for:

- accuracy,
- effect of porewater matrix,
- cross-reactivity with TPs.

4.1 The atrazine immunoassay

4.1.1 Precision test

The precision and accuracy of the Aquascreen kit for analysing atrazine in chalk pore-water was tested by running octuplicate assays of chalk porewater blank, and spiked at 0.025, 0.05, 0.073, 0.1 and 0.15 µg l^{-1} along with the associated calibration standards needed to perform the assay. The instructions of the Guildhay Aquascreen kit were followed when carrying out the IA. The results of this test are included in Table 4.1.

Table 4.1 Accuracy of immunoassay for atrazine in porewater

Spiking level (µg l^{-1})	Mean conc. (µg l^{-1})	Sw	RSDw (%)	Recovery (%)
0	0.046	0.009	19	N/A
0.025	0.074	0.007	10	112
0.05	0.096	0.018	19	100
0.073	0.116	0.019	17	96
0.1	0.132	0.013	10	86
0.15	0.164	0.014	9	80

Sw: within-batch standard deviation
RSDw: relative within-batch standard deviation
N/A = not applicable

The results indicate that the chalk porewater used to test the assay already had some low levels of atrazine present. However, the results show sufficient accuracy (for the purpose of carrying out a rough profile of atrazine concentrations within a chalk core) with relative standard deviations of less than 20% and recoveries of better than 79%. This compares with the

accuracy achieved for the Aquascreen kit at similarly low concentrations in model drinking water (Gale *et al.* 1994). Accuracy at higher concentrations is likely to be better (with RSDs of less than 10%) if the results of Gale *et al.* (1994) are followed.

4.1.2 Effect of porewater matrix

The effect of the porewater matrix was tested by carrying out quadruplicate atrazine assays of the atrazine IA kit calibration standards (blank, 0.02, 0.06, 0.12, 0.25, 0.75 and 2 $\mu g\, l^{-1}$), atrazine standards made up in deionised water (the same concentrations) and atrazine standards made up in porewater (the same concentrations).

The optical density data were divided by the highest optical density data point, logit transformed and plotted against the log of concentration (Figure 4.1). The data were statistically analysed and it was found that the calibration line for the standards made in deionised water and the calibration line for the standards made in chalk porewater were adequately described by two parallel lines, allowing the lines to deviate from parallel did not significantly improve the fit with the data. This means (according to Jung *et al.* 1989) that the porewater matrix has no significant effect on the performance of the atrazine immunoassay.

4.1.3 Cross-reactivity test

The cross-reactivity of the TPs was tested as follows: An atrazine calibration was carried out by running all the atrazine IA kit calibration standards in quadruplicate. At the same time quadruplicate assays were performed on a 1 $\mu g\, l^{-1}$ atrazine standard plus 1 and 100 $\mu g\, l^{-1}$ standards of DEA, DIA, DAA, and HYA in deionised water. The concentrations (or equivalent concentrations) of atrazine in the deionised water standards were calculated using the software associated with the Anthos Labtec HtII plate-reader used for the assay. The cross-reactivity of the TPs was then calculated by dividing the atrazine equivalent concentration of the 100 $\mu g\, l^{-1}$ atrazine TP by the detected concentration of the 1 $\mu g\, l^{-1}$ atrazine standard (1.04 $\mu g\, l^{-1}$) and then dividing by one hundred. The 100 $\mu g\, l^{-1}$ standards were used in the calculations since the cross-reactivities were too low to use the 1 $\mu g\, l^{-1}$ standards. The cross reactivities calculated in this way are shown in Table 4.2.

Table 4.2 Cross-reactivities of atrazine TPs

Transformation product	Cross-reactivity (%)
DEA	1
DIA	0.3
DAA	0.04
HYA	0.2

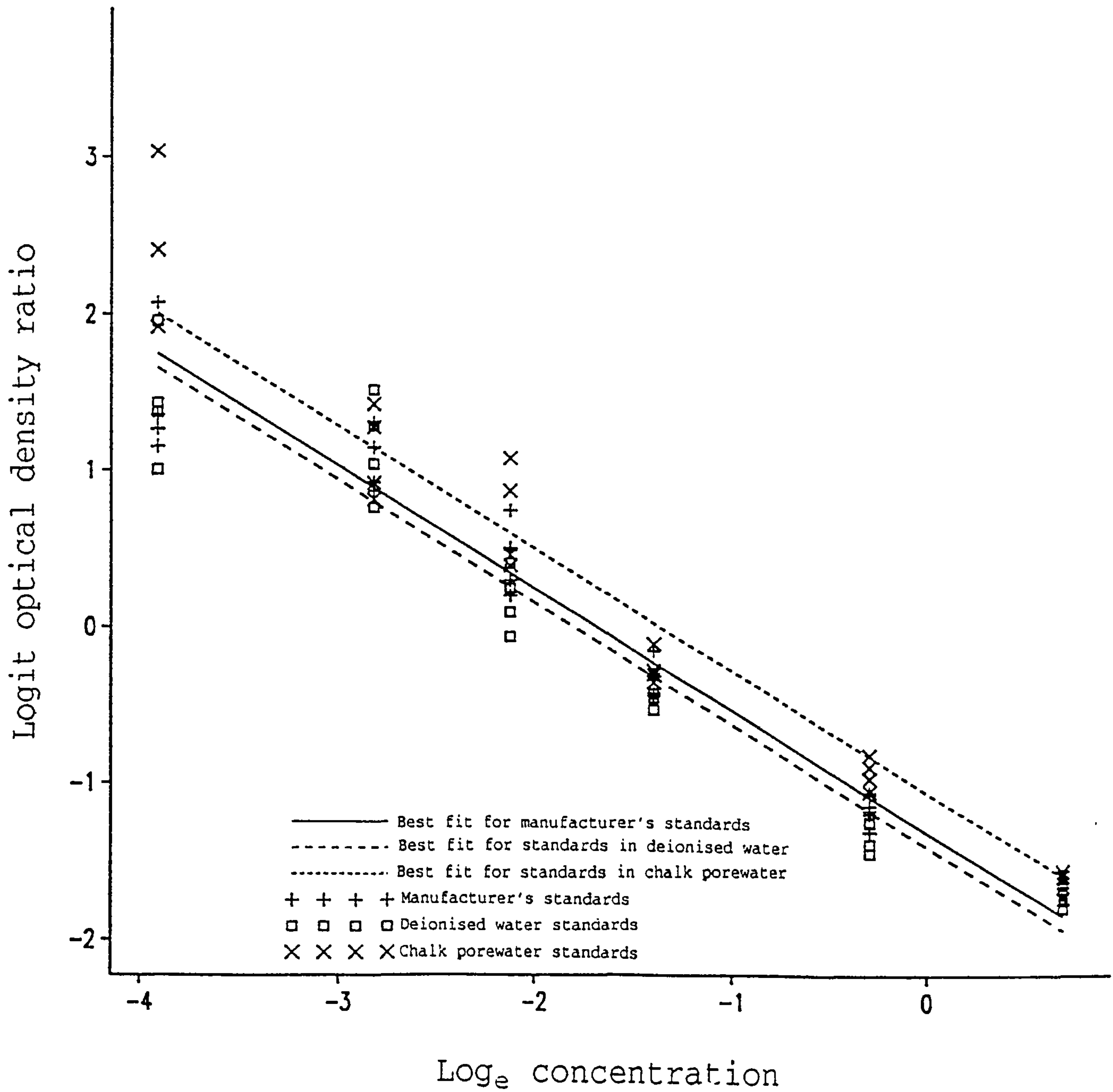


Figure 4.1 Immunoassay of atrazine in porewater

The cross reactivity for DEA (1%) compares with that determined by Gale *et al.* (1994) also using the Aquascreen kit (0.72%). The cross-reactivities with the TPs are very low, showing that this IA is very specific to atrazine. Any IA results obtained using the Aquascreen kit will have little bias due to atrazine TPs present in the sample. This also means the Aquascreen kit is not suitable for monitoring atrazine TPs.

4.2 The isoproturon immunoassay

4.2.1 Precision test

The precision and accuracy of the Aquascreen kit for analysing isoproturon in chalk pore-water were tested by running three batches of quadruplicate assays, at the same concentrations as for atrazine. The instructions of the Guildhay Aquascreen kit were followed when carrying out the IA. The results of this test are included in Table 4.3. They indicate a performance poorer than expected from previous experience (Gale *et al.* 1994).

Table 4.3 Accuracy of immunoassay for isoproturon in chalk porewater

Spiked ($\mu g\ l^{-1}$)	Mean ($\mu g\ l^{-1}$)	Sw	Sb	St	RSDt (%)	Recovery (%)	Degs F
0	0.005	0.017	0.000	0.017	330	-	11
0.02	0.016	0.024	0.015	0.029	175	80	7
0.06	0.015	0.018	0.000	0.018	121	25	10
0.12	0.049	0.030	0.029	0.042	84	41	5
0.25	0.095	0.046	0.047	0.066	70	38	5
0.75	0.423	0.107	0.096	0.144	34	56	5
2	1.887	0.264	0.707	0.755	40	94	2

Mean - Mean concentration of IA results
Sw - Within batch standard deviation
Sb - Between batch standard deviation
St - Total standard deviation
RSDt - Relative total standard deviation
Degs F - Degrees of freedom
N/A - Not applicable

4.2.2 Cross-reactivity test

The cross-reactivities of the isoproturon TPs for the kit were tested, in the same manner as the atrazine kits. These are shown in Table 4.4. The cross-reactivities with the TPs are very low apart from DMI and therefore this IA is fairly specific to isoproturon. Any IA results obtained using the Aquascreen kit may have a small bias if DMI concentrations are approaching the

same concentrations as the isoproturon present. The Aquascreen isoproturon kit is not suitable for monitoring isoproturon TPs since the kit is more sensitive to isoproturon, which is likely to be present in samples at higher concentrations than the TPs.

Table 4.4 Cross-reactivities of isoproturon TPs

Transformation product	Cross-reactivity (%)
DMI	20
DDMI	1
IPA	0.01

5. FIELD TRIALS

5.1 Methodology

Large areas of Europe, for example in Italy and parts of Spain, are covered by alluvial deposits, which represent major aquifers, and can be hundreds of metres in depth. The upper horizons (or surficial deposits) of this material effectively represents the agricultural soil. In the UK the two main aquifers consist of the Chalk and the Sandstone rock. The former outcrops in large areas of the south and south-east of England, the latter occupies major tracts of the north and central England. These regions are all typified by very shallow sub-soils; usually less than 20 cm. There is effectively no barrier between the 'ploughed soil horizon' and the pure consolidated (or semi-consolidated) chalk and sandstone aquifer material, these conditions are somewhat atypical of those in many other European countries.

The unsaturated zone above the water table is made up almost entirely of semi-consolidated rock matrix. In Europe it is therefore possible to investigate a very shallow depth of the unsaturated zone by the excavation of trial pits. In the UK a similar experiment would often necessitate the use of a drilling rig, the advantage of which is that it is possible to investigate a large depth of the unsaturated zone. The costs incurred by these two methods are obviously vastly different and adoption of the latter methodology severely restricts the number of sites which can be investigated. This is not considered to be a problem in this project however, since the prime aim is to obtain samples of the unsaturated zone to validate methodology, both analytical and sampling, rather than undertake a long-term monitoring programme. It is therefore quite sufficient to undertake **one** drilling exercise to obtain a small number of boreholes (drilled through the full extent of the unsaturated zone and into the water table), rather than repeatedly revisit a given site over the period of the contract.

The UK interpretation of the nomenclature for the study is shown in Figure 5.1. A major problem identified is the ability to undertake analyses of pesticide in solid aquifer material from the unsaturated zone. A study of the attenuation of pesticides within the soil horizon, was not required under the original aims of this Project. This type of work would obviously entail the use of suction samplers, together with soil chemical and physical measurements, and would obviously be important if the aim of the work were to establish the quantities of a given pesticide which could leach into the underlying aquifer. WRc are not therefore including such work within the field trials, since it is considered outside the remit of the Project.

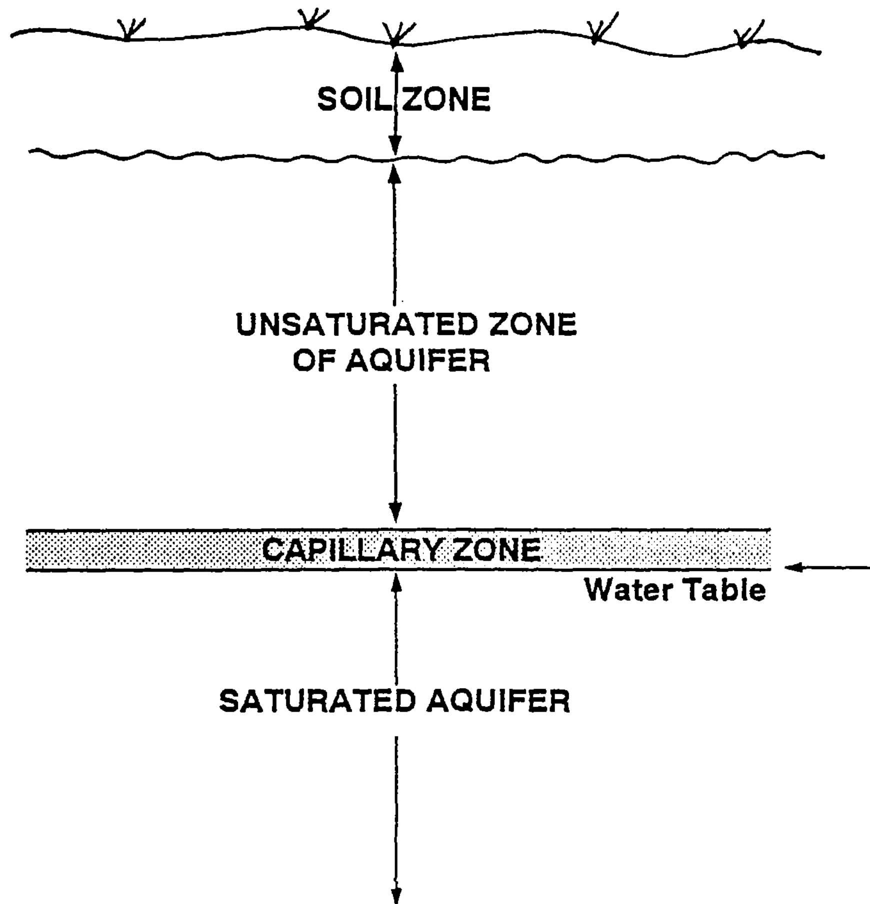


Figure 5.1 Aquifer zones

Table 5.1 Herbicide loading data for Field 57, Institute for AFRC, Compton, Berkshire

Harvest Year	Crop	Herbicide Brand name	Manufacturer	Active ingredients	Quantity active ingredient	Area under crop (and sprayed) (ha)	Loading rate of herbicide (ha^{-1})	Amount active ingredient ha^{-1} (kg)
1989	Maize	Gesaprim 500L	Ciba Geigy	atrazine	$500 g l^{-1}$	13.37	3 l	1.5
1990	Maize	Gesaprim 500L	Ciba Geigy	atrazine	$500 g l^{-1}$	13.37	3 l	1.5
1991	Maize	Gesaprim 500L Lentagran	Ciba Geigy	atrazine pyridate	$500 g l^{-1}$	13.37	1.5 l 1.5 kg	0.75
1992	Maize	Gesaprim 500L Lentagran	Ciba Geigy	atrazine pyridate	$500 g l^{-1}$	13.37	1.5 l 1.5 kg	0.75
1993	Maize	Gesaprim 500L Lentagran	Ciba Geigy	atrazine pyridate	$500 g l^{-1}$	13.37	1.5 l 1.5 kg	0.75
1994	Beans			none				

Figure 5.3 General location map showing AFRC Compton

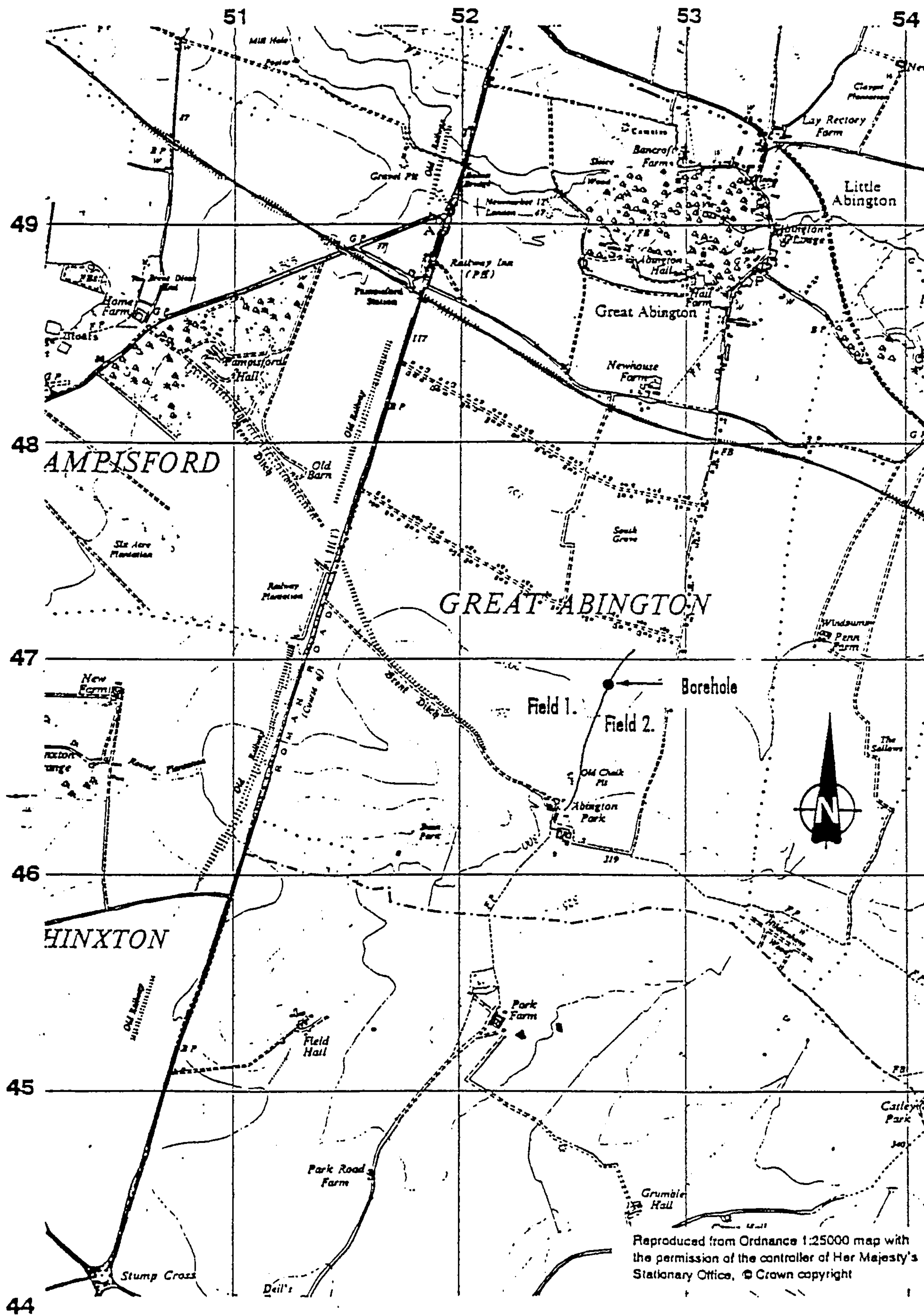


Figure 5.6 Location of borehole at Great Abington

Table 5.2 Herbicide loading data for Fields 1 and 2, Abington Park Farm, Great Abington, Cambridge

Harvest Year	Crop	Active Ingredients	Date applied	Active ingredient applied ha^{-1}
		FIELD 1		
1986	Winter Wheat	triallate	14.11.86	1.6 kg
		isoproturon	15.11.86	1.75 kg
1987	Winter Wheat	triallate	18.11.87	1.6 kg
		isoproturon		1.5 kg
1988	Spring Wheat	thifensulfuron methyl	25.05.88	0.35 kg
		metsulfuron methyl		3.4 kg
1989	Sweet Peas	terbutyrin	14.03.89	1.0 kg
		trietazine		1.0 kg
1990	Winter Wheat	triallate	01.12.90	1.6 kg
		isoproturon		0.9 kg
		trifluralin		0.6 kg
1991	Winter Barley	triallate	28.11.91	1.9 kg
1992	Set aside	-	-	-
1993	Set aside	-	-	-
1994	Winter Barley	triallate	-	1.9 kg
		FIELD 2		
1986	Winter Barley	triallate		1.0 kg
		isoproturon		1.0 kg
1987	Winter Barley	triallate		2.0 kg
1988	Winter Barley	isoproturon		0.6 kg
		trifluralin		0.4 kg
1989	Spring Barley	glyphosate		0.9 kg
		triallate		1.5 kg
1990	Winter Barley	isoproturon		1.2 kg
		trifluralin		0.8 kg
1991	Winter Beans	carbetamide		1.8 kg
		simazine		0.5 kg

Note: Run-off from fields 1 and 2 collect in the soakaway ditch where the drilling was located.

5.3 Drilling

5.3.1 Drilling Methodology

Drilling work in the Chalk was completed using unsaturated zone core-profiling techniques. The drilling works were subcontracted to Wimpey Environmental Limited. A 'U-100' percussion drilling rig was used to remove samples, using aluminium core barrel liners to the full depth of each borehole. One hundred percent chalk recovery was specified in the drilling contract. Two boreholes were sited at Compton and one at Abington Park Farm; all three were drilled to a depth of 22 metres. The two boreholes at Compton were finished approximately 14 metres below the water table, borehole casing was then installed to allow future groundwater monitoring The borehole at Abington was completed within the unsaturated zone of the aquifer.

At the Compton site a replicate of the first borehole was drilled approximately 10 metres from the first. This distance was selected to keep natural variations in herbicide concentrations between the two boreholes as small as possible, whilst at the same time minimising the effects that pumping one borehole would have on the other. The borehole selected for the piezometer installation was logged geophysically for DT, calliper and conductivity, to identify fissures and flow patterns within the borehole. The logging was then used to set the depths of the three piezometers in the nested borehole, so that future monitoring could take into account both fissure (fast flow) water quality and matrix (slow) flow.

The use of drilling muds and water was avoided to prevent contamination of the recovered material. All drilling equipment and casing was steam cleaned prior to commencing drilling work. To reduce the risk of cross-contamination as each sample was cored by the drilling rig, it was extruded into aluminium foil, wrapped in a double layer of polythene layflat tubing, then sealed and frozen on-site. The samples were then transported to the laboratory for analysis.

5.4 Validation of analytical methods

LCMS and immunoassay techniques were used to analyse the solid aquifer material obtained during drilling. Whilst the LCMS extraction is undertaken on a sample of the solid aquifer material, immunoassay requires a sample of porewater to be extracted from the core. Kits for atrazine and isoproturon were available for use in the field trials. Each of the '60 cm' cores from Compton and Abington Park was subsampled and a sample of porewater collected for analysis by immunoassay using centrifugation at 4 °C. Details of the sampling and laboratory protocols used are given in Appendices B and C. ELISA kits are not currently available specifically for TPs of the pesticides concerned, although the presence of these compounds may enhance the readings obtained for the parent compound.

A total of 12 core samples were analysed by LCMS for each parent pesticide. Spiked and blank samples were included for Quality Assurance/Quality Control purposes for each borehole and these samples were additional to these numbers. The sub-samples taken from each core for mass spectrometry analysis were selected on the basis of the immunoassay data.

5.4.1 Results of profiling Isoproturon and its major transformation products

The results of the profiling undertaken by immunoassay and LCMS are detailed in Table 5.3. Figure 5.7 and Figure 5.8, provide a graphical representation of the data. As explained previously the two test sites have been used in previous (but limited) pesticide profiling studies. In the work undertaken in 1991, a number of cores from a borehole sited within the soakaway ditch at Abington Farm (approximately 5 metres from the new borehole) were analysed by LCMS. Figure 5.7 provides a comparison of the data obtained at that time and the new profiling data obtained using immunoassay. It is known that isoproturon has not been re-applied to either Fields 1 or 2 in the period between 1991 and 1994. The profiles appear to indicate that the main 'peak' concentration of isoproturon has moved by approximately 2-3 metres, down the profile. Figure 5.8 presents a comparison of the LCMS and immunoassay data obtained in 1994. There is very good agreement between the two sets of data, with both low and high values confirmed by each method. The benefits of undertaking profiling using immunoassay are clearly demonstrated. A high level of detail is possible with this technique for a fraction of the cost involved in undertaking LCMS analysis. Although the field site has been subject to repeated applications of isoproturon over a period of at least six years, no detectable quantities of the main transformation products (DDMI and DMI) were identified. This would seem to suggest that either degradation of the parent compound has not occurred within the conditions prevalent within the unsaturated zone of the aquifer, or that the half-life of the TP is extremely short.

The work at Abington has provided quality material for successful validation of the new analytical method at low levels of analytical detection. The validity of using immunoassay as an accessory tool to more expensive and time consuming LCMS has been clearly shown.

5.4.2 Profiling Atrazine and its major transformation products

The results of the profiling undertaken by immunoassay and LCMS are detailed in Table 5.4, Table 5.5 and Table 5.6. For easy comparison with borehole depth, LCMS and immunoassay results have been appended to the borehole logs where appropriate. LCMS analysis was undertaken on each core for atrazine, and its major TPs. Immunoassay analysis was undertaken on the chalk core for 'total triazines' although the kit used was particularly sensitive to atrazine. (cross-reactivities with other triazines were very low).

Table 5.5 Drilling and Analysis Records for Borehole 2 AFRC, Compton, Berkshire 1994

Core No	Depth (m)	% spun porewater BH1(weight)	Atrazine in porewater in BH1 by .A (µg l^{-1})	% spun porewater (weight)	Atrazine in porewater in BH2 by I.A (µg l^{-1})
1	0 - 0.6	3.9	0.18	3.9	0.58 /0.52 Rpt
2	0.6 - 1.2	3.4	0.37	3.4	1.09
3	1.2 - 1.8	0.2	0.24	0.2	0.78
4	1.8 - 2.4	3.2	0.70	3.2	0.41 / 0.43 Rpt
5	2.4 - 3.0	5.9	0.75	5.9	0.71
6	3.0 - 3.6	3.7	0.56	3.7	0.37
7	3.6 - 4.2	5.5	0.59	5.5	0.35 / 0.33 Rpt
8	4.2 - 4.8	5.6	0.65	5.6	0.27
9	4.8 - 5.4	5.2	0.67 / 0.60Rpt	5.2	0.08 / 0.07 Rpt
10	5.4 - 6.0	5.5	0.56 / 0.56 Rpt	5.5	0.08 / 0.03 Rpt
11	6.0 - 6.6	7.1	0.47	7.1	0.02
12	6.6 - 7.2	9.2	0.66	9.2	0.47
	Duplicate	9.5	0.74	9.5	0.43 / 0.44 Rpt
13	7.2 - 7.8	12.2	0.58	12.2	0.87
14	7.8 - 8.4	12.6	0.82	12.6	1.08
15	8.4 - 9.0	15.2	0.67	15.2	1.11
16	9.0 - 9.6	13.8	0.77	13.8	1.35
17	9.6 - 10.2	12.6	0.51	12.6	1.70
18	10.2 - 10.8	14.5	0.33	14.5	1.09
19	10.8 - 11.4	15.3	0.34	15.3	1.21 / 1.15 Rpt
20	11.4 - 12.0	14.5	0.35	14.5	0.83
	Duplicate	15.1	0.34	15.1	0.89
21	12.0 - 12.6	15.4	0.38	15.4	0.85
22	12.6 - 13.2	15.1	0.32	15.1	0.85
23	13.2 - 13.8	14.7	0.36	14.7	0.65
24	13.8 - 14.4	16.6	0.33	16.6	0.59
25	14.4 - 15.0	13.3	0.29	13.3	0.55
26	15.0 - 15.6	13.7	0.26	13.7	0.62 / 0.58 Rpt
27	15.6 - 16.2	11.7	0.22	11.7	0.56
28	16.2 - 16.8	15.6	0.21	15.6	0.54
29	16.8 - 17.4	13.0	0.25	13.0	0.51
30	17.4 - 18.0	16.2	0.29	16.2	0.45
31	18.0 - 18.6	14.5	0.33	14.5	0.41
31	Duplicate	13.9	0.36	13.9	0.41
32	18.6 - 19.2	11.8	0.42	11.8	0.40
33	19.2 - 19.8	10.1	0.40	10.1	0.40
34	19.8 - 20.4	12.2	0.29	12.2	0.30

Core No	Depth (m)	% spun porewater BH1(weight)	Atrazine in porewater in BH1 by .A (µg l^{-1})	% spun porewater (weight)	Atrazine in porewater in BH2 by I.A (µg l^{-1})
35	20.4 - 21.0	3.8	0.37	3.8	0.36
36	21.0 - 21.6	9.8	0.35	9.8	0.36
37	21.6 - 22.2	14.9	0.25	14.9	0.29

Table 5.6 Analysis of BH2 at Compton by Immunoassay and LCMS

Depth (m)	Atrazine in porewater in BH2 by I.A (µg l^{-1})	Atrazine in Chalk in BH2 by LCMS (µg kg^{-1})	DEA in Chalk in BH2 by LCMS (µg kg^{-1})	D1A in Chalk in BH2 by LCMS (µg kg^{-1})	DAA in Chalk in BH2 by LCMS (µg kg^{-1})	Simazine in Chalk in BH2 by LCMS (µg kg^{-1})
0 - 0.6	0.58 /0.52					
0.6 - 1.2	1.09	0.44	0.61	0.97	0.76	<0.02
1.2 - 1.8	0.78					
1.8 - 2.4	0.41 / 0.43	0.13	0.23	0.43	<0.34	<0.02
2.4 - 3.0	0.71	0.16	0.40	0.19	0.57	<0.02
3.0 - 3.6	0.37					
3.6 - 4.2	0.35 / 0.33					
4.2 - 4.8	0.27					
4.8 - 5.4	0.08 / 0.07	0.08	<0.15	0.07	0.41	<0.02
5.4 - 6.0	0.08 / 0.03					
6.0 - 6.6	0.02	0.13	0.23	0.10	0.34	<0.02
6.6 - 7.2	0.47					
Duplicate	0.43 / 0.44					
7.2 - 7.8	0.87					
7.8 - 8.4	1.08	0.43	0.60	0.15	0.47	<0.02
8.4 - 9.0	1.11					
9.0 - 9.6	1.35	0.44	0.54	0.17	0.47	<0.02
9.6 - 10.2	1.70	0.47	0.71	0.12	0.51	<0.02
10.2 - 10.8	1.09					
10.8 - 11.4	1.21 / 1.15	0.38	0.18	0.10	0.38	<0.02
11.4 - 12.0	0.83					
Duplicate	0.89					
12.0 - 12.6	0.85					
12.6 - 13.2	0.85	0.24	0.47	0.16	0.41	<0.02
13.2 - 13.8	0.65					

Depth (m)	Atrazine in porewater in BH2 by I.A ($\mu g\ l^{-1}$)	Atrazine in Chalk in BH2 by LCMS ($\mu g\ kg^{-1}$)	DEA in Chalk in BH2 by LCMS ($\mu g\ kg^{-1}$)	D1A in Chalk in BH2 by LCMS ($\mu g\ kg^{-1}$)	DAA in Chalk in BH2 by LCMS ($\mu g\ kg^{-1}$)	Simazine in Chalk in BH2 by LCMS ($\mu g\ kg^{-1}$)
13.8 - 14.4	0.59					
14.4 - 15.0	0.55					
15.0 - 15.6	0.62 / 0.58					
15.6 - 16.2	0.56	0.26	0.65	0.12	0.45	<0.02
16.2 - 16.8	0.54					
16.8 - 17.4	0.51					
17.4 - 18.0	0.45					
18.0 - 18.6	0.41					
Duplicate	0.41					
18.6 - 19.2	0.40					
19.2 - 19.8	0.40	0.15	0.23	0.29	0.30	<0.02
19.8 - 20.4	0.30					
20.4 - 21.0	0.36					
21.0 - 21.6	0.36					
21.6 - 22.2	0.29					
Chalk Cntrl Spike 0.5 $\mu g\ kg^{-1}$		0.37	0.31	0.21	0.32	0.30
Chalk Spike FD (1.0 $\mu g\ kg^{-1}$ DAA, Others 0.2 $\mu g\ kg^{-1}$)		0.19	0.23	0.33	0.58	0.19
Detection limits determined by performance testing		**0.04**	**0.15**	**0.06**	**0.34**	**0.023**

The two boreholes drilled at AFRC Compton in 1994 were sited at the edge of the field, which drains excess runoff and field drainage water. It was expected that recharge pathways to the unsaturated zone should therefore be fairly well developed. The analysis by immunoassay of the porewater extracted from the chalk supports such a case. Figure 5.9 provides a comparison of the immunoassay results obtained from one of these boreholes and an earlier borehole drilled approximately twenty metres away in 1993. The results from this work and that carried out on the two boreholes drilled in 1994 (Figure 5.10), provide an extremely detailed picture of atrazine migration from the soil surface through the unsaturated zone and into the groundwater. The profiles represents five years of repetitive atrazine application, during this time the atrazine has moved through 11 metres of relatively unfractured chalk. The persistence of atrazine in the surface soil after application (1993) is clearly shown. However, it is interesting to note that despite receiving large quantities of drainage water from the surrounding field over a period of two years after its application atrazine has not been completely leached from the surface and is persisting at levels of 0.6 $\mu g\ l^{-1}$ (approximately 1.6 $\mu g\ kg^{-1}$). The profiles produced by I.A in 1993 and then subsequently in 1994 exhibit very

good correlation. In 1989 and 1990, application rates were double those presently used; the large peaks of atrazine detected near the water table between 8 and 11 metres are thought to represent the atrazine applied at this time. The data clearly shows that atrazine is persisting in the unsaturated zone and moving through the profile as discrete peaks, adsorption and degradation rates are not sufficient to cause 'smoothing' of the profile. Although the results obtained by LCMS (which represent $\mu g\ kg^{-1}$ of solid aquifer material) can not be compared directly with those of immunoassay, the LCMS results mimics the trend of the immunoassay profile (Figure 5.11). The core samples obtained from the two boreholes provided excellent material for testing the LCMS method, as with the isoproturon method low levels of detection have been achieved. Of interest to pesticide fate and behaviour studies is that concentrations of DAA and DIA actually exceeded that of the parent compound, and also mimic the concentration profile of atrazine itself.

Atrazine herbicides are increasingly detected in groundwaters around the UK. The results from the profiling work undertaken in the Chalk aquifer corroborate the theory that atrazine herbicides and some of its major TPs are sufficiently persistent to migrate through a considerable thickness of unsaturated zone through the intergranular pore spaces to the water table and enter the groundwater. Degradation is sufficiently slow that applications can be mapped as distinct pulses of product moving down the profile.

The successful validation of the LCMS methodology for atrazine and its TPs has been demonstrated. The very valuable input of immunoassay techniques to pesticide profiling has also been proven.

Monitoring of the boreholes was undertaken over a 7 month period, beginning in November 1994 and finishing in May 1995. Groundwater samples were initially collected on a monthly basis, this was followed by sample collection every two months. It was important that the boreholes were sampled over a reasonable period of time to identify any annual fluctuations or patterns in water quality, and to determine whether the results from such precise monitoring is different from more coarse monitoring. Analytical data from the groundwater sampling programme is shown in Table 6.1. Groundwater samples were analysed for atrazine and its major transformation products.

Table 6.1 Analysis of groundwater, Compton by GCMS and LCMS

Determinand	DHA				DAA			
Date	Nov 94	Dec 94	Mar 95	May 95	Nov 94	Dec 94	Mar 95	May 95
BH1	nd	nd	nd	nd	0.029	<0.01	<0.01	<0.01
BH2 - 11m	nd	nd	nd	nd	0.059	0.05	<0.01	<0.01
BH2 - 17m	nd	nd	nd	nd	0.072	<0.01	<0.01	<0.01
BH2 - 22m	nd	nd	nd	nd	0.048	<0.01	0.02	<0.01
Determinand	**Simazine**				**DIA**			
Date	Nov 94	Dec 94	Mar 94	May 94	Nov 94	Dec 94	Mar 95	May 95
BH1	0.123	0.23	0.04	0.04	0.047	0.04	<0.01	<0.01
BH2 - 11m	0.016	<0.01	0.01	0.03	0.039	0.04	<0.01	<0.01
BH2 - 17m	<0.016	<0.01	<0.01	0.03	0.035	0.03	<0.01	<0.01
BH2 - 22m	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	<0.01
Determinand	**Atrazine**				**DEA**			
Date	Nov 94	Dec 94	Mar 95	May 95	Nov 94	Dec 94	Mar 95	May 95
BH1	0.376	0.37	0.37	0.53	1.037	0.89	0.91	1.31
BH2 - 11m	2.295	2.09	2.04	0.57	2.165	2.64	2.57	2.08
BH2 - 17m	0.791	0.85	0.84	1.05	1.681	1.85	1.72	2.50
BH2 - 22m	0.454	0.43	0.42	0.38	1.210	1.42	1.07	1.83

Key: All results in $\mu g\ l^{-1}$, nd - not detected, DHA not detected in any sample or standard

6.1 Sampling methodology

Of critical importance when compiling and interrogating water quality data is that sampling methodology is consistent over the period of sampling. It is important that stagnant water in the boreholes is removed prior to collecting the sample for analysis and that collection vessels and storage methods are suitable to minimise chemical and bacteriological changes in the sample prior to analysis. If attention is not paid to such detail, variability in concentration levels introduced during the sampling process could exceed those caused by variation in the aquifer system. To minimise data errors caused by poor sampling techniques WRc have devised and thoroughly tested a sampling methodology for use in narrow diameter observation boreholes. The same methodology could be used in larger diameter boreholes, however, it may then be appropriate to use a larger diameter pump to ensure comprehensive purging of the borehole prior to sampling. A copy of this protocol is provided in Appendix D.

Extensive water level data (Figure 6.2) has been obtained for a borehole located approximately 3 km along the valley (East Ilsley) from the test site. The borehole is at a similar elevation to those at Compton. The data shows annual variation in water levels for a period covering thirty years and indicates that annual movement in the water table often exceeds 14 metres. During the period of this study water levels have risen by nearly 8 metres (green squares on the graph show Compton water level data in relation to that at East Ilsley). The detected variations in water quality data for atrazine and its TPs are plotted in Figure 6.3, a comparison of the concentrations of atrazine detected in the chalk porewater by immunoassay and in the groundwater at similar depths (LCMS) is shown in Figure 6.4.

6.2 Water Quality Data

A number of broad observations can be made:

- The rising water table has produced some stark differences in water quality data and shows the importance of undertaking any monitoring programme over at least an annual cycle. One-off concentration measurements can be extremely misleading.

- Levels of atrazine and its TPs detected in the 'standard borehole' design (BH2) provide bulk water quality data that is comparable with an average of the levels detected in the three piezometers, although it closely mimics the levels detected in the lower piezometer in BH1, which was set at a depth in the borehole thought to be controlled by matrix flow.

- The nested design of BH1 provides specific information on pesticide movement within a given depth profile, and not just a bulk 'average' value.

- Concentration data clearly varies with the changing water table and shows the importance of the interaction between the solid aquifer and the groundwater.

7. CONCLUSIONS

Prior to the commencement of this project there has been very little published work in the UK which deals with the fate of pesticides, in particular in the unsaturated zones of the aquifer. This study of pesticides and their transformation products would have been impossible without the development and validation of new analytical methodology as part of the project. Immunoassay has been shown to be a powerful semi-quantitative tool for profiling a given pesticide in the unsaturated zone of an aquifer. The low volumes of porewater required for each analysis mean it often provides the only method of analysis available for analysing water held in-situ in the rock matrix.

7.1 Review of transformation products and formulation chemicals

Environmental TPs have been identified of many high-usage pesticides. Several have been reported in water sources in the UK at significant levels (close to or exceeding 0.1 $\mu g\ l^{-1}$), for example, deethylatrazine and other TPs of atrazine and simazine. Many others have been detected in surface and ground waters in other countries.

Little information is available on the physico-chemical properties of TPs, and it has not been possible to assess their potential to contaminate drinking water sources. More information on the physico-chemical properties of these compounds is needed. Information on the ecotoxicological properties of the TPs is also generally unavailable. It is likely that, if a systematic assessment of the environmental effects of pesticide TPs is to be undertaken, the task will need to be reduced in complexity and magnitude through the use of simplifying assumptions or prioritisation procedures to identify the most significant TPs.

General information on the chemicals used in FCs was obtained, as well as usage data for adjuvant active ingredients. A detailed review of the FCs used in pesticide products was not possible because the information required is held in confidence by regulatory authorities and the pesticide manufacturers. It is suggested that the Environment Agency should access this information through involvement in the regulatory approval mechanisms. FCs include some widely used chemicals, so the environmental impact associated with pesticide use may not be significant. However, it is not possible to confirm this without detailed compositional and usage information.

7.2 Isoproturon Profiling

Isoproturon is subject to relatively rapid degradation in the soil and beneath fields to which pesticides have been applied at recommended rates and under normal conditions very low concentrations of pesticides are detected below the soil in the unsaturated zone of the Chalk (Chilton *et al.* 1993). Previous profiling work in the Chalk (Clark *et al.* 1995) has shown that in normal loading and flux conditions uron pesticides would not be expected to move very deep (2 - 4 m) in the profile. The main mechanism or primary flow path for movement in this scenario would be via intergranular flow paths, i.e. slow matrix movement.

The borehole as previously noted was drilled through the bottom of a blind ditch which drains excess run-off and field drainage water. It might be expected, therefore, that recharge pathways from the ditch would be more highly developed than from the surface of a continually cultivated field. The results support such a case with isoproturon finding relatively quick *routes* to the unsaturated zone and penetrating to a depth of greater than twenty metres. It appears that whilst adsorption and degradation processes are active in the top few metres of the profile (where organic matter levels and oxygen allow for richer bacterial populations and enhanced degradation rates); profiled concentrations of isoproturon are low. Under the right conditions slugs of pesticide are moving to greater depths in the and discrete peaks, which then effectively bypass the microbiological processes that are active in the top few metres of the aquifer. Equally the top few metres of the profile may have been flushed by field run-off and rainfall that has occurred in the twelve months between the last application and the drilling programme. By either of these two mechanisms, pesticides relatively near the surface may have been removed leaving higher concentrations at depths below 3 metres. The occurrence of discrete peaks in the pesticide profile and the lack of the type of a smooth profile that is the norm, for example in nitrate unsaturated zone work, can be explained by the different nature of the non-conservative pesticide compounds. It is suggested that they may be concentrated where organic carbon contents lead to greater adsorption, and may be expected to be present in relatively large doses in microfissures.

7.3 Atrazine Profiling

Beneath the soil and root zone the data shows that atrazine is mobile and persistent in its own right and the profiling data has shown that many of its major TPs share the same properties that enable them, in areas of high hydraulic flux to migrate into groundwater supplies. In particular DEA and DAA are present in the lower depths of the profile, at concentrations that are greater than the parent product.

There are some differences between the two profiles at Compton, despite the fact that they were only drilled ten metres apart. This is thought to be due to a combination of lateral inhomogeneities in the Chalk (noted during drilling), soil conditions and application and uptake of the pesticides.

The mass of pesticide which is leached from the soil gravitates vertically down toward the water table, the solute moving through the fissure systems subjected to minimal delay. Solute moving through the Chalk matrix will, however, move very slowly, at a rate of about 0.4 metres per annum (Clark *et al.* 1992). It will be subject to further biochemical decay, dependent on the time taken to reach the water table and the effective half life of the pesticide. Once the pesticide has reached the water table it is assumed to move with water through the fissure system, and be subject to diffusive exchange between the fissure water and pore water. There is currently very little data available on pesticide decay processes in groundwater. Bacteria have been detected in Chalk at depths up to 50 m below surface (Whitelaw and Rees 1980), though in significantly fewer numbers than near the surface. It is likely that although biochemical decay will continue during transit through the unsaturated and saturated zones, it will be at significantly slower rates than in the surfical deposits. This is supported by the profiles of atrazine and its TPs in the Chalk.

The profiles show that atrazine is moving down the depth profile by means of vertical solute transport through both the fissured and porous Chalk. The profiling work at both sites shows that this transport is occurring in discrete pulses. As you move down the profile dilution processes caused diffusion between the mobile fissure water and the matrix water will account for the general reduction in concentration observed with depth. Transport in the matrix is governed largely by molecular diffusion and is slow as explained above it is likely that biodegradation serves to further reduce the concentration of pesticide in the matrix. The levels of atrazine detected by immunoassay in the Chalk associated with those depths in the profile where matrix flow is thought to predominate are lower than the levels detected in those regions of the aquifer that were identified by geophysical logging as containing major fracture zones. This effect is particularly noticeable at a depth of 11 metres in the borehole; just below the water table. The profiling of atrazine and isoproturon seems to indicate that multiple pulses can move through the profile in the same way as a single pulse with minimal diffusion of the solute slugs.

7.4 In Conclusion

The field demonstration segment of the project has served to provide real material to validate the analytical methodology for priority pesticides and relevant transformation products in both the saturated and unsaturated zones of the aquifer.

Monitoring of the unsaturated zone of aquifers for pesticides is in its infancy. The work undertaken as part of this project has served to provide invaluable data on concentrations of agricultural herbicides and their TP that can persist within the aquifer environment and potentially contaminated groundwater. Indeed the data has important implications to current recommendation for pesticide use in the agricultural environment. It has been clearly demonstrated that atrazine and isoproturon herbicides persist at detectable concentrations at depth for many years after an application. The data produced for aquifer and porewater material in the unsaturated zone produced by this project are vital for the validation of pesticide transport models.

The work undertaken by WRc as part of this project has provided a successful field demonstration of the sampling and analytical methodology for atrazine and isoproturon pesticides, and their major TPs in solid aquifer material. The project provides the Environment Agency with a valuable capability for producing detailed profiles of parent pesticide and TP movement in the unsaturated and saturated zones of the aquifer.

Profiling has served to show shown that agricultural herbicides have the potential to migrate through significant thickness of unsaturated material and infiltrate groundwater where there is karstic flow (fissure flow), or high flux rates. The profiling data has served to show that the triazine herbicides are mobile and persistent and that when preferential field conditions exist they can play a major role in groundwater contamination.

The importance of fissure flow in the unsaturated zone has been demonstrated, and would appear to be the main route for pesticide movement to the saturated zone. The groundwater monitoring data clearly shows that concentrations of atrazine and its TPs are highest in the piezometers associated with fracture zones within the borehole.

The profiles provide previously unavailable data on pesticide persistence and migration, in particular in the unsaturated zone. The work has specifically identified that the major TPs of atrazine can, in the presence of favourable hydrogeological conditions, present a real risk to groundwater quality. The profiling data when used in conjunction with off the shelf modelling tools now make it possible to quantify herbicide fluxes moving toward the water table. The analytical methodology will allow further detailed profiling work to be completed in other aquifers, and the data so produced will have important consequences to land management and aquifer protection, since the accuracy of any modelling of pesticide transport depend heavily on these measurements of pesticide behaviour in the subsurface.

The recent profiling work has improved current understanding of the fate of agriculturally applied pesticides and their impact on water quality. The current project could be used as a basis of repeat studies to study the rate of decay of pesticides and their TPs in aquifers and further improve pesticide transport models.

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APPENDIX A A REVIEW OF TRANSFORMATION PRODUCTS AND FORMULATION CHEMICALS

A.1 TRANSFORMATION PRODUCTS

Table A.1 lists the principal TPs of the 68 pesticides most widely used in the UK. The discussion that follows is based largely on that of Cable *et al.* (1994), up-dated as appropriate. The main TPs are in bold print, where it has been possible to identify these.

A.1.1 Transformation products of *s*-triazine herbicides

The five most widely used triazine herbicides in the UK are atrazine, cyanazine, simazine, terbutryn and trietazine.

Atrazine

The principal environmental transformation products of atrazine appear to be the three *N*-dealkylated species: deethylatrazine, deisopropylatrazine and deisopropyldeethyl- atrazine (dealkylatrazine, diaminoatrazine), plus the four hydrolysis products: hydroxyatrazine, deethylhydroxyatrazine, deisopropylhydroxyatrazine and deisopropyl- deethylhydroxyatrazine. Further environmental transformation products have been reported, and these are listed in Table A1. Cyanuric acid, carbamylurea, and urea are the final organic compounds identified before mineralisation (Pelizzetti *et al.* 1990).

Hydroxyatrazine is suspected to be the major environmental transformation product, particularly from biodegradation in soils (Muir and Baker 1978). Some authors suggest that it can also be produced, but only abiotically, in aqueous solutions (Kolpin and Kalkhoff 1993). However, this TP tends to be bound strongly to soil particles, or, alternatively, be readily precipitated on water-borne particles. Moreover, it is apparently less persistent than atrazine (Goswami and Green 1971), being readily subject to further degradation.

The two mono *N*-dealkylated TPs, deethylatrazine, and deisopropylatrazine, both retain some of the pesticidal properties of their parent. Both of these, and particularly deethylatrazine, which is the more persistent of the two and has been suggested to be the major source of long-term ecotoxicity of atrazine residues in the environment (Sirons *et al.* 1973), appear to be at least as persistent as atrazine itself. They have both been widely reported in water sources (Kolpin and Kalkhoff 1993).

In the present study, deethyl- and deisopropylatrazine have been detected in chalk aquifer materials following agricultural use of atrazine.

Simazine

Simazine differs from atrazine only in one of its *N*-alkyl side chains. Hence, *N*-deethylation gives the TP, deethylsimazine, which is identical to deisopropylatrazine. Other TPs are either the same as atrazine TPs, or analogous.

Trietazine

The difference between trietazine and atrazine is again, in the *N*-alkyl side chains. Hence, a series of similar or analogous TPs is expected.

Cyanazine

Cyanazine can degrade to give similar TPs to those formed from simazine. However, additional TPs are possible, involving derivatisation of the cyanomethylamino side chain (Beynon *et al.* 1972). The nitrile function may be oxidised; first to an amide, and then to a carboxylic acid. The more common TPs are listed in Table A1.

Terbutryn

Terbutryn has a methylthio group in place of the chlorine in the other triazine pesticides. It is this moiety that is most susceptible to transformation, either, by oxidation, to sulphoxide, or by hydrolysis, to hydroxyterbutryn. These two TPs may each give rise to a further range of products, via loss of the *N*-alkyl groups, etc. However, all of these products (by analogy with the -OH containing atrazine and simazine TPs) will presumably sorb strongly to soil particles and consequently be of little significance to water resources.

A.1.2 Transformation products of phenoxyalkanoic acid herbicides

The six phenoxyalkanoic acid (chlorophenoxy acid) herbicides appearing on the list of high-use UK pesticides are 2,4-D, 2,4-DB, dichlorprop, MCPA, MCPB and Mecoprop (MCPP or CMPP). Mecoprop appears twice on the list, as mecoprop-p (optically pure) and as the racemic mixture. As far as the consideration of TPs is concerned, these are effectively the same compound.

The transport and transformation of phenoxyalkanoic acids is made more complex because they can be applied in the form of either alkyl esters, amine salts or alkali metal salts. In the case of esters, hydrolysis to the parent acids, an abiotic process, appears to be relatively facile, typically taking several days. (Smith 1989, Sandmann *et al.* 1988). For this reason, the free acids are not usually considered to be TPs.

There are few reports of TPs of phenoxyalkanoic acid herbicides being detected during routine monitoring of water resources. However, the degradation behaviour of this class of herbicides has been well studied during field trials and under simulated soil conditions.

The phenoxybutanoic acids (2,4-DB and MCPB) owe their pesticidal activity to bioconversion, in the target plants, to the corresponding phenoxyacetic acid herbicides (2,4-D and MCPA respectively). This biotransformation step can also be mediated by soil-living bacteria.

Either 2,4-dichlorophenol (from 2,4-D, 2,4-DB and dichlorprop) or 4-chloro-2-methyl- phenol (from MCPA, MCPB and mecoprop) appear to be the most frequent initial biotransformation products of the phenoxyalkanoic acids. 4-Chloro-2-methylphenol was determined as a TP of mecoprop (Cable *et al.* 1994), and it is thought that a cresol or phenol of this type would have potential to reach water supplies (Somasundaram *et al.* 1990), even though it is less mobile

than its parent (Somasundaram *et al.* 1991). However, there is evidence that such compounds are readily subject to further biotransformation (Gibson and Suflita 1990). Methylation of the phenols to the corresponding anisole has also been observed (Smith 1989). Other TPs, formed by loss of the aryl chlorines and substitution by hydroxyl groups, have been reported (see Table A.1).

A.1.3 Transformation products of substituted urea herbicides

The substituted urea herbicides include the five major-use UK compounds chlorotoluron, diuron, isoproturon, linuron and methabenzthiazuron.

Transformation of these compounds in the environment appears to proceed with *N*-demethylation or *N*-demethoxylation. Transformation to the corresponding substituted anilines has also been observed (Agrochemicals Handbook 1991). In addition, halogenated aryl moieties may lose halogen atoms to give the corresponding phenols and the isopropyl group of isoproturon may be converted to a 1-hydroxy-1-methylethyl group. In the present study, the principal TPs of isoproturon were monitored in chalk aquifers and were not detected.

The diuron TP, *N'*-(3,4-dichlorophenyl)-*N*-methyl urea (DCPMU, also known as demethylated diuron) has been reported in groundwaters in Sweden, where its appearance is associated with the use of diuron on railway tracksides. It is apparently similar in mobility and persistence to diuron (Torstensson 1993). This is significant in view of the increasing UK usage of diuron as a replacement for atrazine.

The photochemical transformation of isoproturon has been studied (Kulshrestha and Mukerjee 1986). The dimers, 4,4'-diisopropylazobenzene and 4,4'-diisopropylazoxy- benzene were formed. It is not clear, however, whether such compounds can be formed under field conditions.

A.1.4 Transformation products of the ethylenebisdithiocarbamate (EBDC) pesticides

Of the six ethylenebisdithiocarbamate (EBDC) fungicides approved for use in the UK, only maneb and mancozeb are in the list identified as high-use pesticides. However, the discussion below can equally apply to the other four, cufraneb, metiram, nabam and zineb.

On contact with water, the EBDC fungicides are unstable and form the major degradation product, ethylenethiourea (ETU), though transformation to this compound is not complete after several months. The EBDC fungicides are less stable in soils, suggesting that ETU is also a metabolite of microbiological degradation (Engst 1977; Howard 1991; Agrochemicals Handbook 1991). Further, ETU is present in significant quantities as an impurity in formulations of the EBDC fungicides, and continues to be formed during storage. ETU, and perhaps related compounds, appear to be the active pesticides; the physical properties of the EBDC fungicides limits them to the top layer of soil, and apparently prolonged pesticidal activity is achieved by the continuing release of key degradation products (Howard 1991). ETU has been reported in the water resources, including drinking water sources, and it is a known carcinogen; consequently, its presence in water has provoked some concern (Somasundaram and Coats 1991; Miles 1991).

Other degradation products formed directly from the EBDC parent compounds include ethylene thiuram disulphide (ETD), ethylenediisothiocyanate and ethylene thiuram monosulphide (ETM) [also known as 5,6-dihydroimidazo-1,2,4-dithiazole-3-thione (DIDT)] (Agrochemicals Handbook 1991; Howard 1991, Hogendoorn *et al.* 1991).

The ETU formed as a TP is subject to further transformation to give a range of compounds including ethylene urea (EU), 2-imidazole, hydantoin, ethylenediamine and Jaffe's base [*N*-(dihydro-2-imidaz)ethylene thiourea] formed from the condensation of two molecules of ETU, or the co-condensation of one molecule of EU and one molecule of ETU (Engst 1977).

Some of the listed EBDC TPs are probably of little concern, as they are either produced in low quantities, or else are naturally occurring in the environment. However, some of them may not yet have been reported in water resources owing to the lack of suitable analytical techniques.

A.1.5 Transformation products of hydroxybenzonitrile (HBN) herbicides (bromoxynil and ioxynil)

The two HBN herbicides, bromoxynil and ioxynil, are widely used in the UK, either as octanoate esters or potassium or sodium salts. Both appear to be degraded in the environment by hydrolysis of ester and nitrile groups, and dehalogenation. TPs, such as 4-hydroxybenzoic acid have been reported in environmental microcosm studies, but not during routine field monitoring. The potential of such compounds to reach water resources appears to be low. Additional photolysis TPs are listed in the table. These are not thought to be very significant (Agrochemicals Handbook 1991; Kolchany *et al.* 1990).

A.1.6 Transformation products of the bipyridylium contact herbicides, Diquat and Paraquat

The TPs of the contact herbicides diquat and paraquat are largely cations (Table A.1) and are thought to have little potential to leach to water resources. In this respect, they resemble the parent pesticides.

A.1.7 Transformation products of other pesticides

Aldicarb

Aldicarb (AS) has been reported to give rise to a range of transformation products that have been detected in water resources (Agrochemicals Handbook 1991). The TPs with the strongest ecotoxicological properties are aldicarb's stepwise oxidation products, aldicarb sulphoxide (ASO) and aldicarb sulphone (ASO_2). AS, ASO and ASO_2 are often referred to collectively as the total toxic residue of aldicarb (Priddle *et al.* 1989, Miles and Delfino 1985). Most of the total toxic residue reported in ground and surface water is in the form of either ASO or ASO_2, accompanied by comparatively low concentrations of unconverted aldicarb (Hansen and Spiegel 1983, Ritter 1990). All three of these compounds can be hydrolysed in the environment to the corresponding oxime, and subsequently dehydrated to produce the nitrile, giving a total of eight possible degradation products. The `non-toxic' TPs have been the subject of comparatively little research.

Amitrole

Of the two amitrole degradation products identified (see Table A.1), urea is unlikely to constitute a problem as it is naturally occurring. Aminonitrile may have potential to contaminate water resources. The formation of addition products between pesticides and plant biochemicals is presumably common and the risk of contamination of water is low.

Bentazone

Anthranilic acid and *N*-isopropylanthranilamide (*N*-isopropyl-2-aminobenzamide) have been observed as TPs of bentazone (Lee *et al.* 1988; Booth *et al.* 1973), though more recent reports suggest mineralisation is rapid (Huber and Otto 1994, BASF 1995).

Captan

All transformation reactions affect the $>NSCCl_3$ moiety. Such TPs as trithiocarbamate, thiophosgene or tetrahydrophalimide are possible (Agrochemicals Handbook 1991; Kolchany *et al.* 1990).

Carbendazim

Carbendazim, itself a transformation product of the fungicides benomyl and thiophanate-methyl, degrades via hydrolysis of its amide group to 2-aminobenzimidazole, 5-hydroxy-2-aminobenzimidazole and 5-hydroxy-2-aminobenzimidazolecarbamate (Helweg 1977).

Dicamba

Conversion of the anisole group to a phenol group gives the only reported TP (Smith 1974).

1,3-Dichloropropene

The soil fumigant and nematicide, 1,3-dichloropropene has been observed to give rise to the degradation products, *cis*- and *trans*-1-chloro-1-propen-3-ol (3-chloroallyl alcohol) which have been detected in groundwaters [Fielding (ed) 1991]. Although additional related compounds have been detected, it is probable that these are either impurities in the parent pesticide, or else TPs of these impurities. (1,3-dichloropropene used to be available for use as a pesticide in a much cruder form) (Maddy *et al.* 1982).

Diclofop-methyl

Diclofop-methyl is initially hydrolysed to its free acid form (diclofop). Breakage of the phenoxyether bonds and incorporation of hydroxy groups on the benzene rings leads to further TPs (Agrochemicals Handbook 1991, Headley *et al.* 1994).

Dimethoate

Dimethoate is transformed by oxidation of the phosphorodithioate group to phosphorothioate, producing dimethoxon. Loss of the *N*-methyl group, and oxidation of the amide group are further possibilities. Demethylation of the ester group is also possible (Agrochemicals Handbook 1991).

Ethirimol

Transformation is by loss of the *N*-ethyl side-chain (Agrochemicals Handbook 1991).

Flamprop-S-isopropyl

The main reported TP is the free acid form (Agrochemicals Handbook 1991), though other TPs have been reported (Hitchings and Roberts 1979).

Fluroxypyr

Degradation in soils is to the corresponding hydroxy and methoxy compounds (Agrochemicals Handbook 1991).

Glyphosate

Glyphosate is sorbed strongly by soil particles, and is unlikely to leach into water sources. It is not clear how much of the soil-bound glyphosate is degraded to simpler compounds or eventually mineralised. Degradation owing to microbiological activity in soils is a possible degradation pathway, leading to the formation of a major metabolite, aminomethylphosphonic acid (AMPA). Several minor metabolites have also been identified but in experiments these always constitute much less than 1% of the glyphosate applied. AMPA can also be degraded by microbiological means, but apparently less readily than glyphosate, so it may accumulate. However, it sorbs to soil particles even more strongly than glyphosate, and so is unlikely to constitute a problem in water contamination. None of the glyphosate TPs identified retain the herbicidally active N-phosphonomethylglycine moiety (Torstensson 1985).

Iprodione

3,5-Dichloroaniline has been reported as a TP. Additional TPs, all substituted ureas, can be produced by cleavage of the hydantion ring structure of the parent pesticide.

Lindane (γ-HCH, γ-hexachlorocyclohexane)

Lindane is one of the few organochlorine pesticides still used. Its toxicity is lower than is typical for this class of pesticides, and this is reflected in the relatively high health related WHO Guideline Value. It is important that any TPs that have potential to reach water resources are identified, as these may not have received the same scrutiny and could have significant ecotoxicities.

The appearance of a-HCH in environmental samples could be as a result of the historical use of technical grade HCH (which contains 55-60% α-HCH). However, there is evidence that photochemical conversion of γ-HCH to α-HCH is a possibility (Plimmer and Johnson 1991; Lubkowski *et al.* 1991).

Other TPs identified include pentachlorocyclohexenes, tetrachlorocyclohexenes and tetrachlorobenzenes (Tu 1976). The ability of some of these TPs to leach to water resources may be similar to that of lindane.

Metazachlor

2,6-Dimethylaniline has been reported as an environmental TP of metazachlor (European Institute for Water 1988).

Methyl bromide

It has been assumed that the soil fumigant, methyl bromide is converted to hydrogen bromide and methanol in the environment, neither of which would be expected to constitute a problem for water contamination. This has been supported by experimental evidence (Gentile *et al.* 1989).

Pendimethalin

Pendimethalin is transformed in soils by oxidation of the 4-methyl group on the benzene ring to the carboxylic acid, via the phenol (Agrochemicals Handbook 1991). Degradation is also possible by loss or fragmentation of the *N*-alkyl group, and by oxidation of the amino group (Barua *et al.* 1990).

Propachlor

Propachlor is presumably subject to cleavage of its C-N bonds to give products including aniline and chloroacetic acid. However, isopropylamine is the only product to have been identified (Novick *et al.* 1986).

Propyzamide

The TPs identified involve reaction of the alkyne group (Agrochemicals Handbook 1991; Rouchard *et al.* 1987).

Triazophos

The TP included in Table A.1 involves hydrolysis of the thiophosphonate ester. Presumably, a similar hydrolysis involving loss of an ethyl group is also possible (Agrochemicals Handbook 1991).

Trifluralin

Trifluralin has been shown to be subject to a complex range of transformations caused either by soil biofilms, or by chemical processes in soil and water (Golab *et al.* 1979). These include reduction of one, or both, nitro groups to amino groups, *N*-depropylation, heterocyclic ring formation (to give benzimidazoles), coupling to give azo compounds and hydrolysis (especially of the trifluoromethyl function, at the benzene ring positions and of the *N*-propyl side-chains). Eleven of the most common TPs are shown in Table A.1. It has been noted that the total TPs commonly account for no more than 3% of the trifluralin residue in soils and flood water. Further, they tend to be bound strongly to soils, where they appear to be subject eventually to mineralisation.

A.1.8 TPs of pesticides, not on the high-use UK list

While the pesticides listed below are not among the 68 high-use pesticides in the UK, their use is on the increase and their TPs have been widely detected in other countries during routine monitoring.

Dichlobenil

Dichlobenil (2,6-dichlorobenzonitrile) is a herbicide used in the UK. In the Netherlands, where it has wide application, a transformation product, 2,6-dichlorobenzamide (BAM) has been detected in groundwaters by routine monitoring. This degradation has been shown to be the result of microbial or alkali-catalysed hydrolysis in soils. BAM, in turn, can be degraded to 2,6-dichlorobenzoic acid [Fielding (ed), 1991; Lagas *et al.* 1989; Agrochemicals Handbook 1991].

Benomyl

The fungicide, benomyl, is known to degrade to methyl 2-benzimidazolecarbamate. The TP is the compound usually detected in the environment (Agrochemicals Handbook 1991). The fungicide carbendazim is also a known TP of benomyl.

Chlorthal-dimethyl (DCPA)

Chlorthal-dimethyl (dimethyl tetrachloroterephthalate, DCPA) is often known by its trade-name, Dacthal. This herbicide is used to control weeds in turf, ornamentals and market gardening. Residues have recently been perceived to be a problem in North America (Somasundaram and Coats 1991a) and its use is increasing in the UK.

Degradation in the environment is by hydrolysis, first to the mono-acid, and then the di-acid. There is evidence that the first of these steps is mediated by microbial activity (and not by chemical hydrolysis). Although the mono-acid is not very persistent, the potential for further degradation of the di-acid metabolite is unknown, but it appears to be very persistent. Because chlorthal di-acid is more soluble and less volatile than the parent pesticide, it has far more potential to contaminate water resources, and it is this TP, rather than the parent, that has been detected during routine monitoring (Wettasinghe and Tinsley 1993).

It should be stressed that the half-life of chlorthal-dimethyl (*ca.* 90 days) implies that the hydrolysis of this ester is a completely different case to the hydrolysis of esters of HBN and phenoxyalkanoic acids, where the free phenols/acids are not generally thought of as TPs.

Metham-sodium

Methyl isothiocyanate (MITC or MIT) has been reported in Dutch groundwaters. This appears as a TP of the soil fumigant metham-sodium. However, because metham-sodium is applied as the precursor of MITC, which is the active soil fumigant, it is questionable whether MITC can be regarded as a genuine transformation product. The soil fumigant, dazomet also produces MITC as a TP.

Table A.1 Transformation products of high-usage pesticides

Pesticide	Systematic chemical name	Principal transformation products
Aldicarb	2-Methyl-2-(methylthio)propanal *O*-[(methylamino)carbonyl]oxime Nanogen Index (1975), Agrochem. HBk. (1991), European Institute for Water (1988), Canton *et al.* (1991), Priddle *et al.* (1989), Miles and Delfino (1985), Hansen and Spiegel (1983), Ritter (1990), Marvin *et al.* (1991), Moye and Miles (1988), Howard (1991), Ou *et al.* (1985), Somasundaram and Coats (1991)	**Aldicarb sulphoxide** {a.k.a. 2-methyl-2-(methylsulphinyl)propanal *O*-[(methylamino)carbonyl]oxime} **Aldicarb sulphone** {a.k.a. 2-methyl-2-(methylsulphonyl)propanal *O*-[(methylamino)carbonyl]oxime} Aldicarb oxime Aldicarb sulphoxide oxime Aldicarb sulphone oxime Aldicarb nitrile Aldicarb sulphoxide nitrile Aldicarb sulphone nitrile
Amitrole	1-*H*-1,2,4-Triazol-3-ylamine Carter (1975), Agrochem. HBk. (1991), Somasundaram and Coats (1991)	**Aminonitrile** **Urea** Also forms adducts in plants with glucose, serine and other plant biochemicals; e.g. with serine;3-(3-Amino-*s*-triazole-1-yl)-2-aminopropanoic acid (3-ATAL)
Atrazine	2-Chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine Muir and Baker (1978), Erickson and Lee (1989), Muir and Baker (1976), Somasundaram *et al.* (1991), Goswami and Green (1971), Agrochem. HBk. (1991), Pereira *et al.* (1992), Jones *et al.* (1982),	**2-Chloro-4-amino-6-isopropylamino-1,3,5-triazine** (a.k.a. deethylatrazine) **2-Chloro-4-ethylamino-6-amino-1,3,5-triazine** (a.k.a. deisopropylatrazine, deethylsimazine) **2-Chloro-4,6-diamino-1,3,5-triazine** (a.k.a. dealkylatrazine, deisopropyldeethylatrazine) **2-Hydroxy-4-ethylamino-6-isopropylamino-1,3,5-triazine** (a.k.a. hydroxyatrazine)

Pesticide	Systematic chemical name	Principal transformation products
	Winkelmann and Klaine (1991a, 1991b), Nanogen Index (1975), Kross *et al.* (1992), Kaufman and Blake (1970), Skipper *et al.* (1978), Esser *et al.* (1975), MAFF (1992a)	**2-Hydroxy-4-amino-6-isopropylamino-1,3,5-triazine** (a.k.a. deethylhydroxyatrazine) **2-Hydroxy-4-ethylamino-6-amino-1,3,5-triazine** (a.k.a. Deisopropylhydroxyatrazine) **2-Hydroxy-4,6-diamino-1,3,5-triazine** (a.k.a. deisopropyldeethylhydroxyatrazine) Further degradation products, similar or analogous to those for simazine degradation, including cyanuric acid, carbamylurea and urea.
Bentazone	3-Isopropyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide Lee *et al.* (1988), Booth *et al.* (1973), Huber and Otto (1994), BASF (1995)	**Anthranilic acid** (a.k.a. *o*-aminobenzoic acid) ***N*-Isopropylanthranilamide** (a.k.a. *N*-isopropyl anthranilic acid amide) *N*-2-Carboxyphenyl-*N*'-isopropylsulphodiamide Rapid humification and mineralisation
Bromoxynil	3,5-Dibromo-4-hydroxybenzonitrile Agrochem. HBk. (1991), Kolchany *et al.* (1990)	**4-Hydroxybenzoic acid** also: 3-Bromo-4-hydroxybenzonitrile 3-Chloro-4-hydroxybenzonitrile 3-Bromo-5-chloro-4-hydroxybenzonitrile 4-Hydroxybenzonitrile (all photolysis products on soils - presence of NaCl is implied)

Pesticide	Systematic chemical name	Principal transformation products
Captan	1,2,3,6-Tetrahydro-*N*-(trichloromethylthio)-phthalimide Agrochem. HBk. (1991), Buyanovsky *et al.* (1988)	Unstable with respect to hydrolysis in aqueous media, especially under alkaline conditions. Product(s) of hydrolysis include: Tetrahydrophthalimide Tetrahydrophthalic acid *o*-Aminotetrahydrophthalic acid
Carbendazim	2-Aminobenzimidazole Helweg (1977), Agrochem. HBk. (1991), Nanogen Index (1975), Somasundaram and Coats (1991b)	**2-Aminobenzimidazole** **5-Hydroxy-2-aminobenzimidazole** **5-Hydroxy-2-aminobenzimidazolecarbamate** Carbendazim is also a TP of the pesticide benomyl
Chloridazon	5-Amino-4-chloro-2-phenylpyridazin-3(2*H*)-one Eberspracher and Lingens (1981)	**5-Amino-4-chloro- 3(2*H*)- pyridazinone**
Chlormequat chloride	2-Chloroethyltrimethylammonium chloride BASF (1995)	Rapid mineralisation; no degradation products identified
Chlorothalonil	Tetrachloroisophthalonitrile Katayama *et al.* (1992)	**2,5,6-Trrschloro-4-methylthioisophthalonitrile**
Chlorotoluron	*N*-(3-Chloro-4-methylphenyl)-*N'*,*N'*-dimethylurea European Institute for Water (1988)	**3-Chloro-4-methylaniline** (a.k.a. 5-chloro-*p*-toluidine)

Pesticide	Systematic chemical name	Principal transformation products
Cyanazine	2-Chloro-4-(1-cyano-1-methylethylamino)-6- ethylamino)-1,3,5-triazine Agrochem. HBk. (1991), Muir and Baker (1978), Beynon *et al.* (1972a, 1972b), Nanogen Index (1975), Sirons *et al.* (1973)	**2-Chloro-4-(1-carbamoyl-1-methylethylamino)-6- ethylamino-1,3,5-triazine** **2-Hydroxy-(1-carboxy-1-methylethylamino)-6- ethylamino-1,3,5-triazine** 2-Chloro-4-amino-6-(1-carbamoyl-1-methylamino)- 1,3,5-triazine **2-Chloro-4-(1-carboxy-1-methylethylamino)-6- ethylamino-1,3,5-triazine** 2-Chloro-4-(1-cyano-1-methylethylamino)-6-amino-1,3,5-triazine 2-Chloro-4-(1-carboxy-1-methylethylamino)-6-amino-1,3,5-triazine 2-Hydroxy-4-(1-carboxy-1-methylethylamino)-6-amino-4,3,5-triazine 2-Chloro-4-amino-6-ethylamino-1,3,5-triazine (i.e. deisopropylatrazine/deethylsimazine)
2,4-D	(2,4-Dichlorophenoxy)acetic acid Agrochem. HBk. (1991), Smith (1989), Somasundaram *et al.* (1991), European Institute for Water (1988), Gibson and Suflita (1990), Mikesell and Boyd (1985), Loos (1975), Crosby and Tutass (1966), Audus (1960), Somasundaram and Coats (1991), Smith and Muir (1984)	**2,4-Dichlorophenol** **2,4-Dichloroanisole** 2-Chlorophenol 4-Chlorophenol also: 4-Chloro-*o*-catechol 2-Hydroxy-4-chlorophenoxyacetic acid 1,2,4-benzenetriol (photolysis products) As with the other phenoxyalkanoic acids, formulated as alkali metal salt, amine salt or alkyl ester. Parent amines or alcohols could constitute additional TPs.

Pesticide	Systematic chemical name	Principal transformation products
2,4-DB	4-(2,4-Dichlorophenoxy)butanoic acid Agrochem. HBk. (1991), Gibson and Suflita (1990)	**4-(2,4-Dichlorophenoxy)acetic acid (i.e. 2,4-D)** **2,4-Dichlorophenol** Obviously, all the degradation products of 2,4-D are also possible degradation products of 2,4-DB. As with the other phenoxyalkanoic acids, the formulation can be as alkali metal salt, amine salt or alkyl ester. The parent amine or alcohol could constitute an additional TP.
Demeton-S-methyl	S-2-Ethylthioethyl *O,O*-dimethyl phosphorothioate	No data available
Dicamba	3,6-Dichloro-2-methoxybenzoic acid Nanogen Index (1975), Agrochem. HBk. (1991), Smith (1974), Smith and Muir (1984)	**3,6-Dichlorosalicylic acid** (a.k.a. 3,6-dichloro-2-hydroxybenzoic acid)
1,3-Dichloropropene	a.k.a. 1,3-Dichloro-1-propene (E & Z isomers) Agrochem. HBk. (1991), Maddy *et al.* (1982), Castro and Belser (1966), Connors *et al.* (1990)	**1-Chloro-1-propen-3-ol (E & Z isomers)** (a.k.a. 3-Chloroallyl alcohol) 3-Chloropropenoic acid (E & Z isomers) Formylacetic acid Also used to be supplied in impure form (before 1984 in NL); admixture contained up to 34 % 1,2-dichloropropane (now ~ 0.5 % permitted). TPs of active pesticide may thus be confused with impurities and TPs of impurities.

Pesticide	Systematic chemical name	Principal transformation products
Dichlorprop	2-(2,4-Dichlorophenoxy)propanoic acid Agrochem. HBk. (1991)	**2,4-Dichlorophenol**
Diclofop-methyl	Methyl (±)-2-[4-(2,4-dichlorophenoxy)phenoxy]- propanoate Agrochem. HBk. (1991), Headley et al. (1994), MAFF (1991)	**2-[4-(2,4-Dichlorophenoxy)phenoxy]propanoic acid** (a.k.a. diclofop acid, diclofop) 4-(2,4-Dichlorophenoxy)phenol 4-(2,4-Dichlorophenoxy)dehydrophenetole 4-(2,4-Dichlorophenoxy)phenetole
Difenzoquat	1,2-Dimethyl-3,5-diphenylpyrazolium methyl sulphate	No data available
Diflufenican	*N*-(2,4-Difluorophenyl)-2-(3-trifuoromethyl-phenoxy)pyridine-3-carboxamide Rouchaud *et al.* (1991)	***N*-(2,4-Difluorophenyl)-2-hydroxy-3-pyridine-carboxamide** 2-[3-(trifuoromethyl)phenoxy]-3-pyridine-carboxylic acid 2-hydroxy-3-carboxypyridine
Dimethoate	*O,O*-Dimethyl *S*-methylcarbamoylmethyl phosphorodithioate Nanogen Index (1975), Steller and Brand (1974), Howard (1991), Kolbe *et al.* (1991), MAFF (1993b)	***O,O*-Dimethyl *S*-methylcarbamoylmethyl phosphorothioate** (a.k.a. omethoate, dimethoxon) De-*N*-methyl dimethoate De-*N*-methyl dimethoxon *N*-Hydroxymethyl dimethoate *N*-Hydroxymethyl dimethoxon ***O,O*-Dimethyl phosphorothioic acid** ***O*-Desmethyl dimethoate** Dimethoate carboxylic acid Dimethyl dithiophosphoric acid Dimethyl phosphoric acid Phosphoric acid

Pesticide	Systematic chemical name	Principal transformation products
Diquat	1,1'-Ethylene-2,2'-dipyridylium dibromide Howard (1991), Smith and Grove (1969), Agrochem. HBk. (1991), Grover and Cessna (1991)	**Picolinic acid** (a.k.a. 2-pyridinecarboxylic acid) **Picolinamide** (a.k.a. 2-pyridinecarboxylamide) **1,2,3,4-Tetrahydro-1-oxopyrido(1,2-a)-5-pyrazinium salt (TOPPS)** 1,2,3,4-Tetrahydro-1-oxopyrido(1,2-a)-5-pyrid-6-one 1,2,3,4-Tetrahydro-1-oxopyrido(1,2-a)-8-hydroxy-5-pyrid-6-one. Diquat monopyridone salt (oxo group ortho to nitrogen) (photochemical decomposition products only)
Dithianon	2,3-Dicyano-1,4-dithia-anthraquinone	No data available
Diuron	*N'*-(3,4-Dichlorophenyl)-*N*,*N*-dimethylurea Agrochem. HBk. (1991), Geissbühler (1969), Nanogen Index (1975), Attaway *et al.* (1982a and 1982b), Ellis and Camper (1982), Stepp *et al.* (1985), Somasundaram and Coats (1991)	***N'*-(3,4-Dichlorophenyl)-*N*-methylurea (DCPMU)** ***N*-(3,4-Dichlorophenyl)urea (DCPU)** **3,4-Dichloroaniline (DCA)** *N'*-(3-Chlorophenyl)-*N*,*N*-dimethylurea
Ethirimol	5-Butyl-2-ethylamino-6-methylpyrimidin-4-ol Agrochem. HBk. (1991).	**2-Amino-5-butyl-6-methylpyrimidin-4-ol**
Ethofumesate	(±)-2-Ethoxy-2,3-dihydro-3,3-dimethyl-benzofuran-5-yl methanesulphonate McAuliffe and Appleby (1984), Terreni *et al.* (1994), Agr Evo (1995)	**2,3-Dihydro-3,3-dimethyl-2-oxobenzofuran-5-yl methanesulphonate** 2,3-Dihydro-2-hydroxy-3,3-dimethyl- benzofuran-5-yl methanesulphonate 2-(2-hydroxy-5-methanesulfoxyphenyl)-2-methylpropionic acid

Pesticide	Systematic chemical name	Principal transformation products
Fenpropidin	(±)-1-[3-(4-*tert*-Butylphenyl)-2-methylpropyl]piperidine	No data available
Fenpropimorph	(±)-*cis*-4-[3-(4-*tert*-Butylphenyl)-2-methyl-propyl]-2,6-dimethylmorpholine Dieckmann *et al.* (1993), BASF (1995)	Fenpropimorphic acid (aka 4-(3-[4-(2-carboxypropyl)phenyl)-2-methyl-propyl-2,6-cis-dimethylmorpholine) 2,6-dimethylmorpholine
Fentin hydroxide	Triphenyltin hydroxide	No data available
Flamprop-m-isopropyl	Isopropyl (±)-*N*-Benzoyl-*N*-(3-chloro-4-fluorophenyl)-2-aminopropionate Agrochem. HBk. (1991), Hitchings and Roberts (1979)	**Flamprop free acid** 3-Chloro-4- fluoroaniline *N*-Benzoyl-3-chloro-4- fluoroaniline *N*-(3-chloro-4- fluorophenyl)-2-aminopropionate
Fluroxypyr	4-Amino-3,5-dichloro-6-fluoro-2-pyridyloxyacetic acid Agrochem. HBk. (1991)	**4-Amino-3,5-dichloro-6-fluoropyridin-2-ol** **4-Amino-3,5-dichloro-6-fluoro-2-methoxypyridine**
Flusilazole	*bis*-(4-Fluorophenyl)methyl(1*H*-1,2,4-triazol-1-ylmethyl)silane MAFF (1989)	***bis*-(4-Fluorophenyl)methyl-silanol** **1*H*-1,2,4-triazole**
Flutriafol	(±)-a-(4-Fluorophenyl)-1*H*-1,2,4-triazole-1-ethanol	No data available

Pesticide	Systematic chemical name	Principal transformation products
Glyphosate	*N*-(Phosphonomethyl)glycine Tortensson (1985), Cowell *et al.* (1986)	**Aminomethylphosphonic acid (AMPA)** *N*-Methylaminomethylphosphonic acid *N*,*N*-Dimethylaminomethylphosphonic acid Hydroxymethylphosphonic acid Glycine
Imazamethabenz-methyl	Methyl 6-(4-isopropyl-4-methyl-5-oxo-2--imidazolin-2-yl)-*m*- & -*p*-toluate (mixture) Rouchaud *et al.* (1994)	**Imazamethabenz free acid** (aka 6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-*m*- & -*p*-toluate (mixture)) 2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-1,4-benzene dicarboxylic acid, in mixture with the 1,5-benzene dicarboxylic acid 1,2,4-benzene tricarboxylic acid, in mixture with the 1,2,5-benzene tricarboxylic acid
Ioxynil	4-Hydroxy-3,5-diiodobenzonitrile Agrochem. HBk. (1991), Grover and Cessna (1991)	**4-Hydroxybenzoic acid** 4-Hydroxy-3,5-diphenylbenzonitrile
Iprodione	3-(3,5-Dichlorophenyl)-*N*-isopropyl-2,4-dioxoimidazolidine-1-carboxamide Agrochem. HBk. (1991), Belafdal *et al.* (1986), Gomez *et al.* (1982)	**3,5-Dichloroaniline** (reported as plant metabolite only) 3-Isopropylcarbamoyl-5(3,5-dichlorophenyl)hydantoic acid -a range of disubstituted ureas have also been observed.

Pesticide	Systematic chemical name	Principal transformation products
Isoproturon	*N*-(4-Isopropylphenyl)-*N'*,*N'*-dimethylurea Kulshrestha and Mukerjee (1986), Agrochem. HBk. (1991), Geissbühler *et al.* (1976), Mudd *et al.* (1983)	***N*-[4-(1-Hydroxy-1-methylethyl)phenyl]-*N'*-methyl-urea** ***N*-(4-Isopropylphenyl)-*N'*-methylurea** ***N*-(4-Isopropylphenyl)urea** **4-Isopropylaniline** *N*-[4-(1-Hydroxy-1-methylethyl)phenyl]urea 3,4-(2'-hydroxy-2'-propyl)aniline 4,4'-Diisopropylazobenzene 4,4'-Diisopropylazoxybenzene
Lindane	1α,2α,3b,4α,5α,6β-Hexachlorocyclohexane (a.k.a. γ-HCH, γ-BHC, benzene hexachloride) Howard (1991), Tu (1976), Plimmer and Johnson (1991), Lubkowski *et al.* (1991) N.B. Technical grade HCH is α-, β-, γ-, δ- and ε-HCH mixture.	γ-2,3,4,5,6-Pentachloro-1-cyclohexene α-3,4,5,6-Tetrachloro-1-cyclohexene β-3,4,5,6-Tetrachloro-1-cyclohexene γ-3,4,5,6-Tetrachloro-1-cyclohexene Pentachlorobenzene 1,2,3,4-Tetrachlorobenzene 1,2,4,5-Tetrachlorobenzene It is not clear whether *alpha*-HCH, found especially in atmospheric samples, is a TP of lindane or a component of technical grade HCH.
Linuron	*N'*-(3,4-Dichlorophenyl)-*N*-methoxy-*N*-methylurea European Institute for Water (1988), Geissbühler (1969), Nanogen Index (1975), Stepp *et al.* (1985)	**3,4-Dichloroaniline** ***N'*-(3,4-Dichlorophenyl)-*N*-methylurea** *N'*-(3,4-Dichlorophenyl)-*N*-methoxyurea *N*-(3,4-Dichlorophenyl)urea *N'*-(3-Chlorophenyl)-*N*-methoxy-*N*-methylurea

Pesticide	Systematic chemical name	Principal transformation products
Mancozeb and Maneb	Manganese-zinc ethylenebis(dithiocarbamate) Ethylenebis(dithiocarbamato)manganese Agrochem. HBk. (1991), Hogendoorn *et al.* (1991), Howard (1991), Marshall (1977), Nanogen Index (1975), Engst (1977), Somasundaram and Coats (1991a and 1991b)	**Ethylenethiourea (ETU)** (a.k.a. 2-imidazolidinethione) Ethylene thiuram monosulphide (ETM) [a.k.a. 5,6-dihydro-3H-imidazo (2,1-c)-1,2,4- dithiazole-3-thione (DIDT)] [a.k.a. ethylene bis(isothiocyanate) sulphide (EBIS)] Ethylenediisothiocyanate Ethylene thiuram disulphide (ETD) (a.k.a. ethylenebisdithiocarbamate polymer) Ethylene urea (EU) (a.k.a. 2-imidazolidinone) 2-Imidazoline 2,4-Imidazolidinedione (a.k.a. hydantoin) Glycine (a.k.a. 2-aminoacetic acid) 3-(2-Imidazolin-2-yl)-2-imidazolidinethione (a.k.a. Jaffe's base) Ethylenediamine (EDA) Carbon disulphide
MCPA	(4-Chloro-2-methylphenoxy)acetic acid European Institute for Water (1988), Agrochem. HBk. (1991), Sattar (1982), Smith and Aubin (1991)	**2-Methyl-4-chlorophenol** (a.k.a. 4-chloro-*o*-cresol) **5-Chloro-3-methyl-*o*-catechol** (a.k.a. 2-hydroxy-5-chloro-3-methylphenol) Formulated as alkali metal salt, amine salt or ester. Alcohol or amine could constitute additional by-products. Ring hydroxylation and ring opening are other possible degradation pathways.

Pesticide	Systematic chemical name	Principal transformation products
MCPB	4-(4-Chloro-2-methylphenoxy)butanoic acid Agrochem. HBk. (1991), Sattar (1982)	**4-Chloro-2-methylphenol** (a.k.a. 4-chloro-*o*-cresol) **5-Chloro-3-methyl-*o*-catechol** (a.k.a. 2-hydroxy-5-chloro-3-methylphenol) **4-Chloro-2-methylphenoxyacetic acid (i.e. MCPA)** - initial product of metabolism in plants (active pesticide), and probably also initial product of micro-biological degradation in soils. Obviously, the TPs identified for MCPA will also be possible TPs of MCPB. As in the case of the other phenoxyalkanoic acids, MCPB can be formulated as alkali metal salt, amine salt or alkyl ester. The parent amine or alcohol could degrade to give additional TPs.
Mecoprop **(+ Mecoprop-p)**	(±)-2-(4-Chloro-2-methylphenoxy)propanoic acid (*R*)-(+)-2-(4-Chloro-2-methylphenoxy)propanoic acid Agrochem. HBk. (1991), Smith (1985, 1989), Lappin *et al.* (1985)	**4-Chloro-2-methylphenol** (a.k.a. 4-chloro-*o*-cresol) Formulated as alkali metal salt, amine salt or ester. Alcohol or amine could constitute additional by-products. Ring hydroxylation and ring opening are other possible degradation pathways.
Metamitron	4-Amino-4,5-dihydro-3-methyl-6-phenyl-1,2,4-triazin-5-one	**Deamino-metamitron** 2,2'-Azinodiphenylacetate 3-Methyl-6-phenyl-1,2,4,5-tetrazine 5-Methyl-2-phenyl-1,3,4-oxadiazole Benzoic acid

Pesticide	Systematic chemical name	Principal transformation products
Metazachlor	2-Chloro-N-(pyrazol-1-ylmethyl)acet-2',6'-xylidide European Institute for Water (1988)	**2,6-Dimethylaniline** (a.k.a. 2,6-xylidine)
Methabenzthiazuron	1,3-Dimethyl-3-(2-benzothiazolyl)urea	**3-Methyl-3-(benzothiazol-2-yl)urea**
Methyl bromide	a.k.a. Bromomethane Gentile *et al.* (1989)	**Methanol** (a.k.a. methyl alcohol) **Hydrogen bromide** (a.k.a. hydrobromic acid) - either by hydrolysis directly or via formation of a methyl radical.
Paraquat	1,1'-Dimethyl-4,4'-bipyridylium dichloride Agrochem. HBk. (1991), Grover and Cessna (1991), Somasundaram and Coats (1991)	**1-Methyl-4-carboxypyridinium chloride** **Methylamine (hydrochloride)** (photochemical decomposition products only - formed on leaf surfaces, not in aqueous solution).
Pendimethalin	*N*-(1-Ethylpropyl)-2,6-dinitro-3,4-xylidine Barua *et al.* (1990), Agrochem. HBk. (1991), Scheunert *et al.* (1994)	*N*-Propyl-3-methyl-4-hydroxy-2,6-dinitroaniline *N*-(1-Ethylpropyl)-2-amino-6-nitro-3,4-xylidine 2,6-Dinitro-3,4-xylidine *N*-(1-Ethylpropyl)-2,6-dinitro-3-methyl-4- hydroxymethyl)- aniline 4-(1-Ethylpropyl)amino-3,5-dinitro-2-methylbenzoic acid 3-(2,6-Dinitro-3,4-xylidino)-2-pentanol
Phenmedipham	Methyl 3-(3-methylcarbaniloyloxy)-carbanilate Knowles and Benezet (1981), Agr. Evo. (1995)	**Methyl N-(3-hydroxyphenyl)carbamate**

Pesticide	Systematic chemical name	Principal transformation products
Pirimicarb	2-Dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate Honig and Barcelo (1994)	2-Dimethylamino-5,6-dimethyl-4-hydroxypyrimidine 2-Methylamino-5,6-dimethyl-4-hydroxypyrimidine 2-Amino-5,6-dimethyl-4-hydroxypyrimidine
Prochloraz	*N*-Propyl-*N*-[2-(2,4,6-trichlorophenoxy)ethyl]- imidazole-1-carboxamide Agr. Evo. (1995)	*N*-Formyl-*N'*-Propyl-*N'*-[2-(2,4,6-trichlorophenoxy)ethyl]urea *N'*-Propyl-*N'*-[2-(2,4,6-trichlorophenoxy)ethyl]urea 2,4,6-trichlorophenol
Propachlor	2-Chloro-*N*-isopropylacetanilide Novick *et al.* (1986)	**Isopropylamine**
Propiconazole	(±)-1-[2-(2,4-Dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole	No data available
Propyzamide	3,5-Dichloro-*N*-(1,1-dimethyl-2-propynyl)benzamide Agrochem. HBk. (1991), Rouchaud *et al.* (1987)	**2-(3,5-Dichlorophenyl)-4,4-dimethyl-5-methyleneoxazoline** **3,5-Dichloro-*N*-(1,1-dimethylacetonyl)benzamide**

Pesticide	Systematic chemical name	Principal transformation products
Simazine	2-Chloro-4,6-*bis*(ethylamino)-1,3,5-triazine Erickson and Lee (1989), Nanogen Index (1975)	**2-Chloro-4-amino-6-ethylamino-1,3,5-triazine** (a.k.a. deethylsimazine or deisopropylatrazine) **2-Chloro-4,6-diamino-1,3,5-triazine** (a.k.a. dealkylsimazine) **2-Hydroxy-4,6-*bis*(diethylamino)-1,3,5-triazine** (a.k.a. hydroxysimazine) 2-Hydroxy-4,6-diamino-1,3,5-triazine 2-Hydroxy-4-amino-6-ethylamino-1,3,5-triazine 2,4-Dihydroxy-6-amino-1,3,5-triazine 2,4-Dihydroxy-6-ethylamino-1,3,5-triazine 2-Chloro-4-amino-6-hydroxy-1,3,5-triazine 2-Chloro-4,6-dihydroxy-1,3,5-triazine (a.k.a. ammelide) 2,4,6-Trihydroxy-1,3,5-triazine (a.k.a. cyanuric acid) Carbamylurea Urea
TCA	Sodium trichloroacetate	No data available
Terbutryn	2-*tert*-Butylamino-4-ethylamino-6-methylthio-1,3,5-triazine Agrochem. HBk. (1991), Muir and Yarechewski (1982)	**2-*tert*-Butylamino-4-ethylamino-6-hydroxy-1,3,5-triazine** (a.k.a. hydroxyterbutryn) **N-deethylhydroxyterbutryn** **Terbutryn sulphoxide**

Pesticide	Systematic chemical name	Principal transformation products
Triadimenol	1-(4-Chlorophenoxy)-3,3-dimethyl-1- (1*H*-1,2,4-triazol-1-yl)butan-2-ol Clark et al (1991), Haque (1983), Petrovic *et al.* (1994)	**4-(4-Chlorophenoxy)-3-hydroxy-2,2-dimethyl-4- (1*H*-1,2,4-triazol-1-yl)butanoic acid** Epimerisation occurs to give a different mix of enantiomers. Triadimenol is also a TP of the pesticide triadimefon.
Tri-allate (triallate)	*S*-(2,3,3-Trichloroallyl) diisopropyl-thiocarbamate Smith (1969, 1970, 1971), Smith and Muir (1984)	No data on specific TPs
Triazophos	1-Phenyl-1,2,4-triazolyl-3-(*O,O*-diethylthiono-phosphonate Agrochem. HBk. (1991), MAFF (1993)	**1-Phenyl-3-hydroxy-1,2,4-triazole** *O,O*-diethyl-*O*-(1-Phenyl-1*H*-1,2,4-triazol-3-yl)phosphate urea
Tridemorph	4-Alkyl-2,6-dimethylmorpholine (mixture, alkyl = C_{11} - C_{14}) BASF (1995)	**2,6-dimethylmorpholine**
Trietazine	2-Chloro-4-diethylamino-6-ethylamino-1,3,5-triazine Agrochem. HBk. (1991)	**2-Hydroxy-4-diethylamino-6-ethylamino-1,3,5-triazine**

Pesticide	Systematic chemical name	Principal transformation products
Trifluralin	2,6-Dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)-aniline European Institute for Water (1988), Agrochem. HBk. (1991), Nanogen Index (1975), Leitis and Crosby (1974), Golab *et al.* (1979), Smith and Muir (1984), Kearney *et al.* (1976, 1977), Fox *et al.* (1991)	**2-Amino-6-nitro-4-(trifluoromethyl)aniline** **2-Ethyl-7-nitro-5-(trifluoromethyl)benzimidazole** **2-Ethyl-7-nitro-5-(trifluoromethyl)benzimidazole-3-oxide** 2-Ethyl-7-nitro-1-propyl-5-(trifluoromethyl)benzimidazole 2,6-Dinitro-4-(trifluoromethyl)aniline **2,6-Dinitro-*N*-propyl-4-(trifluoromethyl)aniline** **2-Amino-6-nitro-*N*,*N*-dipropyl-4-(trifluoromethyl)-aniline** 2,2'-Azoxybis(trifluoro-6-nitro-*N*,*N*-dipropyl-toluidine) 2,2'-Azoxybis(trifluoro-6-nitro-*p*-toluidine) 2-Amino-6-nitro-*N*-propyl-4-(trifluoromethyl)aniline 2,6-Diamino-*N*,*N*-dipropyl-4-(trifluoromethyl)aniline

a.k.a. = also known as
Agrochem. Hbk. = Agrochemical Handbook
Transformation products in bold are the principal ones formed by the pesticide.

A.2 FORMULATION CHEMICALS

The information on FCs is divided into categories of solvents, surfactants and adjuvants, for which modern usage data were obtained from Central Science Laboratory.

A.2.1 Solvents

Solvents used in the preparation of pesticide products include hydrocarbons such as kerosene, toluene, xylenes, naphthenes and diesel oil. Other solvents such as acetone, methanol and trichloroethylene are also used (Fielding 1991).

Table A.2 lists of solvents used in pesticide formulations, taken from the Nanogen Index (1975).

Table A.2 Solvents used in pesticide formulations (Nanogen Index 1975)

Acetone	Acetonitrile
Acetic anhydride	Amyl acetate / Isoamyl acetate (Banana oil)
Benzene	Butendiol (But-2-en-1,4-diol)*n*-Butanol
g-Butyrolactone	a-Chloronaphthalene
Cumeme (Isopropylbenzene)	Cyclohexanone
Diacetone alcohol (2-Hydroxy-2-methyl-4-pentanone)	Diethanolamine
Dimethylsulphoxide (DMSO)	1,4-Dioxane
Dipropylene glycol	Dipropylene glycol monomethyl ether
Ethanol	Ethyl acetate
Ethylene glycol (Ethan-1,2-diol)	Ethylene glycol monobuyl ether
Ethylene glycol monomethyl ether	Ethyl methacrylate
Glycerol (Propan-1,2,3-triol)	Hexane
n-Hexyl alcohol	Isobornyl acetate
Isophorone (3,5,5-trimethyl-2-cycloheptenone)	Isopropyl alcohol (Propan-2-ol)
Mesityl oxide (4-Methyl-3-penten-2-one)	Methanol
N-Methylpyrrolidone	Methyl ethyl ketone (Butan-2-one)
Methyl isoamyl ketone (5-Methyl-2-hexanone)	Methyl isobutyl ketone (4-Methyl-2-pentanone)
Methyl naphthalene	Mineral oil
Naphtha	Pentane
Petroleum ether (kerosene)	Tetrahydrofurfuryl alcohol
Toluene	Triacetin (Glyceryl triacetate)
Triethanolamine	Triethylene glycol
Xylene (*o*-, *m*- and *p*- isomers)	

Some indication of the maximum concentrations of certain solvents in pesticide formulation can be had from the guidance relating to the labelling of pesticides (Working Document 8/10 of the 'Data requirements for approval under the Control of Pesticides Regulations (1986)'). Based on an assessment of their toxicity, solvents are assigned to one of five categories. Solvents in a given category may be included in formulations up to a maximum stated concentration without being named. If a solvent is present at a higher level, it must be identified on the label.

The categories and concentrations are given as follows in Tables A.3-A.7.

Table A.3 Toxic solvents, that must be named on the label if their concentration in the formulation exceeds 0.2% by weight

Carbon disulphide	Benzene
Carbon tetrachloride	1,1,2,2-tetrachloroethane
Pentachloroethane	Nitrobenzene
Aniline	1,1,2,2-tetrabromoethane
2-propen-1-ol-(Allyl alcohol)	1,2-dibromoethane
2-chloroethanol	Bis (2-chloroethyl) ether
Phenol	Cresol
2-Furaldehyde (Furfural)	Piperidine
1-Bromopropane	Methanol
Acetronitrile	Hexan-2-one

Table A.4 Harmful solvents, that must be named on the label if their concentration in the formulation exceeds 3% by weight

1,1,2-trichloroethane	Furfuryl Alcohol
4-Methyl-3-penten-2-one (Mesityl oxide)	Pyridine
Chloroform (Trichloromethane)	1,2-Dichlorobenzene
1-Nitropropane	2-Nitropropane
1,2-Dichloroethane (Ethylene Dichloride)	Chlorobenzene
1-Chloro-1-nitropropane	Hexane - Mixture of isomers containing > 5% n-hexane
1,4-Dioxane	

Table A.5 Harmful solvents, that must be named on the label if their concentration in the formulation exceeds 6% by weight

Dibromomethane (Methylene dibromide)	1,1-Dichloroethane (Ethylidine chloride)
Dichloropropane	1,1-Dichloroethylene
1,2-Dichloroethylene	Trichloroethylene
Tetrachlorethylene (Perchloroethylene)	N,N-Dimethylformamide
Nitromethane	Nitroethane
N,N-Dimethylacetamide	2-Butoxyethanol

Table A.6 Harmful solvents, that must be named on the label if their concentration in the formulation exceeds 10% by weight

o-Methylstyrene	2-Methylcyclohexanone
2-Ethylbutan-1-ol	Ethylbenzene
Benzyl alcohol	Pentane-2,4-dione
1,1,1-Trichloroethane	Chloropropane (Propyl chloride)
Chloropentane	Xylene
Toluene	Dimethyl carbonate
Cyclohexanone	Turpentine
2-Methoxyethanol	

Table A.7 Harmful solvents, that must be named on the label if their concentration in the formulation exceeds 20% by weight

Dichloromethane (methylene chloride)	Butanol, except tert-Butyl alcohol
1,2-Ethanediol (Ethylene glycol)	Amyl alcohol, except tert-Pentanol
2-Methylbutan-2-ol	Cyclohexanol
2-Methylcyclohexanol	2-Methylpropan-2-ol
1-(2-Butoxypropoxy)propan-2-ol	2-Ethoxyethyl acetate (Ethyl glycol acetate)
2-Butoxyethyl acetate (Butyl glycol acetate)	Hexan-1-ol
2-Heptan-2-one	2-Methoxyethylacetate
Tetrahydrothiophene-1,1-dioxide	2-Isopropoxyethanol

A.2.2 Surfactants

The Weed Science Society of America has attempted to standardise the terminology and nomenclature for adjuvants used in herbicide formulations (Colby *et al.* 1989). The stated aims of this work were two-fold:

1. To avoid the possibility of identical or similar compounds, used by various manufacturers, being mistakenly regarded as completely different.

2. To avoid the need to refer to compounds by their trade-name.

The adjuvants that have been treated in this way are non-ionic and anionic surfactants. Some of these surfactants are of indeterminate composition, being complex mixtures of related compounds or homologues. Commonly, the composition of a reaction mixture is selected to produce a surfactant with the desired properties, without further purification. As well as mixtures of homologues, the final product will, typically, also contain unreacted starting materials and a range of side-products.

Among the surfactants catalogued, and given standardised trivial names by the Weed Science Society of America are those in Table A.8.

A.2.3 Adjuvants

Adjuvants are products co-applied with pesticides to enhance their efficacy. A survey on usage of active ingredients in adjuvants was conducted by the Pesticide Usage Survey Group, Central Science Laboratory, from the data were obtained. Table A.9 lists the trade names of the products for which data were available. The usage data by crop group, in kg and in spray hectares, are presented in Tables A.10 and A.11 respectively. 'Spray-hectares' represent the area to which the adjuvants are applied with pesticides. The same data, in kg, broken down by month are presented in Table A.12.

The names of the chemicals in Tables A.10 to A.12 is as used by the manufacturers and suppliers. There is clearly overlap between the various substances on the list, for example the alkyl phenol ethoxylates, but without more information from the manufacturers it is not possible to identify the specific similarities or differences.

Table A.8 Systematic chemical names of surfactants

Trivial name	Systematic chemical name
Albenate	Alkyl (straight chain, C_{18}-C_{24}) benzenesulphonic acids and salts.
Alfos	α-Alkyl(C_{10}-C_{16})-ω-hydroxypoly(ethylene glycol) - in the form of dihydrogen phosphate esters.
Allinate	α-Lauryl-ω-hydroxypoly(ethylene glycol) sulphate.
Allinol	α-Alkyl(C_{11}-C_{15})-ω-hydroxypoly(ethylene glycol).
Diocusate	Sodium dioctyl sulphosuccinate.
Dooxynol	α-(*p*-Dodecylphenyl)- ω-hydroxypoly(ethylene glycol).
Ligsolate	NH_4, K, Na, Ca, Mg and Zn salts of lignosulphonic acids.
Nofenate	α-(*p*-Nonylphenyl)-ω-hydroxypoly(ethylene glycol) sulphate - NH_4, Ca, Mg, K, Na and Zn salts.
Nonfoster	α-(*p*-Nonylphenyl)-ω-hydroxypoly(ethylene glycol) - dihydrogen and monohydrogen phosphate esters.
Nonoxynol	α-(*p*-Nonylphenyl)-ω-hydroxypoly(ethylene glycol).
Octoxynol	α-[*p*-(1,1,3,3-Tetramethyl)butylphenyl)- ω-hydroxypoly(ethylene glycol).
Oxycastol	Polyoxyethylated caster oil.
Oxysorbic	Polyoxyethylated sorbitol fatty acid esters.
Talloil, tall oil	Fatty acids and rosin acids
Thalestol	Polyglyceryl phthalate ester of coconut oil fatty acid.

Table A.9 List of tradenames of adjuvants used on arable crops in Great Britain, 1994

Actipron	Activator 90	Adder
Adstem	Agral	Ashlade Adjuvant Oil
Ashlade Non-Ionic Wetter	Atlas Adherbe	Atlas Adjuvant Oil
Axiom	Barclay Actol	Barclay Dryfast XL
Booster	Citowett	Codacide Oil
Conka	Cropoil	Cropspray 11E
Cutback	Cutinol	Cytozyme
Desikote	Emerald	Enhance
Ethokem	Farmon Blue	Frigate
Fyzol 11E	GS 800	Headland Guard
Headland InTake	High Trees Galion	Hyspray
Intracrop BLA	LI-700	Libsorb
Lo Dose	Lyrol	Minder
Non-Ionic 90	Non-Ionic Wetter	Nu Film P
Output	PBI Spreader	Perm-E-8
Q 900	Rapide	SAS 90
SM 99	Signal	Slippa
Sprayfast	Spraymate Activator 90	Spraymate Bond
Spraymate LI 700	Sprayprover	Stick-It
Stimcote	Swirl	Sylgard 309
Team	Topup	Tripart Acer
Tripart Lentus	Tripart Minax	

Table A.10 Usage of adjuvants on arable crops grown in Great Britain by cropgroup, 1994 (weight applied in kg)

	Wheat	Winter barley	Spring barley	Oats	Rye	Triticale	Oilseed rape	Linseed	All potatoes	Peas	Beans	Sugar beet	Set aside	All crops
Alkyl alcohol ethoxylate	405	382	0	145	0	0	1,105	16	0	0	0	0	273	2,327
Alkyl phenol ethoxylate	175	179	14	721	0	0	828	907	0	14	224	16	459	3,537
Alkyl phenol ethylene oxide	14,900	5,800	1,099	927	499	223	12,054	4,923	150	3,417	3,080	11,824	5,005	63,901
Alkyl phenol ethylene oxide condensate	72,879	813	606	12	29	0	2,048	572	515	497	111	449	1,226	79,756
Alkyl phenyl hydroxypolyoxyethylene	1,480	1,064	0	134	0	0	420	2,371	291	95	1,242	615	8	7,719
Alkylarylpolyglycol ether	405	214	57	0	0	0	202	0	0	0	0	0	216	1,095
Alkylphenyl hydroxypolyoxyethylene	30	17	0	0	0	0	332	0	0	0	0	0	0	380
Total alkyl phenol ethoxylate species	**90274**	**8469**	**1776**	**1939**	**528**	**223**	**16,989**	**8789**	**956**	**4023**	**4657**	**12904**	**7187**	**158,715**
Butadiene	415	0	0	0	0	0	313	0	599	0	0	0	0	1,327
Di-1-p-menthene	3,020	1,697	653	0	0	0	4,805	673	193	642	234	0	2,126	14,043
Mineral oil	410,648	16,807	6,979	590	2,092	0	127,228	6,784	6,427	19,767	37,124	149,238	45,106	828,790
Nonyl phenol ethylene oxide	699	111	217	0	0	0	1,453	199	64	190	55	0	619	3,606
Nonyl phenol ethylene oxide condensate	0	0	0	0	0	0	0	0	0	0	69	0	0	69
Organic acids	23,193	5,437	1,176	1,212	0	336	1,524	308	52	134	160	410	4,058	38,001
Organic co-polymer	4,013	2,081	113	0	0	0	588	0	1,690	109	480	394	315	9,782
Paraffinic oil	0	0	0	0	0	0	464	0	0	0	0	0	0	464
Phenol ethylene oxide condensate	1,210	995	0	733	0	0	2,063	820	0	412	495	1,241	880	8,850
Poly-1-p-menthene	0	257	0	0	0	0	48	0	0	0	83	0	45	433
Polyalkyleneoxide modified heptamethyltrisiloxane	0	0	0	0	0	0	21	0	0	0	0	0	0	21
Polymethyl siloxane	0	0	0	0	0	0	181	0	0	0	0	0	0	181
Polyoxyalkylene glycol	9,548	631	223	106	406	0	2,690	0	0	0	0	0	0	13,605
Polyoxyethylene tallow amine	24,903	1,563	4,487	0	0	0	16,782	14,865	989	796	2,503	1,295	44,740	112,924
Propionic acid	313	0	0	0	0	0	0	0	0	0	0	0	0	313
Silyated polyether	238	95	0	0	0	0	336	0	0	0	0	0	76	746
Soyal phospholipids	106,534	19,720	2,923	1,439	0	0	4,118	1,021	184	313	257	1,354	6,417	144,280
Surface active agents	949	828	402	208	0	0	96	0	0	0	0	0	698	3,180

	Wheat	Winter barley	Spring barley	Oats	Rye	Triticale	Oilseed rape	Linseed	All potatoes	Peas	Beans	Sugar beet	Set aside	All crops
Synthetic latex	3,935	851	0	31	0	0	960	0	3,643	221	336	512	319	10,809
Tallow amine ethoxylate	13,910	6,592	2,806	418	722	0	3,278	8,231	57	877	827	1,136	48,392	87,246
Unspecified adjuvants	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vegetable oil	132,001	41,159	29,349	0	0	256	13,451	3,590	1,411	589	6,444	27,531	25,637	281,419
Total	**825803**	**107293**	**51104**	**6676**	**3748**	**815**	**197388**	**45280**	**16265**	**28073**	**53724**	**196015**	**186615**	**1718804**

Table A.11 Usage of adjuvants on arable crops grown in Great Britain by cropgroup, 1994 (spray hectares)

	Wheat	Winter barley	Spring barley	Oats	Rye	Triticale	Oilseed rape	Linseed	All potatoes	Peas	Beans	Sugar beet	Set aside	All crops
Alkyl alcohol ethoxylate	4,789	3,363	0	187	0	0	4,165	60	0	0	0	0	1,370	13,935
Alkyl phenol ethoxylate	3,338	3,572	519	520	0	0	4,708	3,001	0	31	1,573	178	1,452	18,890
Alkyl phenol ethylene oxide	83,462	47,438	6,860	3,511	1,621	2,189	51,459	17,314	2,071	7,806	12,481	22,736	29,215	288,163
Alkyl phenol ethylene oxide condensate	27,426	19,147	3,696	404	319	0	9,455	3,086	4,413	2,877	496	3,147	5,348	79,815
Alkyl phenyl hydroxy polyoxyethylene	7,524	2,148	0	1,893	0	0	3,509	6,242	1,942	1,048	1,542	4,744	27	30,619
Alkylarylpolyglycol ether	6,965	2,782	1,061	0	0	0	1,615	0	0	0	0	0	1,088	13,511
Alkylphenyl hydroxypolyoxyethylene	675	232	0	0	0	0	1,596	0	0	0	0	0	0	2,504
Total alkyl phenol ethoxylates	**134,179**	**78,682**	**12,136**	**6,515**	**1,940**	**2,189**	**76,507**	**29,703**	**8,426**	**11,762**	**16,092**	**30,805**	**38,500**	**447,437**
Butadiene	6,823	0	0	0	0	0	6,098	0	3,725	0	0	0	0	16,646
Di-1-p-menthene	19,104	4,818	3,925	0	0	0	22,440	2,145	2,548	2,044	2,726	0	4,558	64,308
Mineral oil	321,939	29,367	15,624	458	1,220	0	83,627	6,955	5,571	14,255	26,770	125,641	31,172	662,599
Nonyl phenol ethylene oxide	17,747	2,795	3,925	0	0	0	18,992	1,608	2,548	1,533	2,144	0	2,604	53,896
Nonyl phenol ethylene oxide condensate	0	0	0	0	0	0	0	0	0	0	582	0	0	582
Organic acids	67,434	16,594	3,750	3,643	0	972	4,403	289	230	397	338	1,420	9,479	108,949
Organic co-polymer	32,483	8,301	971	0	0	0	4,508	0	10,029	1,141	4,364	286	573	62,657
Paraffinic oil	0	0	0	0	0	0	313	0	0	0	0	0	0	313
Phenol ethylene oxide condensate	12,356	11,114	0	4,275	0	0	9,864	2,944	0	1,936	1,149	6,301	1,209	51,147
Poly-1-p-menthene	0	2,467	0	0	0	0	451	0	0	0	480	0	676	4,074
Polyalkyleneoxide modified heptamethyltrisiloxane	0	0	0	0	0	0	329	0	0	0	0	0	0	329
Polymethyl siloxane	0	0	0	0	0	0	903	0	0	0	0	0	0	903
Polyoxyalkylene glycol	18,466	1,284	743	353	914	0	5,136	0	0	0	0	0	0	26,896
Polyoxyethylene tallow amine	27,644	2,334	3,411	0	0	0	17,866	9,923	1,136	1,603	3,479	1,670	59,655	128,721
Propionic acid	970	0	0	0	0	0	0	0	0	0	0	0	0	970
Silyated polyether	5,299	2,141	0	0	0	0	2,506	0	0	0	0	0	525	10,471
Soyal phospholipids	180,618	35,405	4,245	2,576	0	0	8,835	1,513	233	396	1,132	2,342	9,476	246,772

	Wheat	Winter barley	Spring barley	Oats	Rye	Triticale	Oilseed rape	Linseed	All potatoes	Peas	Beans	Sugar beet	Set aside	All crops
Surface active agents	3,221	3,680	1,190	1,073	0	0	213	0	0	0	0	0	1,551	10,929
Synthetic latex	40,767	10,964	0	404	0	0	8,386	0	31,663	2,243	4,668	3,228	4,474	106,798
Tallow amine ethoxylate	22,461	8,392	4,987	529	914	0	4,880	9,507	121	1,219	1,265	2,186	62,021	118,482
Vegetable oil	73,048	24,041	13,948	0	0	77	7,199	2,144	1,988	310	3,057	13,423	15,568	154,802
Total	**984,559**	**242,379**	**68,855**	**19,826**	**4,988**	**3,238**	**283,456**	**66,731**	**68,218**	**38839**	**68,246**	**187302**	**242041**	**1278681**

Table A.12 Usage of adjuvants on arable crops grown in Great Britain by month, 1994 (weight applied in kg)

Active substance	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Alkyl alcohol ethoxylate	784	.	359	219	326	429	211	.	.	.	.	.	2,327
Alkyl phenol ethoxylate	.	12	6	52	1,098	65	786	612	836	.	71	.	3,537
Alkyl phenol ethylene oxide	21	536	3,595	7,638	23,928	9,253	6,448	3,569	2,507	4,189	2,217	.	63,901
Alkyl phenol ethylene oxide condensate	267	.	212	350	73,485	1,779	1,415	749	702	350	448	.	79,756
Alkyl phenyl hydroxypolyoxyethylene	.	.	865	662	2,274	1,996	556	1,356	10	.	.	.	7,719
Alkylarylpolyglycol ether	.	.	40	52	487	78	38	.	.	216	184	.	1,095
Alkylphenyl hydroxypolyoxyethylene	.	.	.	.	9	30	.	284	56	.	.	.	380
Total alkyl phenol ethoxylate species	**1072**	**548**	**5077**	**8973**	**101607**	**13630**	**9454**	**6570**	**4111**	**4755**	**2920**		**158,715**
Butadiene	.	.	.	262	191	365	269	239	.	.	.	.	1,327
Di-1-p-menthene	.	180	738	789	2,509	2,433	3,207	1,603	2,336	.	247	.	14,043
Mineral oil	3,852	25,366	111,299	222,482	283,059	112,469	11,273	1,837	15,235	22,758	18,195	966	828,790
Nonyl phenol ethylene oxide	.	60	245	262	507	355	944	468	683	.	82	.	3,606
Nonyl phenol ethylene oxide condensate	.	.	.	.	69	.	.	.	.	.	.	.	69
Organic acids	.	557	8,978	11,817	10,568	3,106	1,610	981	.	190	195	.	38,001
Organic co-polymer	.	.	95	402	2,013	6,410	510	161	118	73	.	.	9,782
Paraffinic oil	.	.	.	.	.	.	.	.	.	.	464	.	464
Phenol ethylene oxide condensate	.	.	113	662	2,581	2,046	1,084	1,884	47	48	355	28	8,850
Poly-1-p-menthene	.	.	.	45	257	131	.	.	.	.	.	.	433
Polyalkyleneoxide modified heptamethyltrisiloxane	.	.	.	21	.	.	.	.	.	.	.	.	21
Polymethyl siloxane	.	.	.	.	.	.	181	.	.	.	.	.	181
Polyoxyalkylene glycol	.	.	4,453	2,429	3,902	1,998	171	.	470	.	181	.	13,605
Polyoxyethylene tallow amine	.	454	2,138	13,600	26,584	7,305	19,010	18,467	15,780	7,279	2,307	.	112,924
Propionic acid	.	.	.	313	.	.	.	.	.	.	.	.	313
Silyated polyether	55	.	69	393	153	.	.	.	.	76	.	.	746
Soyal phospholipids	82	2,031	28,859	56,903	39,652	10,687	1,675	1,955	987	518	931	.	144,280
Surface active agents	.	.	24	1,679	531	402	96	449	.	.	.	.	3,180

Active substance	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Synthetic latex	.	.	299	423	1,683	5,003	1,955	1,051	249	115	30	.	10,809
Tallow amine ethoxylate	.	71	1,347	11,335	19,373	11,282	9,191	15,560	15,699	2,856	448	82	87,246
Vegetable oil	137	6,236	30,155	63,956	100,212	61,190	11,601	4,462	480	912	.	2,079	281,419
Total	**5198**	**35503**	**193889**	**396746**	**595451**	**238812**	**72231**	**55687**	**56195**	**39580**	**26355**	**3155**	**1718,804**

APPENDIX B SAMPLING PROTOCOL FOR AQUIFER MATERIAL

Title: Drilling, subsampling and preservation of aquifer core material for laboratory analysis

This sampling protocol provides guidance on sampling aquifer material, obtained from a drilling rig for laboratory analysis.

Office procedures

1. Obtain permission for site access, check site access is suitable for the drilling rig, cabins and other vehicles. Check Health and Safety and clothing requirements for site.

2. Obtain fresh sample bottles and labels from analytical services. Arrange for a freezer to be located on-site. A cool box filled with frozen packs should also be available for returning samples to the laboratory. Inform the laboratory of the intended schedule for return of samples.

Field Procedures

1. Ensure that the rig and all casing has been steam cleaned or jet washed prior to arriving on-site. work. Pay particular attention to any coring devices that will be in contact with the aquifer material to be sub-sampled. Ensure that Heath and Safety, and the requirements specified in the Drilling Contract Bill of Quantities have been satisfied. The equipment will require further cleaning between each new borehole.

2. Inform the drilling crew of your requirements for sampling. Reiterate that the use of water, foam and drilling muds is prohibited.

3. Set out a clean area in the site cabin (cover any bench's with Benchkote™ or similar material) and ensure the freezer is sufficiently chilled to accept samples (at least -10 °C).

4. Immediately on recovery take the core of aquifer material and place on a clean and dry surface open (in case of a split spoon sampler, hollow stem auger) or extrude (U100 core, percussion) as appropriate and make a visual geological log of the core in the field note book.

5. Screen the core using Thermo Electron portable PID (fitted with 10.4 EV lamp) to detect the presence of volatile compounds (e.g. chlorinated solvents and petroleum hydrocarbons).

6. Take three sample from the bottom of each extracted core (1 metre intervals for a split spoon sampler and 45 cm intervals standard U100 core) using a stainless steel spatula or trowel taking care to discard the outer material that has been in contact with the sampling device. If working at a contaminated site ensure that all waste material is stored in drums or a skip for licensed disposal.

7. Place the samples into clean glass jars fitted with a screw cap. Line one of the glass jar lids with aluminium foil, this should be the sample submitted for any organic analysis. Retain one sample for inorganic analysis and one as a spare in the event of subsequent analytical problems. Thoroughly clean the spatula between samples using RO water.

8. If geotechnical tests are required take a further sample in a sealable polythene bag.

9. In the absence of specified labelling procedure all samples should be clearly marked with both a paper label and indelible marker with Project number, site, borehole identifier, depth and date. Place all samples into the freezer.

10. Lay the remaining core material in a labelled core box (care should be taken to place the bottom of succeeding cores against the top of the preceding one. Use a new box for each new borehole.

11. Complete sample custody forms and return samples to the analytical laboratory as appropriate in a cool box containing frozen cool packs.

12. Return to the office, file all paper work in project record file. Clean all equipment using a 10% solution of Decon cleaning fluid and rinse thoroughly in RO water.

Equipment List

Benchkote™
Clean glass jars
Aluminium foil
Stainless steel spatula or trowel
Self seal polythene bags
RO water in sealed glass aspirators
Cool box and cooler packs
Freezer
Core boxes

APPENDIX C EXTRACTION OF POREWATER FROM CORE MATERIAL USING CENTRIFUGATION

Title: Extraction of porewater from core material using centrifugation

Apparatus:

Decon® and methanol for equipment cleaning
RO water for cleaning and rinsing pots between samples
250 ml Teflon® pots, lids, stainless steel liners, and Teflon® filter discs
Glass microfibre filters (5 or 8 cms diameter)
Metal spatula
Analytical balance (2 decimal place)
8 ml amber glass vials, with black screw caps and Teflon® faced silicone septa (available from Phase Separations Ltd.)

Method:

1. Cores should all be stored at -10 °C in the freezer double wrapped in plastic layflat tubing and the ends tied with bags ties to effectively seal the sample. Select the appropriate number of core samples from the freezer and defrost for 12 hrs at room temperature, prior to spinning. The maximum number of spins which can be completed in an average working day is 5, therefore 30 cores is the maximum which should be defrosted for any one working day. Once removed from the freezer the samples must be spun on the next day and the extracts stored in a refrigerator.

2. All centrifuge equipment should be soaked in 10% Decon® solution for 24 hours prior to use. When soaking is complete rinse three times in Reverse Osmosis (RO) water. Then rinse once in methanol or acetone, and dry in the warm air cabinet.

   Note: When rinsing and drying pots always place on clean tissue or benchcote. Plastic gloves should be worn at all times when handling the samples and equipment.

3. Make-up a table in the laboratory notebook (across a clean double page) with the following headings:

   - Sample reference;
   - Pot number (make sure pots are clearly numbered with an indelible marker pen);
   - Weight of pot (g) = A;
   - Weight of pot + liner + lid + filter paper (g) = B;
   - Weight of sample (g) = C - B = D;
   - Weight of pot + porewater (g) = E;
   - Weight porewater (g) = E - A = F; note F ÷ D x 100 = approximate % moisture
   - content (by weight);
   - Comments.

4. Weigh the pot (A) and record the weight, then weigh the pot + liner +lid + filter paper (B) and record the weight.

5. Select a core for spinning and record the core reference number against the pot weight *in* the laboratory note book.

6. The core sample should be labelled 'top and bottom',. When taking a sample for spinning always take the sample from the bottom of the core (the drilling process means the depth of removal can be estimated more accurately for the bottom of the core than the top). Open up the core and using a metal spatula (plastic if sampling for trace metals) sample approximately 200 g of material. Discard the material which has been in contact with the wrapping material and take the sample from the centre. If the aquifer material is very dry first try doing multiple spins to obtain sufficient porewater. If this is unsuccessful add 5 mls of RO water prior to spinning. This must be noted down in the 'Comments' column in the laboratory notebook.

7. Record the total weight of pot + lid + liner + filter paper + sample (C)

8. Place the sample in the stainless steel inserts and then into the Teflon shells. The maximum number of pots per run is six although four or two can be run if there is less than a full batch. However, the pots (with liner, sample and lid) must all be balanced to within ± 0.1g.

9. Spin the pots at a maximum speed of 6000 rev/minute for 30 minutes at a temperature of 4 °C.

10. The porewater will drain through the porous insert and collect in the Teflon® /PTFE® shell. Remove inner liner and re-weigh pot and porewater (E). Pour a small amount of the spun porewater into a clean labelled amber glass vial (pre-rinsed in RO water), screw on cap and septa and shake and discard this aliquot. Pour the remaining spun sample into the vial replace lid and septa and at 4 °C.

11. After each spin, the set of shells and inserts must be cleaned as in (2) before re-use.

Additional Notes

1. The septa in the amber vials are designed to go with the thin Teflon® (shiny plastic) side towards the sample.

2. Each vial should be labelled using adhesive labels with the following information

   - sample reference number;
   - site location;
   - date of spinning;
   - name of spinner;
   - Contract number;

APPENDIX D SAMPLING PROTOCOLS FOR MONITORING GROUNDWATER

Title: General protocol for sampling groundwater from an observation borehole (After Clark 1992)

Equipment/apparatus

The following list is not exhaustive but includes the main elements:

- Site map and borehole diagram (background information on the monitoring array is highly desirable);
- Tool kit (to serve the monitoring equipment as well as the closure cover of the borehole);
- pH meter and probe;
- Conductivity meter and probe;
- Dissolved oxygen (DO) meter and probe;
- Eh meter and probe (optional);
- Flow through cell for pH, conductivity, Eh and DO measurements (optional);
- Sample bottles;
- Plastic sheet;
- Groundwater level dipper;
- Sample recovery equipment;
- Totalising water meter (optional);
- Deionised or distilled water for rinsing equipment.

Preparation for sampling

1. The requirements of the sampling exercise will be documented in the Sampling Plan. Before developing the Sampling Plan, the objectives of the exercise must be defined. This protocol only covers the basic sampling methodology, but the following check list will assist the development of the Sampling Plan and hence the preparations for the sampling exercise.

2. Read the Company/organisation health and safety policy statement and prepare a Site Operating Procedure (SOP) for inclusion in the Sampling Plan. (NOTE: The SOP should take account of the employer's responsibility with respect to the UK Control of Substances Hazardous to Health (COSHH) Regulations 1988. Each SOP should be assigned a specific hazard/risk code which can be used to identify appropriate Personal Protective Equipment (PRE) for the task.)

3. Check the access route and ground conditions for the field vehicle and discuss with the site owner or other responsible person. Agree conditions of entry to the site in writing and add these to the Sampling Plan.

4. Discuss the sample analytical requirements with the analyst (e.g. determinands, sample type and condition, bottles, sample storage, reception arrangements) and collect the prepared bottles in good time for the sampling exercise. (NOTE: Other sample requirements like filtration, preservation, bottle head space should be discussed at this stage).

5. Obtain all information relating to borehole construction and rest water levels. (NOTE: a geophysical log of the borehole provides additional valuable information, if available.)

6. Calculate the volume of water to be pumped from the borehole in order to remove at least three borehole volumes of water. (NOTE: It is often helpful at this stage to create a quick look up table for later use in the field.)

7. Decide on the depth at which the pump is to be set. (NOTE: the decision will be based on the borehole characteristics, the position of the screen, the type of pump and the objectives of the exercise. Always check the Sampling Plan and discuss with the supervisor.)

8. Before packing the sample recovery equipment, check the cleaning procedure records and repeat to the appropriate standard, if not satisfied.

9. Check the calibration of the pH, temperature, conductivity, Eh and DO probes. (NOTE: Ensure that calibration and standard solutions are taken on the sampling exercise.)

10. Procedure for taking a groundwater sample from an observation borehole

11. Open the observation borehole and check the depth to the water table and the total depth of the borehole using a groundwater level dipper. Record the results using field documentation.

12. Lay out all the sample recovery equipment on a clean plastic sheet.

13. Check the volume of water to be pumped (see Preparation for Sampling, item 5) and set up arrangements for disposing of the purged groundwater (see the Sampling Plan).

14. Assemble the sample recovery equipment, tape all cables and rising main together to avoid tangling and damage, and lower the assembly into the borehole. For large diameter observation boreholes a tripod and winch assembly may be required. (NOTE: Secure any loose cables to the rising main to avoid tangling and damage.)

15. Slowly lower the assembly to the required depth and secure in position (e.g. by locking the cable drum or by using a catch-plate). The sample inlet should be 1-2 meters above the screen (if present).

16. Connect the discharge hose to the top of the rising main. A totalising water meter can be fitted onto the discharge hose to aid the measurement of discharge volume. (NOTE: The discharge hose should be sufficiently long to prevent water returning to the borehole head works.)

17. Consult the Sampling Plan for the required purge volume, start the pump and run until the borehole has been purged. (NOTE: see item 3 above.)

18. Check the calibration of probes for on-site determinations. Measurements of temperature, pH, conductivity, Eh, and DO should all be carried out in a flow-through cell connected to the reduced-flow discharge line, after removing air bubbles from the cell. Alternatively pH, temperature and conductivity can be measured in a clean beaker full of groundwater, but on no account should Eh and DO be measured in this way. Record the results in the field log, with any comments on appearance and odour.

19. Fill the sample bottles direct from the discharge tubing where possible. Rinse the bottles which do not contain preservative with groundwater and fill to the top. Bottles containing preservatives should not be rinsed and only filled to the 'fill-to-mark'. Filter the samples for metal determinations through 0.45 µm membrane filters (after discarding the first aliquot of filtered sample).

20. Reduce the pumping rate to $<2\ l\ min^{-1}$ when sampling for volatile determinands. Fill the glass vial to the brim and screw on the cap with PTFE-lined septum. There should be no headspace within the vial. Store the vials upside-down in a coolbox to minimise the loss of volatiles.

21. Check that the sample bottles are labelled correctly, then pack them into a coolbox containing ice packs for transport.

22. When QA/QC samples are needed, 'trip' blanks should remain unopened and 'field' blanks should be transferred from their bottles into fresh bottles containing the relevant preservative. 'Equipment' blanks are prepared by running organic-free/deionised water through the sampling equipment.

23. Rinse the sampling accessories with organic-free, deionised water before packing them away.

24. Slowly withdraw the sample recovery equipment from the borehole so as to avoid damage to the rising main or any cables. Disassemble the equipment on the plastic sheet, rinse with deionised or distilled water and pack the equipment away.

25. Secure the closure cover of the borehole.

26. Deliver the sample bottles to the laboratory, completing sample custody forms.

27. All field equipment should be thoroughly cleaned using a proprietary cleaning fluid on return to the laboratory (NOTE: It is prudent practice to set up a record of this activity and get a colleague to authorise the completion of the cleaning before the equipment is returned to storage.)

ADDITIONAL INFORMATIVE NOTES

1. Pump sets and dippers used for contaminated water should be appropriately marked and must not be used for routine groundwater monitoring.

2. Where dedicated sampling equipment for each borehole is not available, and previous monitoring data demonstrate that a range of levels of contamination will be encountered during a sampling exercise, attempt to commence the sampling exercise with the least contaminated borehole, finishing with the most heavily contaminated borehole.

3. Where piezometers are being sampled, the 'active' water column will be equal to the distance from the sample inlet of the recovery equipment to the bottom of the borehole.

4. For large diameter observation boreholes, a dual pump array for purging and sampling may be required.

5. Conditions in the borehole (e.g. presence of silt or other heavy particulates) may affect the temporal variations in the data, or be responsible for systematic trends. Where changes in borehole conditions are encountered, the field technician must discuss his observations with his supervisor and any agreed changes in monitoring strategy logged in the Sampling Plan.

6. The principle of removing three well volumes to purge boreholes is a good general guide. However, detailed knowledge obtained during a monitoring programme might indicate that a change to this strategy is appropriate.

REFERENCE

Clark, L. (1992) Methodology for monitoring and sampling groundwater. NRA R&D Note 126.

Title: Sampling from nested or multiple observation boreholes by pumping (After Clark 1992)

This sampling protocol is for sampling narrow diameter (50 mm) observation boreholes. These boreholes may be in the form of nested or solitary slim boreholes. The diameter of the boreholes would be a maximum of 50 mm (2 inches) and the screened length between 3 and 10 meters.

Office procedures

1. Obtain permission for site access, check site access is possible, check health & safety and clothing requirements for site.

2. Obtain all information relating to borehole construction, rest water levels and if a geophysical log of the borehole is available this should also be obtained.

3. Identify depth at which the pump is to be set.

4. Obtain appropriate field equipment, submersible pump*, rising main and power supply. Clean to standard appropriate to project.

5. Obtain and calibrate pH, redox, temperature dissolved oxygen and conductivity probes. Ensure that calibration solutions are taken on sampling exercise.

6. Obtain fresh sample bottles from analytical services. Identify whether a cool box is required for sample storage.

* see equipment list

Field procedures

1. Open the observation borehole and check the depth to the water table and the total depth of the borehole using an appropriate dipper**.

2. Lay out all sampling equipment to be used onto a clean plastic sheet.

3. Calculate the volume of water to be pumped from the borehole in order to purge the borehole with four borehole volumes of water.

4. Assemble the submersible pump and flexible rising main on plastic sheet.

5. Lower the pump assembly into the borehole. fasten all cables to rising main to avoid tangling and damage to cables.

6. Slowly lower the pump to the required depth, this ideally should be 1-2 meters above the screen and anchor.

7. Connect the discharge hose to the top of the rising main; the discharge hose should be sufficiently long to prevent discharged water returning to the borehole head works. A bucket will be sufficient in instances where a battery-operated pump is used. Consideration into where the purged water should be disposed of should be taken *into* account.

8. Connect the pump to the power source, then switch power on. Measure discharge rate from the pump and calculate the purge time required.

9. Calibrate water quality meters and insert into flow-through cell. Connect flow-through cell to low flow discharge port and remove all air bubbles from the cell. Wait for meter readings to stabilise and take readings, this should ideally be undertaken immediately before sampling.

10. Turn meters off and disconnect flow-through cell. Rinse sample bottles three times with purged water before collecting sample, using the final rinse water to rinse the cap. Fill each sample bottle to the brim to eliminate headspace.

11. When sampling for volatile compounds the discharge rate from the sampling port should be <100 ml per minute. The sample vial should always be made from glass and the cap should have a Teflon lined insert, the vial should be filled to eliminate headspace.

    NB Samples to undergo metals analyses should be filtered first using a 0.45 µm cellulose acetate membrane. Bottles already containing preservatives should not be rinsed with the sample water and only filled to the 'fill-to-mark'.

12. Check that the sample bottles are labelled correctly then pack into a cool box.

13. When QA/QC samples are needed, 'trip' blanks should remain unopened and 'field' blanks should be transferred from their bottles into fresh bottles containing the relevant preservative. 'Equipment' blanks involve running organic-free/deionised water through the sampling equipment.

14. Rinse flow-through cell and accessories with organic-free/deionised water before packing away.

15. Withdraw submersible pump from borehole slowly to avoid damage to cables. Disassemble the pump on the plastic sheet, rinse the pump with organic-free/deionised water and pack away.

16. Lock the borehole.

17. Deliver sample bottles to the laboratory, completing all sample custody forms.

18. Return to office. All field equipment should be thoroughly cleaned using DECON cleaning fluid.

NOTE

(a) Pump sets used for polluted boreholes should be appropriately marked and must not be used for routine groundwater monitoring.

(b) Pump sets designated 'pesticides' or 'clean boreholes only' should never be used for monitoring potentially contaminated sites.

(c) Where piezometers are being sampled the 'active' water column will be equal to the distance from the top of the pump to the bottom of the borehole.

Equipment	**Comments**
Submersible pump	Use submersible pump* appropriate to sampling exercise.
Rising main	Use rising main appropriate to pump and determinants to be sampled for.
Power supply	Battery for Duplo pump, generator for electric submersible pump.

Miscellaneous

Tool kit	
Cool box	
Petrol can	4*/unleaded.
Dipper	Use appropriate dipper**.
* Pump selection	
Grundfos MP1	This is a specialist pump suitable for sampling boreholes of 50 mm diameter although it may be used in larger boreholes if necessary. It has a maximum head of approximately 90 metres as well as having a variable flow rate - ideal for purging a borehole followed by sampling.
Duplo pump	These have a maximum head of 30 metres. A 24 volt supply, i.e. two batteries in series will be required to achieve this otherwise a 12 volt supply will sufficient to pump to heads of 10 to 15 metres. Duplo pumps are not designed for continuous use, therefore run the pumps for 15 minutes maximum before resting them for 15 minutes.
** Dipper selection	All dippers are marked with their intended use.
Batteries	Two of. The RS batteries are recommended; these offer extended battery life over car batteries. However, they cannot be charged off a car battery charger; they must be returned to WRc for charging. Previous experience suggests they are more than capable of lasting a weeks field work.

REFERENCE

Clark, L. (1992) Methodology for monitoring and sampling groundwater. NRA R&D Note 126.

APPENDIX E ANALYTICAL METHOD FOR ATRAZINE, SIMAZINE AND THEIR TRANSFORMATION PRODUCTS IN CHALK AQUIFER MATERIAL

1 Performance Characteristics of the method			
	1.1	Substances determined	Atrazine, simazine, dealkylated atrazine (DAA) deisopropylatrazine (DIA) deethylatrazine (DEA). This method may be suitable for the determination of other triazine herbicides but has only been tested for the compounds listed above.
	1.2	Types of samples	Chalk aquifer materials. May also be applicable to other aquifer materials but not tested for this purpose.
	1.3	Basis of method	Extraction of sample with acetone, concentration, filtration and determination by liquid chromatography - mass spectrometry (LC-MS).
	1.4	Range of application	Up to at least 10 $\mu g\ kg^{-1}$
	1.5	Calibration curve	Has been shown to be linear over the range 0.05 to 10 $\mu g\ kg^{-1}$ apart from DAA which is linear from 0.5 to 10 $\mu g\ kg^{-1}$.
	1.6	Standard deviation	See Table 1.
	1.7	Limit of detection	See Table 1.
	1.8	Sensitivity	Dependent on the determinand and the instrument in use.

1.9 Bias — No significant biases were detected ($\alpha = 0.05$). The mean concentrations detected in spiked samples are given in Table 1.

1.10 Interferences — See 3 below.

1.11 Time required for analysis — About six samples in seven days - total time.

Table 1 Performance characteristics of the analytical method for atrazine, simazine and TPs in chalk

Performance characteristic	Spiking level ($\mu g\ kg^{-1}$)	Value of performance characteristic ($\mu g\ kg^{-1}$) for				
		Atrazine	Simazine	DEA	DIA	DAA
LOD		0.040	0.023	0.158	0.063	0.341
Mean	0.05	0.051	0.041	0.034	0.048	0.422
S_w	(DAA 0.5)	0.006	0.004	0.024	0.009	0.051
Mean	0.2	0.190	0.196	0.207	0.168	0.994
S_w	(DAA 1)	0.007	0.015	0.016	0.022	0.102
S_b		0.003	0.020	0.027	0.051	0.271
S_t		0.008	0.025	0.031	0.056	0.290
RSD_t (%)		4	13	15	33	29
Degs F		5	3	3	2	2
Mean	2	1.989	2.219	2.173	2.092	5.200
S_w	(DAA 5)	0.089	0.544	0.550	0.529	1.652
S_b		0.125	0.291	0.142	0.103	1.670
S_t		0.154	0.617	0.568	0.539	2.349
RSD_t		8	28	26	26	45
Degs F		3	4	5	5	3

LOD - Limit of detection as defined by Cheeseman and Wilson (1989)
Mean - Mean concentration of analytical results
S_w - Within batch standard deviation
S_b - Between batch standard deviation
S_t - Total standard deviation
RSD_t - Relative total standard deviation
Degs F - Degrees of freedom

2 Principle

Chalk aquifer material is freeze dried, ground to a powder and extracted with acetone. The acetone extract is filtered to remove the chalk, concentrated by Turbo-Vap technique, passed through a 0.2 µm syringe filter to remove particulate material. The concentrations of the determinands in the final extract are determined by liquid chromatography-mass spectrometry (LC-MS) in selected ion recording mode.

3 Interferences

No specific interferences have been noted (apart from for DAA) , however the occurrence of compound(s) which are extracted and both co-elute with and contain the quantitation ion of a determinand will interfere. The use of confirmatory ions and ion ratios are able to reduce the number of false positive results. DAA elutes on the side of a large interfering peak, hence the larger detection limit and smaller linear range. If interferences are suspected, additional work, not described here, may be necessary. This might include the use of MS-MS techniques.

4 Hazards

All reagents must be COSHH assessed before use and the recommended control procedures implemented. The solvents are harmful and flammable, and ammonium acetate is an irritant. Caution must be exercised when preparing the stock and working calibration standard solutions, skin contact, ingestion and inhalation must be avoided. Good laboratory procedure must be followed at all times.

5 Reagents

All reagents must be of sufficient purity that they do not give rise to significant interfering peaks in the LC-MS analysis of extracts.

5.1 Methanol, High-performance liquid chromatography (HPLC) grade.

5.2 Acetone, Glass distilled grade.

5.3 Distilled or deionised water, pesticide free.

5.4 Ammonium acetate solution 0.1M. Dissolve 0.77 ± 0.01 g Analar grade ammonium acetate in ~50 ml of HPLC quality water (e.g. glass double distilled) in a 100-ml volumetric flask and make up to mark. Prepare fresh for each batch of samples.

5.5 HPLC eluent for reconstituting extracts. Pipette 4.5 ± 0.1 ml 0.1M ammonium acetate solution into a 10-ml volumetric flask and make up to volume with methanol.

5.6 Nitrogen gas, dry oil-free passed through an activated carbon filter.

5.7 Standard solutions. Store in a refrigerator. Prepare fresh solutions every six months from pure or certified materials.

5.7.1 Atrazine, simazine and TPs stock solution (100 mg l^{-1}). Accurately weigh 10 mg of atrazine, simazine, DIA, and DEA into a 100-ml volumetric flask, dissolve and make up to volume with methanol.

5.7.2 DAA stock solution (100 mg l^{-1}). Accurately weigh 10 mg of DAA into a 100-ml volumetric flask, dissolve and make up to volume with methanol.

5.7.3 Internal standard (d_5-atrazine) stock solution (100 mg l^{-1}). Accurately weigh 10 mg of d_5-atrazine into a 100 ml volumetric flask, dissolve and make up to volume with methanol.

WARNING
CAUTION MUST BE EXERCISED WHEN PREPARING THE STOCK SOLUTIONS. SKIN CONTACT, INGESTION AND INHALATION MUST BE AVOIDED.

5.7.4 Atrazine, simazine and TPs spiking solution (1 mg l^{-1}). Add 1 ± 0.01 ml of stock solution (see 5.7.1) to 50 ml of methanol in a 100 ml volumetric flask and make up to volume with methanol.

5.7.5 DAA spiking solution (1 mg l^{-1}). Add 1 ± 0.01 ml of stock solution (see 5.7.2) to 50 ml of methanol in a 100 ml volumetric flask and make up to volume with methanol.

5.7.6 Internal standard spiking solution (1 mg l^{-1}). Add 1 ± 0.01 ml of internal standard stock solution (see 5.7.3) to about 50 ml of methanol in a 100 ml volumetric flask and make up to volume with methanol.

5.7.7 Calibration standards. Prepare duplicate standards by adding the appropriate volumes of atrazine, simazine and TPs spiking solution (from 5.7.4); DAA spiking solution (from 5.7.5) and internal standard spiking solution (from 5.7.6) to 5 ml of deionised water in a measuring cylinder. Pour the spiked water onto a 250 ± 0.5 g sample of powdered pesticide free chalk. Rinse the measuring cylinder with 5 ml of deionised water and add this to the chalk sample also. These calibration standards should then be put through the full analytical procedure at the same time as the samples to be analysed.

Volume of solution 5.7.4 added (µl)	Volume of solution 5.7.5 added (µl)	Volume of solution 5.7.6 added (µl)	Concentration of simazine, atrazine DEA and DIA in standards (µg kg^{-1})	Concentration of DAA in standards (µg kg^{-1})	Concentration of d_5-atrazine in standards (µg kg^{-1})
40	200	125	0.16	0.8	0.5
550	1300	125	2.2	5.2	0.5

5.7.8 LC-MS standard solution (0.2 µg kg^{-1} equivalent). Add 500 µl of solutions 5.7.1 and 5.7.2, and 1250 µl of solution 5.7.3 to a 100 ml volumetric flask containing 45 ml of 0.1 M ammonium acetate solution (see 5.4). Make up to volume with methanol.

6 Apparatus

Glassware should be clean (soaked in a suitable proprietary laboratory detergent solution, rinsed with tap water, soaked in dilute orthophosphoric acid solution, rinsed with deionised or distilled pesticide free water and finally rinsed with acetone) and dry.

6.1 Reagent preparation - Syringes 25, 50, 100, 250 µl and 1 ml. Volumetric flasks 10 ml and 100 ml, pipettes for measuring 4.5 and 45 ml. Analytical balance (5 place), Top pan balance (3 place).

6.2 Chalk aquifer material preparation - Bricklayers hammer and bolster. Freeze drier (Edwards Mini-fast 3400 is suitable). Impeller type cutting mill with 4 mm sieve (Glen Creston type SM1 mill is suitable). Glass or stainless steel containers..

6.3 Extraction and extract filtration - Quickfit widemouth stoppered conical flasks (1 l), Buchner flasks (2 l), Buchner funnels (12.5 cm), vacuum line fitted with a suitable solvent vapour trap, Whatman glass fibre filters (GF/F and GF/D, 12.5 cm), orbital shaker fitted with a platform suitable for 1 l conical flasks, (Gallenkamp model is suitable). Top pan balance (3 place).

6.4 Concentration and concentrate preparation - Zymark Turbo-Vap 500 or similar evaporator, 3 ml vials (Wheaton are suitable), 2 ml syringe with luer fitting, Anotop 10 0.2 µm syringe filters, low dead volume disposable syringe needles with Luer fittings, 1 ml LC-MS autosampler vials.

6.5 Liquid chromatograph - mass spectrometer (LC-MS)

A LC-MS system with data system operated in accordance with the manufacturer's instructions.

Suitable conditions are:
LC conditions: Column Spherisorb S5 C8 250 x 4.6 mm column. Isocratic eluent, methanol:0.1 M aqueous ammonium acetate (55:45), flow rate 1 ml $minute^{-1}$. Injection loop 20 µl. In-line filters, 0.2 µm, fitted between the injector and column, and column and interface, to prevent blockages.

MS conditions: Plasmaspray ion source operated at 200 °C with 600 µA discharge current. Other MS conditions are tuned by optimising the response of a LC-MS standard (0.2 µg kg^{-1} equivalent, as prepared in step 5.7.8) injected post-column. The mass spectrometer is calibrated for selected ion recording using a solution of polyethylene glycol 200 in methanol, injected post column.

Typical retention times and ions monitored:

Compound	Retention time (minutes)	m/z monitored quantification	m/z monitored confirmation
dealkylatrazine	3:15	146	148
deisopropylatrazine	4:20	174	176
deethylatrazine	5:18	188	190
simazine	8:17	202	204
d_5-atrazine	11:25	221	223
atrazine	11:45	216	218

6.6 Moisture determination - Porcelain dishes (2), Analytical balance (5 place), Microwave oven (suitable power rating 700 W). Desiccator and tongs.

7 Sample Storage and Preservation

Core samples are taken using a percussion drilling rig with steam cleaned aluminium U100 core barrel liners. The sample, complete with aluminium core barrel liner, is enclosed in double lined polythene sleeving, sealed, and placed in a freezer for transit. Samples should be analysed as soon as possible upon receipt at the laboratory, if not they should be stored in a freezer.

8 Analytical Procedure

Step	Procedure	Notes
8.1	Sample pretreatment.	
8.1.1	Using a core pusher the core sample is removed from the aluminium liner. Between 25 and 50 mm of each end of the core sample is removed and discarded (see Note a).	a. If after steps 8.1.1, 8.1.2 or 8.1.3 no further work is to be immediately carried out, the sample must be sealed in a glass or stainless steel container and returned to the freezer.
8.1.2	Sections of the core sample are broken into small lumps (see Note b) with a bricklayers hammer and bolster (see Notes a and c).	b. To facilitate grinding the maximum size of the lumps should be about 50 mm.
8.1.3	The broken core sample is freeze dried until the moisture content is negligible (i.e. <0.3%) (see Note d).	c. The bricklayers hammer and bolster are cleaned between samples by brushing free of chalk, washing with tap water, then drying.
8.1.4	The sample is ground to a homogeneous powder in a knife mill fitted with a 4 mm sieve (see Notes a and e). The powder is then shaken in its container to homogenise the sample.	d. In the freeze drier used to obtain the performance data this required freeze drying over a weekend. e. The knife mill must be cleaned in between samples by brushing away all the loose chalk with a stiff bristled brush and washing the sieve with tap water, then drying it. In between batches of samples the knife mill is cleaned more thoroughly by brushing and washing with water (deionised

or distilled) to remove chalk from inside the housing, rotary head, blades and sieve. The knife mill should then be dried before use.

With this cleaning procedure it is estimated that the maximum cross contamination that could possibly occur is 0.4%. As an extra precaution when profiling chalk cores, the deepest sections of the cores are always knife milled first, as these should have lower levels of determinands.

8.2 Extraction

8.2.1 Accurately weigh 250 ± 0.5 g of powdered sample into a wide mouthed 1 l conical flask. Record the weight (W) in kg.

8.2.2 Add internal standard spiking solution, 125 µl of solution from step 5.7.6 (125 ng of d_5-atrazine is equivalent to 0.5 µg kg^{-1} in chalk) to 5 ml of deionised water in a 10 ml measuring cylinder then add to the flask. Rinse the measuring cylinder with 5 ml of deionised water then add this to the flask and mix well (see note f). Leave sample overnight in the dark at room temperature to allow equilibration.

f. Be careful to add the spiked deionised water to the chalk without any spillage.

8.2.3 Add 500 ± 10 ml acetone to the flask, stopper and mix well. Place the flask on an orbital shaker and shake at about 180 revolutions per minute for 4 hours.

8.3 Extract filtration

8.3.1 Filter the sample under vacuum through a GF/D glass fibre prefilter and a GF/F glass fibre filter. Wash all the solid sample into the Buchner funnel using two 50 ± 5 ml portions of acetone. Add each washing to the filter

g. When filtering and washing do not allow the filter cake to go completely to dryness, until the last wash has passed through.

cake after the extract has passed through, so that it is also washed (see Notes g and h).

h. If chalk particles are observed in the filtrate the filtrate must be filtered again.

8.3.2 Transfer the filtrate and washings into a clean 1 l wide mouth conical flask. Wash the Buchner flask twice with the minimum amount of acetone (about 10 ml) and add it to the flask.

8.4 Concentration

8.4.1 A Zymark Turbo-Vap 500 (or similar evaporator) at a water bath temperature of 40 °C and fan speed C is used to concentrate the extracts to a final volume of about 1 ml. Add about 300 ml of extract at first. When this is concentrated to about 10 ml add the rest and wash the flask twice with the minimum amount of acetone (about 10 ml) and add it to the Turbo-Vap.

8.4.2 After Turbo-Vap concentration transfer the extract to a 3 ml vial using a pasteur pipette. Wash the Turbo-Vap tube sides with three aliquots of about 0.5 ml of acetone and transfer the washings to the vial.

8.4.3 Evaporate the extract to about 0.2 ml using a gentle stream of nitrogen and warming (45 ± 3 °C).

8.5 Concentrate preparation

8.5.1 Pass the extract through a 0.2 µm syringe filter (previously washed with 2 ml of acetone). Wash the vial out four times with 0.2 ml of acetone, passing each aliquot through the syringe filter (see note i). Use the last vial washing to wash the syringe barrel.

i. Use a low dead volume disposable syringe needle to direct the filtrate from the filter to the LC-MS autosampler vial.

8.5.2 Evaporate the extract to dryness using a gentle stream of nitrogen and warming (45 ± 3 °C).

8.5.3 Reconstitute the extract with 100 ± 1 µl of HPLC eluent (as prepared in step 5.5).

8.5.4 If the extracts are not to be analysed immediately by LC-MS they must be stored in a freezer.

8.6 LC-MS

8.6.1 In conjunction with step 6.5 the LC-MS conditions should be optimised to give the best signal to noise ratio.

8.6.2 Inject the sample extracts, calibration standards and AQC samples in random order with a repeat LC-MS standard (see 5.7.8) after each extract.

8.6.3 Check that there has been no significant drift in the calibration by monitoring the peak area ratios for the repeat LC-MS standards. If the relative standard deviation for any of the determinands peak area ratios is over 20% then repeat the LC-MS analysis of the batch of samples.

8.7 Moisture determination.

Accurately (±0.01 g) weigh lumps of sample from step 8.1.2 (about 50 g) into a tared shallow porcelain dish, in duplicate (see Note j). Place the sample and dish in a microwave oven and heat on maximum for 30 minutes. Cool the sample and dish in a desiccator and reweigh. Return the sample and dish to the microwave oven and heat on maximum for a further 10 minutes. Cool the sample and dish in a desiccator and reweigh. If the difference in weight between the first and second weighing after drying is

j. Accurate weight is required as the moisture content may be low.

greater than 0.01 g then return the sample to the oven for a further 10 minutes, and repeat until the difference is less than 0.01 g.

Calculate the % moisture content:

Moisture content (M) = 100x(1-[dry weight/ wet weight])

8.8 Calculation of Concentration

8.8.1 For each calibration standard, control and sample, divide the pesticide or TP quantification ion peak area by the internal standard peak area to obtain the peak area ratio.

Determine the linear regression of the calibration data from the peak area ratio and concentration for each pesticide and TP. Obtain the gradient and intercept.

The concentration of pesticide or TP in the freeze dried sample can be calculated using the following equation:

$$C_{FD} = \frac{(A_R - I)}{G}$$

where C_{FD} = concentration of pesticide or TP in freeze dried chalk (μg kg^{-1})

A_R = area ratio

I = intercept

G = gradient.

8.8.2 The concentration needs to be corrected for moisture content using the following equation:

$$C = \frac{C_{FD}\ (100 - M)}{100}$$

Where C = concentration of pesticide or TP in original chalk before freeze drying (µg kg^{-1})

C_{FD} = concentration of pesticide or TP in freeze dried sample (µg kg^{-1})

M = % moisture content in original chalk

8.9 Blanks and Control samples

To check the accuracy of the analyses and presence of contamination and interferences, at least one blank and one spiked control sample (0.2 µg kg^{-1}) should be analysed by the entire procedure with each batch of analyses.

To check if the chalk sampled has a different matrix effect to the chalk used in the performance test, one replicate sample should be spiked at 2 µg kg^{-1} and analysed with each batch.

A replicate of one of the samples in the batch should be analysed in order to check sample homogeneity.

APPENDIX F ANALYTICAL METHOD FOR ISOPROTURON AND ITS TRANSFORMATION PRODUCTS IN CHALK AQUIFER MATERIAL

1 Performance Characteristics of the method

1.1 Substances determined	Isoproturon, demethylisoproturon (DMI) didemethylisoproturon (DDMI). This method may be suitable for the determination of other uron pesticides but has only been tested for the compounds listed above.
1.2 Types of samples	Chalk aquifer materials. May also be applicable to other aquifer materials but not tested for this purpose.
1.3 Basis of method	Extraction of sample with acetone, concentration, filtration and determination by liquid chromatography - mass spectrometry (LC-MS).
1.4 Range of application	Up to at least 10 µg kg^{-1}.
1.5 Calibration curve	Has been shown to be linear over the range 0.05 to 10 µg kg^{-1}.
1.6 Standard deviation	See Table 1.
1.7 Limit of detection	See Table 1
1.8 Sensitivity	Dependent on the determinand and the instrument in use.
1.9 Bias	No significant biases were detected ($\alpha = 0.05$). The mean concentrations detected in spiked samples are given in Table 1.
1.10 Interferences	See 3 below.

1.11 Time required for analysis	A batch of six samples plus associated standards and quality control samples in seven days from sample collection to obtaining results of analysis.

Table 4.1 Performance characteristics of the analytical method for isoproturon and TPs in chalk

Performance characteristics	Spiking level (µg kg^{-1})	Value of performance characteristic (µg kg^{-1}) for		
		Isoproturon	DMI	DDMI
LOD		0.094	0.198	0.090
Results from 'Blank' samples				
Mean	0.02	0.017	0.045	0.045
S_w		0.014	0.030	0.014
Results from low level spiked samples				
Mean	0.2	0.208	0.205	0.212
S_w		0.012	0.004	0.024
S_b		0.014	0.013	0.000
S_t		0.018	0.014	0.024
RSD_t (%)		9	7	11
Degs F		3	2	5
Results from high level spiked samples				
Mean	2	2.063	1.932	1.900
S_w		0.060	0.252	0.352
S_b		0.069	0.000	0.194

Performance characteristics	Spiking level (µg kg^{-1})	Value of performance characteristic (µg kg^{-1}) for		
		Isoproturon	DMI	DDMI
S_t		0.092	0.252	0.402
RSD_t		4	13	21
Degs F		3	5	4

LOD - Limit of detection as defined by Cheeseman and Wilson (1989)
Mean - Mean concentration of analytical results
S_w - Within batch standard deviation
S_b - Between batch standard deviation
S_t - Total standard deviation
RSD_t - Relative total standard deviation
Degs F - Degrees of freedom

2 Principle

Chalk aquifer material is freeze dried, ground to a powder and extracted with acetone. The acetone extract is filtered to remove the chalk, concentrated by Turbo-Vap technique, and passed through a 0.2 µm syringe filter to remove particulate material. The concentrations of the determinands in the final extract are determined by liquid chromatography-mass spectrometry (LC-MS) in selected ion recording mode.

3 Interferences

No specific interferences have been noted, however the occurrence of compound(s) which are extracted and both co-elute with and contain the quantitation ion of a determinand will interfere. If interferences are suspected, additional work, not described here, may be necessary. This might include the use of MS-MS techniques.

4 Hazards

All reagents must be COSHH assessed before use and the recommended control procedures implemented. The solvents are harmful and flammable, and ammonium acetate is an irritant. Caution must be exercised when preparing the stock and working calibration standard solutions, skin contact, ingestion and inhalation must be avoided. Good laboratory procedure must be followed at all times.

5 Reagents

All reagents must be of sufficient purity that they do not give rise to significant interfering peaks in the LC-MS analysis of extracts.

5.1 Methanol, High-performance liquid chromatography (HPLC) grade.

5.2 Acetone, Glass distilled grade.

5.3 Distilled or deionised water, pesticide free.

5.4 Ammonium acetate solution 0.1M. Dissolve 0.77 ± 0.01 g Analar grade ammonium acetate in ~ 50 ml of HPLC quality water (e.g. glass double distilled) in a 100-ml volumetric flask and make up to mark. Prepare fresh for each batch of samples.

5.5 HPLC eluent for reconstituting extracts. Pipette 3.7 ± 0.1 ml of 0.1M ammonium acetate solution into a 10-ml volumetric flask and make up to volume with methanol.

5.6 Nitrogen gas, dry oil-free passed through an activated carbon filter.

5.7 Standard solutions. Store in a refrigerator. Prepare fresh solutions every six months from pure or certified materials.

5.7.1 Isoproturon and TPs stock solution (100 mg l^{-1}). Accurately weigh 10 mg of isoproturon, DMI and DDMI into a 100-ml volumetric flask, dissolve and make up to volume with methanol.

5.7.2 Internal standard (d_3-isoproturon) stock solution (100 mg l^{-1}). Accurately weigh 10 mg of d_3-isoproturon into a 100 ml volumetric flask, dissolve and make up to volume with methanol.

WARNING

CAUTION MUST BE EXERCISED WHEN PREPARING THE STOCK SOLUTIONS. SKIN CONTACT, INGESTION AND INHALATION MUST BE AVOIDED.

5.7.3 Isoproturon and TPs spiking solution (1 mg l^{-1}). Add 1 ± 0.01 ml of stock solution (see 5.7.1) to 50 ml of methanol in a 100 ml volumetric flask and make up to volume with methanol.

5.7.4 Internal standard spiking solution (1 mg l^{-1}). Add 1 ± 0.01 ml of internal standard stock solution (see 5.7.2) to

about 50 ml of methanol in a 100 ml volumetric flask and make up to volume with methanol.

5.7.5 Calibration standards. Prepare duplicate standards by adding the appropriate volumes of isoproturon and TPs spiking solution (from 5.7.3) and internal standard spiking solution (from 5.7.4) to 5ml of deionised water in a measuring cylinder. Pour the spiked water onto a 250 ± 0.5 g sample of powdered pesticide free chalk. Rinse the measuring cylinder with 5ml of deionised water and add this to the chalk sample also. These calibration standards should then be put through the full analytical procedure at the same time as the samples to be analysed.

Volume of solution 5.7.3 added (µl)	Volume of solution 5.7.4 added (µl)	Concentration of isoproturon, DMI and DDMI in standards (µg kg^{-1})	Concentration of d_3-isoproturon in standards (µg kg^{-1})
40	125	0.16	0.5
550	125	2.2	0.5

5.7.6 LC-MS standard solution (0.2 µg kg^{-1} equivalent). Add 500 µl of solution 5.7.1 and 1250 µl of solution 5.7.2 to a 100 ml volumetric flask containing 37 ml of 0.1 M ammonium acetate solution (see 5.4). Make up to volume with methanol.

6 Apparatus

Glassware should be clean (soaked in a suitable proprietary laboratory detergent solution, rinsed with tap water, soaked in dilute orthophosphoric acid solution, rinsed with deionised or distilled pesticide free water and finally rinsed with acetone) and dry.

6.1 Reagent preparation - Syringes 25, 50, 100, 250 µl and 1 ml. Volumetric flasks 10 ml and 100 ml, pipettes for measuring 4.5 and 45 ml. Analytical balance (5 place), Top pan balance (3 place).

6.2 Chalk aquifer material preparation - Bricklayers hammer and bolster. Freeze drier (Edwards Mini-fast 3400 is suitable). Impeller type cutting mill with 4 mm sieve (Glen Creston type SM1 mill is suitable). Glass or stainless steel containers.

6.3 Extraction and extract filtration - Stoppered Quickfit wide neck conical flasks (1 l), Buchner flasks (2 l), Buchner funnels (12.5 cm), vacuum line fitted with a suitable solvent vapour trap, Whatman glass fibre filters (GF/F and GF/D, 12.5 cm), orbital shaker fitted with a platform suitable for 1 l conical flasks, (Gallenkamp model is suitable). Top pan balance (3 place).

6.4 Concentration and concentrate preparation - Zymark Turbo-Vap 500 or similar evaporator, 3 ml vials (Wheaton are suitable), 2 ml syringe with luer fitting, Anotop 10 0.2 µm syringe filters, low dead volume disposable syringe needles with Luer fittings, 1 ml LC-MS autosampler vials.

6.5 Liquid chromatograph - Mass spectrometer (LC-MS)

A LC-MS system with data system operated in accordance with the manufacturer's instructions.

Suitable conditions are:

LC conditions: Column Spherisorb 5µm ODS-1 250 x 4.6 mm column. Isocratic eluent, methanol : 0.1 M aqueous ammonium acetate (63:37), flow rate 1 ml $minute^{-1}$. Injection loop 20 µl. In-line filters, 0.2 µm, fitted between the injector and column, and column and interface, to prevent blockages.

MS conditions: Plasmaspray ion source operated at 200°C with 600 µA discharge current. Other MS conditions are tuned by optimising the response of a LC-MS standard (0.2 µg kg^{-1} equivalent, as prepared in step 5.7.6) injected post-column. The mass spectrometer is calibrated for selected ion recording using a solution of polyethylene glycol 200 in methanol, injected post column.

Typical retention times and ions monitored:

Compound	Retention time (min:sec)	m/z monitored quantification
DDMI	6:50	179
DMI	8:10	193
d_3-isoproturon	10:08	210
isoproturon	10:12	207

6.6 Moisture determination - Porcelain dishes (2), Analytical balance (5 place), Microwave oven (suitable power rating 700 W). Desiccator and tongs.

7 Sample Storage and Preservation

Core samples are taken using a percussion drilling rig with steam cleaned aluminium U100 core barrel liners. The sample, complete with aluminium core barrel liner, is enclosed in double lined polythene sleeving, sealed, and placed in a freezer for transit. Samples should be analysed as soon as possible upon receipt at the laboratory, if not they should be stored in a freezer.

8 Analytical Procedure

Step	Procedure	Notes
8.1	Sample pretreatment.	
8.1.1	Using a core pusher the core sample is removed from the aluminium liner. Between 25 and 50 mm of each end of the core sample is removed and discarded (see note a).	a. If after steps 8.1.1, 8.1.2 or 8.1.3 no further work is to be immediately carried out, the sample must be sealed in a glass or stainless steel container and returned to the freezer.
8.1.2	Sections of the core sample are broken into small lumps (see note b) with a bricklayers hammer and bolster (see Notes a and c).	b. To facilitate grinding the maximum size of the lumps should be about 50 mm.

c. The bricklayers hammer and bolster are cleaned between samples by brushing free of chalk, washing with *tap* water, then drying.

8.1.3 The broken core sample is freeze dried until the moisture content is negligible (i.e. <0.3%)(see note d).

d. Using the Edwards Mini-fast 3400 freeze drier this required freeze drying over a weekend.

8.1.4 The sample is ground to a homogeneous powder in a knife mill fitted with a 4 mm sieve (see notes a and e). The powder is then shaken in its container to homogenise the sample.

e. The knife mill must be cleaned in between samples by brushing away all the loose chalk with a stiff bristled brush and washing the sieve with tap water, then drying it. In between batches of samples, the knife mill is cleaned more thoroughly by brushing and washing with water (deionised or distilled) to remove chalk from inside the housing, rotary head, blades and sieve. The knife mill should then be dried before use.

With this cleaning procedure it is estimated that the maximum cross contamination that could possibly occur is 0.4%. As an extra precaution when profiling chalk cores, the deepest sections of the cores are always knife milled first as these should have lower levels of determinands.

8.2 Extraction

8.2.1 Accurately weigh 250 ± 0.5 g of powdered sample into a 1 l wide neck conical flask. Record the weight (W) in kg

8.2.2 Add internal standard spiking solution, 125 µl of solution from step 5.7.4 (125 ng of d3-isoproturon is equivalent to 0.5 µg kg^{-1} in chalk), to 5ml of deionised water in a 10 ml measuring cylinder, then add this to the flask (see note f). Rinse the measuring cylinder with 5 ml of deionised water, then add this to the flask and mix well. Leave the sample overnight in the dark at room temperature, to allow equilibration.

f. Be careful to add the spiked deionised water to the chalk without any spillage.

8.2.3 Add 500 ± 10 ml acetone to the flask, stopper and mix well. Place the flask on an orbital shaker and shake at about 180 revolutions per minute for 4 hours.

8.3 Extract Filtration

8.3.1 Filter the sample under vacuum through a GF/D glass fibre prefilter and a GF/F glass fibre filter. Wash all the solid sample into the Buchner funnel using two 50 ± 5 ml portions of acetone. Add each washing to the filter cake after the extract has passed through, so that it is also washed. (see notes g and h).

g. When filtering and washing do not allow the filter cake to go completely to dryness, until the last wash has passed through.

h. If chalk particles are observed in the filtrate, the filtrate must be filtered again.

8.3.2 Transfer the filtrate and washings into a clean 1 l wide neck conical flask. Wash the Buchner flask twice with the minimum amount of acetone (about 10 ml) and add it to the flask.

8.4 Concentration

8.4.1 A Zymark Turbo-Vap 500 (or similar evaporator) at a water bath temperature of 40°C and fan speed C is used to concentrate the extracts to a final volume of about 1 ml. Add about 300 ml of extract at first. When this is concentrated to about 10 ml, add the rest and wash the flask twice with the minimum amount of acetone (about 10 ml) and add it to the Turbo-Vap.

8.4.2 After Turbo-Vap concentration, transfer the extract to a 3ml vial using a Pasteur pipette. Wash the Turbo-Vap tube sides with three aliquots of about 0.5 ml of acetone and transfer the washings to the vial.

8.4.3 Evaporate the extract to about 0.2 ml using a gentle stream of nitrogen and warming (45 ± 3 °C).

8.5 Concentrate preparation

8.5.1 Pass the extract through a 0.2 μm syringe filter (previously washed with 2 ml of acetone). Wash the vial out four times with 0.2 ml of acetone, passing each aliquot through the syringe filter (see note i). Use the last vial washing to wash the syringe barrel.

i. Use a low dead volume disposable syringe needle to direct the filtrate from the filter to the LC-MS autosampler vial.

8.5.2 Evaporate the extract to dryness using a gentle stream of nitrogen and warming (45 ± 3°C).

8.5.3 Reconstitute the extract with 100 ± 1 µl of HPLC eluent (as prepared in step 5.5).

8.5.4 If the extracts are not to be analysed immediately by LC-MS they must be stored in a freezer.

8.6 LC-MS

8.6.1 In conjunction with step 6.5 the LC-MS conditions should be optimised to give the best signal to noise ratio.

8.6.2 Inject the sample extracts, calibration standards and AQC samples in random order with a repeat LC-MS standard (see 5.7.6) after each extract.

8.6.3 Check that there has been no significant drift in the calibration by monitoring the peak area ratios for the repeat LC-MS standards. If the relative standard deviation for any of the determinands peak area ratios is over 20% then repeat the LC-MS analysis of the batch of samples.

8.7 Moisture determination.

Accurately (± 0.01 g) weigh lumps of sample from step 8.1.2 (about 50 g) into a tared shallow porcelain dish, in duplicate (see Note j). Place the sample and dish in a microwave oven and heat on maximum for 30 minutes. Cool the sample and dish in a desiccator and reweigh. Return the

j. Accurate weight is required as the moisture content may be low.

sample and dish to the microwave oven and heat on maximum for a further 10 minutes. Cool the sample and dish in a desiccator and reweigh. If the difference in weight between the first and second weighing after drying is greater than 0.01g then return the sample to the oven for a further 10 minutes, and repeat until the difference is less than 0.01 g.

Calculate the % moisture content:

Moisture content (M) = 100x(1-[dry weight/ wet weight])

8.8 Calculation of Concentration

8.8.1 For each calibration standard, control and sample, divide the pesticide or TP peak area by the internal standard peak area to obtain the peak area ratio.

Determine the linear regression of the calibration data from the peak area ratio and concentration for each pesticide and TP. Obtain the gradient and intercept.

The concentration of pesticide or TP in the freeze dried sample can be calculated using the following equation:

$$C_{FD} = \frac{A_R - I}{G}$$

where

C_{FD} = concentration of pesticide or TP in freeze dried chalk ($\mu g\ kg^{-1}$)

A_R = area ratio

I = intercept

G = gradient.

8.8.2 The concentration needs to be corrected for moisture content using the following equation:

$$C = \frac{C_{FD}\,(100-M)}{100}$$

Where C = concentration of pesticide or TP in original chalk before freeze drying ($\mu g\ kg^{-1}$)

C_{FD} = concentration of pesticide or TP in freeze dried sample ($\mu g\ kg^{-1}$)

M = % moisture content in original chalk

8.9 Quality control samples

To check the accuracy of the analyses and presence of contamination and interferences, at least one blank and one spiked control sample (0.2 $\mu g\ kg^{-1}$) should be analysed by the entire procedure with each batch of analyses.

To check if the chalk sampled has a different matrix effect to the chalk used in the performance test, one replicate sample should be spiked at 2 $\mu g\ kg^{-1}$ and analysed with each batch.

A replicate of one of the samples in the batch should be analysed in order to check sample homogeneity.