

# **Chapter One**

## **Introduction**

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## 1.1 Aim and Objectives

This research aims to improve analytical methodologies for monitoring significant and persistent agrochemicals, named in the Water Framework Directive (2000). The study investigates the development and application of rapid monitoring techniques for selected priority agrochemicals in river water samples obtained from several rivers in South Wales, specifically concentrating on the development and application of a single method for the simultaneous determination of specific triazine, phenyl-urea and chloroacetanilide groups of agrochemicals. These chemically different compounds were analysed through the application of multi-mode solid phase extraction (SPE) followed by Ultra Performance Liquid Chromatography and tandem mass spectrometry (UPLC-MS/MS).

Pollution monitoring often determines the presence of harmful pollutants in the environment both qualitatively (where the identity of a substance is proposed) and quantitatively (where the concentration is determined). The main purpose of monitoring is to identify potential threats to humans and to ecosystems, to assess the effectiveness of pollution control measures and to investigate trends in pollution and identify future problems.

The analysis of samples from selected rivers in South Wales using the method developed will determine whether or not action programmes to reduce the output of agrochemicals listed as priority pollutants in the Water Framework Directive have been effective.

The method should also have the potential to be applied in the field of Environmental Forensics to investigate environmental incidents which may involve these compounds.

## 1.2 The Water Framework Directive

The European Union (EU) and United Kingdom Governments are involved in various programmes to monitor and control the use of environmental pollutants. The EU Water Framework Directive (WFD) 2000/60/EC sets out a strategy against pollution and protection of inland surface water, ground water, transitional water, coastal water and groundwater making it one of the most significant pieces of European water legislation for over 20 years. The WFD, first established in 2002, is amended and updated periodically; the latest amendment was in June 2009 [1].

One of the Directive's requirements under Article 16 is the management of chemical pollutants and requires the identification and review of priority substances and priority hazardous substances through Environmental Quality Standards and Emission Controls. EU Decision number 2455/2001/EC [2] establishes a list of thirty-three priority substances that appear as Annex X (under Article 16) of the WFD and includes heavy metals, polyaromatic hydrocarbons and pesticides, Table 1.1 lists the compounds contained within Annex X. The WFD requires that the 'Priority Substances' list be reviewed regularly as new and emerging substances replace or supplement older ones as they are controlled and pose less of an environmental risk.

To this end, laboratories and analytical instrument manufacturing will need to meet the demands of such legislation due to challenges in limits of detection (LOD) of older analytical instruments and by developing more efficient and cheaper methods for generating the information needed to manage the water environment more effectively and efficiently. Traditional laboratory methods that were developed and have been used successfully over

past decades may now not be capable of analysing these compounds at the low concentrations posed, so new and innovative techniques are required to meet current and future demands.

**Table 1.1** Environment Agency list of priority organic chemicals listed under the EU Water Framework Directive [3]

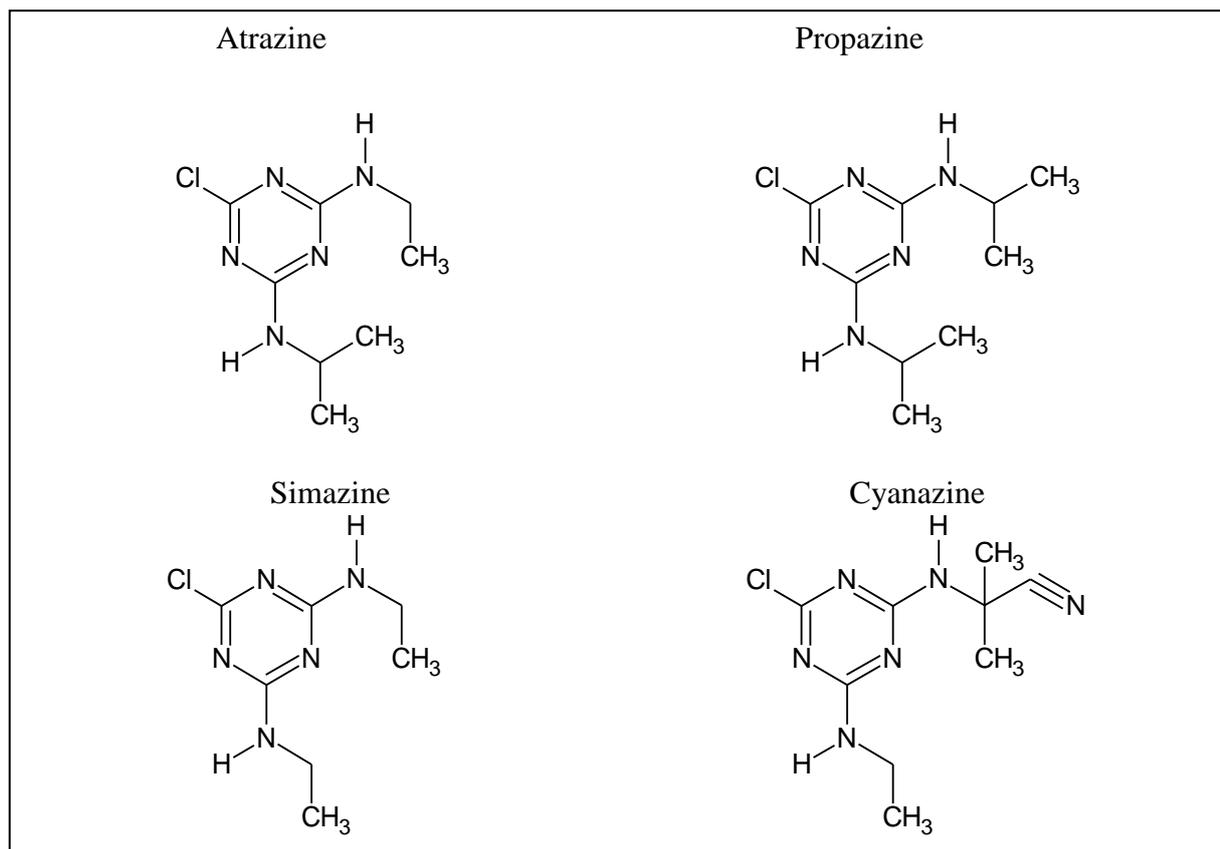
Name	Type	IUPAC name
Chlorfenvinphos	Organophosphate insecticide	2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate
Atrazine	Chlorotriazine herbicide	6-chloro- <i>N</i> <sup>2</sup> -ethyl- <i>N</i> <sup>4</sup> -isopropyl-1,3,5-triazine-2,4-diamine
Diuron	Phenylurea herbicide	3-(3,4-dichlorophenyl)-1,1-dimethylurea
Isoproturon	Phenylurea herbicide	3-(4-isopropylphenyl)-1,1-dimethylurea
Simazine	Chlorotriazine herbicide	6-chloro- <i>N</i> <sup>2</sup> , <i>N</i> <sup>4</sup> -diethyl-1,3,5-triazine-2,4-diamine
Trifluralin	Dinitroaniline herbicide	$\alpha,\alpha,\alpha$ -trifluoro-2,6-dinitro- <i>N,N</i> -dipropyl- <i>p</i> -toluidine
Chloropyrifos	Organophosphate insecticide	<i>O,O</i> -diethyl <i>O</i> -3,5,6-trichloro-2-pyridyl phosphorothioate
Alachlor	Chloroacetanilide herbicide	2-chloro-2',6'-diethyl- <i>N</i> -methoxymethylacetanilide
Endosulphan	Cyclodiene insecticide /acaricide	(1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylenebismethylene) sulfite
PCP	Organochlorine herbicide	Pentachlorophenol C <sub>6</sub> Cl <sub>5</sub> OH
HCH	Organochlorine herbicide	1,2,3,4,5,6-hexachlorocyclohexane
HCB	Organochlorine fungicide	Hexachlorobenzene
TBT	Organotin fungicide	bis(tributyltin) oxide
Diazinon	Organothiophosphate insecticide	<i>O,O</i> -diethyl <i>O</i> -2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate
Cypermethrin	Pyrethroid ester insecticide	( <i>RS</i> )- $\alpha$ -cyano-3-phenoxybenzyl (1 <i>RS</i> ,3 <i>RS</i> ;1 <i>RS</i> ,3 <i>SR</i> )-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
Flumethrin	Pyrethroid ester acaricide	( <i>RS</i> )- $\alpha$ -cyano-4-fluoro-3-phenoxybenzyl (1 <i>RS</i> ,3 <i>RS</i> ;1 <i>RS</i> ,3 <i>SR</i> )-3-( $\beta$ ,4-dichlorostyryl)-2,2-dimethylcyclopropanecarboxylate
Amitraz	Formamidine antiparasitic	<i>N</i> -methylbis(2,4-xilyliminomethyl)amine

### 1.3 Target Analytes for Method Development

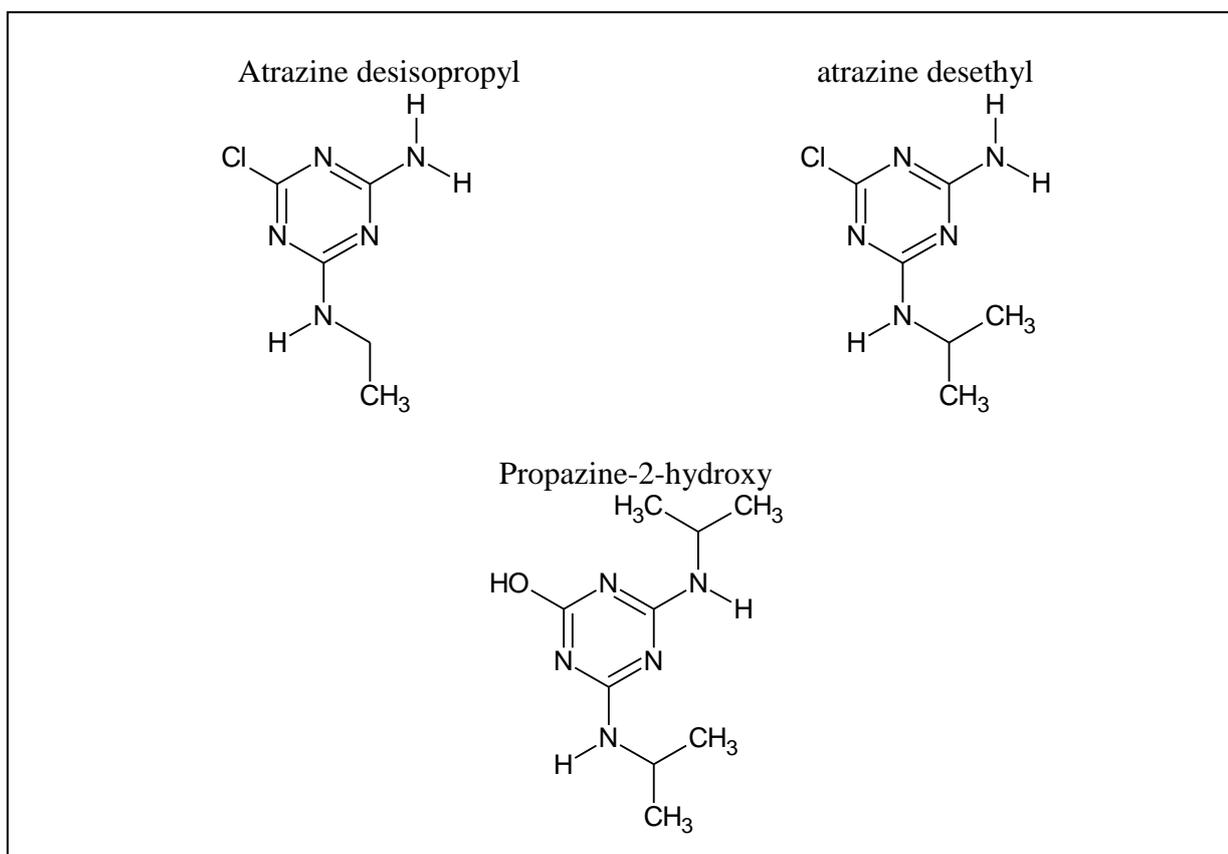
Agrochemicals account for seventeen of the thirty-three persistent substances listed in Annex X of the Water Framework Directive. The Pesticide Action Network [4] has catalogued a list of agrochemicals and identified those associated with harmful health or environmental impact.

The sources of these compounds include regular agricultural use, authorised discharges, veterinary medicines, anti-fouling treatments, amenity pesticides and public hygiene.

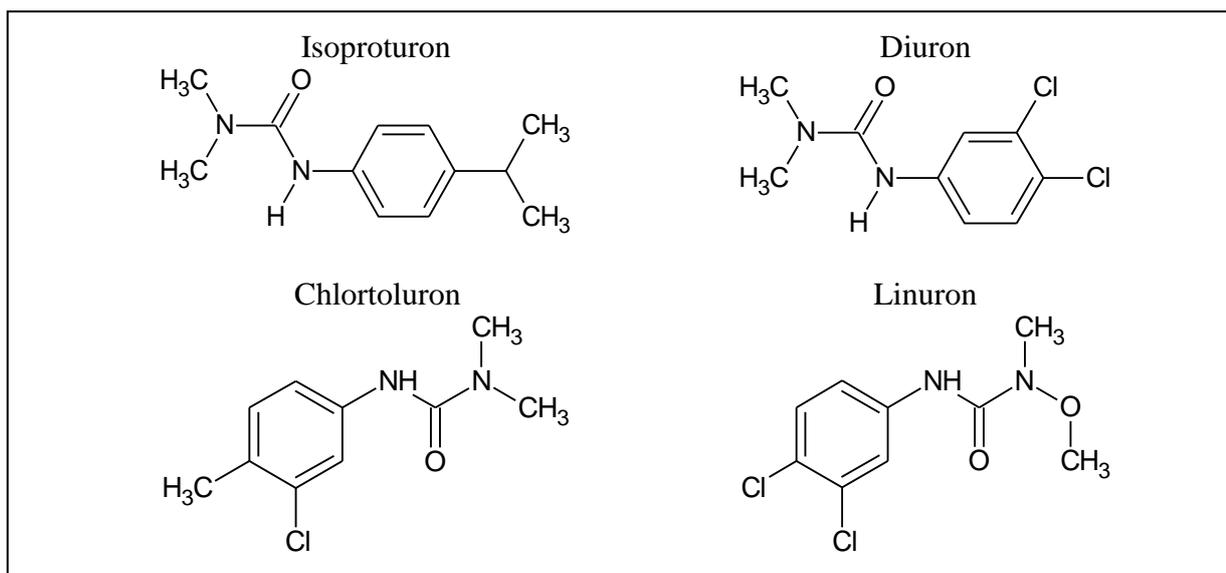
Twelve of these agrochemicals were chosen to be representatives of the major classes of agrochemicals listed on the WFD. These were used to assess the potential of UPLC-MS/MS for rapid robust multi-compound analysis through simultaneous detection using a single method. The compounds chosen were the triazine herbicides and triazine metabolites of atrazine, propazine, simazine, cyanazine (Figure 1.1a), atrazine-desisopropyl, atrazine-desethyl, propazine-2-hydroxy (Figure 1.1b), phenylurea compounds of isoproturon, diuron, chlortoluron and linuron (Figure 1.1c) and chloroacetanilide compound alachlor (Figure 1.1d).



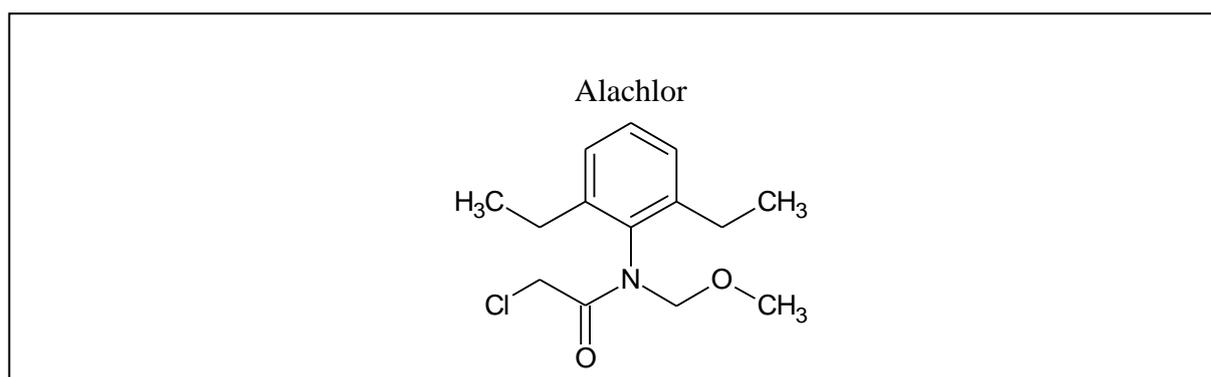
**Figure 1.1a** The chemical structures of the selected triazine herbicides in this study



**Figure 1.1b** The chemical structures of the selected triazine metabolites in this study



**Figure 1.1c** The chemical structures of the selected phenyl urea analytes in this study



**Figure 1.1d** The chemical structures of the chloroacetanilide analyte in this study

Atrazine is a man-made, selective triazine herbicide, which is toxic to aquatic organisms and highly persistent in soil [5] and is believed to cause reproductive, mutagenic and carcinogenic problems in humans [4]. Under the EU Commission Decision 2004/248/EC authorisation for plant protection products containing atrazine was withdrawn as from 10 September 2004 and the use of atrazine was totally banned by 31 December 2007 [6].

Isoproturon and diuron are both phenylurea herbicides which function by interfering with plant photosynthesis. Excessive exposure in humans is believed to affect the red blood cell count in humans and may also cause cancer [7, 8]. Both compounds have been classified as “priority hazardous substance under review” in the WFD.

Three triazine metabolites, atrazine-desethyl, atrazine- desisopropyl and propazine-2-hydroxy were also chosen for investigation. Figure 1.2 shows the degradation route of atrazine in the environment. Figure 1.3 shows the degradation of triazine compounds to their metabolites [9]. This shows the metabolite atrazine desisopropyl could be a degradation product of atrazine, cyanazine and/or simazine whilst the metabolite atrazine desethyl could be a degradation product of atrazine and/or propazine.

These metabolites were chosen for investigation as representative compounds of possible indicators for presence of the parent compound being previously released into the environment.

Table 1.2 lists the compounds studied in this programme and indicates whether they are considered as target analytes, metabolites or deuterated standards.

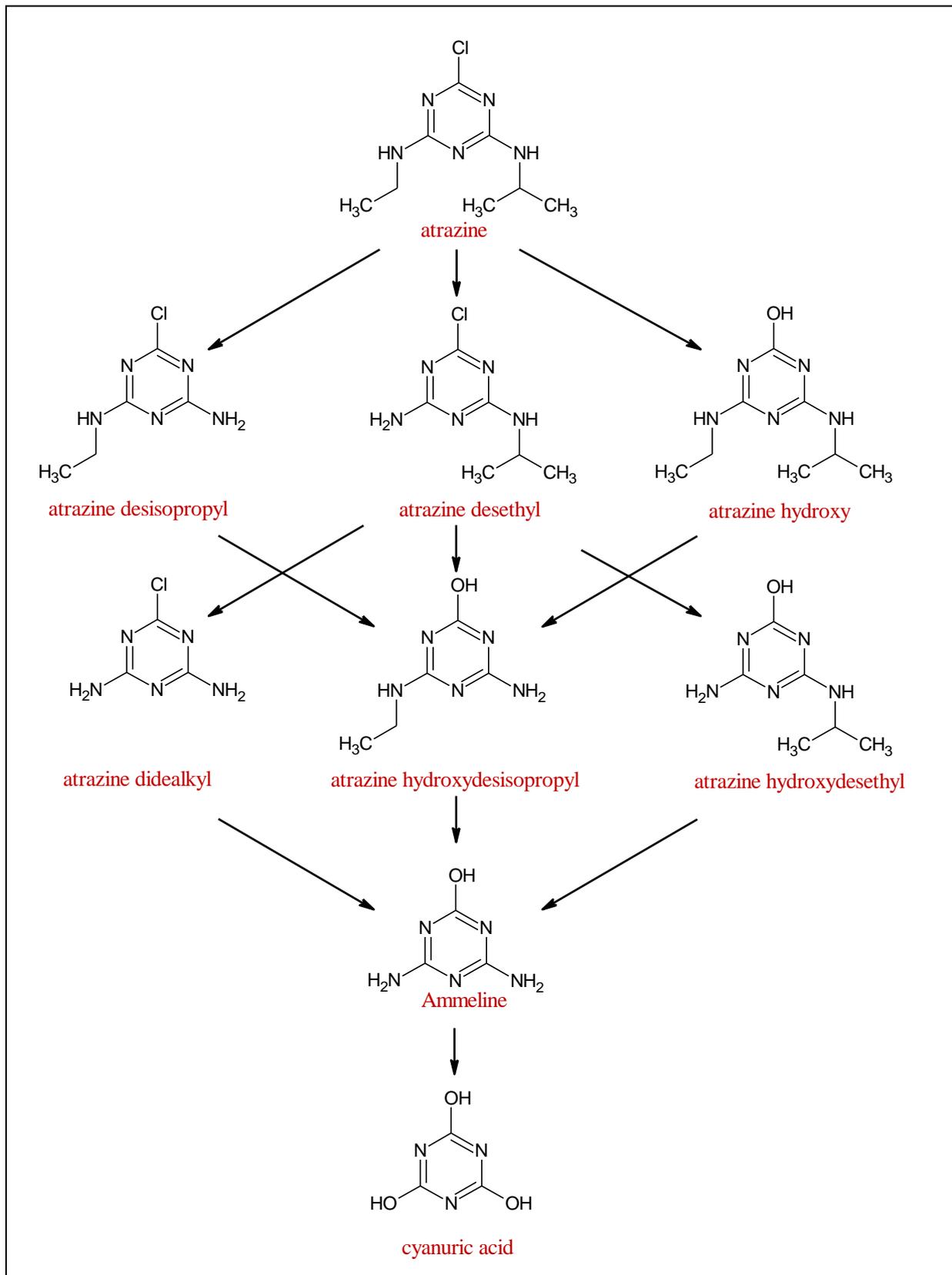
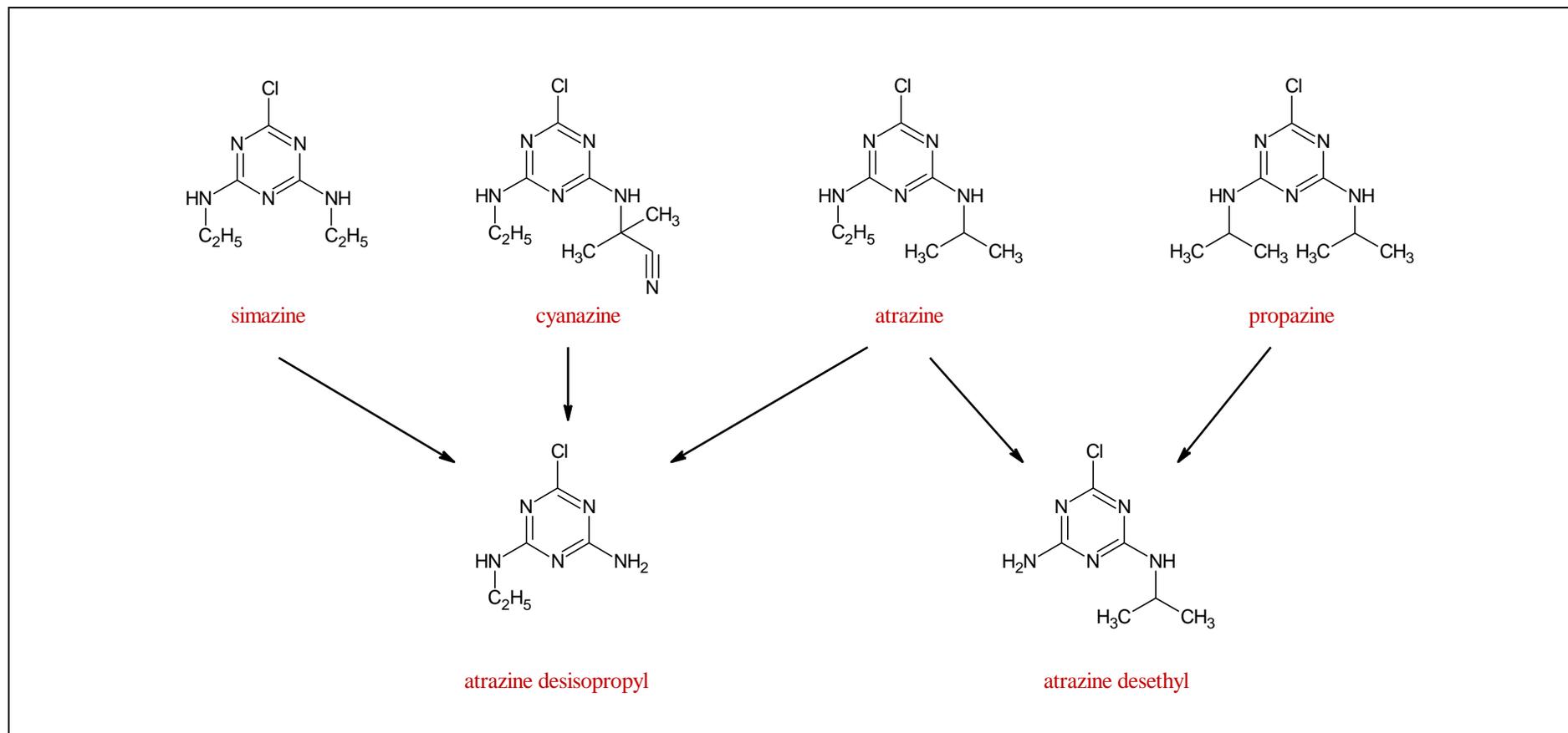


Figure 1.2 Degradation of atrazine to its metabolites in the environment [9]



**Figure 1.3** Breakdown of triazine compounds to its metabolites in the environment [9]

**Table 1.2** Compounds used in this study, showing agrochemical compounds, metabolites, and internal and external standards

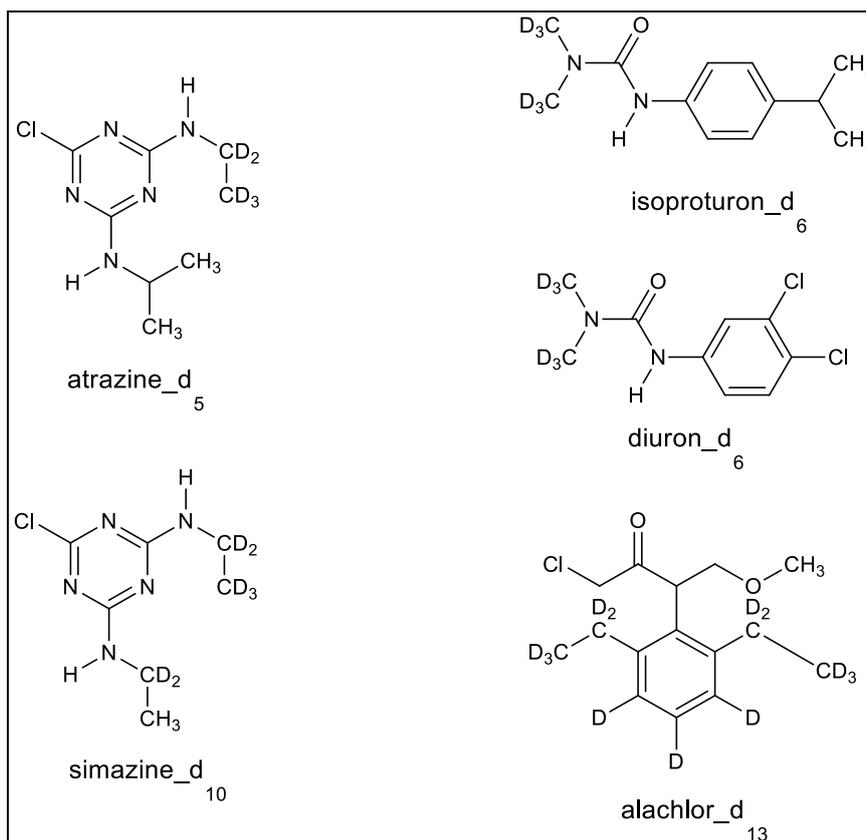
Compound Name	IUPAC name	CAS No.	Compound Class	Formula	Molecular Weight (g mol <sup>-1</sup> )
Atrazine	6-chloro- <i>N</i> <sup>2</sup> -ethyl- <i>N</i> <sup>4</sup> -isopropyl-1,3,5-triazine-2,4-diamine	1912-24-9	Triazine	C <sub>8</sub> H <sub>14</sub> ClN <sub>5</sub>	215.68
Propazine	6-chloro- <i>N</i> <sup>2</sup> , <i>N</i> <sup>4</sup> -diisopropyl-1,3,5-triazine-2,4-diamine	139-40-2	Triazine	C <sub>9</sub> H <sub>16</sub> ClN <sub>5</sub>	229.71
Isoproturon	3-(4-isopropylphenyl)-1,1-dimethylurea	34123-59-6	Phenylurea	C <sub>12</sub> H <sub>18</sub> N <sub>2</sub> O	206.28
Diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea	330-54-1	Phenylurea	C <sub>9</sub> H <sub>10</sub> Cl <sub>2</sub> N <sub>2</sub> O	233.09
Simazine	6-chloro- <i>N</i> <sup>2</sup> , <i>N</i> <sup>4</sup> -diethyl-1,3,5-triazine-2,4-diamine	122-34-9	Triazine	C <sub>7</sub> H <sub>12</sub> ClN <sub>5</sub>	201.66
Cyanazine	2-(4-chloro-6-ethylamino-1,3,5-triazin-2-ylamino)-2-methylpropionitrile	21725-46-2	Triazine	C <sub>9</sub> H <sub>13</sub> ClN <sub>6</sub>	240.69
Alachlor	2-chloro-2',6'-diethyl- <i>N</i> -methoxymethylacetanilide	15972-60-8	Chloroacetanilide	C <sub>14</sub> H <sub>20</sub> ClNO <sub>2</sub>	269.77
Chlortoluron	3-(3-Chloro-4-methyl)-1,1-dimethylurea	15545-48-9	Phenylurea	C <sub>10</sub> H <sub>13</sub> ClN <sub>2</sub> O	212.68
Linuron	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea	330-55-2	Phenylurea	C <sub>9</sub> H <sub>10</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>2</sub>	249.09
Atr-desisopropyl	2-Amino-4-chloro-6-ethylamino-1,3,5-triazine	1007-28-9	Triazine metabolite	C <sub>5</sub> H <sub>8</sub> ClN <sub>5</sub>	173.60
Atr-desethyl	2-Amino-4-chloro-6-isopropylamino-1,3,5-triazine	6190-65-4	Triazine metabolite	C <sub>6</sub> H <sub>10</sub> ClN <sub>5</sub>	187.63
Propazine-2-hydroxy	2-Hydroxy-4,6-bis(isopropylamino)-1,3,5-triazine	7374-53-0	Propazine metabolite	C <sub>9</sub> H <sub>17</sub> N <sub>5</sub> O	211.26
Atrazine_d5	2-Chloro-4-pentadeuteroethylamino-6-isopropylamino-1,3,5-triazine	163165-75-1	Deuterated standard	C <sub>8</sub> H <sub>9</sub> D <sub>5</sub> ClN <sub>5</sub>	220.71
Isoproturon_d6	3-(4-Isopropylphenyl)-1,1-dimethylurea-d <sub>6</sub>	217487-17-7	Deuterated standard	C <sub>12</sub> H <sub>12</sub> D <sub>6</sub> N <sub>2</sub> O	212.32
Diuron_d6	3-(3,4-Dichlorophenyl)-1,1-dimethylurea-d <sub>6</sub>	1007536-67-5	Deuterated standard	C <sub>9</sub> H <sub>4</sub> D <sub>6</sub> Cl <sub>2</sub> N <sub>2</sub> O	239.13
Simazine_d10	2,4-Bis(pentadeuteroethylamino)-6-chloro-1,3,5-triazine	220621-39-6	Deuterated standard	C <sub>7</sub> H <sub>2</sub> D <sub>10</sub> ClN <sub>5</sub>	211.72
Alachlor_d13	2-Chloro-2',6'-diethyl- <i>N</i> -(methoxymethyl)acetanilide-d <sub>13</sub>	1015856-63-9	Deuterated standard	C <sub>14</sub> H <sub>7</sub> D <sub>13</sub> ClNO <sub>2</sub>	282.85

### 1.3.1 Deuterated Standards

An internal standard is a compound of known concentration added to an analyte before analysis. The signal response from the internal standard and analyte are then compared to determine the concentration of analyte in the sample.

The European Union describes an internal standard as a substance not contained in the sample with the same physio-chemical properties as similar as possible to the analyte that has to be measured which is added to each sample. The internal standard should preferably be related with a retention time similar to the analyte under the same experimental conditions and under mass spectrometry conditions it will have similar molecular and fragmentation ions masses.

Deuterated standards are used in this study as they are unlikely to be found in the environment and have elution times and fragment ions similar to the analytes under investigation. In this study five deuterated standards atrazine\_d5, isoproturon\_d6, diuron\_d6, simazine\_d10, and alachlor\_d13 were used for quantification of analytes. Structures are presented in Figure 1.4.



**Figure 1.4** The deuterated internal standards used in this study

#### 1.4 Current Safety Concerns

The compounds under investigation in this study have been classified by various international organisations and government bodies in terms of their toxicity, carcinogenic effects and endocrine disrupting properties. Table 1.3 summarises the classification given to these compounds by the World Health Organisation (WHO) [10], the International Agency for Research on Cancer (IARC) [11], the United States Environmental Protection Agency (US EPA) [12] the European Union (EU) [13,16], the United Kingdom Environmental Agency (UK EA) [14], the Federal German Environmental Agency (Ger EA) [15], the World Wide Fund for nature (WWF) [18] and the Oslo Paris Commission (OSPAR) [17,19].

The endocrine system in a body is a collection of glands that produces these hormones which are necessary for bodily functions. An endocrine disruptor interferes with this system and can affect hormonal growth control by inhibiting the testosterone and oestrogen production upsetting the balance of the two, the reproductive system, the body's metabolism and the immune system. This can then lead to reproductive cancers such as prostate cancer in males, breast cancers in females, bladder and kidney cancers and inhibit pituitary hormones necessary for ovulation [4].

**Table 1.3** Summary of Toxicity, Endocrine Disrupting and Carcinogen Properties of selected compounds

Category	Pesticide	Group	Classification Group	Classification Comment
WHO classification	2,4-D	WHO	II	Moderately hazardous toxin
Pesticides and Cancer	Atrazine	IARC	3	Unclassifiable
	Propazine	EU	3	Possible risks of irreversible effects
	Isoproturon	EU	3	Possible risks of irreversible effects
	Diuron	US EPA	known	Known/likely tumour effects.
		EU	3	Possible risks of irreversible effects
	Cyanazine	US EPA	C	Possible human carcinogen
	Linuron	US EPA	C	Possible human carcinogen
EU		3	Possible risks of irreversible effects	
Endocrine disruptors	Atrazine	UK EA	Yes	
		Ger. EA	Yes	
		EU	Yes 1	At least one study providing evidence
		OSPAR	Yes	
		WWF	Yes	
	2,4-D Diuron	EU	Yes 2	Potential for endocrine disruption
	Diuron	Ger. EA		
		EU	Yes 2	Potential for endocrine disruption
	Linuron	EU	Yes 2	Potential for endocrine disruption
Simazine	EU	Yes 2	Potential for endocrine disruption	
OSPAR commission [11]	Atrazine	OSPAR	Yes	Substance of concern
European Regulations WFD[12]	Atrazine	WFD 2000/60.EC	On list as:	Possible priority hazardous substance
	Diuron	WFD 2000/60.EC	On list as:	Possible priority hazardous substance
	Isoproturon	WFD 2000/60.EC	On list as:	Possible priority hazardous substance
European Regulations DSD [13]	Atrazine	DSD 76/464/EC	Was list I	Now list II
European Regulations	Atrazine	EU directive 91/414	Severe restriction	Restricted on 10/2004

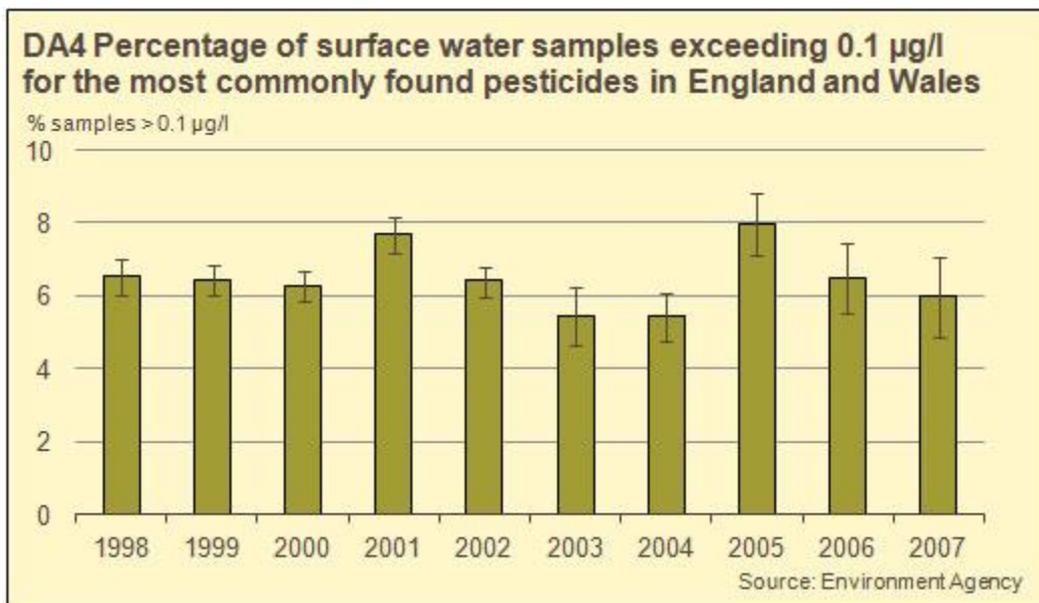
## 1.5 Uses of Agrochemicals and Pesticides

The term agrochemical is often used to mean all synthetic substances manufactured or natural products sold in agriculture to increase production yield. It includes all mineral fertilisers and pesticides. Urine, lime, soap suds, vinegar and tobacco have all been used as pesticides at some time [20].

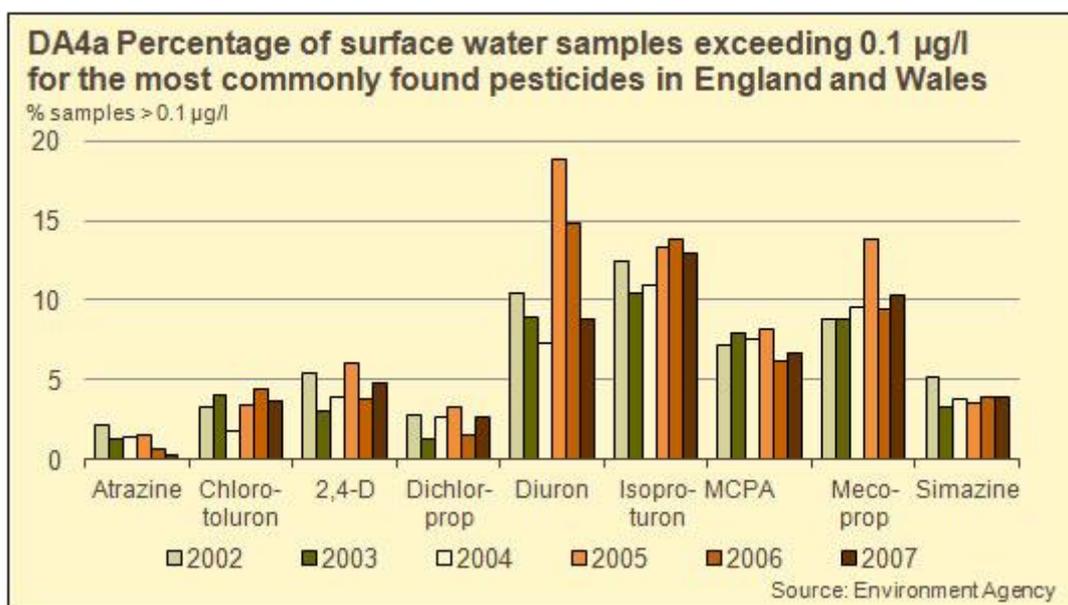
Pesticides are used to protect crops from insect pests, weeds and fungal diseases whilst they are growing and prevent rats, mice, flies and other insects from consuming and contaminating foods whilst they are being stored and to safeguard human health, by stopping food crops being contaminated by fungi, harmful microbes and moulds. Pesticide use can harm people, wildlife and the environment, and as a result strict controls are in place over their sale and use. The manufacture, storage and use of pesticides cause many issues such as residues in food, human health and safety, and the effects on wildlife and environmental effects [21].

The UK Environment Agency [22] monitored a range of pesticides year on year including herbicides, insecticides, sheep dips, timber preservatives and antifoulants and there is evidence of their presence in the aquatic environment. In 1998 the European Union Council adopted Council Directive 98/83/EC on the quality of water intended for human consumption. This directive follows the World Health Organisation Guidelines for Drinking Water Quality, Geneva 1993. The EC Drinking Water Directive sets Maximum Allowable Concentrations of  $0.1 \mu\text{g l}^{-1}$  for any pesticide and  $0.5 \mu\text{g l}^{-1}$  for total pesticides in drinking water, these figures are arbitrary and not based on ecotoxicological evaluation.

Figure 1.5 shows the percentage of surface water samples exceeding this limit for the most common pesticides in England and Wales from 1998 to 2007. Figure 1.6 displays the individual pesticides monitored by the Environment Agency from 2002 to 2007. From this data there appears to be little change to concentration levels following bans or restrictions to the agrochemicals from 1998 to 2007 except for atrazine where concentrations do appear to fall year on year from 2002 to 2007. Atrazine use was restricted from 2002 and banned in 2004. Due to a change to the monitoring programme by the Environment Agency, this indicator is no longer updated [22].



**Figure 1.5** Percentage of surface water samples exceeding  $0.1 \mu\text{g l}^{-1}$  for the most common pesticides in England and Wales from 1998 to 2007 [22]



**Figure 1.6** Percentage of surface water samples exceeding  $0.1 \mu\text{g l}^{-1}$  for the most commonly found pesticides in England and Wales from 2002 to 2007 [22]

Under the Food and Environment Protection Act (FEPA), a pesticide is “any substance, preparation or organism prepared or used, among other uses, to protect plants or wood or other plant products from harmful organisms; to regulate the growth of plants; to give protection against harmful creatures; or to render such creatures harmless [23]. The term pesticide therefore has a very broad definition, which embraces herbicides, fungicides, insecticides, rodenticides, soil-sterilants, wood preservatives and surface biocides among others. However these groups can be further divided, and the pesticide specifically designed for its target.

### 1.5.1 Use of Pesticides

About 300 chemical compounds are approved for use as pesticides in the UK [24]. Agricultural use accounts for approximately 80% of the whole of pesticide manufacture [21, 26]. Pesticides can be used for the pre-planting of crops, as they are growing, maturing or after they have been harvested and stored. Pesticides are also used on farm animals to control lice, mange or flies and other insects on livestock [20, 26].

Non-agricultural use of pesticides includes local authority use in parks, lawns, golf courses and other recreational areas. Motorway embankments and other roads are regularly treated with herbicides as are canals and waterways to remove excessive weeds. [20-21, 26]. Modern timber frame housing uses wood preservative treatments [25, 26] as well as in all stages of forest management [26] and are also used widely in domestic gardens. Pesticides are also present in other manufactured items including wall paper pastes, marine and anti-fouling paints, wooden furniture, DIY products and natural-fibre textiles [26].

### **1.5.2 Classification of pesticides**

Pesticides can be classified in a number of ways depending on the insects, animals, plants or other targets pest to be controlled [26]. The main types of classes of pesticides are by Target organisms; mode of action; type of formulation; and class of chemical [20].

**Target organism.** Pesticides may selectively target specific organisms, for example those acting as insecticides, herbicides and fungicides. Certain pesticides offer a greater degree of selectivity towards the target organism, including rodenticides, nematicides, acaricides, miticides and ovicides.

**Mode of action.** Some pesticides can kill by affecting cell metabolism in animals or insects, or acting as neurotoxins. The majority of pesticides are aimed to kill, but some can act as attractants, repellents or affect the ability of males to reproduce.

**Type of Formulation.** Pesticides come in different formulations depending on their application. Pesticides for large crops will usually be in a liquid form to be sprayed over wide areas of land, whilst aerosols or powders are used for more domestic applications in insecticides or repellents.

### **1.5.3 Class of Chemical**

The class of chemical to which a pesticide belongs to depends on its chemical structure and form such as pyrethroids, carbamates, thio/dithiocarbamates, organophosphate and ureas. For instance, herbicides can be divided into more than 60 sub groups containing approximately 500 different compounds in total. A complete list of pesticides contains at least 1800 compounds.

#### **1.5.3.1 Triazines and Triazoles**

These are grouped together, the triazine compounds are herbicides and most of the triazole compounds are fungicides. However, the structures of the pesticides in both groups are similar in that they consist of a single ring structure with three nitrogen atoms.

The triazine herbicides were discovered in 1954. The first compound in the group, Simazine, was manufactured in 1958 [27]. Triazine herbicides are among the most widely used herbicides in the U.S. [28]. Triazoles are similar to triazines, different side chains that branch off the central ring structure determine the activity and potency of the compound [29].

#### **1.5.3.2 Other Chemical Groups**

Pyrethroids and other botanical pesticides are grouped together because they all have biological origins. Carbamates are derivatives of carbamic acid and are effective as insecticides, herbicides, and fungicides. Organochlorines also known as chlorinated hydrocarbons were widely used widely agricultural pest control and for malarial control programs [29]. Organophosphates are the most widely used insecticides though they are also used as herbicides and fungicides. They are much less persistent in the environment than the

organochlorines. Phenoxy and benzoic herbicides are similar in structure and mechanism. The most controversial phenoxy compound is 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) [29]. Substituted urea compounds are widely used as herbicidal agents and to a lesser degree as insecticides [30, 31]. The phenylurea compounds include some of the most commercially important herbicides with at least 20 different compounds within this subgroup.

## 1.6 Regulatory Controls

The control and risk assessment of agrochemicals are organised by various European member state Governments, European committees and agencies. These bodies control the manufacturing, storage, usage and registration approval in member states.

The following list shows the organisations involved in regulatory controls of agrochemicals:

- European Commission
- The Council of the European Union
- European Food Safety Authority (EFSA)
- Pesticides Safety Directorate (PSD)
- Biocides and Pesticides Assessment Unit of the Health and Safety Executive (HSE)
- Advisory Committee on Pesticides (ACP)
- Biocides Consultative Committee as well as other committees and European Union agencies
- Department for Environment, Food and Rural Affairs (DEFRA)
- Department for Transport and Local Regions of the Health and Safety Executive
- Department of Health
- Welsh Government (WG)
- Department of Agriculture and Rural Development in Northern Ireland

- Regulatory Committees and Scientific Communities.
- The Food Standards Agency
- Food and Environment Protection Act 1985 (FEPA)
- Control of Pesticides Regulations 1986 (COPR)
- Plant Protection Products Regulations 2002 (PPPR)
- Plant Protection Products (Basic Conditions) Regulations 1997
- Biocidal Products Regulations 2001
- Maximum Residue Levels in Crops, Food and Feeding Stuff (MRL) Regulations 1999
- The Control of Substances Hazardous to Health 2002 (COSHH)
- The Statutory Codes of Practise

## 1.7 Toxicity of Agrochemicals

The toxicological effects of pesticides have been investigated and reported over many years, and may be measured in a quantitative and qualitative context through the following factors:-.

Acute toxicity: LD<sub>50</sub> Oral, Dermal, Intravenous, Inhalation, Eyes.

Chronic toxicity: LD<sub>50</sub> Teratogenic effects, Mutagenic effects, Carcinogenic effects,  
Reproductive effects, Organ toxicity

Ecological effects: Fate in humans, Wildfowl, Domestic, caged birds, aquatic life, Honey  
bees, Non target animals

Degradation: Soil, Water environments, Groundwater/surface water,  
Vegetation, Air

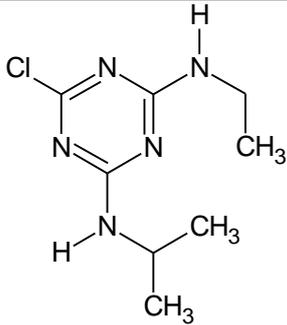
The importance of the various toxicity and ecological effects are dependant on the location, concentration and specific activity being conducted whilst using these compounds and the overall environment in which they are being used.

The following sections (1.7.1 to 1.7.8) present toxicity information for each of the agrochemical compounds presented in this study. Atrazine metabolites and deuterated internal / external standards are not presented here.

### 1.7.1 Atrazine (ATR)

Atrazine, a man-made selective triazine herbicide, was withdrawn as from 10 September 2004 and the use of atrazine was totally banned on 31 December 2007. Atrazine has been widely used and the main route of release is to soil, surface water and groundwater through its use as a herbicide in agriculture. It is highly persistent in soil (up to 1 year) and chemical breakdown is particularly slow in neutral soils. It has a high potential for ground water contamination. Excessive exposure to atrazine may affect the digestive system, eye, heart, kidney, liver and skin. [32]. It has been found in some samples of drinking water and groundwater and there are believed to be no natural sources of release to the environment [33].

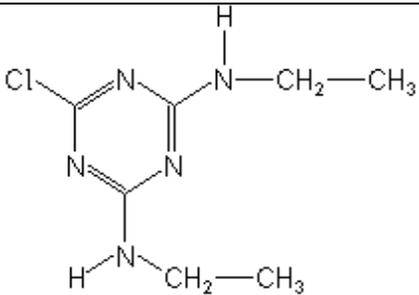
**Table 1.4** Chemical properties of atrazine

Chemical Structure	
IUPAC Name	6-chloro- <i>N</i> <sup>2</sup> -ethyl- <i>N</i> <sup>4</sup> -isopropyl-1,3,5-triazine-2,4-diamine
Chemical Formula:	C <sub>8</sub> H <sub>14</sub> ClN <sub>5</sub>
Chemical class/use:	triazine / herbicide
Solubility in water:	33 ppm at 25 °C
Solubility in other	soluble in most organic solvents
Melting Point:	173-175 °C

### 1.7.2 Simazine (SIM)

Simazine is a selective triazine herbicide. It is used to control broad- leaved weeds and annual grasses in field. It is a selective systematic herbicide, absorbed principally through the roots but also foliage [34].

**Table 1.5** Chemical properties of simazine

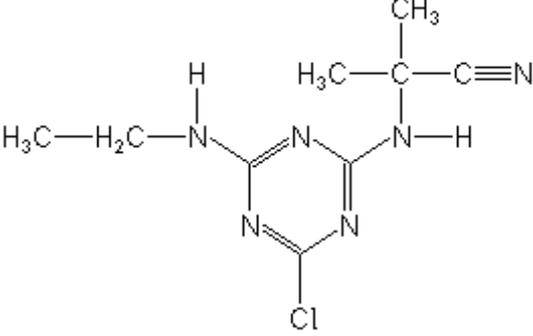
Chemical Structure	
IUPAC Name	6-chloro- <i>N</i> <sup>2</sup> , <i>N</i> <sup>4</sup> diethyl-1,3,5-triazine-2,4-diamine
Chemical Formula:	C <sub>7</sub> H <sub>12</sub> ClN <sub>5</sub>
Chemical class/use:	Algicide / chlorotriazine herbicides
Solubility in water:	0.5 mg l <sup>-1</sup> at 20 °C, or 5 ppm at 20-22 °C
Solubility in other solvents:	Solubility at 20 °C Chloroform            900 ppm Light petroleum    2 ppm Methanol              400 ppm
Melting Point:	225-227 °C

Simazine has a long half life (several months) in water and is likely to be removed by bacterial action or reactions with light or by hydrolysis; it has been found in ground water in a number of locations. It is resistant to breakdown at very low concentrations, trace levels of simazine (below 0.1 µg l<sup>-1</sup>) have been found to occur in the environment at sites many miles away from its point of use [35].

### 1.7.3 Cyanazine (CYA)

Cyanazine is a triazine herbicide used as a pre and post-emergent to control annual grasses and broadleaf weeds. Cyanazine is classified by the EPA as a Restricted Use Pesticide (RUP) because of its teratogenicity and because it has been found in groundwater [36].

**Table 1.6** Chemical properties of cyanazine

Chemical Structure	
IUPAC Name	2-(4-chloro-6-ethylamino-1,3,5-triazin-2-ylamino)-2-methylpropionitrile
Chemical Formula:	C <sub>9</sub> H <sub>13</sub> ClN <sub>6</sub>
Chemical class/use:	chlorotriazine herbicides
Solubility in water:	171 mg l <sup>-1</sup> at 25 °C
Solubility in other solvents:	soluble in benzene, chloroform, alcohol, and hexane. Slightly soluble (<10%) in xylene, ethanol, chlorobenzene. Soluble (21%) in chloroform and methyl-cyclohexanone.
Melting Point:	167 °C

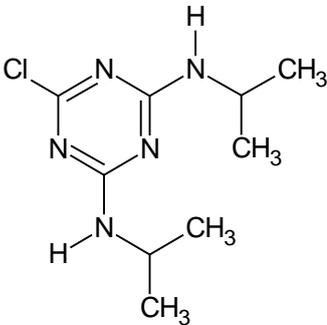
Cyanazine has been found in numerous groundwater samples at very low concentrations (1 - 80 ppb) [37]. Cyanazine quickly degrades in many soil types mostly due to the action of microbes [38].

### 1.7.4 Propazine (PRO)

Propazine is a herbicide used for control of broadleaf weeds and annual grasses. It does not adsorb as strongly to soil particles as other commercial triazine herbicides.

Propazine is persistent, moderately mobile in most soils, and it is resistant to breakdown by hydrolysis, photolysis or biodegradation and is therefore considered by the EPA to have the greatest potential for leaching into groundwater [39].

**Table 1.7** Chemical properties of propazine

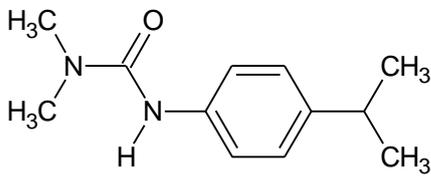
Chemical Structure	
IUPAC Name	6-chloro- <i>N</i> <sup>2</sup> , <i>N</i> <sup>4</sup> -diisopropyl-1,3,5-triazine-2,4-diamine
Chemical Formula:	C <sub>9</sub> H <sub>16</sub> ClN <sub>5</sub>
Chemical class/use:	triazine herbicide
Solubility in water:	8.6 ppm at 20 °C
Solubility in other solvents:	Difficult to dissolve in organic solvents
Melting Point:	212-214 °C

### 1.7.5 Isoproturon (IPU)

Isoproturon is taken up by the roots and through absorption through the leaves of plants and owes its action to inhibition of photosynthesis. It is used as a pre- and post-emergence herbicide against grasses and broad-leaved weeds in spring and winter cereal crops.

Isoproturon is also released into the environment through its use as an algicide. There are no natural sources of isoproturon [40].

**Table 1.8** Chemical properties of isoproturon

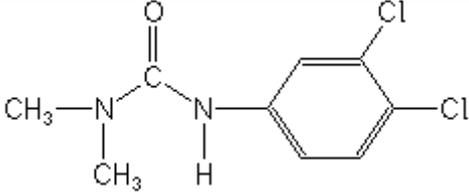
Chemical Structure	
IUPAC Name	3-(4-isopropylphenyl)-1,1-dimethylurea
Chemical Formula:	C <sub>12</sub> H <sub>18</sub> N <sub>2</sub> O
Chemical class/use:	phenylurea herbicide
Solubility in water:	65 mg/l (22°C).
Solubility in other solvents:	methanol 75 g l <sup>-1</sup> , 20°C acetone 38 g l <sup>-1</sup> , 20°C
Melting Point:	153-156°C

Isoproturon is classed as very toxic to aquatic organisms and may cause long-term effects on the aquatic environment. Excessive exposure to isoproturon may affect the blood, and may cause cancer. The Environment Agency aims to ensure that environmental exposures are too low to harm human health [40].

### 1.7.6 Diuron (DRN)

Diuron is a systemic herbicide which interferes with how plants make use of sunlight in the photosynthesis process. It is a pre-emergence herbicide used for general weed control on non-crop areas. It is a strongly adsorbed, highly persistent chemical and if released in soil will remain in the upper 5-10 cm of soil and have a half-life of about 330 days. If released into water, diuron will adsorb to the sediment where it will slowly biodegrade after acclimation, the accelerated degradation of one pesticide induced by the presence of another. Diuron is classed as very toxic to aquatic organisms and may cause long-term effects on the aquatic environment [41].

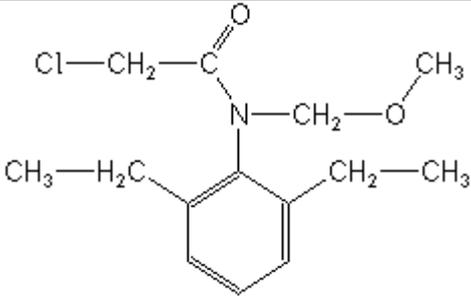
**Table 1.9** Chemical properties of diuron

Chemical Structure	
IUPAC Name	3-(3,4-dichlorophenyl)-1,1-dimethylurea
Chemical Formula:	C <sub>9</sub> H <sub>10</sub> Cl <sub>2</sub> N <sub>2</sub> O
Chemical class/use:	substituted urea / herbicide
Solubility in water:	42 ppm at 25 °C
Solubility in other solvents:	acetone 5.3 g/100 g; benzene 0.12 g/100 g; butyl stearate 0.14 g/100 g

### 1.7.7 Alachlor (ALA)

Alachlor is an aniline herbicide used to control annual grasses and certain broadleaf weeds in field corn, soybeans and peanuts. It is a selective systemic herbicide, absorbed by germinating shoots and by roots. The compound works by interfering with a plants ability to produce (synthesize) protein and by interfering with root elongation [42, 43].

**Table 1.10** Chemical properties of alachlor

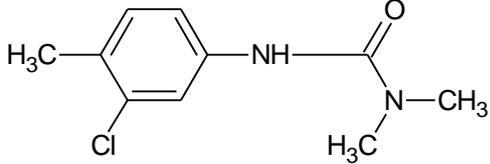
Chemical structure	
IUPAC Name	2-chloro-2',6'-diethyl-N-methoxymethylacetanilide
Chemical Formula:	C <sub>14</sub> H <sub>20</sub> Cl NO <sub>2</sub>
Chemical class/use:	aniline/herbicide
Solubility in water:	240 mg/l at 25 °C
Solubility in other solvents:	soluble in most organic solvents
Melting Point:	39.5-41.5 °C

Alachlor persists in soil from two weeks to a month, depending on soil type and climate. About half of the compound remains in soil after eight days [44]. It rapidly metabolises to water-soluble products with the acid product accumulating in the plant [45].

### 1.7.8 Chlortoluron (CHL)

Chlortoluron is a phenylurea herbicide and widely used for pre- or post-emergence selective control of grasses and weeds [46]. It is normally soil-applied and relatively soluble in water, it readily accumulates in crops. Studies have demonstrated that accumulation of chlortoluron by plants resulted in stunted growth and metabolic disorder [47].

**Table 1.11** Chemical properties of chlortoluron

Chemical Structure	
Iupac Name	3-(3-chloro-4-methyl)-1,1dimethylurea
Formula	C <sub>10</sub> H <sub>13</sub> ClN <sub>2</sub> O
Chemical class	phenylurea herbicide
Water solubility	74 mg/l
Solubility in other solvents	54 g/kg acetone 24 g/kg benzene 51 g/ kg dichloromethane
Melting point	142 - 144 °C

## 1.8 Previous Related Work

Very little previous work has been published on the use of UPLC-MS/MS for the analysis of the set of compounds forming this study. However, UPLC-MS/MS has gained wide acceptance in the field of environmental analysis over the last few years, and papers have been published in fields such as the analysis of pharmaceuticals and illicit drugs in river water.

Several studies have also compared UPLC to HPLC, and have outlined the benefits in terms of resolution, speed of analysis and sensitivity towards analytes.

Wren and Tchelitcheff [48] compared UPLC to standard HPLC to measure three pharmaceuticals in water. They found the speed of analysis increased by between four and seven times and that there was a solvent saving of approximately 80% when using the Waters UPLC system which is not only economically beneficial, but also potentially beneficial to the environment in terms of waste disposal.

The United States Environmental Agency [49] has well documented methods for the analysis of a wide range of agrochemicals. The methods use a variety of extraction methods including microextraction, liquid-solid extraction and solid phase extraction. Gas chromatography and liquid chromatography are used in conjunction with the extraction procedures. None of these methods are capable of measuring the broad range of compounds targeted in this study in a single analysis. USEPA method 508 [50] uses a liquid-solid gas chromatography method to analyse atrazine, simazine and alachlor amongst others. The method elutes alachlor in approximately 15 minutes whilst atrazine and simazine co-elute at 13 minutes. Method 536

[51] 2007 determines triazine herbicides on drinking water by LC-MS/MS, simazine, cyanazine, atrazine and propazine elute between 31 and 43 minutes. USEPA method 532 [52] determines diuron and linuron by HPLC and UV detection, their results show elution times are eight minutes for diuron and 12 minutes for linuron.

Compared to other research, the elution times achieved in the research presented in this thesis are significantly faster. Bichon et. al. [53], developed an HPLC - electro-spray ionisation – tandem mass spectrometry (HPLC-ESI-MS/MS) method and analysed six of the compounds used in this study, namely atrazine, simazine, diuron, isoproturon, chlortoluron and linuron. In each case, the analysis time was more than 2.4 times when compared to results achieved in this research and in the case of simazine, more than 3.0 times longer (9.1 minutes compared to 3.0 minutes).

Vega et. al., analysed 32 pesticides including isoproturon and diuron by LC-MS with electrospray ionisation [54]. The total analysis time was in excess of 27 minutes. Rodrigues et. al., analysed several pesticides by LC-MS/MS, which included isoproturon and diuron. The co-elution of these two pesticides occurred between 13 and 15 minutes [55], neither study achieved baseline separation of the two compounds.

During method development (Chapter 2), two of the phenyl urea compounds in this study, isoproturon and diuron have been difficult to separate with baseline resolution. They have similar chemical structures (Figure 1.1c) that make the separation of these compounds difficult to achieve. Several previous attempts by other researchers have failed to separate these two compounds in the same chromatographic elution by HPLC [54, 56, 57]. De la Pena et. al., analysed four phenylurea herbicides including isoproturon and diuron but could not

separate these two, instead they analysed them independently [56] and the same authors used a complicated process of derivatisation and photochemically-induced fluorescence detection incorporating a post-column photoreactor knitted around a xenon lamp [57].

Kasprzyk-Hordern et. al. [58], successfully developed a multi-residue method for environmental monitoring of basic/neutral pharmaceuticals by SPE, UPLC and tandem mass spectrometry. The method achieved high recovery and reproducibility rates from solid-phase extraction and detected fifteen compounds in river water at levels ranging from single nanograms to single micrograms per litre. The highest concentrations were determined in river water samples collected post-wastewater treatment plants.

## **1.9 Research Outline**

Environmental analyses demands sophisticated, sensitive and rapid analytical methods. Results and data are often considered and investigated to comply with environmental and quality standards.

The development of new monitoring methods in this subject area poses some analytical challenges. The concentrations of analytes in river water samples may be close to or below the detection limits of some analytical instruments. Low analyte concentrations require sample preparation strategies to concentrate up the sample and reduce naturally occurring matrix interferences or environmental pollution. The complexity of environmental matrices can be a major problem as aqueous samples may range from highly polluted wastewaters, to run off water or very clean rain or river water. In some instances there may be no suitable method of analysis available or interferences occur with existing techniques and new methods may have to be developed.

The standard method used to analyse organic environmental pollutants for many years has been by gas chromatography (GC). This method requires that the samples must be volatile at relatively low temperatures, or else a derivatisation stage is necessary to reduce polarity and increase volatility, and is often a time consuming part of the process. Liquid chromatography can overcome these problems.

To accomplish the analytical challenges presented by this research, hyphenated liquid chromatography-mass spectrometry in the form of Ultra High Performance Liquid Chromatography (UPLC) coupled to a tandem mass spectrometer (MS/MS) working in the electrospray ionisation mode (ESI) was utilised, with matrix clean-up and sample extraction achieved through solid phase extraction (SPE).

High Performance Liquid Chromatography (HPLC) has been the method of choice for many applications in the field of pharmaceutical quality control, forensic investigation and environmental analysis over the last 30 years due to its versatility. The development of UPLC in 2003/4 by Waters Corporation offers much improved speeds of analysis, sensitivity towards targeted analytes and chromatographic resolution, achieved through the use of significantly smaller column particle sizes. A smaller 1.7  $\mu\text{m}$  particle size stationary phase required the design of a new system to deliver the sample through the column. The UPLC system achieves this by being able to deliver solvents under high pressure, up to 15,000 psi, without damaging the system's pumps, valves, delivery line, sampling needles and sample injection ports.



This research will simultaneously determine a unique combination of analytes listed in Table 1.1 through a single procedure, producing desirable chromatographic resolution and sensitivity in the shortest time possible. Evaluation of chromatographic data was performed by investigating the limits of detection, limits of quantification, method detection limits and peak area selectivity.

The multi-residue method was applied to analyse river water samples from rivers rising in Mid and South Wales and flowing into the Severn Estuary. The rivers chosen were the Rivers Wye, Ely, Ogmore and Taff. These were chosen as examples of rivers passing through a mixture of agricultural, industrial and semi-rural areas, and areas of outstanding beauty (AONB) where tourism and leisure pursuits are important to the economy.

Legislation brought in by European Union and implemented by the UK Government under the WFD attempts to reduce and ultimately stop harmful pesticides reaching the environment. To monitor these pesticides this research investigates a new, faster method of analysis to determine the concentrations of specific pesticides in river water with a potential to indicate whether or not the governmental procedures in place to restrict pesticide use and to stop them reaching the environment are effective.

## REFERENCES

- [1] European Union Water Framework Directive 2000/60/EC. Directive of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy.
- [2] EU Decision number 2455/2001/EC. Decision of the European Parliament and of the Council of 20 November 2001 establishing the list of priority substances in the field of water policy.
- [3] <http://www.environment-agency.gov.uk/research/planning/40309.aspx> (accessed on 13/01/2011)
- [4] <http://www.pan-uk.org/default.htm> (accessed on 13/01/2011)
- [5] <http://www.environment-agency.gov.uk/business/444255/446867/255244/substances/16/> (accessed on 11/12/2005).
- [6] EU Commission Decision 2004/248/EC. Commission decision of 10 March 2004 concerning the non-inclusion of atrazine in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing this active substance.
- [7] <http://www.environment-agency.gov.uk/business/444255/446867/255244/substances/1533/> (accessed on 11/12/2005)
- [8] <http://www.environment-agency.gov.uk/business/444255/446867/255244/substances/1530/> (accessed on 11/12/2005)
- [9] Elisabeth A. Scribner, E.M. Thurman, Lisa R. Zimmerman. Analysis of selected herbicides metabolites in surface and ground water of the United States. *Science of the Total Environment* 248 (2000) 157-167
- [10] The World Health Organisation recommended classification by hazard and guidelines to classification: 2004 [http://www.who.int/ipcs/publications/pesticides\\_hazard/en/](http://www.who.int/ipcs/publications/pesticides_hazard/en/) (accessed on 13/01/2011)
- [11] International Agency for Research on Cancer. Overall evaluation of carcinogenicity to humans. Evaluated in IARC monographs volumes 1-88.
- [12] United States Environmental Protection Agency [www.epa.gov/pesticides/carlist/](http://www.epa.gov/pesticides/carlist/) (accessed on 13/01/2011)
- [13] European Union Commission Directive 2001/59/EC adapting council directive 67/548/EEC
- [14] Endocrine disrupting substances in the environment <http://www.environment-agency.gov.uk/commondata/acrobat/39909> (accessed on 11/12/2005)
- [15] Priority list of EDC (category 1) Federal German Environmental Agency (Umweltbundesamt) <http://www.umweltbundesamt.org/fpdf-1/2530.pdf> (accessed on 11/12/2005)
- [16] The implementation of the Community strategy for Endocrine Disruptors, a range of substances suspected of interfering with the hormone systems of humans and wildlife (COM(1999)706)

- [17] Identified as possible EDC under the Oslo Paris Commission, endocrine disrupting pesticide, Gwynne Lyons, Pesticide News 46 Dec. 1999
- [18] World Wide Fund for Nature list of reported pesticides to have reproductive and/or endocrine effects
- [19] The Oslo Paris Commission is the current instrument guiding international cooperation on the protection of the marine environment of the North-East Atlantic. It combined and updated the 1972 Oslo Convention on dumping waste at sea and the 1974 Paris Convention on land-based sources of marine pollution.  
*[http://www.ospar.org/documents/dbase/publications/p00554\\_inventory\\_monitoring\\_2011.pdf](http://www.ospar.org/documents/dbase/publications/p00554_inventory_monitoring_2011.pdf)* (accessed on 30/08/2011)
- [20] Applied Ecotoxicology, J Rombke, JF Moltmann, CRC Press Inc. 1996, ISBN 0-56670-070-1
- [21] <http://www.pesticides.gov.uk/garden.asp?id=219> (accessed on 09/08/2005)
- [22] [http://archive.defra.gov.uk/evidence/statistics/foodfarm/enviro/observatory/indicators/d/da4\\_data.htm](http://archive.defra.gov.uk/evidence/statistics/foodfarm/enviro/observatory/indicators/d/da4_data.htm) 329 (accessed on 09/08/2005)
- [23] <http://www.pesticides.gov.uk/garden.asp?id=329> (accessed on 09/08/2005)
- [24] [http://www.environment-agency.gov.uk/commondata/acrobat/r\\_agri\\_pest\\_t\\_v1\\_1008146.pdf](http://www.environment-agency.gov.uk/commondata/acrobat/r_agri_pest_t_v1_1008146.pdf) (accessed on 11/12/2005)
- [25] Woods, A., Pest Control, McGraw-Hill, London, 1974 p.82
- [26] <http://www.foresight-preconception.org.uk/summaries/frames/agrochem-nf.html> (accessed on 10/08/2005)
- [27] Draber, W. Structure-activity studies of photosynthesis inhibitors, In Rational Approaches to Structure, Activity and Ecotoxicology of Agrochemicals. Draber, W. and Fujita, T., Eds.
- [28] Ware, C. W. Fundamentals of Pesticides: A Self-Instruction Guide, 2nd ed. Thomson Publications, Fresno, CA, 1986
- [29] Pesticide Profiles, Toxicity, Environmental Impact and Fate. ed MA Kamrin CRC Press 1997. ISBN 0-56670-190-2
- [30] Retnakaran, A. and Wright, J. F., Eds. Control of insect pests with benzoylphenyl urea. In Chitin and Benzoylphenyl Ureas (Series Entomologica Vol. 38), Wright, J. F. and Retnakaran, A., Eds. Kluwer Academic, Dordrecht, The Netherlands, 1987.
- [31] Jordan, L. S. and Cudney, D. W. Herbicides. In Fate of Pesticides in the Environment, Biggar, J. W. and Seiber, J. N., Eds. Agricultural Experiment Station, Division of Agriculture and Natural Resources, University of California, CA, 1987.
- [32] <http://www.environment-agency.gov.uk/business/444255/446867/255244/substances/16/> (accessed on 11/12/2005)
- [33] US EPA. 1988 (August). Atrazine: Health Advisory. Office of Drinking Water, US EPA, Washington, DC
- [34] U.S. Environmental Protection Agency. 1990 (fall). National Pesticide Survey: Simazine. Office of Water, Office of Pesticides and Toxic Substances, US EPA, Washington DC
- [35] <http://www.environment-agency.gov.uk/business/444255/446867/255244/substances/229/> (accessed on 11/12/2005)

- [36] Walker, M.M. and L.H. Keith. 1992. EPA's Pesticide Fact Sheet Database. Lewis Publishers, Chelsea, MI.
- [37] U.S. Environmental Protection Agency. Office of Drinking Water. "Cyanazine Health Advisory." Draft Report. August 1987.
- [38] U.S. Environmental Protection Agency. Office of Pesticides and Toxic Substances. Cyanazine. Special Review Position. Document 1. Washington DC, April 1985
- [39] U. S. Environmental Protection Agency. 1988 (Dec.) Guidance for the Reregistration of Pesticide Products Containing Propazine as the Active Ingredient. Office of Pesticides and
- [40] <http://www.environment-agency.gov.uk/business/444255/446867/255244/substances/1533/>  
(accessed on 11/12/2005)
- [41] <http://www.environment-agency.gov.uk/business/444255/446867/255244/substances/1530/>  
(accessed on 11/12/2005)
- [42] National Library of Medicine (1992). Hazardous Substances Databank. TOXNET, Medlars Management Section, Bethesda, MD
- [43] Walker, Mary M. and Lawrence H. Keith. (1992). EPA's Pesticide Fact Sheet Database. Lewis Publishers. Chelsea, MI
- [44] The Agrochemicals Handbook. (1991) The Royal Society of Chemistry, Cambridge, England
- [45] Food and Drug Administration (1986). The FDA Surveillance Index. Bureau of Foods, Dept of Commerce, National Technical Information Service, Springfield, V
- [46] Lechón, C. Sánchez-Brunete and J.L. Tadeo, Influence of the laboratory incubation method on chlorotoluron and terbutryn degradation in soil. *Journal of Agricultural and Food Chemistry*, **45** (1997), pp. 951–954.
- [47] ( F. Pérés, D. Florin, T. Grollier, A. Feurtet-Mazel, M. Coste, F. Ribeyre, M. Ricard and A. Boudou, Effect of the phenylurea herbicide IPU on periphytic diatom communities in freshwater indoor microcosms. *Environmental Pollution*, **94** (1996), pp. 141–152)..
- [48] Use of ultra-performance liquid chromatography in pharmaceutical development. Stephen A.C. Wren, Pierre Tchelitcheff. *Journal of Chromatography A*, 1119 (2006) 140
- [49] [National Exposure Research Laboratory, Office of Research and Development. U.S. Environmental Protection Agency, Cincinnati, Ohio. 45268
- [50] Analysis of Organohalide Pesticides and Commercial Polychlorinated Biphenyl Products in Water by Microextraction and Gas Chromatography.
- [51] Determination of Triazine Pesticides and their Degradates in Drinking Water by Liquid Chromatography Electrospray ionisation tandem mass spectrometry (LC/ESI-MS/MS
- [52] Determination of phenylurea compounds in drinking water by Solid Phase Extraction and High Performance Liquid Chromatography with UV Detection.
- [53] E. Bichon, M. Dupuis, B. Le Bizec, F. Andre, *LC-ESI-MS/MS determination of phenylurea and triazine herbicides and their dealkylated degradation products in oyster*. *Journal of Chromatography B*, **838** (2006) 96-106
- [54] Belmonte Vega, A., A. Garrido Frenich, and J.L. Martinez Vidal, *Monitoring of pesticides in agricultural water and soil samples from Andalusia by liquid chromatography coupled to mass spectrometry*. *Analytica Chimica Acta*, 2005. 538(1-2): p. 117.

- [55] Rodrigues, A.M., et al., *Determination of several pesticides in water by solid-phase extraction, liquid chromatography and electrospray tandem mass spectrometry*. Journal of Chromatography A, 2006.
- [56] Munoz de la Pena, A., M.C. Mahedero, and A. Bautista-Sanchez, *High-performance liquid chromatographic determination of phenylureas by photochemically-induced fluorescence detection*. Journal of Chromatography A, 2002. 950(1-2): p. 287.
- [57] Munoz de la Pena, A., M.C. Mahedero, and A. Bautista-Sanchez, *Monitoring of phenylurea and propanil herbicides in river water by solid-phase-extraction high performance liquid chromatography with photoinduced-fluorimetric detection*. Talanta, 2003. 60(2-3): p. 279.
- [58] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, *Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra performance liquid chromatography–positive electrospray ionisation tandem mass spectrometry*. Journal of Chromatography A, 1161 (2007) 132–145
- [59] Introduction to Modern Liquid Chromatography, 3rd Edition  
Lloyd R. Snyder, Joseph J. Kirkland, John W. Dolan