

HSL, Harpur Hill, Buxton, SK17 9JN
Telephone +44 (0) 1298 218 000
Facsimile +44 (0) 1298 218 570



**New Instrumental Techniques for Toxic and
Harmful Substances**

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Project Leader: **John White**
Author(s): **John White, Helen Corns, Katherine Jones
Ian Pengelly, John Cuthbert**

Science Group: **Environmental Sciences Group
Health Sciences Group**

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EXECUTIVE SUMMARY

Aims

To develop and apply new instrumental techniques for the assessment of risks from toxic and harmful substances in the workplace.

This work has been sub-divided into 5 blocks;

1. Cytotoxic Drugs by LC/MS/MS and LC/MS
2. Isocyanates-in-Air by LC/MS/MS
3. Evaluation of an On-Site monitor for Benzene
4. Biocide and Pesticide determination by LC/MS and SPME
5. Pyrolysis GC/MS of isocyanate containing materials.

The areas of application and HSE interest covered by this report are;

Cytotoxic drugs - hospitals

Isocyanates - motor vehicle repair shops, adhesives, coatings and polyurethane industries

On-Site monitoring of benzene – off-shore oil industry, general workplace use of benzene, filling stations, printing industry

Biocides and Pesticides – farming, agriculture and public amenity use of biocides.

Each of the five blocks represents research into methods that will offer some or all of the following benefits;

- Ease of use
- Portability
- Increased sensitivity
- Increased specificity
- More rapid sample preparation
- More rapid analysis
- Improved reproducibility

Main Findings

1. Cytotoxic Method Development – LC/MS/MS

- Liquid chromatography/tandem mass spectrometry (LC/MS/MS) has been developed for the determination of a range of cytotoxic (anti-cancer) drugs used in hospitals.
- A surface wipe method has been developed for the cytotoxic drugs, cyclophosphamide, ifosfamide and mitomycin. The method uses the same wiping solution as required for methotrexate and platinum-containing drugs, allowing all five of these anti-cancer compounds to be sampled together.
- Quantitation limits for all three drugs was 0.5 µg/l. Recoveries from most surfaces were generally acceptable (> 70%) although the lab floor sample gave poor recoveries for all three compounds and gloves also gave lower recoveries for cyclophosphamide and ifosfamide. These lower recoveries should be borne in mind when interpreting results.
- Initial work on developing a method for 5-fluorouracil has not been successful. Although initial LC/MS conditions were developed, the results of spiking experiments indicate that the extraction procedure is very ineffective, with an estimated recovery of approximately 10%. Furthermore the instrument response diminished 10 fold for the direct analysis of the working solution on two different days, indicating problems with reproducibility possibly caused by contamination of the detector. In addition the analytical method shows inadequate sensitivity.

2. Isocyanates-in-Air Method Development – LC/MS/MS

- Positive ion mode atmospheric pressure chemical ionization (APCI+) and electrospray ionization (ES+) MS modes have been applied to the determination of isocyanate-in-air samples from workplace atmospheres (motor vehicle repair shops (MVR) and spraying of polyurethane foam insulation) and methods using these various MS modes developed. Particular emphasis has been placed on development of methods for the isocyanates used in the MVR industry as this is HSE's main area of concern with regard to isocyanate use.
- The precursor ion APCI+ method gave excellent spectral data but was not deemed to be sensitive enough for HSE requirements.
- The precursor ion ES+ mode method gives excellent spectral data; this is invaluable for unambiguous identification of the isocyanate (NCO) species present. This is particularly useful for the complex NCO aerosols found in paints used in motor vehicle repair body-shops. This

method has similar sensitivity to the existing method used at HSL (MDHS 25/3).

- The ES+ specific positive ion multiple reaction mode (MRM+) transition mode method gives extremely sensitive analysis for target NCO species with low part per billion to medium part per trillion sensitivity depending on NCO species. This is approximately 10x to 1000x more sensitive (depending on the isocyanate species) than the existing HSL method.
- This work has proved the extremely powerful nature of LC/MS/MS as an analytical technique for workplace analysis of NCO.

3. On-Site Monitoring Techniques – Benzene Monitor

- A direct reading monitor for benzene, for potential use on off-shore oil-rigs, has been evaluated in the laboratory in the presence of an interfering compounds (toluene and petrol). The instrument gave mean indicated concentrations of benzene between about 70% and 110% of the true concentration.
- The direct reading monitor is suitable for real time, non-critical measurement of benzene i.e. to verify that the benzene concentration from a process is ~ 10 x below the WEL.
- The chip measuring system (CMS) is an elegant design and is portable and easy to use but it has not been found to give an accurate or repeatable measurement of benzene in the presence of other materials. It should therefore not be used when precise or accurate measurements of complex workplace atmospheres is essential i.e. measurement of benzene levels at or near the WEL or in complex atmospheres.

4. Biocide and Pesticide Method Development – LC/MS and SPME

- Quaternary ammonium herbicides are widely used for agriculture and public amenity purposes. The initial work carried out on various quaternary ammonium herbicides suggests that LC/MS will be able to give good sensitivity for these compounds. More work is required to fully validate this method.
- For the recovery work using the quaternary ammonium herbicide LC/MS method, the results from the high level spikes show a marked improvement in % recovery and variability on the methods previously used at HSL. However, the low level spikes were subject to interference from some co-eluting component from the grass. The use the more specific LC/MS/MS method should improve this situation. Further work is required to fully develop this method.

- Solid phase micro-extraction (SPME) has been investigated for a variety of biocides (timber treatment, anti-fouling paints and organo-phosphorus pesticides) from water samples. More work is required to fully evaluate this technique but it has shown promise as a simple extraction technique for biocides from water samples.

5. Pyrolysis GC/MS of Isocyanates

- Pyrolysis GC/MS has proved to be a rapid technique for the identification of the more volatile derivatized isocyanates produced during thermal degradation of polyurethane foams and coatings.
- It is an excellent complimentary technique to those already in use at HSL and has been particularly useful for isocyanates arising from thermal degradation e.g. heating of polyurethane foams, welding of painted surfaces, application of stoved isocyanates etc.

Recommendations

1. The wipe sampling and LC/MS methods developed for cytotoxic drugs should be applied to workplace (hospital) samples and these methods expanded as necessary to meet HSE needs.

2. The LC/MS/MS methods developed for isocyanates and biocides should be developed further and used for the routine analysis of workplace enforcement samples so improving HSL's analytical service to HSE.

3. The LC/MS instrumentation now available at HSL should be given greater application to the determination of analytes of interest to HSE i.e. rodenticides, disinfectants etc.

4. The evaluation of on-site methods offers the possibility of real time measurement of hazardous workplace chemicals in challenging environments and should be continued in line with HSE requirements.

5. SPME has shown itself to be a rapid and simple extraction technique for pesticides and biocides in water and should be investigated further for other application areas e.g. pesticide spray drift incidents and confirmation of composition.

6. Pyrolysis GC/MS has proved to be an excellent complimentary technique for the analysis of isocyanates from the heating of polyurethanes and other materials. The use of this technique should be expanded to other workplace chemicals and processes as appropriate.

7. The evaluation of new instrumental techniques and new method development work presented in this report and similar work will be continued in the new core activity call-off contract agreed in mid 2005.

1 INTRODUCTION

This report covers the work carried out under the "Novel Instrumentation 2003-2005" call-off contract. The aim of the contract was "to develop and apply new instrumental techniques for the assessment of risks from toxic and harmful substances". This work has been divided into 5 sections;

- Cytotoxic Method Development – LC/MS/MS
- Isocyanates-in-Air Method Development – LC/MS/MS
- On-Site Monitoring Techniques – Evaluation of a Benzene Monitor
- Biocide and Pesticide Method Development – LC/MS and SPME
- Pyrolysis GC/MS of Isocyanates.

The main areas of application and HSE interest covered by this report are;

Cytotoxic drugs - hospitals

Isocyanates - motor vehicle repair shops, adhesives, coatings and polyurethane industries

On-Site monitoring of benzene – off-shore oil industry, general workplace use of benzene as a solvent, filling stations, printing industry

Biocides and Pesticides – farming, agriculture and public amenity use of biocides

Each of the 5 sections represents research into methods that offer some or all of the following benefits;

- Ease of use
- Portability
- Increased sensitivity
- Increased specificity
- More rapid sample preparation
- More rapid analysis
- Improved reproducibility.

The emphasis on LC/MS and LC/MS/MS (liquid chromatography/mass spectrometry and liquid chromatography with tandem mass spectrometry) techniques reflects the power of this technique and its novelty to HSL. This technique will provide more specific and sensitive methods than those currently in use at HSL and extends the range of compounds that HSL can determine so enhancing HSL's analytical service to HSE.

The benzene monitor and solid phase micro-extraction (SPME) work represents research into field-methods that are usually more rapid, portable, user friendly and simple methods for the compounds under study (biocides and benzene)

Pyrolysis gas chromatography/mass spectrometry (GC/MS) is a new technique to HSL and offers the potential for simple and rapid analytical methods. Thermal degradation of isocyanates is a subject of much current interest as it has been suggested that thermal breakdown of isocyanate based materials could lead to the exposure to isocyanate of unsuspecting and unprotected workers.

Each section has been written up as a "stand alone" section, with relevant appendices and references.

2 BIOLOGICAL MONITORING

This section describes the work carried out by the Biological Monitoring Section developing methods for the monitoring of cytotoxic drugs in hospitals and other workplaces. These drugs are used in anti-cancer treatment and there is concern that the staff of hospitals who are using these drugs are exposed to these highly toxic materials either through the air or from contaminated surfaces. There is therefore a need for an accurate method for determining these drugs and for sampling them from surfaces

2.1.1 Cytotoxics Method Development

Biological monitoring section has previously developed an LC/MS/MS (liquid chromatography/tandem (triple quadrupole) mass spectrometry) method for cyclophosphamide and ifosfamide in urine (Appendix 1). This method was extended to include mitomycin. LC/MS/MS was used as it should offer an excellent combination of good sensitivity and specificity when compared to more common methods such as LC/ultra-violet-visible (UV) detection. For compounds without a good UV chromophore UV analysis is easily interfered with by co-eluting and background compounds from the drug formulation used or the sample matrix and may not have the sensitivity required. LC/MS/MS is widely used in the pharmaceutical industry for this type of analysis because of these reasons.

HSE have also funded a study (which is due to take place in 2005) looking at surface contamination in a number of pharmacies following a questionnaire survey. Having conducted a number of sampling visits within hospital pharmacies, it became apparent that it would be unlikely that biological monitoring would be routinely used as a monitoring method but that surface wipe testing might be useful to the sector. It would also be useful to extend the suite of cytotoxic drugs that could be tested. Work was therefore undertaken on adapting the existing method for surface wipe testing and a method for determining fluorouracil was also investigated.

2.1.2 Surface Wipe Method

A surface wipe procedure has already been developed using 10 mM sodium bicarbonate. This was chosen because the solvent also had to be suitable for methotrexate (analysed by immunoassay) and platinum-containing drugs (analysed by ICP-MS).

Calibration curves were prepared in 10 mM sodium bicarbonate with standards (prepared in duplicate) in the range 3.3 to 26.6 µg/l. Three millilitres of sample was extracted using a ChemElut cartridge and eluted with diethyl ether. The ether was then evaporated under nitrogen and the residue reconstituted in 50 µl mobile phase.

Using a combined standard of cyclophosphamide, ifosfamide and mitomycin in methanol (10 mg/l), a 10 µl aliquot was spread onto a 10 cm² surface and allowed to dry. Using three wipes (Kleenex), the area was swabbed using 10 mM sodium bicarbonate as a wetting agent. The wipes were then extracted on a rotary tumbler in 50 ml 10 mM sodium bicarbonate. Three milli-litre aliquots were then extracted by ChemElut as above.

A number of surfaces were investigated including:

- Vinyl floor tile
- Smooth ceramic tile
- Textured ceramic tile
- Lab flooring
- Metal tray
- Disposable overall
- Nitrile gloves

In addition to these, blank wipes and blank GFA filters were also spiked with 10 µl of combined cytotoxic solution. Blank samples of all surfaces, gloves, wipes and filters were also extracted. The results of this work are given in table 1 and 2.

2.1.2.1 Results and Discussion

Table 1. Calibration curves in 10 mM sodium bicarbonate.

Compound	Linear Regression coefficient (N)
Cyclophosphamide	R=0.9951 (N=12)
Ifosfamide	R=0.9939 (N=13)
Mitomycin	R=0.9928 (N=10)

Table 2. Recovery results (% of spiked, mean of two samples).

Sample	Recovery (%)		
	Cyclophosphamide	Ifosfamide	Mitomycin
Spiked Wipe	120	110	129
Spiked GFA Filter	137	137	10
Vinyl floor tile	81	71	118
Smooth ceramic tile	93	84	73
Textured ceramic tile	81	79	71
Lab flooring	33	25	30
Metal tray	95	86	77
Disposable overall	76	62	46
Nitrile gloves	51	44	83

Blank samples were all blank for cyclophosphamide apart from one replicate for the smooth ceramic tile (2.2 µg/l). Ifosfamide and mitomycin were present in some of the blank samples but always at a level of less than 0.5 µg/l allowing quantitation down to 0.5 µg/l.

2.1.3 Fluorouracil Method Development

Fluorouracil is an anti-cancer drug widely used in UK hospitals. A method needs to be developed for this compound so that HSE can assess workplace exposure. Attempts to use tandem mass spectrometry in multiple reaction monitoring mode (MRM) were not successful as the fragmentation of the parent ion was poor. Fluorouracil was therefore analysed by ion trap LC/MS using a negative ion mode selected ion monitoring method. The ion trap MS is a different source design to the triple quadrupole used above and has different benefits and weaknesses. The ion trap is a more robust source design that is less affected by interferants from the sample matrix, this is important if biological samples e.g. urine or wipes from dirty surfaces need to be analysed. Another benefit is the rapid mass scanning rates for the ion trap mean that it is very good for taking full spectral data, which is invaluable for unambiguous identification. There have been no reports in the literature of using LC/MS for fluorouracil unless a derivatizing agent is used. The derivatization process is an added analytical step and so a method in which no derivatization was required was investigated as a more simple approach.

A standard curve was prepared from the working solution by the addition of 0, 200, 400, 600, 800 and 1000 µl to GC vials and making up to 1 ml volumes with distilled water. A graph of area response against concentration produced a correlation coefficient of 0.99.

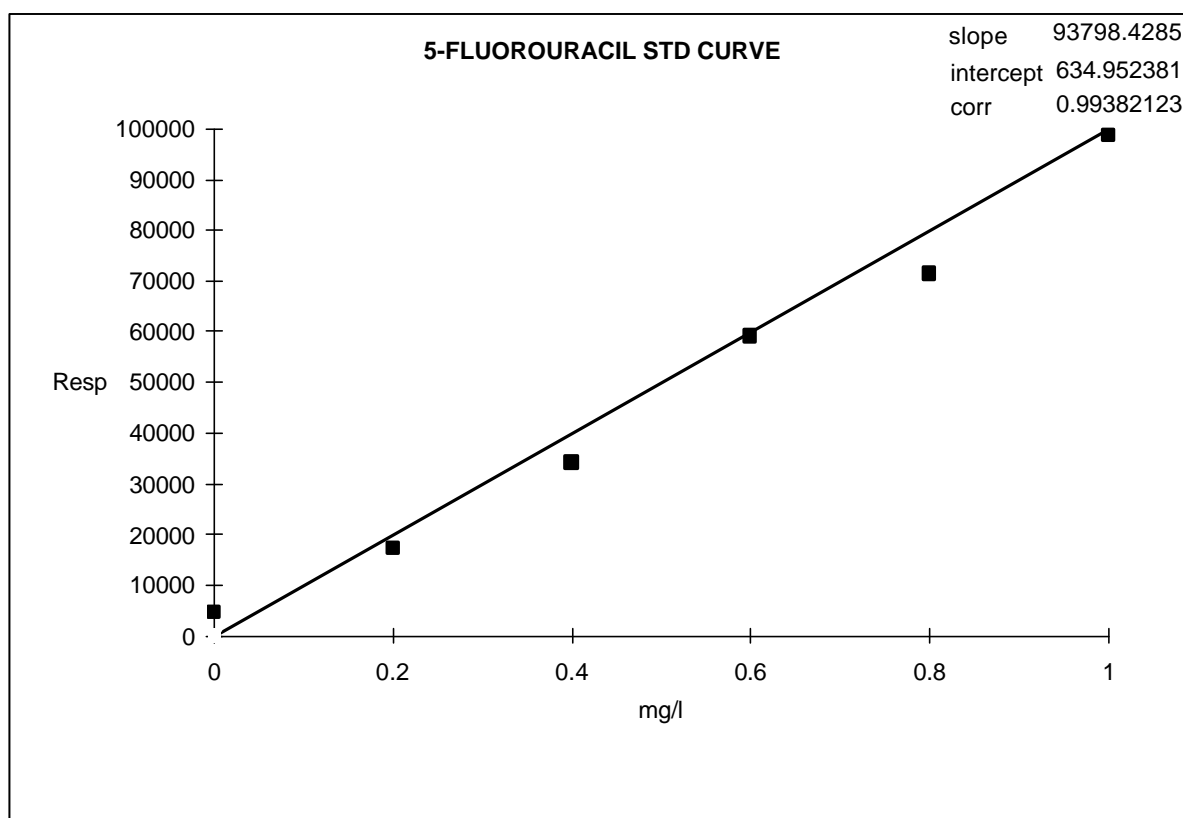
A standard spiked with 100 µl of working solution and extracted as per the sample preparation procedure when analysed gave an area response of 1280

compared with an area response of 8932 for direct analysis of the working solution.

2.1.3.1 Results and Discussion

The results of the calibration work are given in figure 1. Further analytical details are given in Appendix 2 however the general conclusions of this work was that the extraction of fluorouracil was inefficient (recovery about 10%) and that sensitivity was poor with a detection limit in the region of 100 to 200 µg/l (we need to be looking at a detection limit of low µg/l). The derivatisation method mentioned previously may give the required sensitivity and so should be investigated.

Figure 1. Calibration curve for 5-Fluorouracil



2.1.4 Extending the suite of cytotoxic drugs

As well as fluorouracil, other drugs that would be useful to include in a cytotoxic drug suite include the widely used doxorubicin and paclitaxel. Mass spectrometric conditions have been ascertained for these drugs (Appendix 3) but no further work has been undertaken. Papers in the literature report using solid phase extraction (SPE) to extract these and other drugs (separately). It

would therefore be valuable to investigate the possibility of combining these drugs in an SPE extraction method. If successful this could then be automated.

2.1.5 Conclusions

LC/MS has been found to be suitable for the determination of a range of cytotoxics from wipe samples. A surface wipe method has been developed for cyclophosphamide, ifosfamide and mitomycin. The method uses the same wiping solution as required for methotrexate and platinum-containing drugs, allowing all five compounds to be sampled together. Quantitation limits for all three drugs was 0.5 µg/l. Recoveries from most surfaces were generally acceptable (> 70%) although the lab floor sample gave poor recoveries for all three compounds; gloves also gave lower recoveries for cyclophosphamide and ifosfamide. These lower recoveries should be borne in mind when interpreting results.

Initial work on developing a method for fluorouracil has not been successful. The results indicate that the extraction procedure is very ineffective, with an estimated recovery of approximately 10%. Furthermore the area response diminished 10 fold for the direct analysis of the working solution on two different days, indicating problems with reproducibility possibly caused by contamination of the detector. In addition the analytical method appears to show poor sensitivity.

2.1.6 Recommendations

1. The wipe method should be extended and developed to cover the cytotoxic drugs likely to be found in a hospital environment.
2. The development of an LC/MS method for fluorouracil, possibly using a derivatizing agent, should be considered.
3. The methods developed should be applied for the determination of cytotoxic drugs in workplace samples

2.1.7 Appendices to Section 2

Appendix 1 - Analytical Method for Cytotoxic drugs

This describes an analytical method for cyclophosphamide and ifosfamide using solvent extraction and HPLC-MS-MS (triple quadrupole).
Samples are prepared as per OTOP42.

Instrument Set-Up

Analytical Method: Cytotoxic LC/MS/MS SIM – positive ion mode

Mobile phase C: Methanol

Mobile phase D: 20 mM ammonium acetate in 0.1% acetic acid

200 $\mu\text{l}\cdot\text{min}^{-1}$ at 50:50 C: D v/v

Column: 100 x 2.1 mm C18 column; 5 μl injection

MS parameters – positive mode, MRM

Analyte	Ion transition (m/z)	Dwell (ms)
Cyclophosphamide	261.08/154.14	150
Ifosfamide	261.07/139.97	150
Mitomycin	335.05/242.17	150

Analyte	Compound	
Ifosfamide	DP	51
	FP	340
	EP	5
	CEP	18
	CXP	0
Cyclophosphamide	DP	56
	FP	350
	EP	9.5
	CEP	14
	CXP	2
Mitomycin	DP	56
	FP	160
	EP	8
	CEP	19.7
	CXP	2

Source/Gas	
CUR	35
IS	5300
TEM	350
GS1	25
GS2	30

Appendix 2 - Development of an LC-MS Method for 5-Fluorouracil

This describes an analytical method for 5-fluorouracil [5-fluoro-2,4(1H,3H)-pyrimidinedione] using solvent extraction and ion trap LC-MS.

Sample Preparation

Stock solution: 1 mg/ml in methanol

Working solution: 1 mg/l in water

Standards were prepared by spiking 5 ml water with working and stock solutions. Solvent extraction was with ethyl acetate, the organic layer then being dried down under nitrogen and reconstituted in 50 µl mobile phase.

Instrument Set-up

Analytical Method: Fluoracil LC/MS SIM – negative ion mode

Mobile Phase A2: 20 mM ammonium acetate in 0.1% acetic acid

Mobile Phase B1: Methanol

Isocratic flow at 200 µl/min at 50:50 A2:B1 v/v

C18 Genesis column with dimensions 100 x 2.1 mm

5 µl injection volume

MS parameters: negative mode, SIM 129

Mass Range Mode Std/Enhanced

Ion Polarity Negative

Ion Source Type ESI

Dry Temp (Set) 325 °C

Nebulizer (Set) 40 psi

Dry Gas (Set) 12.0 l/min

Trap Drive 32.1

Skim 1 -40.0 Volt

Skim 2 5.0 Volt

Octopole RF Ampl 112.2 Vpp

Capillary Exit -100.7 Volt

Scan Begin 50 m/z

Scan End 170 m/z

Averages 5 spectra

Max. Accu Time 200000 µs

ICC Target 50000

Charge Control on

Appendix 3 – Mass Spectrometry parameters for doxorubicin and paclitaxel from the scientific literature

MS parameters – positive mode, MRM

Analyte	MS mode	Ion transition (m/z)	Dwell (ms)
Doxorubicin	Positive MRM	544.1/130.1	150
Paclitaxel	Negative MRM	852.3/121.1	150

Analyte	Compound	
Doxorubicin	DP	61
	FP	160
	EP	5
	CEP	27
	CXP	2
Paclitaxel	DP	-51
	FP	-330
	EP	-9
	CEP	-40
	CXP	-24

3 LC/MS OF ISOCYANATES

This section reports work on the development of improved methods for the determination of airborne isocyanates (NCO) using triple quadrupole (tandem) liquid chromatography/mass spectrometry (LC/MS/MS).

3.1.1 Introduction

Isocyanates are the largest cause of occupational asthma in the UK and reducing occupational asthma has been identified by HSE as a priority target. The Health and Safety Executive (HSE) has set workplace exposure limits (WELs), for total isocyanate exposure (i.e. all NCO species), of $70 \mu\text{g}/\text{m}^3$ (short term, 15 minute) and $20 \mu\text{g}/\text{m}^3$ (8 hour time weighted average) (HSE, 2005).

Despite general improvements in workplace exposure control, workers are still sensitised by NCO. In some cases this occurs when workplace monitoring has found no observable NCO present. This may be because of poor sampling or analytical performance by the analytical laboratory (Piney et al, 2002). An alternative explanation is that the levels of NCO present are below current detection limits. Most modern methods can detect well below the workplace exposure limits, e.g. the method for airborne isocyanates developed by HSL, MDHS 25/3 (HSE, 1999), uses liquid chromatography (LC) with an electrochemical (EC) detector to quantify the 1-(2-methoxyphenyl)piperazine (MP) derivatized NCO oligomers and polymers and has an estimated limit of detection as routinely used at HSL, using the electrochemical detector (EC) for quantification with the ultra-violet/visible (UV) detector as confirmation, of $\sim 7 \mu\text{g}/\text{m}^3$ for a 15 l air sample. This is significantly lower than the U.K. short-term exposure limit value of $70 \mu\text{g}/\text{m}^3$.

One current theory is that a worker can be sensitised by one exposure above the exposure limit. This exposure may be of very short duration (e.g. 30 sec) and so would be "averaged out" in an 8-hour time weighted average calculation or a long-term sample. After this initial sensitisation event the worker becomes hypersensitive and sensitisation occurs after exposure to extremely low levels of NCO. To prove or disprove this theory the sensitivity of the analytical methods currently used must be improved.

The method for airborne isocyanates developed by HSL, MDHS 25/3 (HSE, 1999), uses liquid chromatography (LC) with an electrochemical (EC) detector to quantify the 1-(2-methoxyphenyl)piperazine (MP) derivatives of NCO oligomers and polymers. The EC detector used at HSL is routinely operated at a range of $\sim 5 \mu\text{A}$ but can be operated at a range of 1 pA i.e. 5×10^6 times more sensitive. Although it is doubtful if all this gain in sensitivity would be achieved because of instrumental factors e.g. pump noise, it is clear that the limit of detection of MDHS 25/3 using the EC detector can be improved quite easily.

This leaves the problem of a confirmatory technique. The EC detector does not give an unambiguous identification that a peak is NCO derived. MDHS 25/3 uses the UV detector (spectral matching), EC/UV response ratio and comparison with a bulk MP derivative to assist in this identification. Unfortunately the UV detectors currently on the LC systems at HSL cannot be made much more sensitive than they are at present and so at very low levels of NCO UV cannot be used as a confirmatory technique. This effectively places a limit on the sensitivity of MDHS 25/3 of $\sim 0.05 \mu\text{g/ml}$ (the limit of detection for the UV detector) which corresponds to $\sim 7 \mu\text{g/m}^3$ for a 15 l air sample as stated above. Typical sensitivity for the EC detector as operated routinely at HSL is $\sim 0.005 \mu\text{g/ml}$ which corresponds to $\sim 0.7 \mu\text{g/m}^3$ for a 15 l air sample.

The obvious choice as confirmatory technique is mass spectrometry (MS). This not only is likely to be more sensitive than UV but also, in scan mode, gives excellent structural information and so should be more selective. The design of MS used in this work was a triple quadrupole. This design is often extremely sensitive and so could be used to replace the EC detector. If the sensitivity and selectivity of the method can be increased, by using LC/MS, the following benefits would occur;

- the ability to measure well below the current exposure limit values e.g. necessary if the WEL is revised downwards or to detect extremely low levels of NCO
- the possibility of taking very short term samples to try to model “burst” (i.e. very short time scale) exposures
- better selectivity of method i.e. better identification of NCO species present in a sample, this may be useful in forensic work
- easier and cheaper analysis because time consuming and complicated identification of NCO species requiring interpretation by a highly skilled analyst is not necessary. The MS will give a mass spectrum or set of ions, which provide unambiguous identification.
- replacement of the EC detector i.e. if an MS method of adequate sensitivity can be developed then the complicated to operate and maintain EC detector can be discarded.

3.1.2 Development of an APCI+ method for NCO

This section describes work carried out to develop a method for NCO based on positive ion mode atmospheric pressure chemical ionization (APCI+). This ionization technique was investigated first instead of the more common electrospray ionization (ESI) because the carbamate class of pesticides, which are chemically similar to the MP urea derivatives produced when sampling by MDHS 25/3, are often analysed using the APCI ionization source. Some of the work described in this section was presented as a poster at the 16th International Mass Spectrometry Conference in Edinburgh, 2003.

Initial work consisted of infusing NCO-MP solutions into the MS to optimise the system. The method developed was a precursor ion scan MS/MS method.

This mode of MS is only available to triple quadrupole or hybrid MS designs. In this mode of MS/MS the third quadrupole (Q3) is fixed to measure the occurrence of a particular fragment ion and the first quadrupole (Q1) is scanned over the required mass range. The second quadrupole (Q2) is the collision cell where the specified daughter ion is produced by fragmentation of the precursor ions. This mode gives a diagnostic spectrum of the parent ions (precursor ions) that fragment to give the particular daughter ion selected. This MS mode combines the selectivity of multiple reaction monitoring (MRM) with the excellent qualitative data (MS spectra) of scan MS. Only those compounds that give the required daughter ion are scanned providing full spectral data for these compounds whilst filtering out any other potential interferants that do not give the required daughter ion.

3.1.2.1 Results and Discussion

Final instrumental conditions are given in Appendix 1. This method was then used to analyse airborne NCO samples taken during a workplace sampling visit (worksheet 03/0133, South Staffordshire Industries) and the results obtained by the LC/MS/MS method compared with those from MDHS 25/3. The results of this work are given in figure 1.

This initial APCI method gave slightly less sensitivity compared to MDHS 25/3 (EC quantification with UV/vis confirmation) but also gave excellent identification data i.e. diagnostic masses and fragmentation patterns for the NCO formulations studied.

Notes for figure 1

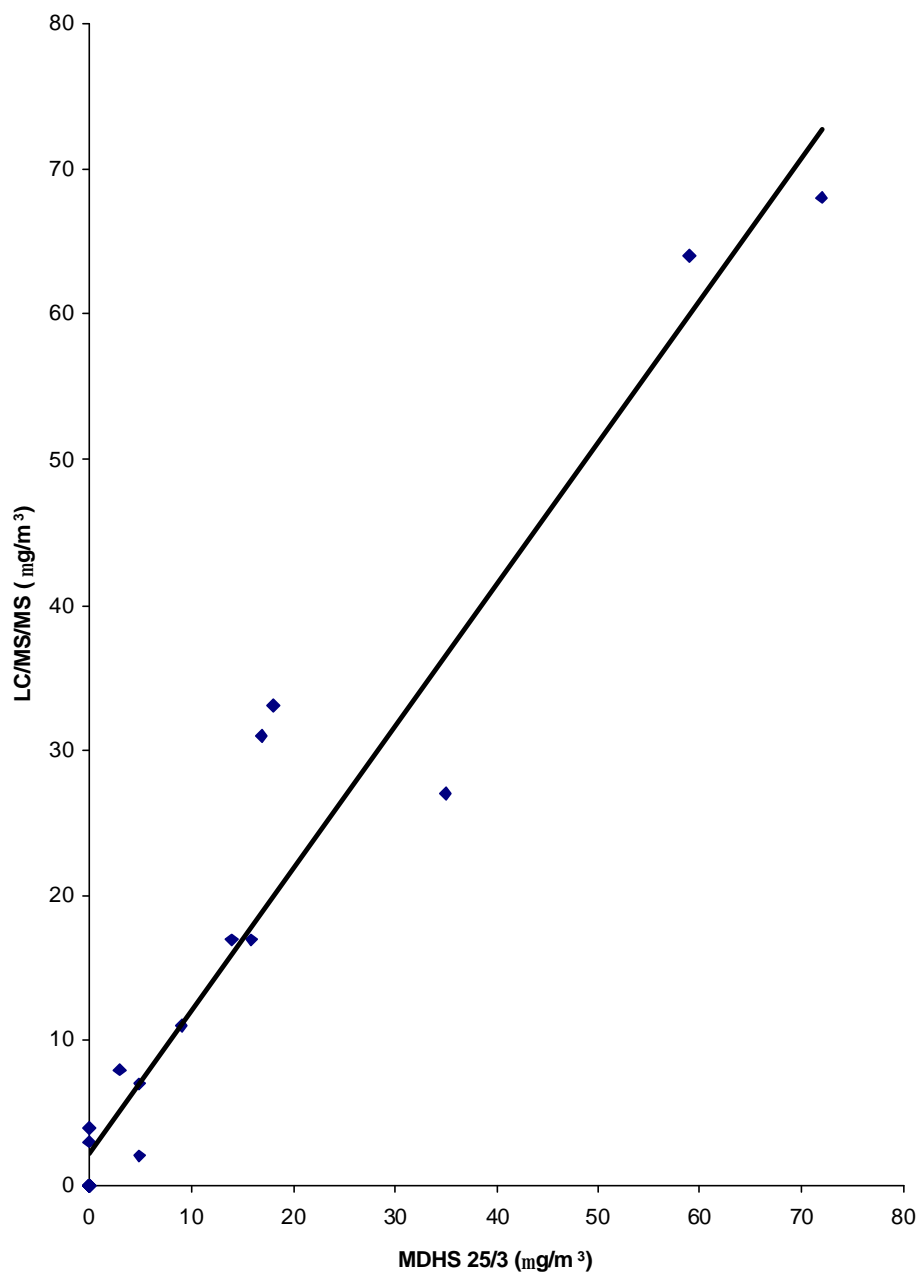
correlation coefficient $r^2 = 0.9671$

Estimated limit of detection (est. LOD) (S/N=3);

HDI-MP ₂	~0.1 µg/ml
HDI-isocyanurate-MP ₃ (HDT)	~0.05 µg/ml

20 pairs of results plotted

Figure 1. Plot of NCO results by MDHS 25/3 against Results by LC/APCI+/MS/MS



3.1.3 Development of an ES+ General method for NCO

Preliminary work aimed at developing a positive ion mode electrospray (ES+) method for NCO suggested that this mode was more sensitive than the APCI+ mode described in section 2.2.2. It was decided to develop a general purpose method for NCO based on the precursor ion scan mode described in section 2.2.2 as this would enable identification of unknown isocyanates and HSL does not always know the nature of NCO sampled. Submission of a sample of the bulk material being used in the workplace greatly assists in the initial characterisation of the NCO. This is particularly important for the HDI based formulations that can be complex mixtures of polymeric and oligomeric HDI.

Initial infusion work was carried out to obtain optimum instrumental conditions. Large contributions from Na⁺ and K⁺ adduct ions were seen in the spectra at first but these were eliminated by storing the LC mobile phases in plastic bottles rather than glass. It was noted that acidification of the samples (100 µl of formic acid to 2 mls of sample) markedly increased the intensity of multiply charged ions for the di-isocyanates and above i.e. 2⁺ ions for MDI, TDI, HDI, 3⁺ ions for HDI-isocyanurate and 3 ring MDI and 4⁺ ions for HDI-diisocyanurate. It was also noticed that injection of high concentration samples led to the presence of dimer peaks especially for MDI. Attempts to yield the more diagnostic [MP+CO]⁺ were unsuccessful so a method was developed using the abundant protonated MP fragment [MP+H]⁺ with a m/z⁺ of 193 Th. The MS and LC parts of this method were optimised and full details of the final method are given in appendix 2.

3.1.3.1 Results and Discussion

The chief advantage of this method is the excellent spectral data it supplies making identification of the NCO species present simple and unambiguous. A variety of MP derivatized NCO formulations were analysed using this method to get a better understanding of the complexity of NCO species likely to be encountered in the workplace. Figures 2 and 3 show examples of chromatograms and MS obtained using this method for a HDI based motor vehicle repair (MVR) bodyshop air sample (figure 2) and an MDI based polyurethane floor screed bulk derivative (figure 3). Figures 4 and 5 show examples of the isocyanate species encountered (MP derivatized and underivatized NCO).

One point of interest is the confirmation of the "n+1" rule tentatively suggested previously (HSL, 2002). This empirically observed rule suggested that the number of NCO groups present on an unknown NCO species could be worked out by calculating the number of MP groups lost (loss of 192 from the molecular ion) and adding one to this number. The rationale for this suggestion was the final MP group on an MP derivatized isocyanate is tightly bound and so will not be removed to generate the parent isocyanate under the "soft" ionization conditions used in LC/MS. This method was used to quantify monomeric and

polymeric HDI samples from the Workplace Analysis Scheme for Proficiency quality assurance scheme (WASP QA). The results of this work are given in table 1, section 2.2.4 where they are compared with those obtained from a "specific" HDI method, the APCI precursor ion method and the electro-chemical detector (MDHS 25/3).

Figure 2. Chromatogram and MS for MVR Bodyshop Air sample (HDI based)

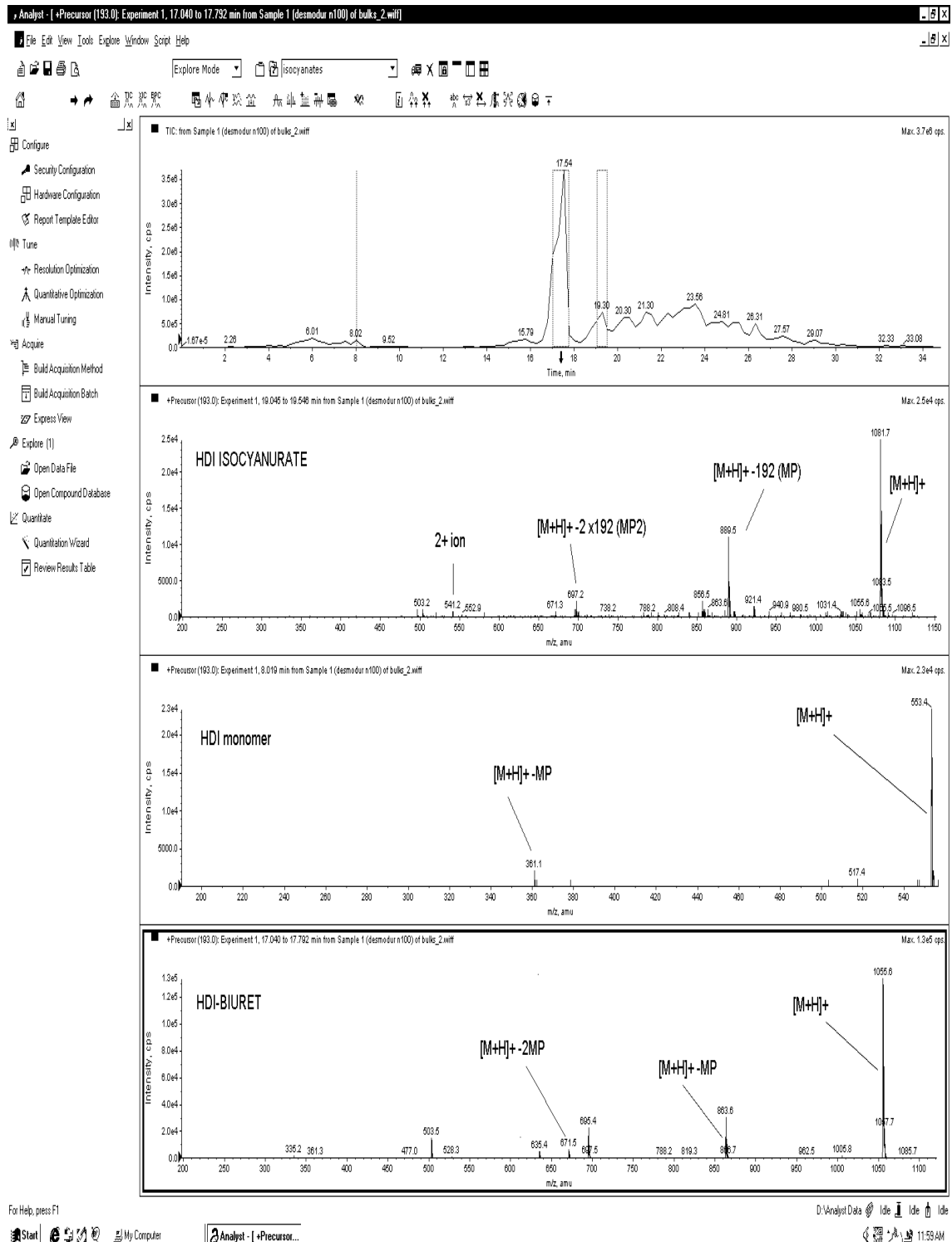


Figure 3. Polyurethane Floor Screed – Bulk NCO "UCRETE" MDI based

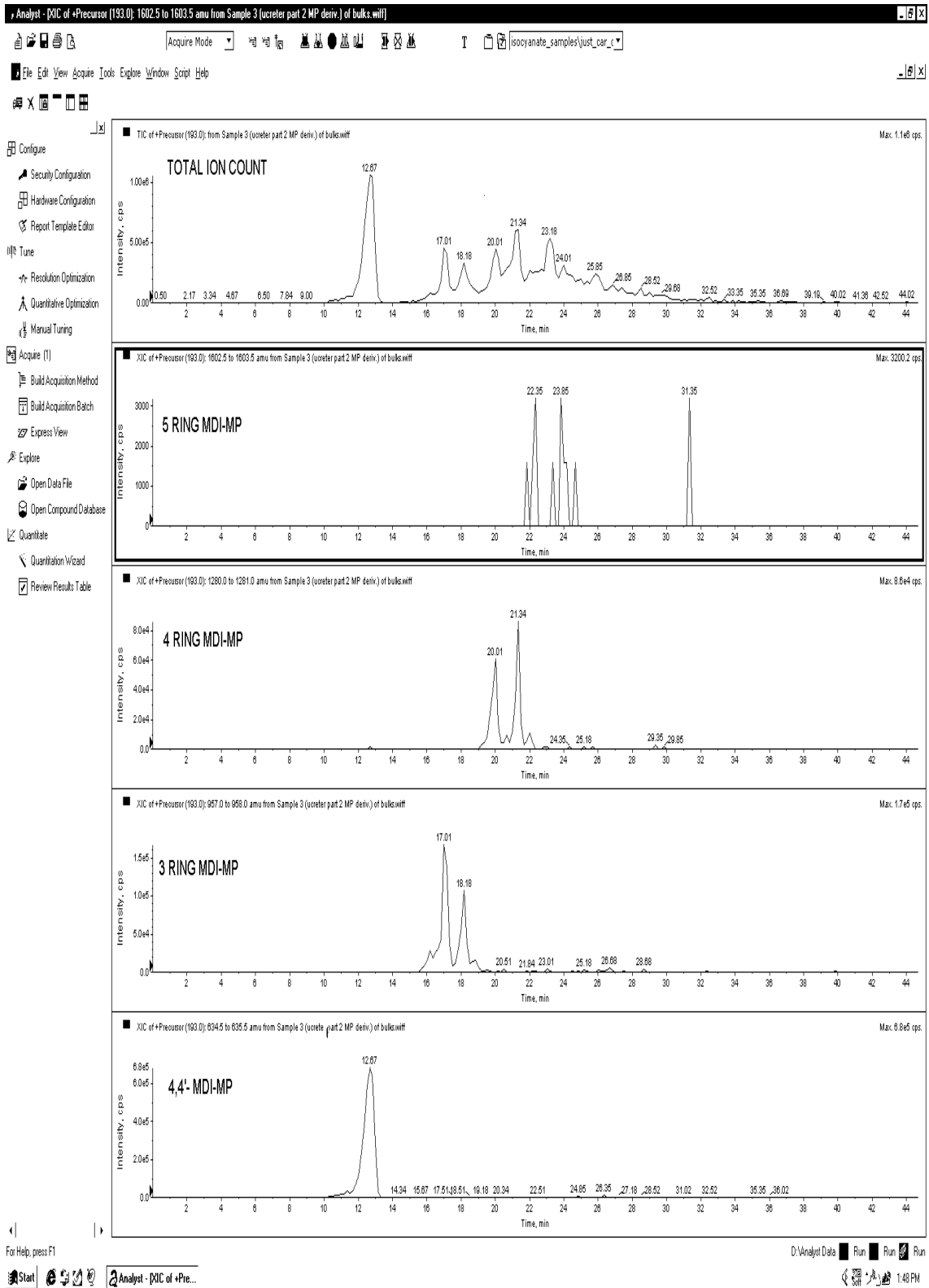


Figure 4. Commonly Occurring HDI oligomer MP derivatives

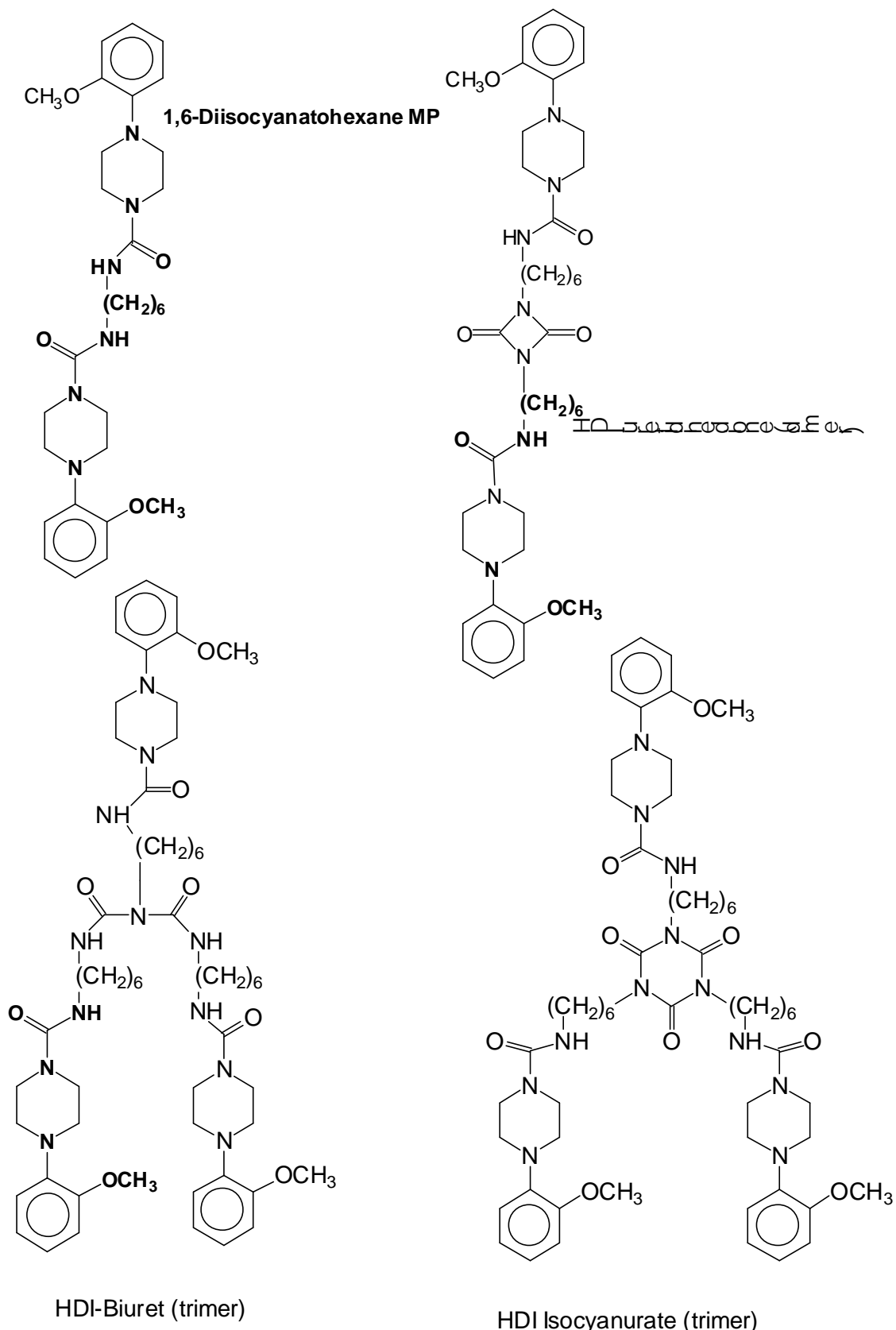
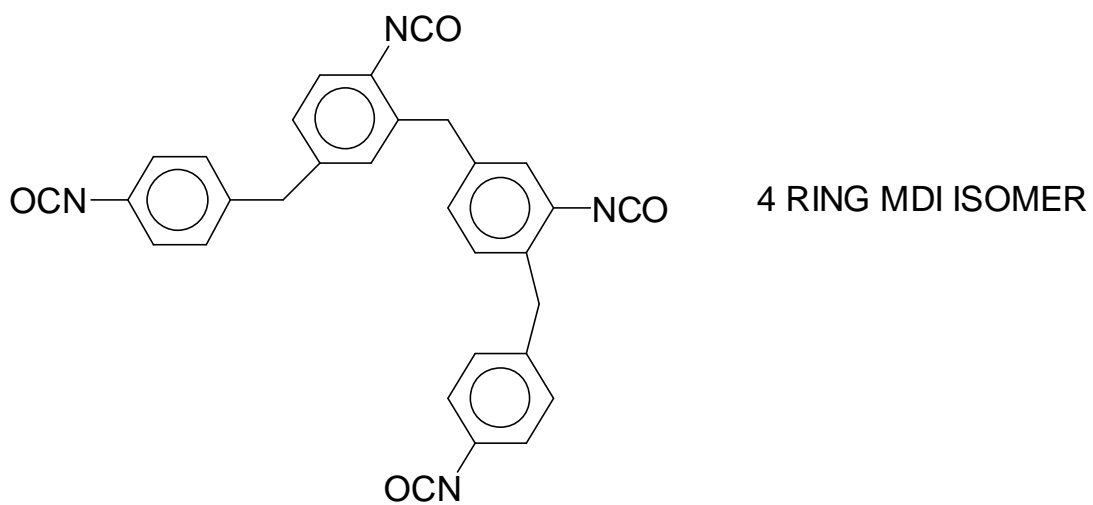
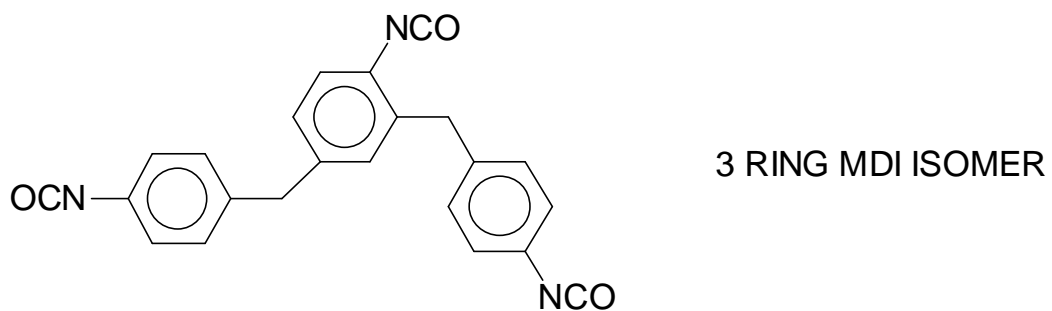
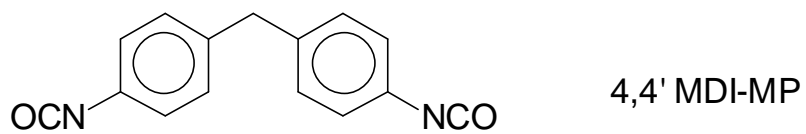


Figure 5. Commonly Occurring poly MDI (underivatized)



3.1.4 Development of an ESI Specific method for HDI based NCO

The precursor ion scan method described in section 2.2.3 is of use if an unknown isocyanate sample is received or if qualitative data is important i.e. unambiguous identification in a forensic case. If a sample of the bulk material is available or the formulation has been characterised previously then it is possible to make assumptions about the NCO species likely to be present and to use a more targeted MS mode resulting in a more sensitive analysis. Multiple reaction monitoring (MRM) is the most sensitive quadrupole MS mode available. In this mode the first quadrupole (Q1) is set allow only a specified ion to pass through. This ion then passes into the second quadrupole (Q2); this is the collision cell. The chosen ion is fragmented by acceleration (collision energy) and subsequent collision with the collision cell gas (nitrogen) to produce fragment ions. One of these fragment ions is then selected by the third quadrupole (Q3) to pass through to the detector. With careful choice of Q1 parent ion, Q2 conditions and Q3 daughter ions it is possible to eliminate nearly all of the background, noise and other interferences resulting in a selective and sensitive analysis.

The most complex isocyanate samples encountered at HSL are aerosols of HDI based formulations used in the MVR industry. Analysis of these samples using the existing method, MDHS 25/3, requires a skilled analyst for correct characterisation of the NCO species present. A method specific for HDI oligomers was developed and applied to the determination of WASP QA filters (HDI monomer and HDI isocyanurate) and to air samples taken during spray room experiments using an MVR bodyshop 2-pack paint carried out at HSL. Full details of the final method are given in appendix 3.

Quantification of the oligomeric HDI is not easy as standards of the oligomer-MP derivatives are not available. There are two options;

- synthesize the MP derivatives of the MP oligomers in house
- use the bulk material to calibrate after characterising it using complimentary techniques.

3.1.4.1 Results and Discussion

The WASP QA sample results presented here are an example of the first approach. A standard of HDI-isocyanurate-MP₃ (HDT) was prepared by derivatizing a bulk formulation (Desmodur N 3390) with MP. The HDI-isocyanurate-MP₃ (HDT) was then purified by preparative LC, fraction collection and repeat recrystallisations. Purity was confirmed by LC/UV and LC/MS. Calibrations for the HDI-MP₂ monomer and HDT were prepared as usual. The WASP samples (round 66) were then analysed by LC/MS/MS. The results of this work and of results obtained by other methods for comparison are given in table 1.

The MVR 2-pack paint samples are an example of the second approach. This paint has been found by previous analysis to be a complex mix of oligomeric HDI species. LC/MS Q1 scan runs on the derivatized bulk identified the major NCO species present and these ions were included in the MS method (appendix 3, MS experiment 1). Analysis using an electro-chemical detector determined the relative concentrations of the HDI oligomers present and the total NCO concentration was determined by titration. Using this information it is possible to construct a calibration curve for each individual MP derivatized HDI oligomer and so quantify the air samples. This approach assumes the relative concentrations of the various NCO species in the bulk and in the air samples are similar. This is probably a valid assumption for slow curing NCO formulation i.e. most aliphatic NCO but may not be valid for faster curing NCO i.e. aromatic NCO.

Air samples were taken during simulated spraying experiments in the HSL spray room and these samples were analysed using MDHS 25/3 (LC/EC/UV) and LC/MS/MS (ES+ Specific HDI method). Spraying was carried out for 2 minutes and sampling for 60 minutes. Full experimental details of this work are given elsewhere (HSL, 2005). The 2-pack paint used was Mirrorcyl MS (hardener and lacquer) which is a topcoat containing ~ 6% NCO (by titration). Figure 6 gives an example chromatogram for one of the samples obtained using the ES+ HDI specific ion method. The results of this work are given in table 2 and figure 7.

Figure 6. Example Chromatograms for HSL Spray Room Simulation Sample Obtained Using the ES+ HDI Specific Ion MS/MS method

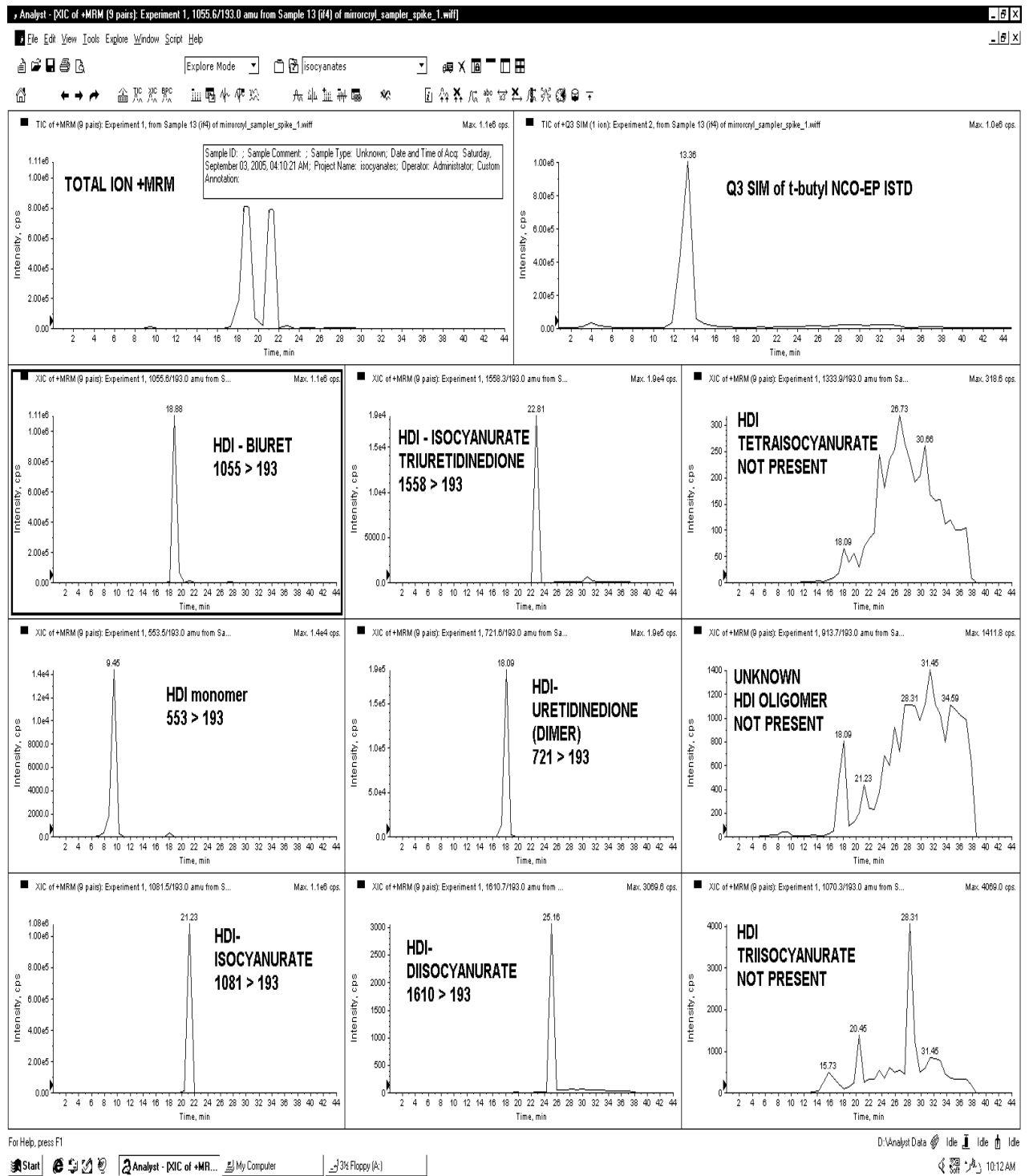


Table 1. Analysis of WASP QA filters – Comparison of methods

Method	WASP 65			WASP 66			Est. LOD	
	1	2	3	1	2	3	HDI ng NCO/ml	HDT ng NCO/ml
ES+ "general" precursor ion scan mode	930	583	N.D.	670	500	N.D.	20	1
ES+ "specifics" MRM mode	826	534	N.D.	675	520	N.D.	2	0.02
EC only MDHS 25/3	846	510	N.D.	696	526	N.D.	12	12
APCI+ "general" precursor ion scan mode	---	---	---	---	---	---	100	50
Nominal (QC result) UV/vis	830	556	blank	689	553	blank	---	---

In general the MS methods give good agreement with the reference method (MDHS 25/3) and the QC result. The general ES method is about as sensitive as the EC detector but with advantage of supplying mass spectra for the two analytes making identification unambiguous. The specific ES method requires more work to set up but it is more sensitive than MDHS 25/3 by a factor of ~10 to ~1000x depending on NCO species. It is possible that further tuning of these methods could improve the sensitivity still further.

One point of interest was noted, the large difference in sensitivity and response factor of the specific method for HDI and HDT, presumably because of the different ionization behaviours of these two compounds. The estimated LOD quoted are also dependant on factors such as instrument cleanliness and sample matrix effects.

Table 2. Comparison of MDHS 25/3 and ES+ HDI oligomer Specific ion methods for HSL Spray Room Simulation Samples

Sample #	MDHS 25/3 ($\mu\text{g NCO}/\text{m}^3$)	ES+ Specific ion ($\mu\text{g NCO}/\text{m}^3$)
I/F 1	271	293
I/F 2	156	156
I/F 3	191	186
I/F 4	154	165
I/F 5	79	74
I/F 6	74	67
GF/B 1	309	294
GF/B 2	166	159
GF/B 3	225	193
GF/B 4	179	185
GF/B 5	75	68
GF/B 6	69	69
PUF 1	289	250
PUF 2	188	190
PUF 3	370	338
PUF 4	184	193
PUF 5	98	91
PUF 6	63	76

Notes

3 sampler types were studied;

I/F = impinger/filter (MDHS 25/3 reference method).

GF/B = MP impregnated GF/B glass fibre filter.

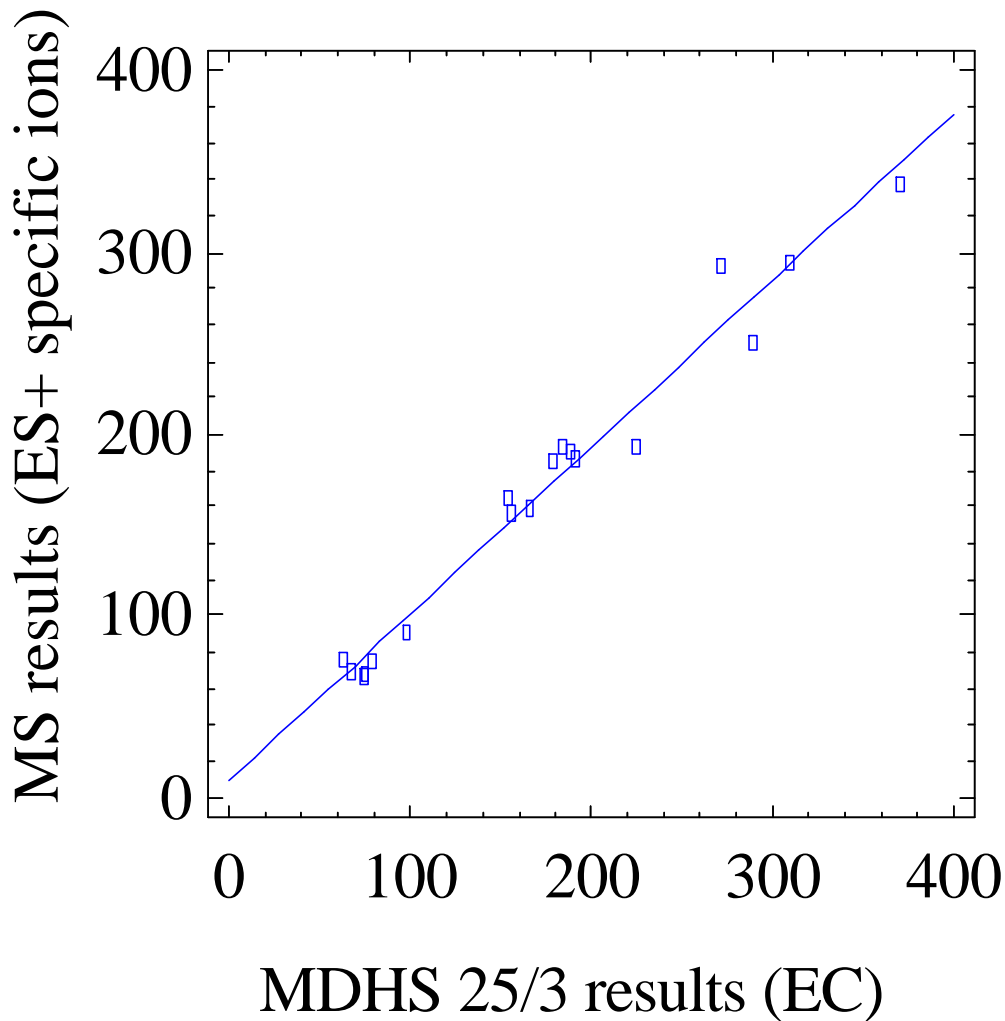
PUF = MP impregnated polyurethane foam and MP impregnated GF/A back-up.

Sampler flow rates:

2l/min for GF/B and PUF

1l/min for I/F

Figure 7. Comparison of MDHS 25/3 results with those from ES+ HDI Specific ion method for HSL Spray Room Work



Notes

Linear regression of y (LC/MS/MS results) on x (MDHS 25/3 results).

Equation is $(LC/MS/MS) = 9.06 \pm 7.73 + 0.919 \pm 0.040 (MDHS\ 25/3)$

Correlation coefficient 0.9855, $r^2 = 97.13\%$.

P value < 0.0001 i.e. a statistically significant relationship exists between these two sets of results at the 99% ($P = 0.01$) confidence level (CL) (actually these results are significant at better than the 99.99% CL).

3.1.5 Lithium Adduct MS/MS

The work reported in sections 2.2.2 to 2.2.4 all use the fact that the bond between the isocyanate carbon and the piperazine nitrogen is easily cleaved in the MS source to give the protonated MP fragment $[MP+H]^+$ with a m/z^+ of 193 Th. Monitoring this fragment gives sensitive MS detection, however any compound that reacts with MP could yield this ion. In practice this has not been found to be a problem for the compounds analysed so far.

One way around this potential difficulty is to monitor a transition specific to the isocyanate species under study, for example for HDI-Biuret the following MRM transition has been used; $M^+ (\sim 1055) > 193 [MP+H]^+$. A more diagnostic transition would be to monitor the other fragment produced in the above scheme; $M^+ (\sim 1055) > [M -MP] \sim 862$ but unfortunately the higher proton affinity of the MP means that the protonated $[M -MP +H]^+$ fragment is not produced in large amounts. MS is a technique for detecting charged particles so the $[M -MP]$ fragment cannot be detected. Work at HSL has confirmed these statements.

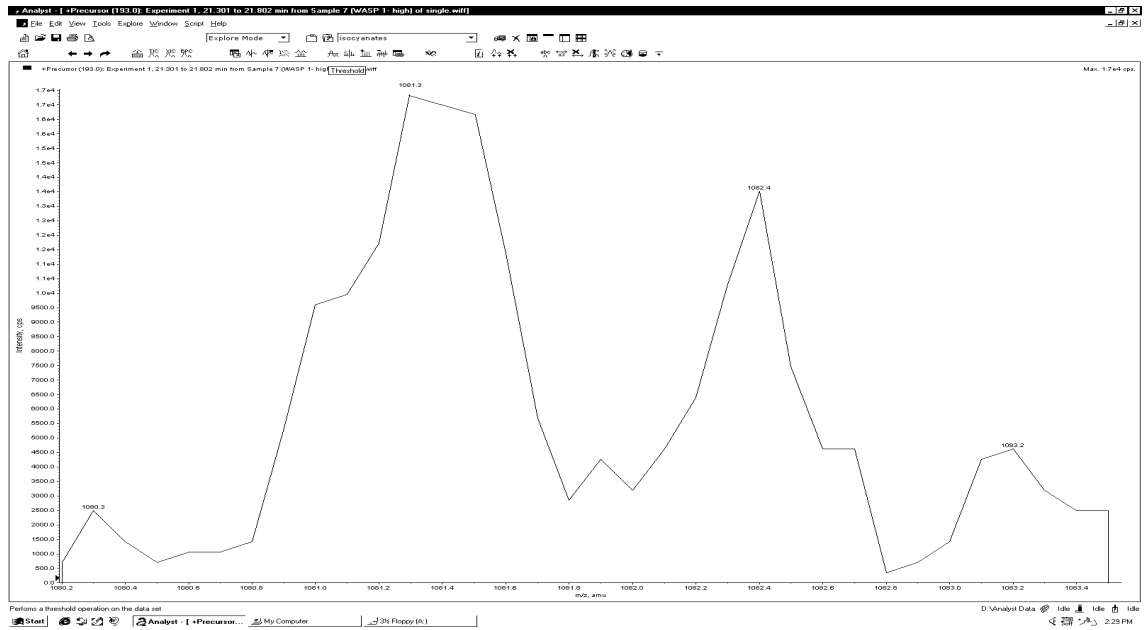
The lithium ion (Li^+) readily forms adducts with the NCO-MP derivatives and because of the highly electropositive nature of the Li^+ cation the positive charge is retained largely on the $[M+ Li]^+$ fragment not on the MP fragment. This means it is possible to monitor the transition; $[M + Li]^+ > [M + Li -MP]^+$. This approach has been applied by Gagné et al (e.g. Gagné et al, 2005) and was investigated at HSL. The methods developed above were used with the following modifications; 10mM lithium acetate used in the mobile phase instead of ammonium acetate and $[M + Li]^+$ adduct ions and $[M + Li -MP]^+$ fragment ions were monitored e.g. for HDI and HDT ions monitored were; HDI 559 > 367, HDT 1087 > 895 – note $Li^+ = +6$ Th.

Initial experiments with lithium ion adduct formation did not show any appreciable increases in sensitivity over the methods described above. In addition, a white precipitate formed on the MS skimmer plates that necessitated cleaning of the instrument. Because of time constraints this approach was not pursued further in this project however the more specific nature of the transitions monitored in the lithium ion adduct method means that further development work is recommended. This mode also may offer the possibility to use the neutral loss mode of the MS to monitor the loss of the neutral MP group.

3.1.6 Comparison of Quadrupole resolution settings

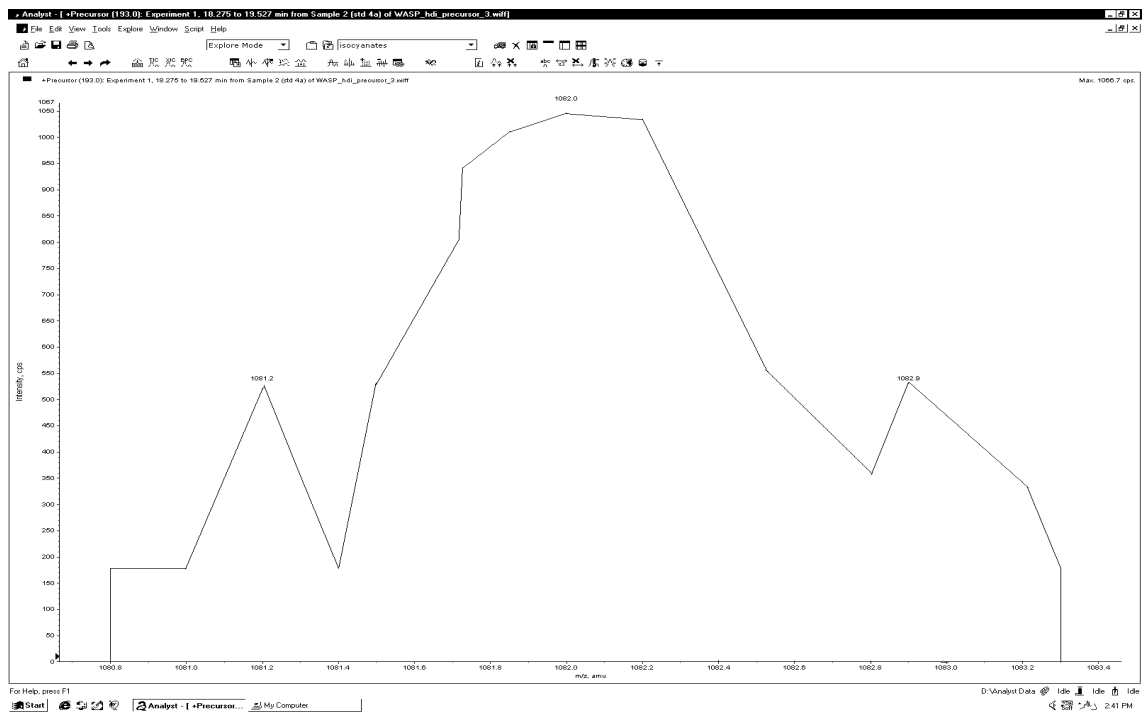
A brief series of experiments to compare the effect of different quadrupole resolution settings was carried out. The results are given in figure 8.

Figure 8. Effect of Quadrupole Resolution Settings (WASP standards)



High/High Q1/Q3 settings

Est. LOD using modified Precursor Ion scan method for HDT ~ 93 ng NCO/g



Low/Low Q1/Q3 settings

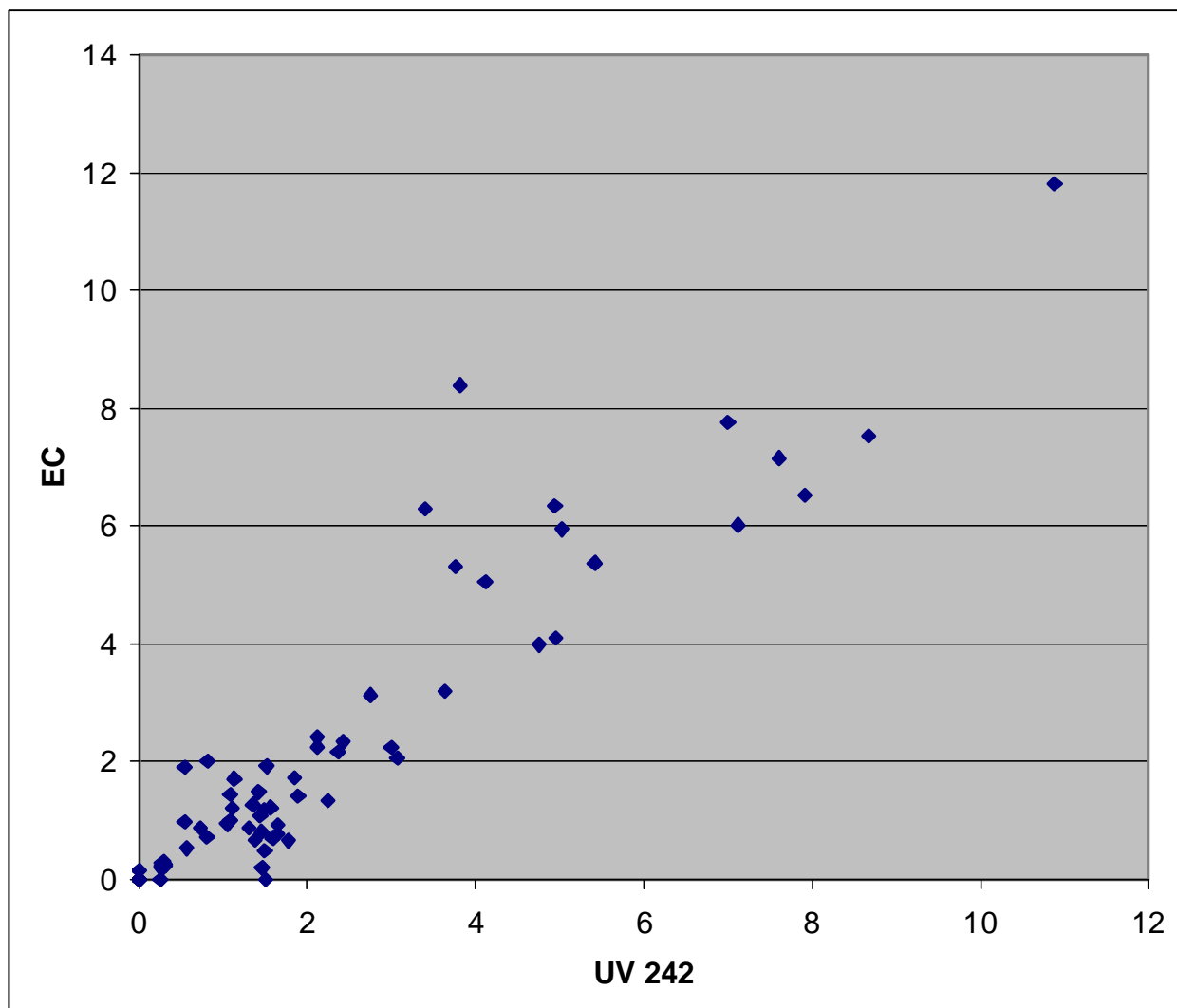
Est. LOD using modified Precursor Ion scan method for HDT ~ 3 ng NCO/g

The high resolution settings (0.5 Th FWHM) gave better spectral data but lower sensitivity in comparison the low resolution (1.1 Th FWHM) settings. The better spectral data obtained from the high resolution setting may be useful for peak identification and determination of charge state (i.e. C13 -C12 difference). The low resolution experiments give better sensitivity.

3.1.7 Comparison of EC and UV detection for Oligomeric HDI-MP Derivatives

Occasionally it would be advantageous to be able to quantify oligomeric HDI MP derivatives using the UV detector i.e. if the EC detector has overloaded. Examining the structures of the HDI oligomers MP derivatives given in figure 4 it can be seen that the major chromophore is the phenyl ring on the MP. It is therefore possible that different oligomeric HDI-MP will have similar UV response factors meaning that they could be quantified using the response factor of monomeric HDI-MP₂. The results of such quantifications, carried out on a variety of HDI based air samples received at HSL, using the EC detector and by UV/vis @ $\lambda = 242$ nm were plotted and are shown in figure 9.

Figure 9. Comparison of EC and UV (242nm) Results for Various Oligomeric HDI-MP Air Samples



Units are $\mu\text{g NCO/ml}$

72 pairs of samples are plotted

Linear regression of y (UV @ 242nm) on x (EC results).

Equation is $(\text{UV}) = -0.0244 + 1.0084(\text{EC})$

Correlation coefficient 0.9323, $r^2 = 86.92\%$.

P value < 0.0001 i.e. a statistically significant relationship exists between these two sets of results at the 99% (P= 0.01) confidence level (CL) (actually these results are significant at better than the 99.99% CL).

These results suggest that it is possible to use the UV/vis response to accurately quantify oligomeric HDI-MP derivatives.

3.1.8 Conclusions

APCI+ and ES+ MS modes have been applied to the determination of isocyanate-in-air samples and methods using these various MS modes developed. The precursor ion ES+ mode gives excellent spectral data, this is invaluable for identification of the NCO species present. The ES+ specific ion MRM transition mode gives extremely sensitive analysis for target NCO species. This work has proved the extremely powerful nature of LC/MS/MS as an analytical technique for workplace analysis of NCO.

3.1.9 Recommendations

1. The APCI+ method should be developed further to see if this technique offers any advantages over the ES+ method developed here. APCI+ has been reported to be more robust i.e. less susceptible to interferences than ES+.
2. The use of ion-trap MS should be investigated to see if this MS source design offers any advantages over the quadrupole MS systems investigated here. Ion trap MS offers the possibility of rapid full scan acquisition and MSⁿ which may be useful for identification purposes.
3. The MS methods developed (Precursor ion scan and MRM) should be optimised further and employed for use in the routine analysis of workplace samples.
4. Further investigation of lithium ion adduct MS methods should be carried out to see if this offers any benefits.

3.1.10 References

S. Gagné, J Lesage, C Ostiguy, Y Cloutier and H-V Tra (2005)
Quantitative determination of HDI, 2,4-TDI and 2,6 TDI monomers at ppt levels in air by alkaline adduct coordination ion spray tandem mass spectrometry, J. Environ. Monit, 7, p 145-150.

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Matthew Coldwell, John Saunders, Derrick Wake and John White, Isocyanate Exposure, Emission and Control in Small Motor Vehicle Repair Premises using Spray Rooms, HSL report – in preparation

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Measuring Isocyanate Exposure – A warning and some guidance, Occupational Hygiene Newsletter, British Institute of Occupational Hygienists, 15/4, p16-18.

3.1.11 Appendices for Section 3

Appendix 1 – APCI method for NCO

Column

Genesis C18 300 4 μ 100 x 2.1 mm?

Mobile phase

time (min.)	equilibration 10'	0	5	10	25	26	35
%A	90	90	90	10	10	90	90
%B	10	10	10	90	90	10	10

A - 10mM ammonium acetate, pH 6 with acetic acid

B - acetonitrile

injection volume 50 μ l

flow rate 500 μ l

API 2000 triple quadrupole

Precursor ion mode - ES+

Precursors of m/z 193 monitored over the range 200 - 1800 Th

Scan time 10 s

declustering potential	10 V	focussing potential	400 V
entrance potential	10 V	exit potential	10 V
collision energy	100 V	collision gas	10 psi
nebulizer current	5	curtain gas	30 psi
gas 1(nebulizer)	50 psi	gas 2 (sheath)	25 psi

Calibrations used HDI and HDI-isocyanurate MP standards prepared by HSL.

Calibration for HDI-MP₂ – extracted 553-554 Th

Calibration for HDI-isocyanurate-MP₃ – extracted 1081-1082 Th

Naphthyl isocyanate-MP was used as the internal standard

Response factor for HDI-MP₂ ~ 3.84 x 10⁻³ counts/relative to the internal standard

Response factor for HDI-isocyanurate-MP₃ ~ 27.60 x 10⁻³ counts/relative to the internal standard

Appendix 2 – ES⁺ screening method for NCO –precursors of 193- scan mode

Column

Genesis C18 300 4 μ 100 x 2.1 mm?

Mobile phase

time (min.)	equilibration 10'	0	5	25	40	41	45
%A	50	50	50	10	10	50	50
%B	50	50	50	90	90	50	50

A - 10mM ammonium acetate, pH 6 with acetic acid

B - acetonitrile

injection volume 50 μ l
 flow rate 250 μ l

API 2000 triple quadrupole
 Precursor ion mode - ES⁺

Experiment 1

Scan type - Precursors of m/z 193 monitored over the range 200 - 1800 Th
 Scan time 15 s

declustering potential	150 V	focussing potential	400 V
entrance potential	10 V	exit potential	10 V
collision energy	50 V	collision gas	10 psi
ionspray current	5200 V	curtain gas	30 psi
gas 1(nebulizer)	35 psi	gas 2 (sheath)	35 psi
Ion heater electronics	on		
interface temperature	550 °C		
Resolution Q1	low		
Resolution Q3	low		

Experiment 2

Scan type – Q3 multiple ion scan

Q3 mass	306	dwell time	25 ms
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(This is the mass of the internal standard used – t-butyl NCO-EP)

declustering potential	80 V	focussing potential	300 V
entrance potential	10 V	exit potential	10 V
ionspray current	5200 V	curtain gas	30 psi
gas 1(nebulizer)	35 psi	gas 2 (sheath)	35 psi
Ion heater electronics	on	RO2	-20 V
interface temperature	550 °C		
Resolution Q3	low		

For the WASP QA standard work calibrations used HDI and HDI-isocyanurate MP standards prepared by HSL.

Calibration for HDI-MP₂ – extracted 553-554 Th
Calibration for HDI-isocyanurate-MP₃ – extracted 1081-1082 Th

Appendix 3 – Specific method for HDI based NCO

Column

Genesis C18 300 4 μ 100 x 2.1 mm?

Mobile phase

time (min.)	equilibration 10'	0	5	25	40	41	45
%A	50	50	50	10	10	50	50
%B	50	50	50	90	90	50	50

A - 10mM ammonium acetate, pH 6 with acetic acid

B - acetonitrile

injection volume 25 μ l

flow rate 250 μ l

API 2000 triple quadrupole

MRM mode - ES+

Experiment 1

Scan type – MRM+

Transitions monitored

All dwell times were 5000 ms

553.5 > 193 HDI-MP₂ (monomeric) *

1081.6 > 193 HDI isocyanurate –MP₃ *

(* These were the only transitions monitored for the WASP QA samples determination)

1055.6 > 193 HDI biuret– MP₃ (HDB)

721.7 > 193 HDI uretidinedione–MP₂ (dimer, HDD)

1610.8 > 193 HDI di-isocyanurate– MP₄ (DIISO)

1558.3 > 193 HDI isocyanurate-triuretidinedione–MP₃ (ISO-TRIURET)

1070.3 > 193 HDI tri-isocyanurate–MP₅ (2+ion monitored) (TRIISO)

1334.5 > 193 HDI tetra-isocyanurate–MP₆ (2+ion) (TETRAISO)

(These ions were monitored for the MVR spray room work in addition to the monomeric HDI and HDT ions)

declustering potential	150 V	focussing potential	400 V
entrance potential	10 V	exit potential	10 V
collision energy	50 V	collision gas	10 psi
ionspray current	5200 V	curtain gas	30 psi
gas 1(nebulizer)	35 psi	gas 2 (sheath)	35 psi
Ion heater electronics	on		
interface temperature	550 °C		

Resolution Q1 low
Resolution Q3 low

Experiment 2

Scan type – Q3 multiple ion scan
Q3 mass 306 dwell time 25 ms

(This is the mass of the internal standard used – t-butyl NCO-EP)

declustering potential	80 V	focussing potential	300 V
entrance potential	10 V	exit potential	10 V
ionspray current	5200 V	curtain gas	30 psi
gas 1(nebulizer)	35 psi	gas 2 (sheath)	35 psi
Ion heater electronics	on	RO2	-20 V
interface temperature	550 °C		
Resolution Q3	low		

4 BENZENE MONITOR

This section describes work carried out by HSL's Organic Measurement Section looking at an on-site monitor for benzene.

Tests were carried out on a commercial instrument (*details can be provided on request*) used in the offshore oil industry to measure benzene. They were undertaken to evaluate the instrument's ability to measure benzene in the presence of other chemicals that may be present in the air on an oil rig. To this end the response to benzene on its own; in the presence of toluene and in petrol vapour; of the instrument was investigated.

The instrument is based on a chip measurement system (CMS) and employs a cartridge (or chip) containing materials for 10 tests. It uses a similar principle to the well-known stain length tubes, but the reading is automatic and it gives an indication of the concentration of a digital display. The instrument is portable, robust and easy-to-use which are important factors to consider when working in the challenging environment of an off-shore oil-rig.

4.1.1 Test atmosphere system

In order to test the monitor a test atmosphere rig was set up. This consisted of a set of mass flow controllers, a humidifier, an evaporator and a test chamber.

The mass flow controllers, under computer control, fed air to the other items. The three streams were then recombined just before entering the chamber to provide a known concentration of the test chemicals and a controlled humidity. The test chamber was temperature controlled by a thermostatically controlled water jacket. The humidity and the temperature of the chamber were also monitored independently to verify that the computer control system was working correctly.

A syringe drive pump, also under computer control, fed a known rate of the chemical, or mixture of chemicals to the evaporator where it was mixed with the air. From the flow rate of the air and the rate at which the test compound is added, the concentration of the test compound in air was calculated.

Overall, this set-up provides a test atmosphere containing a well-defined concentration of an analyte or mixture of analytes in air at known temperature and humidity.

For evaluation, the CMS was placed in the chamber and left to stabilise. It was then set to measure the benzene concentration in the test atmosphere.

4.1.2 Determination of the true benzene concentration.

The test atmosphere system produces a calculated concentration of material in air, but the true concentration might differ from the calculated one because there may be errors in the calibration of the flow rates or leaks in the test chamber. There may also be short-term changes in the concentration due to uneven evaporation.

The first of these can be tested for by taking samples from the atmosphere onto a suitable sorbent and analysing them. This would not show temporal instability of the concentration on a time scale less than the sampling time. The short-term stability of the concentration can be verified by a real-time monitor.

4.1.3 ATD-GC Analysis

For each set of conditions a set of 3 samples were taken onto Tenax filled ATD tubes. These samples were taken at 100+/- 1 ml / min for 10 min thereby sampling 1 litre. These tubes were then thermally desorbed and the vapours analysed by GC to determine the benzene concentration. The conditions are given in appendix 1. The system is calibrated using standard spiked tubes (from the BTX (benzene, toluene and xylenes) WASP scheme) and these tubes are also run throughout the runs QC.

4.1.4 Real-time measurement

The test atmosphere was allowed to equilibrate after the CMS monitor was placed in the chamber. Real time measurements were made both to check that the concentration had equilibrated and to demonstrate that the concentration was constant during testing of the monitor. Initially, a flame ionization detector (FID) was used but as this is a large and fixed instrument this was inconvenient and introduced potential losses through the connecting pipework. For later work the FID was replaced by a photo-ionization detector (PID), which was a hand held instrument. Details of the instruments are given in appendix 2. Because the PID was only being used to check that the concentration was constant, and was being used to monitor a mixture of materials, no attempt was made to calibrate it; the readout was in "equivalent ppm of isobutylene".

For the initial test in air only containing benzene, the concentration is quite low (1 ppm) and this is too low to be measured accurately by the detectors. However it was produced by dilution from a more concentrated stream and the remainder of this gas could be tested before being vented to waste. The flow rates of the gases were shown to be constant so the concentration in the chamber was a fixed fraction of the measured concentration. In subsequent runs, where the total concentration of organic material in the air was much higher, the concentration in the chamber was monitored directly.

4.1.5 Test Conditions

The monitor was tested under 5 sets of conditions

1. Benzene at 1 ppm in air
2. Benzene at 1 ppm in air containing 25 ppm of toluene
3. Benzene at 1 ppm in air containing 50 ppm of toluene
4. Petrol vapour in air, containing 0.4 ppm benzene containing a large number of other compounds
5. Petrol vapour in air, containing 1 ppm benzene, again, containing a large number of other compounds.

These conditions were chosen because the WEL (workplace exposure limit) of benzene is currently 1 ppm and the limit for toluene is 50 ppm.

For each set of conditions the monitor was used to measure the concentration at least 3 times (except the last condition where only 2 samples were taken due to lack of consumables for the CMS). Parallel sampling for GC analysis was undertaken for one of these runs and the PID or FID response was used to ensure that the GC result was representative of the air the CMS sampled i.e. the real time monitoring by PID/FID was in agreement with the ATD-GC result.

The results of this work are given in table 1 and figure 1.

4.1.6 Benzene at 1 ppm

This was prepared by evaporating 0.111 $\mu\text{l}/\text{min}$ of benzene into a flow of 5 l/min of air. 0.5l/min of this mixture were then added 25 l/min of (previously humidified) air to give 1 ppm.

The stability of this concentration was verified by the FID, which gave an indicated concentration between 5.5 and 6 ppm. The FID is calibrated for methane and gives a result roughly proportional to the number of carbon atoms present so 1 ppm of benzene would be equivalent to 6 ppm of methane.

FID measurements of the excess benzene vapour being vented to waste gave an indicated concentration of 300 to 330 ppm where the calculated value would be 306 ppm. The true concentration of benzene in the atmosphere was measured using GC. The 3 tubes gave concentrations of 3.245, 3.267 and 3.298 $\mu\text{g}/\text{l}$. These correspond to an average of 0.999 ppm.

4.1.7 Benzene at 1 ppm in the presence of toluene at 50 ppm.

A mixture of 5.922 g of toluene and 0.6962 g of benzene was prepared. This was fed to the evaporator at 6.735 $\mu\text{l}/\text{min}$ and mixed with 5 l/min of air. This gas stream was diluted with humidified air to produce a total of 30 l/min. The calculated benzene concentration under these conditions is 3.103 mg/m^3 i.e. 0.958 ppm.

The stability of the concentration of this mixture was measured using the PID and found to vary from 88.6 to 94.7 "ppm isobutylene equivalent" i.e. a range of

about +/- 3%. The true concentration was measured by ATD-GC. The 3 tubes gave 3.110, 3.023 and 3.104 µg/l. These correspond to an average of 0.95 ppm.

4.1.8 Benzene at 1 ppm in the presence of toluene at 25 ppm.

A mixture of 2.69982 g toluene and 0.09495 g of benzene was prepared and injected at 3.23 µl/min as before. This corresponds to 0.979 ppm in the test atmosphere. The PID response to this test atmosphere was 42.6 to 48.3 “ppm isobutylene equivalent”, a range of about +/- 6%. The higher apparent variability may be due to inaccuracy of the detector (which has a resolution of 1.3 “ppm”), rather than a real variability. The ATD-GC results were 2.884, 2.979 and 2.753 ppm. These correspond to 0.886 µg/l or 0.906 ppm.

4.1.9 Petrol vapour containing 0.4 ppm benzene in air

In order to test the monitor in conditions that mimic the conditions found on-site i.e. on an oil-rig, it was decided to test the meter using petrol vapour. ATD-GC analysis of the petrol indicated that it contained about 1.35% w/w benzene. Based on this figure and a density of 0.865 we calculated that evaporating 8.23µl/min of petrol into the air and diluting to 30 l/m would give about 1 ppm of benzene.

The test equipment was set up and the CMS used to sample the air. During this test the PID gave an indicated concentration of 41.2 to 42.4 “ppm isobutylene equivalent”.

The ATD-GC results for this run (1.194, 1.265, and 1.467 µg/l) indicated that the true concentration of benzene in the atmosphere was only 0.404 ppm. This is presumably due to the fact that petrol contains a number of rather less-volatile components which did not evaporate but remained as an oil in the injector. Presumably, some of the benzene was trapped in this oil.

4.1.10 Petrol vapour containing 1 ppm benzene in air

Based on the experience of the previous test we raised the injection rate to 20.6µl/min and repeated the measurements. The PID response was 81.1 to 82.5 “ppm isobutylene equivalent”. The ATD-GC gave 3.215, 3.334, and 3.720 µg/l equivalent to 1.056 ppm.

4.1.11 Results and Discussion

The results from the CMS measurements of the benzene concentration are given in table 1 and figure 1.

Table 1. Effect of toluene and other materials on the response of the CMS to benzene

Test Condition	Toluene concn. (ppm)	Calculated benzene concn. (ppm) (ATD-GC result)	CMS result (ppm)	CMS results as % of calc. value	Mean CMS result as % of calc. value	Summary of indicated CMS data		Summary of CMS data as % of calculated concn.
1	0	1 (ATD-GC = 0.999)	0.84	84	109.42			
1	0		1.73	173				
1	0		0.85	85		Mean	1.09	109.42
1	0		0.93	93		SD	0.30	30.01
1	0		1.01	101		CV	27.42	
1	0		1.22	122				
1	0		1.16	116				
1	0		1.21	121				
1	0		1	100				
1	0		0.92	92				
1	0		0.71	71				
1	0		1.55	155				
2	50	0.958 (ATD-GC = 0.950)	0.78	81.41	72.37			
2	50		0.7	73.07		Mean	0.69	72.37
2	50		0.7	73.07		SD	0.071	7.46
2	50		0.57	59.50		CV	10.31	
2	50		0.74	77.24				
2	50		0.67	69.94				
3	25	0.979 (ATD-GC = 0.906)	0.66	67.42	71.30			
3	25		0.77	78.65		Mean	0.70	71.30
3	25		0.7	71.50		SD	0.07	6.67
3	25		0.75	76.61		CV	9.36	
3	25		0.61	62.31				
4	4.8	ATD-GC = 0.404	0.33	81.68	77.97	Mean	0.32	77.97
4	4.8		0.42	103.96		SD	0.09	21.43
4	4.8		0.3	74.26		CV	28.13	
4	4.8		0.21	51.98				
5	11.2	ATD-GC = 1.056	1.04	98.48	100.85	Mean	1.07	100.85
5	11.2		1.09	103.227		SD	0.035	3.35
						CV	3.32	

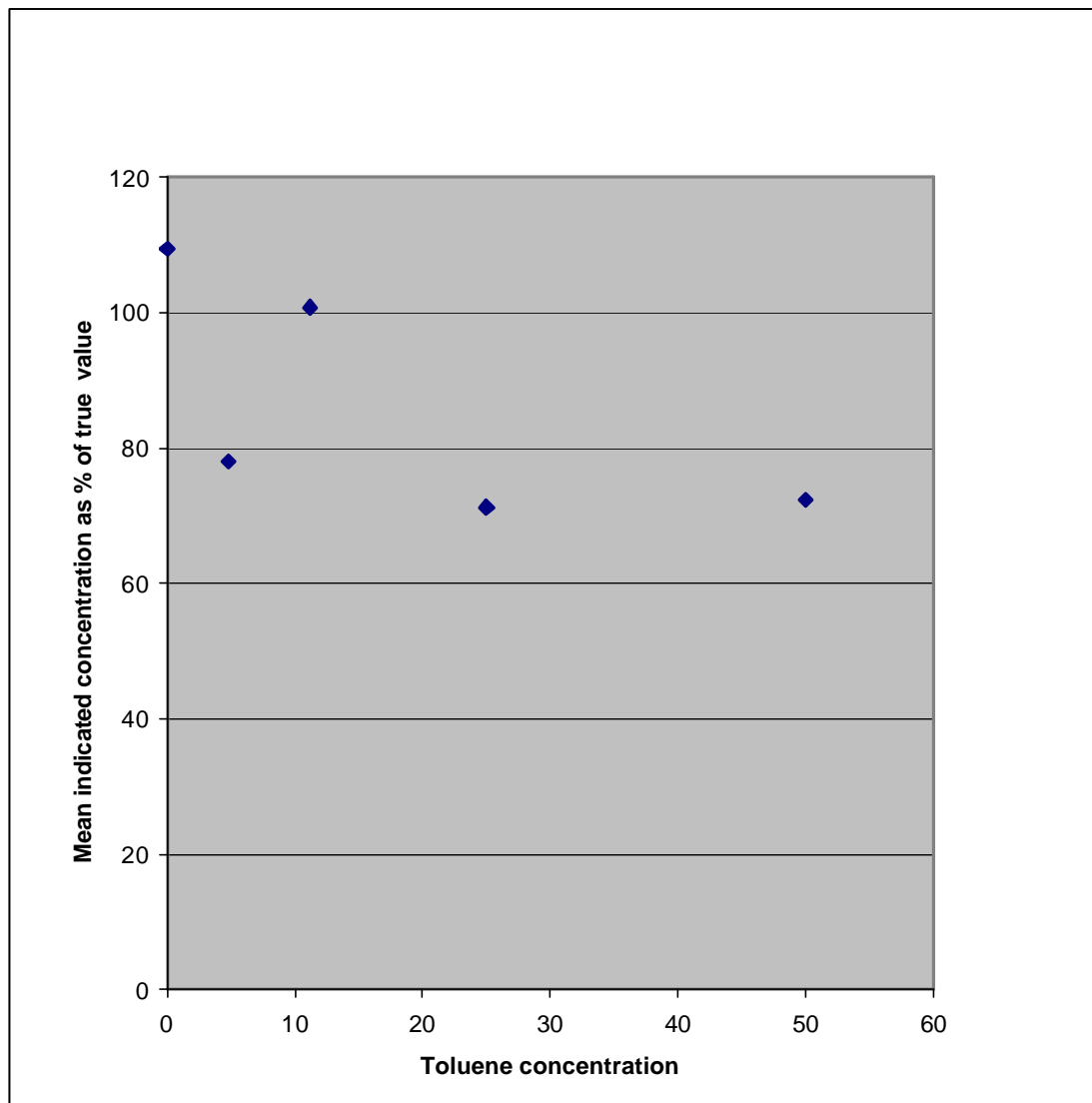
Test conditions 1-3. Calculated benzene concentration was used for %CMS results

Test conditions 4 and 5. Measured benzene concentration (ATD-GC) was used for %CMS results

These data indicate a trend to lower indicated concentration in the presence of toluene. They also show considerable scatter e.g. a coefficient of variation (CV) of 27% for benzene at 1 ppm in otherwise clean air. This is higher than would be expected for standard laboratory based analytical method e.g. < 5% for ATD-GC. This finding suggests that the CMS should not be used if the exact concentration of benzene present is critical i.e. measuring benzene levels at or near the WEL.

On average, the method reads slightly high so it fails safe. However, in the presence of toluene or petrol vapour the response is suppressed and the monitor may indicate a concentration below the WEL, even where the true concentration may be above the limit. The trend is clearer when the mean results are plotted as shown in figure 1 below.

Figure 1. Effect of Toluene on Benzene Determination by CMS



This plot shows that with an interference present (toluene at 25 and 50 ppm) the response for benzene is decreased relative to a zero concentration of toluene. There is not a large enough data set available for non-parametric testing statistics (sign test) to be meaningful. However when the CV of these results is taken into consideration these results are not significantly different from the "100% of true" value. This effect is presumably concentration dependent for toluene but the data gathered does not show that clearly.

A comparison of the points with ~4 and 10 ppm toluene (test conditions 4 and 5 – benzene in petrol vapour) shows that these results, considering the high CV, are not significantly different from each other or the "100% of true" value.

It is possible that if the interfering compounds were known and constant then the CMS could be calibrated to take them into account and so provide quantitative data. It is considered unlikely that this is the case for interfering compounds present in the air on an oil-rig.

4.1.12 Conclusions

The instrument gave mean indicated concentrations of benzene between about ~70% and ~110% of the true concentration with CV of 3 to 28% for the 5 tests undertaken. The range of individual results shown in table 1 is from ~50 to ~170% (n = 29). The presence of toluene was shown to reduce the response to benzene.

The CMS system is an elegant design and is easy to use it but gives measurements of benzene with a high CV relative to standard instrumental techniques and that may be affected by other materials present in the atmosphere under study.

4.1.13 Recommendations

1. This instrument is adequate for use as an indicator of benzene levels in clean air.
2. This instrument should not be used for critical benzene measurements, particularly in the presence of large concentrations of other materials.

4.1.14 Appendices to Section 4

Appendix 1 – ATD-GC Analysis conditions

All analyses were performed using a Perkin-Elmer model ATD-400 thermal desorber with a Tenax TA 80-100 mesh cold-trap at -30°C in forward flush configuration; cold-trap desorption temperature 275°C. Primary desorption is at 250°C (Tenax TA). Typically the split is set in the region of 200:1 for occupational loaded tubes (µg levels) with a primary desorption flow of 25-35 mL/min. and the inlet split at 80 mL/min. A silica transfer line passes the sample to a Perkin-Elmer Autosystem gas chromatograph with outlet splitting (80 ml/min) fitted with twin capillary columns, (BP1 50 m x 0.22 mm with 1µm film thickness and BP10 50 m x 0.22 mm with 0.5µm film thickness), Helium carrier gas at 46.5 psi inlet pressure and twin flame ionization detection. Temperature program, 50°C no hold time, 5°C/min to 200°C. Data acquisition was Perkin-Elmer Turbochrom v4.2.

Appendix 2 – Other Instrumental Conditions

FID

The Flame ionisation detector used in this work was a Signal instrument company 3000HM Heated total hydrocarbon analyser. This was run with hydrogen as the fuel.

PID

The photo ionisation detector used in this work was a thermo environmental instruments inc. OVM / data logger Model 580B. The instrument was set to run from its internal batteries and used a 10.0 eV krypton lamp

5 BIOCIDES AND PESTICIDES

This section describes work by Organic Measurement Section developing analytical methods for commonly encountered biocides and pesticides.

5.1.1 "Quat" Herbicides

HSL provides an analytical service to HSE for the determination of pesticides and other biocides. This work is usually to assist in enforcement activity i.e. forensic work linked to non-approved use and storage, spray drift etc.

Existing methods of analysis for the quaternary ammonium herbicides e.g. diquat, paraquat, chlormequat, mepiquat etc are time consuming and have poor recovery and sensitivity for some complex matrices e.g. soil and vegetation. This section presents preliminary results from work aimed at developing better analytical techniques for these substances. LC/MS methods should give better sensitivity than the LC/UV methods currently in use and unambiguous identification (mass spectra) for the quaternary ammonium herbicides.

5.1.1.1 LC/MS for Quaternary Ammonium Herbicides

This work is based on the method of Takiro et al (Takiro et al, 2000). This method uses an ion-pairing agent to assist the chromatography of the divalent herbicide cations formed in solution. Analytical conditions are given in appendix 1. Initial work consisted of infusing the target compounds into the MS and optimising MS conditions. Instrumental limits of detection (LOD) were obtained by serial dilution of standard solutions and injection into the LC/MS system. The results of this work are given in table 1.

5.1.1.2 Results and Discussion

Table 1. Instrumental Limits of Detection for Quat Herbicides by LC/MS

Compound	Ions monitored	LOD (ng/ml)
diquat	183 (M+H) ⁺ , 157 (M - C ₂ H ₂) ⁺ 92 (M) ²⁺	100
chlormequat	122, 124 (M+H) ⁺ (chlorine isotopes)	10
mepiquat	114 (M+H) ⁺ , 100 (M - CH ₂) ⁺	1
paraquat	185 (M+H) ⁺ , 93 (M) ²⁺	100

5.1.1.3 Recovery Experiments – Paraquat in Grass

Using the LC/UV/vis method previously developed by HSL (see appendix 2) work was carried out to improve recovery for paraquat from vegetation (grass). The revised HSL extraction procedure (based on Worobey et al, 1987) is:

1. 40g of grass, macerate in blender
2. leave for 1 hour
3. add ~10ml of 2M hydrochloric acid
4. sonicate for 5 minutes
5. heat at 100 °C for 1 hour
6. allow to cool
7. centrifuge at ~ 1700 rpm for 10 minutes
8. decant off liquid and re-extract grass (repeat steps 3 to 7)
9. combine extracts
10. pH adjust to 10 with NaOH
11. clean-up through silica gel solid phase extraction column (SPE), elute with de-ionised water (5 ml), methanol (5 ml) and 5M HCl (methanolic).
12. combine extracts
13. dry down
14. resuspend in water

The results of this work are given in table 2. It is intended to apply the LC/MS method developed in section 5.1.1.1 to vegetation samples extracted using the new HSL extraction method. .

5.1.1.4 Results and Discussion

Table 2. Recovery Experiments for Paraquat in Grass

Level	Spike (µg)	%Recovery	%RSD
High spike	480	62	4.3
Low spike	2.3	124	63.5
Previous work (HSE worksheet 048/95)	500	13	---

The method previously in use at HSL involved refluxing in 8M HCl overnight with a complicated work-up procedure post-extraction. The new method gives better recoveries and is more rapid.

5.1.2 Multi-Residue Analysis of Pesticides by LC/MS/MS

A multi-residue method for 97 pesticides using multiple reaction monitoring (MRM) has been obtained from Applied Biosystems (Applied Biosystems, 2003). This method has been installed on the HSL triple quadrupole (Applied Biosystems API 2000). Future work will be carried out to validate this method.

5.1.3 Solid Phase Micro-Extraction of Pesticides

Solid phase micro-extraction (SPME) is a recent technique for the sampling of materials, especially from liquids. An SPME fibre consists of a length of fused silica coated with a polymeric material. This is attached to a stainless steel plunger. The fibre can be used either to sample the headspace of a solid or aqueous sample, or directly submerged in an aqueous sample. The fibre is desorbed by injection of the fibre into a GC injection port, or by HPLC if an SPME/HPLC interface is used (Supelco, 1998). The theory of SPME is given by Wercinski and Pawliszyn (Wercinski and Pawliszyn, 1999).

5.1.3.1 SPME of Biocides (anti-fouling paints)

A magnetic stirrer bar is added to the 40 ml of sample, which is placed on a heater/stirrer at 55 °C. The fibre is exposed to the sample for 60 min. The fibre is then manually injected and desorbed in the injection port of the GC/MS & analysed.

Instrument:	HP 5973 MSD/6890 GC
Column:	HP 5-MS (5 % diphenyl & 95 % dimethyl polysiloxane stationary phase) capillary column. 30 m length x 0.25 µm film thickness x 0.25 mm internal diameter.
Carrier gas:	Helium
Inlet:	250 °C/splitless, purge flow to split vent 50 ml/min @ 4.5 min
Detector:	280 °C
Oven programme:	60 °C (hold 1 min) 60 → 260 °C (@ 10 °C/min) 260 → 300 °C (@25 °C/min) 300 °C (hold 0.4 min)
Run time:	23 min
Flow rate:	1.0 ml/min
Injection volume:	Manual injection of SPME fibre
Retention time/SIM ions:	1. Diuron 14.93 min 187/189 2. Chlorothalonil 15.73 min 266/264 3. Dichlofluanid 17.12 min 224/226 4. Kathon 17.96 min 246/248 5. Irgarol 18.18 min 253/238 6. TCMTB 18.90 min 180/238

5.1.3.2 Results and Discussion

Comparison of Recoveries from Different Water Types:

1 blank and 2 spike experiments were carried out on the following water types; de-ionised, river and sea. The spikes were fortified with the 6 biocides (listed above) at approximately 100 ng/ml.

The method above was followed for each sample, and an 85µm film thickness polyacrylate SPME fibre was used.

All blank water samples did not contain any of the biocides. A calibration curve was calculated using the average results of the de-ionised water extractions and forced through zero because the blank water did not contain the biocides. The average results of the 2 spikes of each water type were compared to the average de-ionised water result. The de-ionised water was taken as 100 % recovery, and the river and sea as a percentage value of that 100 %.

Table 3. Recovery of Biocides from Various Water Types

Recovery of Biocides from River & Sea Water, Compared to Recovery from De-ionised Water						
Water type	Diuron	Chlorothalonil	Dichlofluanid	Kathon	Irgarol	TCMTB
River	0	102	37	80	98	94
Sea	104	130	0.3	10	115	37

The addition of salt to water samples can improve SPME extractions for certain compounds which may explain why the recovery of chlorothalonil and Irgarol are higher from the seawater spikes than the de-ionised water. The response for diuron was considerably lower than the other 5 biocides. This initial work suggested that SPME would be capable of extracting the biocides at the European Union drinking water standard of 100 ng/L and so further work was carried out to find the limits of detection.

5.1.3.3 Limits of Detection

The SPME extractions were carried out as above, using an 85 µm polyacrylate fibre. Solutions of concentration ~ 10 ng/L, ~50 ng/L and ~100 ng/L of each biocide were prepared using de-ionised, river and sea water and then analysed by GC/MS

Table 4. Limits of Detection for Biocides from Various Water Types

Detection of Biocides in De-ionised Water						
Conc	Diuron	Chlorothalonil	Dichlofluanid	Kathon	Irgarol	TCMTB
~ 10 ng/L	X	X	X	✓	✓	X
~ 50 ng/L	X	✓	✓	✓	✓	✓
~ 100 ng/L	X	✓	✓	✓	✓	✓
Detection of Biocides in River Water						
Conc	Diuron	Chlorothalonil	Dichlofluanid	Kathon	Irgarol	TCMTB
~ 10 ng/L	X	X	X	borderline	borderline	X
~ 50 ng/L	X	X	borderline	✓	✓	borderline
~ 100 ng/L	X	✓	✓	✓	✓	✓
Detection of Biocides in Seawater						
Conc	Diuron	Chlorothalonil	Dichlofluanid	Kathon	Irgarol	TCMTB
~ 10 ng/L	X	X	X	✓	borderline	X
~ 50 ng/L	X	borderline	X	✓	✓	✓
~ 100 ng/L	X	✓	✓	✓	✓	✓

Notes

- X = not detected
- ✓ = detected in all the 3 fibres tested per experiment
- borderline = detected in 1 or 2 of the 3 fibres tested per experiment

Five of the six anti-foulant biocides tested could be extracted by SPME at the drinking water limit from all three of the water types tested. Diuron could be detected in none of the spiked samples.

Extractions, carried out under the same conditions used with the polyacrylate fibres, were done using two alternative fibres – a polydimethylsiloxane (PDMS), 100 µm film thickness, non-bonded fibre, and a PDMS, 7 µm film thickness, bonded fibre. These extractions were only carried out on de-ionised water but used the same biocide concentrations, to see whether these fibres offered an improvement on the polyacrylate fibre.

Table 5. Effect of SPME fibre on Limit of Detection

Detection of Biocides in De-ionised Water using a PDMS, 100 µm non-bonded Fibre						
Conc	Diuron	Chlorothalonil	Dichlofluanid	Kathon	Irgarol	TCMTB
~ 10 ng/L	X	X	X	✓	✓	X
~ 50 ng/L	X	X	✓	✓	✓	X
~ 100 ng/L	X	X	✓	✓	✓	X
Detection of Biocides in De-ionised Water using a PDMS, 7 µm Bonded Fibre						
Conc	Diuron	Chlorothalonil	Dichlofluanid	Kathon	Irgarol	TCMTB
~ 10 ng/L	X	X	X	X	X	X
~ 50 ng/L	X	X	X	X	X	X
~ 100 ng/L	-	-	-	-	-	-

Notes

- X = not detected
 ✓ = detected in all the 3 fibres tested per experiment
 borderline = detected in 1 or 2 of the 3 fibres tested per experiment

The ~ 100 ng/L extraction was not carried out using the PDMS, 7 µm bonded fibre because it had not performed well on the two lower concentrations. It appears that the 85 µm polyacrylate fibre is the most suitable for this application because it has the best sensitivity over the range of the biocides.

Chlorothalonil, dichlofluanid, Kathon, Irgarol and TCMTB can all be detected to at least ½ the EU drinking water limit of 100 ng/L in de-ionised water and the drinking water limit in river water. Of these five, only dichlofluanid cannot be seen to the limit in seawater. Previous development work for the analytical methods for booster biocides also saw problems recovering dichlofluanid from seawater, and the reduced recoveries seen here agree with that work. This would lead us to think that an interaction between dichlofluanid and some component/s of the seawater is occurring.

5.1.3.4 Calibration/Quantitation

De-ionised water spiked with known concentrations of the five detectable biocides (~ 10, 50, 100, 250 and 500 ng/L) were analysed using the 85 µm

polyacrylate fibre. The results were used to produce a calibration curve for each of the biocides. The correlation coefficient (r^2) value for the curve indicates how good the curve fit is. The ideal is 1.000. The curves were plotted as linear $y/(0,0)$, the r^2 values are shown in the table below.

Table 6. Correlation Co-efficients for Biocides by SPME

Correlation Coefficients (r^2) of Calibration Curves of Biocides Determined Using SPME					
	Chlorothalonil	Dichlofluanid	Kathon	Irgarol	TCMTB
r^2	0.930	0.954	0.976	0.998	0.978

Only the calibration curve for Irgarol would be of an acceptable correlation to report quantified results. However, the other biocides curves would be good enough to report ballpark/semi-quantitative results with.

The analyses for the biocides were not carried out individually. Some improvement in the curves might be achieved by doing this. It is important to remember that with real samples, matrix effects may occur and these could influence calibrations, because unless we can obtain a matrix matching the sample to prepare calibration standards with, although without biocides present, the interferences caused by the sample matrix cannot wholly be taken into account. Improvements could be made by using an internal standard, which could be a deuterated booster biocide or a chemically similar compound. Because of the wide range of chemical properties of the booster biocides in use deuterated compounds might be a better option as internal standards.

5.1.3.5 Organo-phosphorus Pesticides for the Aquacheck Scheme

Aquacheck is a quality assurance scheme for testing low-levels of contaminants in water. OMS has signed up to 1 of the groups analysing for organo-phosphorus (OP) pesticides. Eleven OP's are included in the mix; azinphos-methyl, azinphos-ethyl, dichlorvos, fenitrothion, malathion, mevinphos, chlorfenvinphos, diazinon, fenthion, parathion-methyl and parathion-ethyl, which are at concentrations ranging between 20 – 120 ng/l in the sample.

Different SPME conditions were tested to ascertain the best conditions for the extraction. A SIM GC/MS method was set-up to detect these pesticides. All experiments were carried out on 40 ml of water, stirred using a magnetic stirrer bar. The waters were all spiked to the same concentration of OP's from a dilution of a stock mix, 20 – 70 ng/l, dependent on the concentration of the pesticide in the stock solution. The following experiments were carried out;

1. 100 μ m PDMS fibre, extraction time 60 min, room temperature, de-ionised water
2. 100 μ m PDMS fibre, extraction time 60 min, 55°C, de-ionised water

3. 85 μm polyacrylate fibre, extraction time 60 min, room temperature, de-ionised water
4. 85 μm polyacrylate fibre, extraction time 60 min, 55°C, de-ionised water
5. 100 μm PDMS fibre, extraction time 60 min, room temperature, 20% (w/w) NaCl in de-ionised water
6. 100 μm PDMS fibre, extraction time 60 min, 55°C, 20 % (w/w) NaCl in de-ionised water

5.1.3.6 Results and Discussion

Table 7. Extraction of Organo-phosphorus Pesticides (Aquacheck) using Various SPME conditions

Pesticide	GC/MS Response					
	Expt1	Expt 2	Expt 3	Expt 4	Expt 5	Expt 6
Dichlorvos	-	-	-	-	2247	2765
Mevinphos	-	-	-	-	915	-
β -Mevinphos	685	1290	-	-	2658	1751
Diazinon	14668	10185	-	910	16078	22436
Parathion-me	8605	3630	3539	5525	28700	20745
Malathion	5663	2643	3381	3768	29516	18082
Fenitrothion	11561	5291	4526	6351	36923	35359
Parathion-et	32243	15701	10857	13416	69421	87000
trans-Chlorfenvinphos	4867	3506	-	1871	7175	8640
cis-Chlorfenvinphos	24133	14841	-	21169	64324	70054
Fenthion	261801	142469	82698	100911	315858	402504
Azinphos-me	2769	1469	-	-	1666	-
Azinphos-et	5643	2060	-	1778	21176	21176

The most favourable conditions for the extraction seems to be those of experiment 5, which allows all the pesticides to be detected. Further work to determine equilibrium time and potential quantification under these conditions would be needed before using the method in the scheme. The use of an internal standard would probably aid quantification e.g. a deuterated pesticide from the mix, or an OP that isn't included, such as chlorpyrifos.

5.1.3.7 Timber Treatment Pesticides

The timber treatment and sheep dip pesticides were two groups of pesticides that had been used when assessing the Twister stir bar sorptive extraction (SBSE) technique. The SBSE work has been reported previously (HSL,

2003a). For comparative purposes, extraction of these pesticides using SPME was briefly assessed.

The following experiments were carried out on the timber treatment pesticides; dieldrin, propiconazole, tebuconazole and permethrin. The responses of the pesticides giving two peaks (propiconazole and permethrin) are given as a combined figure in table 8.

All experiments were carried out on 40 ml of water, stirred using a magnetic stirrer bar. The waters were all spiked to the same concentration from a dilution of a stock mix, 140 – 650 ng/l, depending on the concentration of the pesticide in the stock solution. The following experiments were carried out;

1. 100 µm PDMS fibre, extraction time 60 min, room temperature, de-ionised water
2. 100 µm PDMS fibre, extraction time 60 min, 55°C, de-ionised water
3. 100 µm PDMS fibre, extraction time 60 min, 55°C, 20 % NaCl (w/w) in de-ionised water
4. 100 µm PDMS fibre, extraction time 60 min, room temperature, 20 % NaCl (w/w) in de-ionised water
5. 85 µm polyacrylate fibre, extraction time 60 min, 55°C, de-ionised water

5.1.3.8 Results and Discussion

Table 8. SPME of Timber treatment Pesticides

Pesticide	GC/MS Response				
	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5
Dieldrin	22822	38325	27754	11380	9426
Propiconazole	205160	110822	505772	584874	39173
Tebuconazole	766769	2470899	562126	398261	706245
Permethrin	1906390	1514560	200293	103301	1788070

The conditions of experiment 2 would be best for screening for the presence of timber treatment pesticides. If only one was known to be present, the conditions giving the highest response for that compound could be selected.

5.1.3.9 Sheep Dip Pesticides

The following experiments were carried out on the sheep dip pesticides; diazinon, amitraz, cypermethrin and flumethrin. The responses for the 3 peaks found for cypermethrin were combined and given as one result. Flumethrin does not GC well due to its high boiling point, > 250°C, which requires that the

injection port is set ~ 300°C. During the set-up of the SIM method, flumethrin was not detected, therefore no results were obtained for this compound when using SPME. The analysis of flumethrin may have to be carried out by LC but it may be possible to extract the compound off the fibre into a solvent for LC analysis.

All experiments were carried out on 40 ml of water, stirred using a magnetic stirrer bar. The waters were all spiked to the same concentration from a dilution of a stock mix, 450 – 680 ng/l, depending on the concentration of the pesticide in the stock solution. The following experiments were carried out;

1. 85 µm polyacrylate fibre, extraction time 60 min, 55°C, de-ionised water
2. 100 µm PDMS fibre, extraction time 60 min, 55°C, de-ionised water
3. 100 µm PDMS fibre, extraction time 60 min, 55°C, 20 % NaCl (w/w) in de-ionised water
4. 85 µm polyacrylate fibre, extraction time 60 min, 55°C, 20 % NaCl (w/w) in de-ionised water
5. 100 µm PDMS fibre, extraction time 60 min, room temperature, 20 % NaCl (w/w) in de-ionised water

5.1.3.10 Results and Discussion

Table 9. SPME of Sheep Dip Pesticides

Pesticide	GC/MS Response				
	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5
Diazinon	8874	73687	331343	127219	590930
Amitraz	347608	41081	64011	36075	12728
Cypermethrin	1503363	1835440	1037535	1071918	714747

Experiment 3 conditions would be recommended for screening for these compounds.

Further development of the methods for the timber treatment and sheep dip pesticides would be needed to find limits of detection and investigate quantification of the SPME technique.

5.1.3.11 Summary of Results for 5.1.3.1 to 5.1.3.10

SPME has been used to extract a variety of pesticides and biocides (anti-foulants, organo-phosphorus pesticides, timber treatment pesticides and sheep dip pesticides from de-ionised water samples. The following conditions were found to be best for screening purposes;

Anti-foulants – 85 µm polyacrylate fibre

Organo-phosphorus pesticides – 100 μm PDMS fibre, extraction time 60 minutes, room temperature, 20% (w/w) NaCl

Timber treatment pesticides - 100 μm PDMS fibre, extraction time 60 minutes, 55 $^{\circ}\text{C}$, 20% (w/w) NaCl

Sheep dip pesticides - 100 μm PDMS fibre, extraction time 60 minutes, 55 $^{\circ}\text{C}$, 20% (w/w) NaCl.

5.1.3.12 SPME Equilibrium times of Biocides

To accurately quantify using SPME the pesticide must be at equilibrium partition between the matrix (usually water) and the SPME fibre. This section reports work to calculate the equilibrium times for a variety of biocides and OP pesticides. The results of this work are given in figures 1 to 3.

Figure 1 shows that the booster biocides behave quite differently on the acrylate SPME fibre. For example, extraction of Chlorothalonil and dichlofluoanid has reached equilibrium after ~ 30 minutes but for Irgarol and Kathon equilibrium has not been reached after 70 minutes. Figures 2 and 3 shoe the effect of temperature. At room temperature all the OP pesticides have reached equilibrium after ~ 10 minutes except for fenthion which is still not at equilibrium after 90 minutes. At 55 $^{\circ}\text{C}$ fenthion reaches equilibrium after ~ 30 minutes.

Figure 1. Equilibrium Times for Booster Biocides at Room Temperature

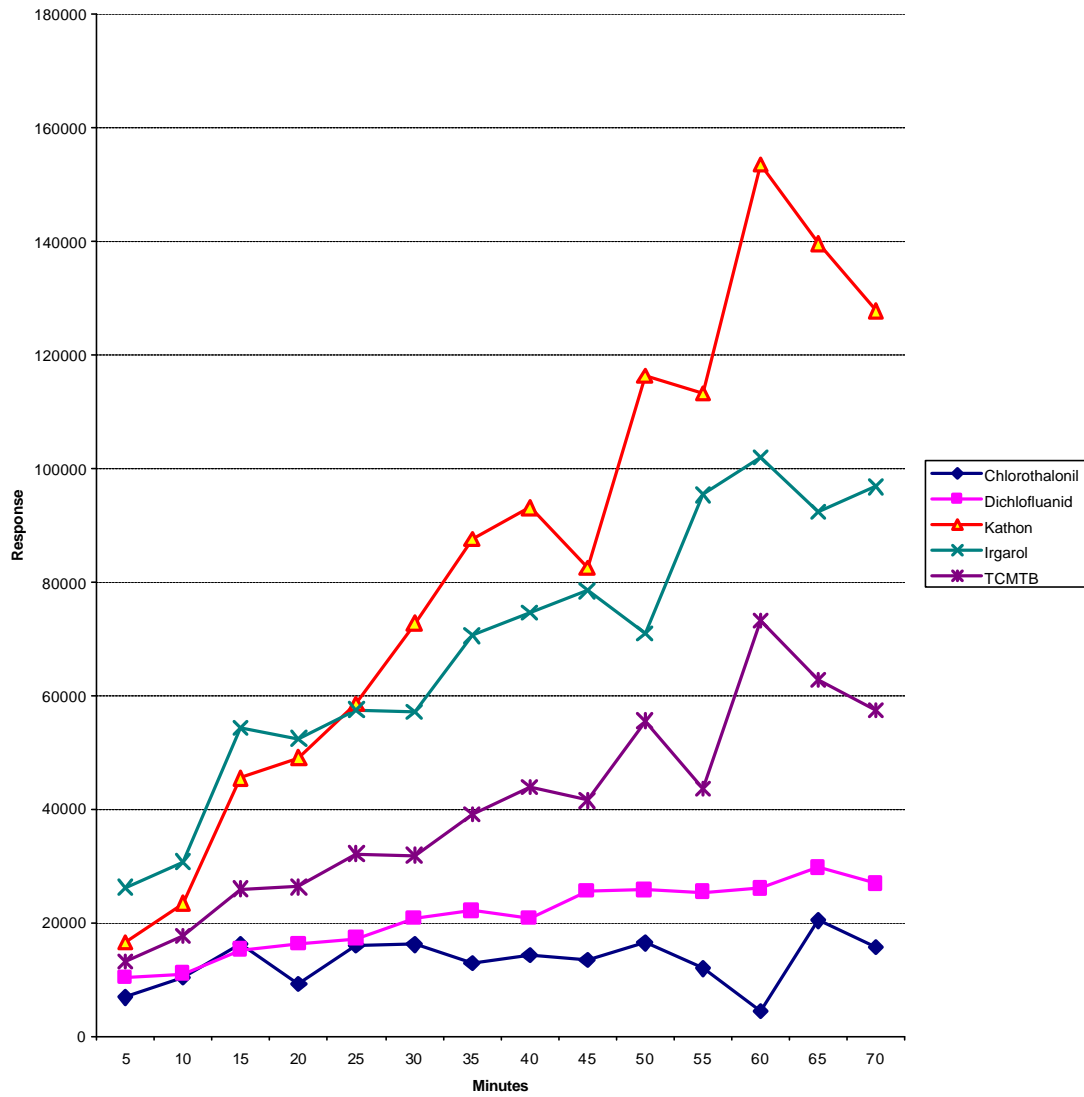


Figure 2. Equilibrium Time for OP Pesticides on a PDMS fibre at Room Temperature

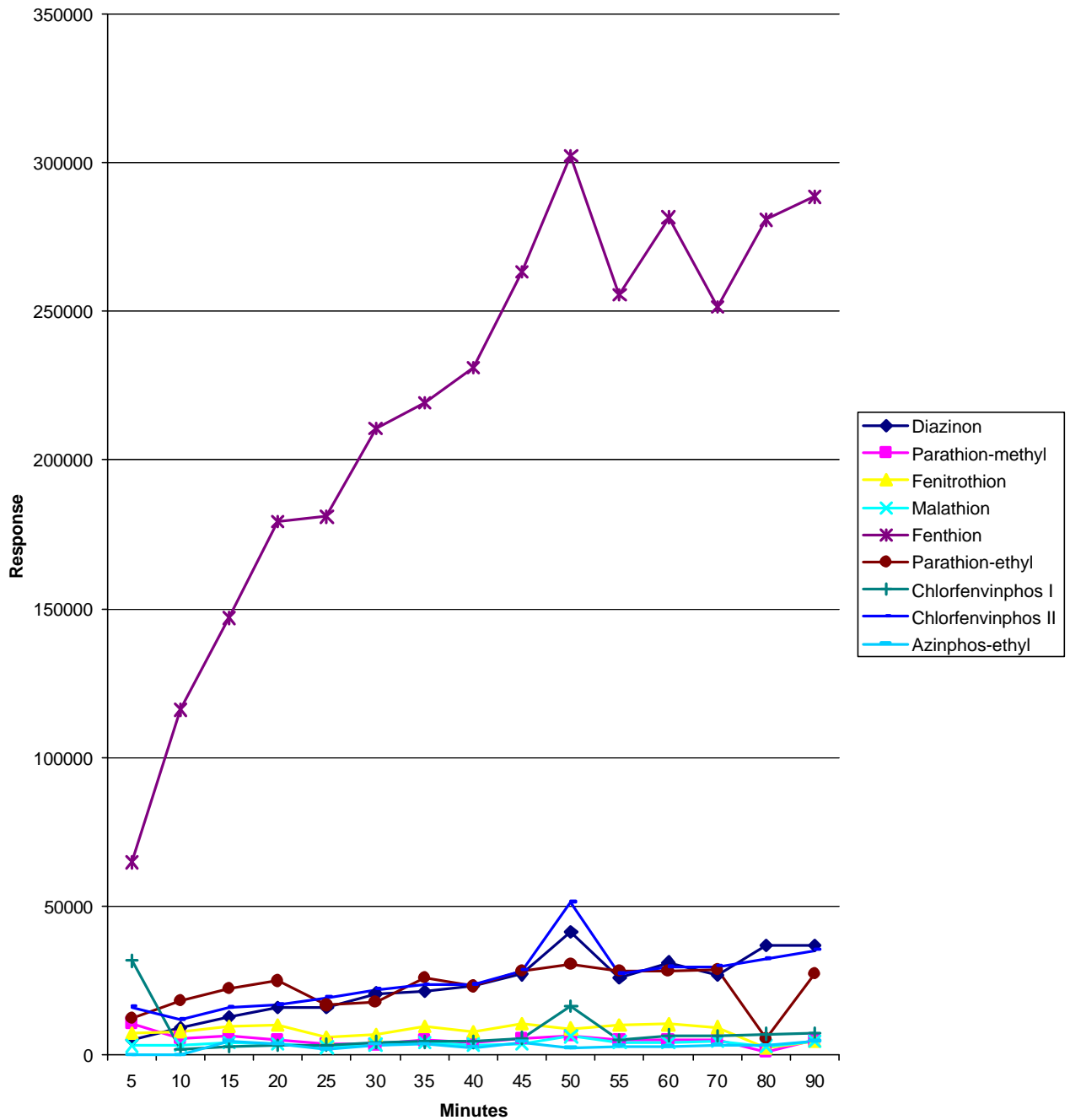
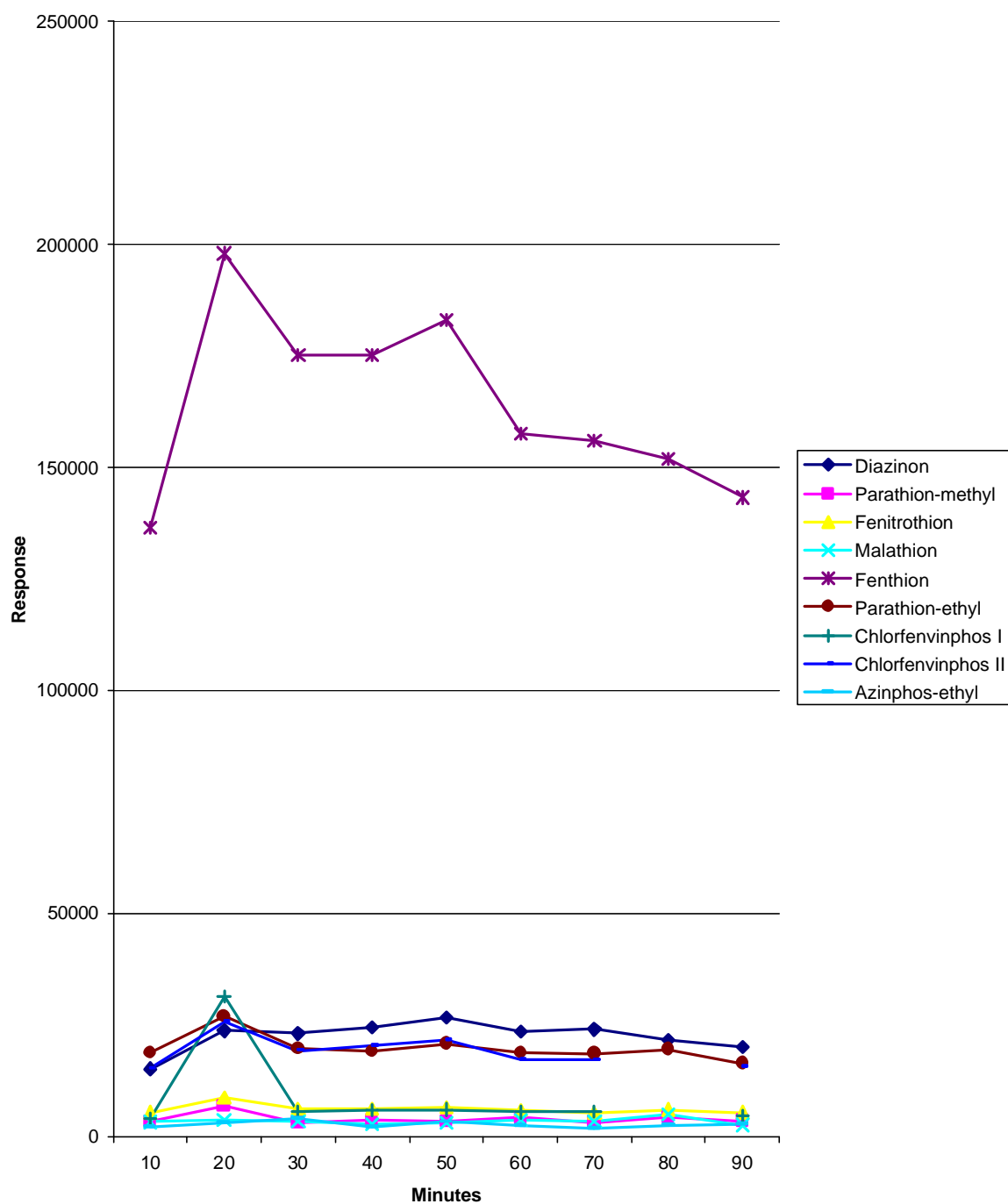


Figure 3. Equilibrium Time of OP Pesticides on a PDMS fibre at 55 °C



5.1.4 Other Pesticide/Biocide related work

The call-off contract was also used to support other instrumental development work in the biocides area. This included attendance at relevant meetings (manufacturer seminars on SPME, SBSE etc and talks/poster presentations at scientific meetings) and small blocks of work concerning the use of new techniques in relation to other project work e.g. method development for rodenticides, biocides, sulphuron herbicides, SBSE and veterinary medicines. This work has been reported fully elsewhere (HSL, 2002a; 2002b, 2003a; 2003b; 2003c; 2004).

5.1.5 Conclusions

The initial work carried out on for Quat herbicides suggests that LC/MS will be able to give good sensitivity for these compounds. More work is required to fully validate this method.

For the recovery work, the results from the high level spikes show a marked improvement on methods previously used at HSL. However, the low level spikes were subject to interference from some co-eluting component from the grass. The use of the more specific LC/MS/MS method should improve this situation. Further work is required to fully develop this method.

SPME has been investigated for a variety of biocides. More work is required to fully evaluate this technique but it has shown promise for the extraction of biocides from water samples.

5.1.6 Recommendations

1. LC/MS and LC/MS/MS methods should be developed to compliment the existing GC/MS methods HSL has for biocides.
2. SPME should be further investigated for the analysis of biocides in water.
3. These developments will enable HSL to respond more rapidly and effectively to HSE survey and enforcement needs.

5.1.7 References

Applied Biosystems (2003)

Application Note – LC/MS

Simultaneous determination of approximately 100 pesticides and metabolites in fruit and vegetables by LC/MS/MS.

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H Corns, P Johnson and J White, Assessment of analytical methods for rodenticides, HSL Report OMS/2002/18

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PD Johnson, H Corns, MR Coldwell and J Higgins, Evaluation of analytical methods for pesticides and biocides, HSL Report OMS/2004/11

Supelco (1998)

Instructions for the use of Supelco SPME fibre holder for manual use.

M Takiro, S Daishima and K Yamaguchi (2000)

Analytical Sciences, 16,p707-711.

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Analytical method for the simultaneous determination of diquat and paraquat residues in potatoes by HPLC, Pestic. Sci., 18, p245-257.

SAS Wercinski and J Pawliszyn (1999)

SPME Theory

From Solid Phase Micro-Extraction - A practical guide, Ed SAS Wercinski, Pub. Marcel Dekker.

5.1.8 Appendices for Section 5

Appendix 1 - LC/MS method for Quats

This describes an analytical method for quaternary ammonium herbicides using solvent extraction and HPLC/MS

Samples are prepared as section 5.1.1.3.

Instrument Set-Up

Analytical Method: QUATS SIM – triple quadrupole

Mobile phase A: 5mM nonafluoropentanoic acid in water

Mobile phase B: 5 mM nonafluoropentanoic acid in acetonitrile

250 $\mu\text{l}\cdot\text{min}^{-1}$ at 50:50 A:B v/v

Column: 100 x 2.1 mm C18 column; 5 μl injection

MS parameters – positive mode, SIM

Analyte	Ions (m/z)	Dwell (ms)
paraquat	185,93	150
diquat	183, 157, 92	150
mepiquat	114,100	150
chlormequat	122,124	150

Analyte	Compound	
paraquat	DP	125
	FP	100
	EP	10
	CEP	10
	CXP	1
	TEM	350
diquat	DP	125
	FP	100
	EP	10
	CEP	10
	CXP	1
	TEM	400
mepiquat	DP	90
	FP	100
	EP	10
	CEP	10
	CXP	1
	TEM	550
chlormequat	DP	70
	FP	100
	EP	10
	CEP	10
	CXP	1
	TEM	450

Source/Gas	
CUR	25
IS	5200
GS1	25
GS2	25

Appendix 2 - LC/UV/vis method for Paraquat

C18 column

Mobile phase

27% acetonitrile

73 water – 10 mM heptane sulphonic acid, sodium salt, (pH 3 with sulphuric acid)

6 IDENTIFYING ISOCYANATES BY PYROLYSIS GC/MS

This section describes work carried out by OMS on the identification of isocyanates released from paints by pyrolysis GC/MS.

6.1.1 Introduction

It is often a requirement at HSL to analyse bulk materials, such as paints, foams and adhesives for the presence of isocyanate material. Currently, this requires the material to be analysed in the same way as air samples ie dissolved in solvent, derivatised with 2-MP and examined by HPLC. However, this technique can be both difficult and time consuming, particularly as the sample material may be difficult to dissolve, contain interfering components and produce chromatograms that are difficult to interpret using the standard UV/vis and electrochemical detectors. The standard technique will also not work for polymerised polyurethane material such as that present in "stoved" paints i.e. paints in which the NCO function is chemically blocked. Thermal degradation of isocyanates is a subject of much current interest as it has been suggested that thermal breakdown of isocyanate based materials could lead to the exposure of unsuspecting and unprotected workers e.g. MVR workers carrying out welding, sanding or grinding processes and workers in other processes where polyurethanes are heated (HSL, 2003).

One solution is LC-MS analysis, however this still requires the sample material to be dissolved/derivatized and will still not work for polymerised material. Another completely different approach is pyrolysis-GC-MS. In this technique a small sample of the bulk material is placed in a pyrolyser which is directly interfaced to the injection port of an otherwise standard GC-MS system. Consequently, it is not necessary to dissolve or derivatize the sample prior to analysis, thus saving time. Pyrolysis also has the advantage of being able to detect and identify polymerised isocyanate material.

Pyrolysis methods have been used at HSL for analysis of a variety of samples, including isocyanates (Pengelly, 2002). However, a new pyrolyser has recently been purchased which is both easier to use and allows faster turnaround. This device, the Pyrola 2000, is illustrated in Figure 1 and works as follows. A small amount of sample material is placed on a platinum filament, which is mounted in a quartz glass cell and sealed inside the pyrolyser. The sample is then rapidly heated in a flow of helium carrier and the resulting pyrolysis products swept into the GC-MS system via a heated transfer line. Typically, pyrolysis is carried out at a temperature of between 500°C and 700°C with a heating time of 2 seconds.

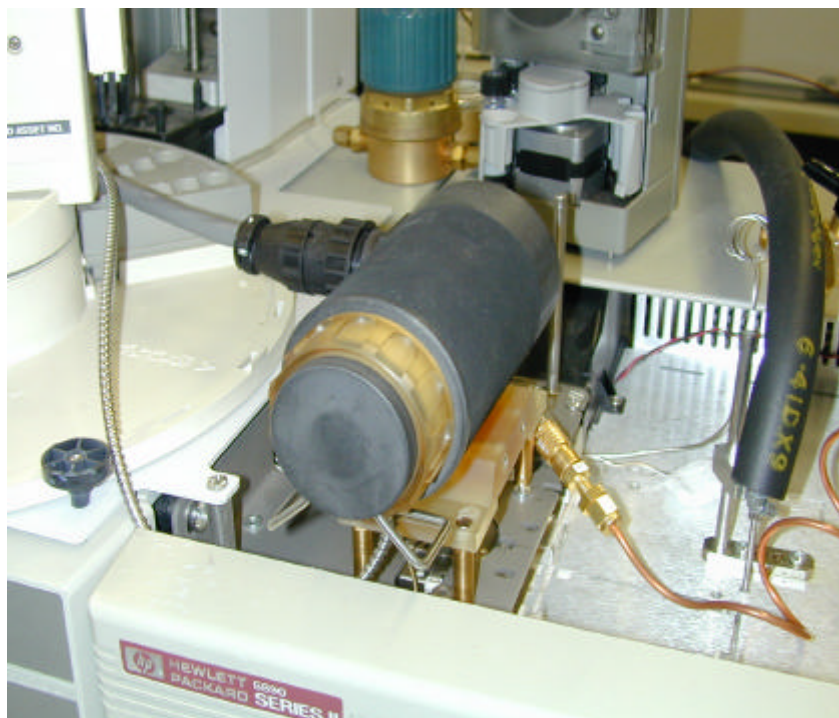


Figure 1: Pyrola 2000 pyrolyser mounted on Agilent GC-MS

6.1.2 Sample Analysis - Results and Chromatograms

Samples of the following three paints were analysed by pyrolysis-GC-MS:-

- Kemple Part 2
- Autocolor 2K
- Beckprim 2000 Primer

The Autocolor sample was pyrolysed at 700°C and the Kemple Part 2 and Beckprim 2000 primer samples were at 800°C. The resulting chromatograms are shown in Figures 2 to 4.

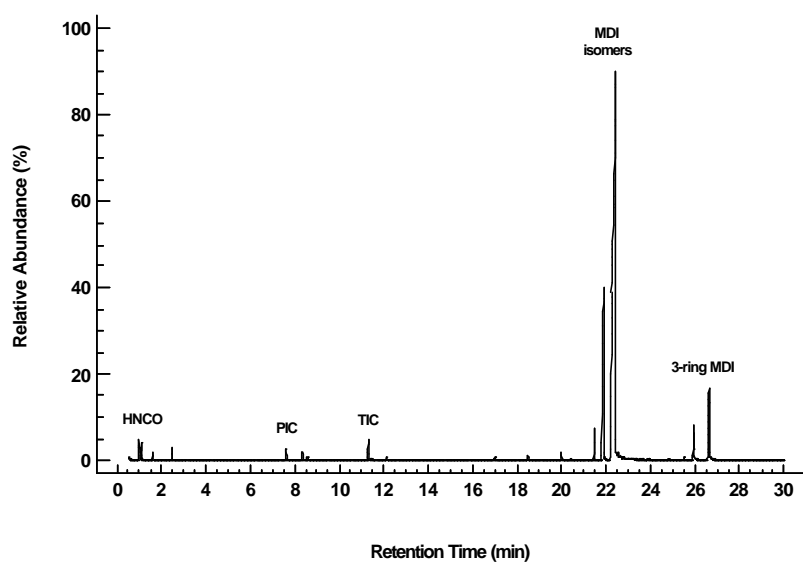


Figure 2: Kemple Part 2 Pyrolysed at 800°C

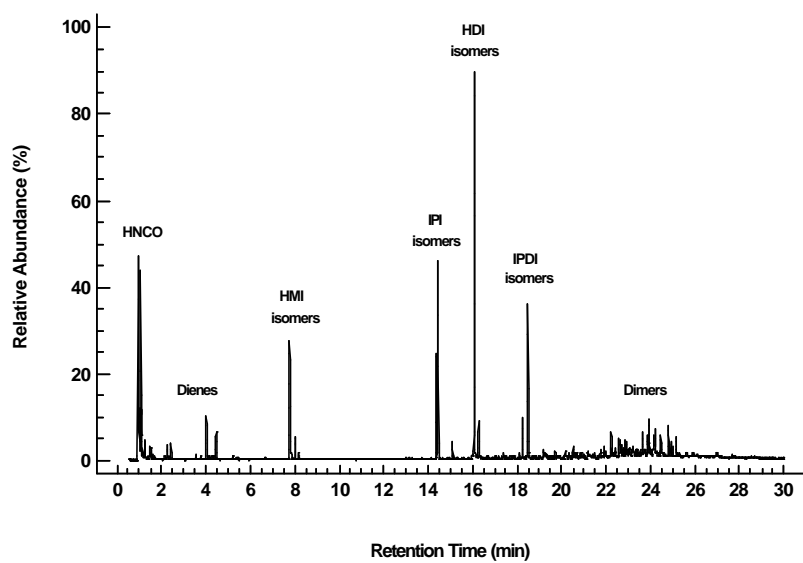


Figure 3: Autocolor 2K Pyrolysed at 700°C

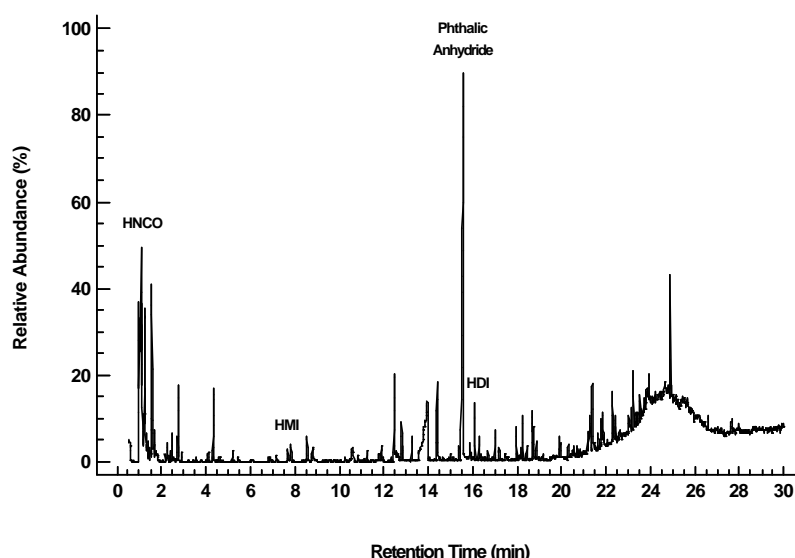


Figure 4: Beckprim 2000 Pyrolysed at 800°C

The chromatogram derived from the Kemple product (Figure 2) was found to contain the following components derived from isocyanate components:-

- Three isomers of methylenebis(phenyl isocyanate) (MDI). The mass spectrum of the main 4,4' isomer is shown in Figure 5.
- Four 3-ring MDI isomers. The mass spectrum of the main isomer is shown in Figure 6.
- Isocyanic acid (HNCO), the mass spectrum of which is shown in Figure 7.
- Minor amounts of phenyl isocyanate (PIC), tolyl isocyanate (TIC) and diphenylmethane isocyanate.

The chromatogram derived from the Autocolor product (Figure 3) was found to contain the following components derived from isocyanate components:-

- Two isomers of isophorone diisocyanate (IPDI). The mass spectrum of the main isomer is shown in Figure 8.
- Two isomers of hexamethylene diisocyanate (HDI). The mass spectrum of the main isomer is shown in Figure 9.
- Two isomers of isophorone isocyanate (IPIC). The mass spectrum of the main isomer is shown in Figure 10.
- Isomers of hexyl isocyanate (HIC) and hexamethylene isocyanate (HMIC). The mass spectrum of the main isomer is shown in Figure 11.
- Isocyanic acid (HNCO), the mass spectrum of which is shown in Figure 7.
- Various dimers of IPDI and HDI.

The chromatogram derived from the Beckprim product (Figure 4) differed from the other two in that most of the main components were not isocyanates. This is because this product is a "stoved" or chemically blocked isocyanate i.e. the isocyanate is only released by heating during the industrial process. Analysis of this product using MDHS 25/3 detected no free isocyanate prior to heating. However, this illustrates how the technique is able to identify the presence of isocyanate material, even when only present as a minor, or even trace, component. In this case the analysis showed the presence of HDI, HMIC and HNCO.

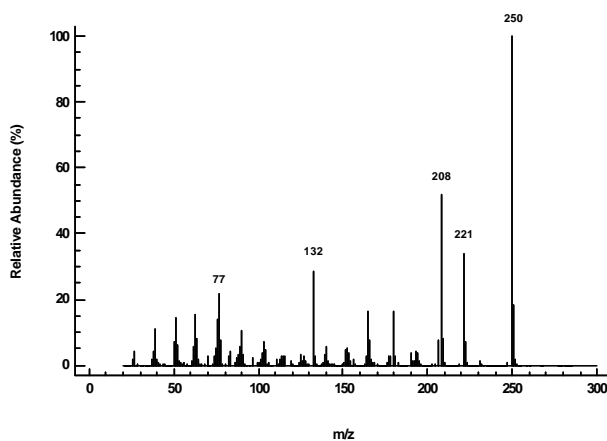


Figure 5: Mass Spectrum of 4,4'-Methylene(bisphenyl isocyanate)

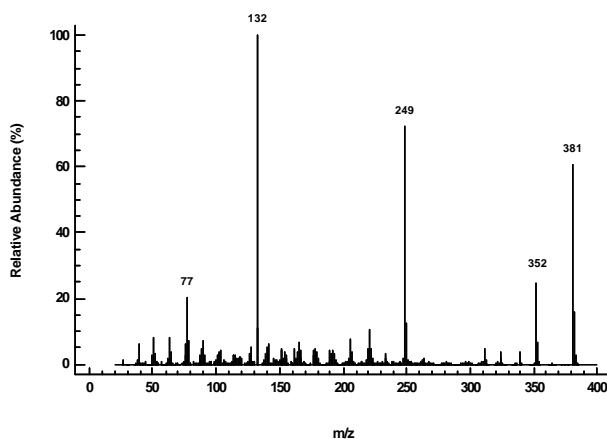


Figure 6: Mass Spectrum of 3-Ring MDI

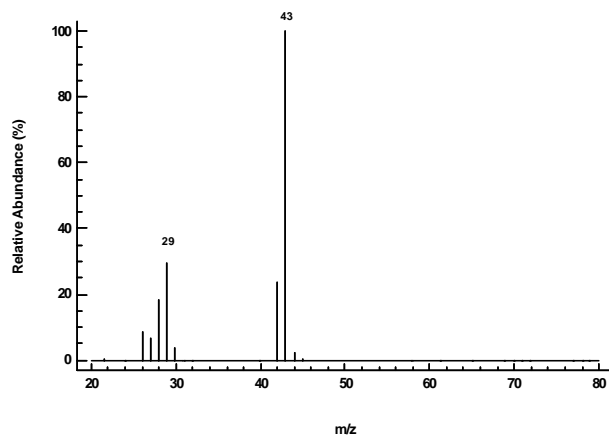


Figure 7: Mass Spectrum of Isocyanic Acid

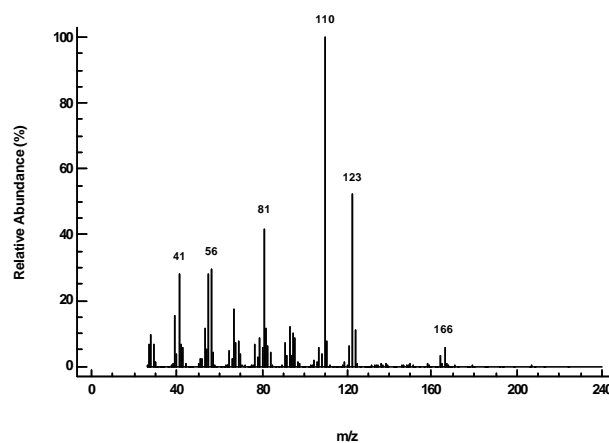


Figure 8: Mass Spectrum of Isophorone Diisocyanate (IPDI)

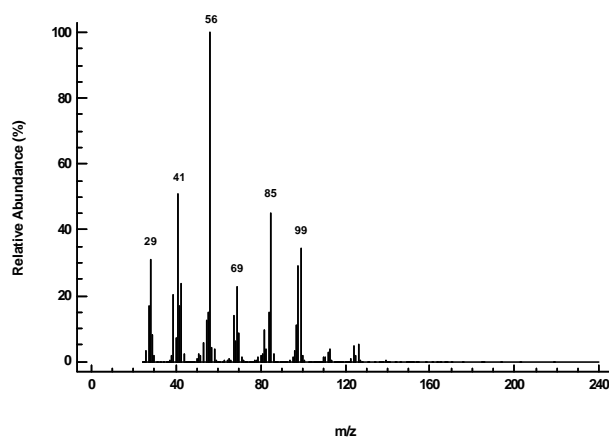


Figure 9: Mass Spectrum of Hexamethylene Diisocyanate (HDI)

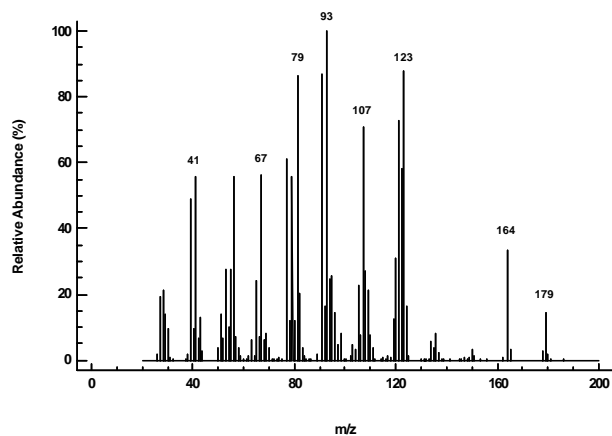


Figure 10: Mass Spectrum of Isophorone Isocyanate (IPIC)

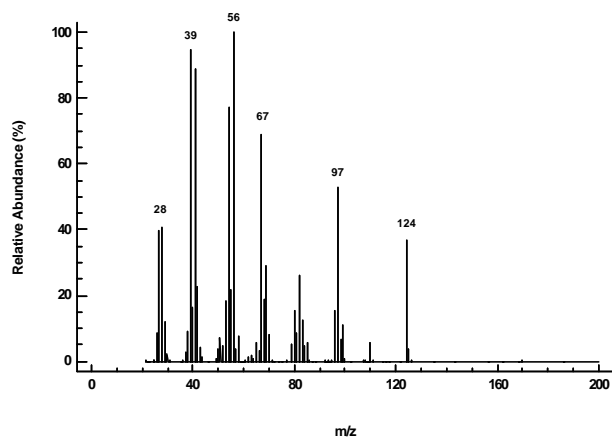


Figure 11: Mass Spectrum of Hexamethylene Isocyanate (HMIC)

6.1.3 Conclusions

Pyrolysis GC/MS has proved to be a rapid technique for the identification of the more volatile isocyanate MP derivatives. It is an excellent complimentary technique to those already in use at HSL. It is particularly useful for isocyanates arising from thermal degradation e.g. heating of polyurethane foams, welding of painted surfaces etc.

6.1.4 Recommendations

1. Further work on the thermal degradation of isocyanate-based and other materials should be carried out. For example the determination of small molecular weight isocyanates (methyl isocyanate and isocyanic acid) produced from the welding of painted surfaces during car body repair shop work.
2. If purchased, HSL will assess the use of a Thermo-Extractor to examine the release of organic vapours at temperatures ranging from 100 to 250 °C i.e. below the temperature range of the current pyrolysis system.

6.1.5 References

HSL (2002)

I Pengelly

Pyrolysis Screening Methods, HSL Report OMS/2002/02.

HSL (2003)

J White

The Determination of Isocyanates in the Presence of Amines, HSL Report OMS/2003/10.

7 TRAINING AND MISCELLANEOUS ACTIVITIES

7.1.1 Ion Trap MS

Four members of staff (Biological monitoring and Organic Measurement section) had two days on-site training on the new Agilent Ion Trap LC-MS and spent some subsequent time familiarising themselves with the system and its operation.

7.1.2 Training on other Systems

Training on the instrumental systems available (GC/MS, ATD-GC, LC/UV/EC and triple quadrupole LC/MS) has been given on an ad-hoc basis as required by operational demands.

7.1.3 Posters and Presentations

Posters

"LC/MS of isocyanates", Poster - 23rd International Mass Spectrometry Society Meeting, Edinburgh, August, 2003

Presentations

SPME of Biocides – Supelco Seminar, May 2004.

Isocyanate Sampling and Analysis talks –

BOHS Wales, HSE, Cardiff, 12th April 2005

BOHS Conference, Isocyanate Workshop, Stratford upon Avon, 22nd April 2004

BOHS London, Society for Chemical Industry, 17th March 2004