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Dioxin Markers Feasibility Study

Environmental Resources Management

R&D Technical Report P61

Dioxin Markers Feasibility Study

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This report examines the feasibility of applying immunoassay techniques to screening dioxin releases from thermal processes and in effluent from VCM manufacture. The information within this document is for use by EA staff and others involved in the operation, management and regulation of these processes.

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EXECUTIVE SUMMARY

1. BACKGROUND

In March 1996 the Environment Agency commissioned Environmental Resources Management (ERM) to undertake consultancy contract No. HMIP/CPR2/41/1/256 entitled Dioxin Markers Feasibility Study. The objectives of the contract as described in the terms of reference were as follows:

(1) *To examine congener profile data of stack emissions samples from processes including those noted below to determine if any particular congener(s) is a suitable marker that could be used as a surrogate for the I-TEQ for that process. Data examined must be relevant to UK operated processes and where possible should include both new plant and old plant modified to meet current new plant standards. The releases are: (a) emissions from municipal waste incinerators; (b) emissions from chemical waste incinerators; (c) emissions from iron and steel making processes; (d) discharge samples from [the manufacture of vinyl chloride monomer].*

(2) *Use the data from (1) and analyse it statistically to draw conclusions on the significance of the results of the evaluation. If appropriate draw up a proposal for further work to verify the conclusions using an independent data set.*

(3) *Undertake a search of the world-wide literature to identify organisations who have the potential to develop antibodies for the measurement of dioxins.*

In this study the term "dioxins" is taken to mean the family of 210 compounds or congeners comprising polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). If both PCDDs and PCDFs are present, they are referred to as PCDD/Fs. The summation of the concentrations of 17 toxic PCDD and PCDF congeners, weighted relative to the toxicity of 2,3,7.8-TCDD, is given in the form of *International Toxic Equivalents*, abbreviated to I-TEQ.

2. STATUS OF IMMUNOASSAY FOR PCDDs AND PCDFs

2.1 Characteristics of Immunoassay for PCDD/Fs

A summary of the strengths and limitations of immunoassay screening for PCDD/Fs is given *Table 1.*

Table 1 Strengths and Limitations of Immunoassay Screening for PCDD/Fs

Strengths	Limitations
Sensitivity	Sensitivity not fully competitive with GC-MS methods. Sensitivity may vary according to environmental medium/matrix.
Speed	Speed is reduced if significant sample cleanup is required.
Simplicity	Probable use of incompatible solvents for sample extraction. Probable need for solvent exchange matrix concentration may be required for adequate sensitivity.
Low cost	Cost is increased if sample cleanup is required.
Parallel processing	Sample preparation less likely to offer parallel processing.
Group recognition of toxic congeners	Recognition of nontoxic congeners ignored by GCMS method. Inability to define level for any specific congener. Need for partial GCMS confirmation to support interpretation.
Potential for TEQ screening	Requires validation by comparison to GCMS-analysed samples. Requires calibration for correct interpretation of results.
Possible use of GCMS extract	Potential conflict with GCMS internal standard protocol.

The commonly recognised ***general strengths*** of immunoassays, such as speed, simplicity, low cost, and parallel processing of many samples, can be applied to any PCDD/F immunoassay if it is correctly configured. These advantages may be partially neutralised if the sample processing requirements are too extreme. The most commonly observed ***limitation*** of immunoassay is that the immunoassay response is comprised of responses to all of the individual chemicals in the sample that are recognised by the antibody. Another general limitation is the requirement for validation against an appropriate set of field samples before the test can proceed with confidence. Some level of confirmation by conventional methodology will also be required as part of the ongoing quality assurance programme.

2.2 Commercial PCDD/F Immunoassay Kits

PCDDs and PCDFs pose special problems in immunoassay because of their low solubility in most common solvents and their extremely low solubility in water. Two kits are available commercially.

The kit sold by EnSys is highly specific for 2,3,7,8-TCDD. However, the test has 4.6% cross reactivity for 2,3,7,8-TCDF, 20% for 2,3,7-TrCDD, and 2.2% for 2,3-DCDD. Cross

reactivity data available for other toxic congeners indicate a high level of specificity which renders the kit unsuitable for toxicity based screening unless the samples are known to contain only 2,3,7,8-TCDD. The test is formatted as a rapid test using antibody-coated tubes and has similar equipment requirements and assay procedure to EnSys' other kits. The limit of detection is approximately 80 pg/tube (2,3,7,8-TCDD). In addition to being used in kits, the antibody is being offered for sale separately by Fitzgerald International Industries, Inc. of Concord MA, USA.

The kit released by Millipore uses the DD3 antibody immobilised on polystyrene tubes. The test is formatted as a rapid tube test. The limit of detection is approximately 100 pg/tube (2,3,7,8-TCDD). This kit exhibits good correlation between cross reactivity and congener toxicity and is therefore appropriate for toxicity-based screening. The I-TEQ screening concept has been examined theoretically by using 43 soil samples to compare predicted immunoassay values to GCMS based TEQ values. No data are available comparing the kit to GCMS using conventional sample preparation methods with field samples. A microplate version of the test, also based on the DD3 antibody, has been prepared and partially characterised. Because full specificity data have not been developed for the microplate test, it is not possible to conclude whether it is as appropriate for I-TEQ screening as the tube kit. However, the improvement in sensitivity to 10-25 pg/well could offer significant advantages over the tube test. The Millipore kits are now under evaluation by EnSys following their purchase of Millipore's EnviroGard product line. The test is subject to licences from FMS and ECOCHEM.

3. STATISTICAL ANALYSIS OF PCDD/F RELEASE DATA

A statistical analysis was performed on PCDD/F concentrations in stack emissions from municipal waste incinerators (MWIs), chemical waste incinerators and iron and steel works, and effluent from the manufacture of vinyl chloride monomer (VCM). PCDD/F release data were obtained from a variety of sources including the Environment Agency's monitoring database; data from specific UK sites provided by plant management; data provided by the University of Bayreuth on PCDD/F emissions from a German MWI; and data on PCDD/F emissions from German and Dutch MWIs obtained from the open literature. A total of 130 data points were collated from these sources.

Statistical analysis using cluster analysis failed to identify sufficiently distinctive profiles of the 2,3,7,8-positional congeners which could be attributed to individual thermal release sources. The VCM data exhibited a pattern distinct from that of the combustion sources, but since the samples derive from a number of related process and effluent streams it is not possible to allocate an unequivocal congener profile specific to VCM manufacture.

For the thermal release sources, the congener 2,3,4,7,8-PeCDF made the greatest contribution to the total I-TEQ concentration of the sample. The concentration of this congener was therefore plotted against the total
I-TEQ of each sample (including VCM effluent), and the resulting correlation tested for linearity. For the three thermal sources excellent linear correlations were obtained. The correlation was less satisfactory for the VCM data set, but the samples derive from a number of different process and effluent streams, albeit related to the manufacture of VCM and other products, and the congener profile in VCM effluent is markedly different from that of the thermal sources.

The 2,3,4,7,8-PeCDF congener was therefore identified as a potential marker for the total I-TEQ of the emission and effluent samples from the release processes considered in this report. Independent research in Germany has also identified this congener as an excellent marker congener for the characterisation of the total I-TEQ concentration in stack emission samples. From an examination of the available data on PCDD/F immunoassays an existing monoclonal anti-dioxin antibody, DD3, has the potential to be applied both in an I-TEQ screen or in an assay that is highly selective towards 2,3,4,7,8-PeCDF, depending on the assay conditions.

4. CONCLUSIONS

4.1 Sampling and Sample Preparation

Because of the need for complete extraction of PCDD/Fs from stack gases, aqueous effluents and their entrained particulates, efficient capture of the sample is essential. The tight binding of PCDD/Fs to solids, either trap adsorbent or sample matrix, indicates that extraction of particulates is critical to both sample types. It is therefore likely that the immediate future of PCDD/F immunoassay will rely upon conventional sample capture and extraction. While this may not ultimately be the best or least costly method of implementing an immunoassay programme, in the short term such a compromise seems necessary to reduce the number of analytical variables. However, research groups are developing promising alternative integrated sampling and cleanup techniques which are in principle compatible with the requirements of immunoassay. Successful validation of an immunoassay using conventionally prepared and cleaned extracts would then allow research to progress confidently to the next step of using these alternative sampling, extraction and/or cleanup methods.

4.2 Sample Cleanup

Successful analysis of crude sample extracts is unlikely to be achievable by immunoassays except for very clean samples. However, the specificity intrinsic to immunoassays suggests that sample extracts do not need to be cleaned to the same degree as for GCMS.

Thus, an adequate partial cleanup of conventionally prepared extracts should exist somewhere between these two extremes. Significant work has been devoted recently to the development of solid phase extraction (SPE) and high pressure liquid chromatography (HPLC) methods for aqueous matrices. These systems have the advantage of potentially coupling directly to the extract cleanup system to provide a one-step extraction and cleanup. In addition, the HPLC technique uses solvents which are compatible with the requirements of immunoassay.

4.3 Antibodies and Test Formats

The statistical value of 2,3,4,7,8-PeCDF as a marker for total I-TEQ of the sample has been identified in this study as a theoretically viable approach to immunoassay screening. Existing antibodies such as the DD3 antibody also appear to be able directly to characterise the sample I-TEQ. New PCDD/F antibodies are currently in development and preliminary indications are for significantly improved sensitivity in response towards sample I-TEQ.

5. RECOMMENDATIONS

5.1 Antibody Development Work

Research effort can proceed along two fronts.

- Firstly, antibodies capable of direct I-TEQ screening should be further characterised and assessed for their potential to be incorporated into an appropriate immunoassay test format. These antibodies include the commercially available DD3 antibody, and antibodies with greatly improved sensitivity currently being developed by ECOCHEM.

- Secondly, since 2,3,4,7,8-PeCDF shows promise as a marker congener, there is merit in considering the development of an antibody for this congener together with the necessary cleanup methods, test procedures and quality assurance.

It is recommended that initial effort be directed towards a fuller evaluation of existing antibodies to directly measure the I-TEQ of a sample, before proceeding with the greater resource requirements of developing a new anti-2,3,4,7,8-PeCDF antibody. However, in the event that research and commercial organisations might independently be developing anti-2,3,4,7,8-PeCDF antibodies, it is recommended that the Environment Agency maintains contact with EnSys, ECOCHEM and the research group at the University of California at Davis to receive regular updates, both on this issue and on progress towards an anti-dioxin antibody of improved sensitivity.

5.2 Sample Extraction and Cleanup

Three areas merit further research. These include the use of solvents such as DMF, DMSO and DMA for solid sample extraction, the use of solid phase extraction adsorbents for aqueous sample extraction, and the further development and characterisation of HPLC systems using elution solvents which are compatible with the requirements of immunoassay. Aprotic solvents have the advantage of being excellent solvents for PCDD/Fs but mediocre solvents for the aliphatic hydrocarbons which frequently accompany PCDD/Fs in far higher concentrations. The utility of such methods would be low unless immunoassay sensitivity were improved more than ten-fold from the present best. However, this level of improvement is entirely possible, so radically different extraction methods should not be discounted.

There is a need for simplified sample cleanup specifically designed for use with immunoassay in order to maximise the utility of a screening test. Work in this area should be directed toward simplification of the current cleanup in a manner that can be packaged more conveniently, but will still remove the inferferences typically found in gaseous emissions and effluent samples. HPLC offers one such route.

5.3 Quality Assurance

It is essential to accommodate adequate QA into an immunoassay which will ultimately be used to inform decisions relating to regulatory control of processes. It is therefore critical to determine an acceptable QA approach as a priority. The format of an immunoassay test can influence the performance of the antibody to a significant extent. An important goal of QA will be to clarify and to standardise as far as is possible an assay format relevant to the application of interest. The format should cover sample preparation, sample cleanup, quality control, assay procedure and data analysis.

5.4 Pilot Study

No field validation data have been generated for the sole I-TEQ screening approach described in the literature and currently marketed by EnSys. This approach appears to have potential, and there is a need to undertake a pilot study for validation of I-TEQ screening using field samples which have been conventionally extracted and cleaned. This can be coupled with the simultaneous development of a simplified sample cleanup which can be applied to conventionally prepared extracts prior to immunoassay analysis, followed by validation of the immunoassay I-TEQ screening test.

5.5 Coordination of Agency Programmes

In order to assist in the coordination of present and future projects in the field of immunoassay it is recommended that the Environment Agency explores means by which the exchange of information on research findings, antibody development, analytical procedures and field validation studies can be facilitated.

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Annex A **Immunoassay Research Activities**
Annex B **Dioxin Congener Profiles and Statistical Analysis**
Annex C **Published Data on Organic Emissions from Municipal and Chemical Waste Incinerator**

1. INTRODUCTION

1.1 Background

In March 1996 the Environment Agency commissioned Environmental Resources Management (ERM) to undertake consultancy contract No. HMIP/CPR2/41/1/256 entitled *Dioxin Markers Feasibility Study*. The objectives of the contract as described in the terms of reference were as follows:

(1) *To examine congener profile data of stack emissions samples from processes including those noted below to determine if any particular congener(s) is a suitable marker that could be used as a surrogate for the I-TEQ for that process. Data examined must be relevant to UK operated processes and where possible should include both new plant and old plant modified to meet current new plant standards.*

(a) emissions from municipal waste incinerators

(b) emissions from chemical waste incinerators

(c) iron and steel making processes

(2) *To examine congener profile data of discharge samples from [the manufacture of vinyl chloride monomer] to determine if any particular congener(s) is a suitable marker that could be used as a surrogate for the I-TEQ for that process.*

(3) *Use the data from (1) and (2) and analyse it statistically to draw conclusions on the significance of the results of the evaluation. If appropriate draw up a proposal for further work to verify the conclusions using an independent data set.*

(4) *Undertake a search of the world-wide literature to identify organisations who have the potential to develop antibodies for the measurement of dioxins.*

The objectives were addressed by the following tasks:

- The technical literature was accessed for reports on the development and application of immunoassay techniques. Research and commercial organisations in the UK, elsewhere in Europe, the US and Canada were contacted and discussions were held with personnel active in this area of research;

- The literature was accessed for data on dioxin releases from the processes of interest. This data was examined for trends in the profile of dioxin congeners, and whether marker congeners could be identified;

- The information obtained on immunoassay techniques was then applied to the releases of interest in order to assess whether a dioxin screening technique could be developed, and if so, the positive aspects and limitations of this procedure;

- Finally, the implications for sample collection, sample cleanup, quality control were assessed.

This report presents the outcome of the study.

1.2 Dioxins and Dibenzofurans

1.2.1 Structure and Composition

The term "dioxin" is often used to denote a family of compounds known chemically as *polychlorinated dibenzo-para-dioxins* (PCDDs) and *polychlorinated dibenzofurans* (PCDFs). Each compound comprises two benzene rings interconnected by oxygen atoms. In the case of PCDDs, the benzene rings are joined by two oxygen bridges, whereas in the PCDFs, the benzene rings are connected by a carbon bond and an oxygen bridge. *Figure 1.2a* depicts the basic structural formula of PCDDs and PCDFs, together with the numbering convention at the positions on the benzene rings where chlorine or other halogen atoms can be substituted.

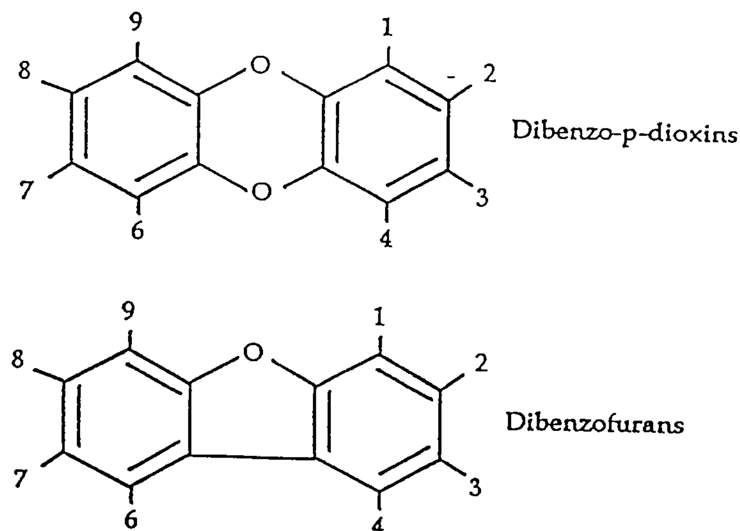


Figure 1.2a Basic Structure of PCDDs and PCDFs

There are 75 PCDDs and 135 PCDFs, each differing in the number and position of the chlorine atoms. Each individual PCDD or PCDF is termed a *congener* (210 in total), while groups of congeners with the same number of chlorine atoms are called *homologues*. The number of congeners in each homologue group is shown in *Table 1.2a*. The homologue groups are often abbreviated for convenience; for example, tetrachloro DDs and DFs are abbreviated to TCDDs and TCDFs, respectively, while the fully chlorinated octachloro congeners are abbreviated to OCDD and OCDF, respectively.

Table 1.2a Homologues and Congeners of PCDDs and PCDFs

Homologue (Abbreviation)	Number of Congeners	
	PCDDs	PCDFs
Monochloro (M)	2	4
Dichloro (D)	10	16
Trichloro (Tr)	14	28
Tetrachloro (T)	22	38
Pentachloro (Pe)	14	28
Hexachloro (Hx)	10	16
Heptachloro (Hp)	2	4
Octachloro (O)	1	1
TOTAL	**75**	**135**

1.2.2 International Toxic Equivalents

PCDD and PCDF congeners with chlorine atoms in the 2, 3, 7 and 8 positions are of particular environmental concern. Of the 17 PCDD and PCDF congeners with chlorine in the 2, 3, 7 and 8 positions, 2,3,7,8-TCDD is the most toxic, and by convention is assigned a toxicity rating (called a *Toxic Equivalent Factor,* or *TEF)* of 1.0. The remaining 2,3,7,8-positional congeners are then assigned lower TEFs, relative to that of 2,3,7,8-TCDD. The toxicity of a mixture of PCDDs and PCDFs, relative to 2,3,7,8-TCDD, can then be expressed by multiplying the concentrations of those 2,3,7,8-positional congeners present in the mixture by their respective TEFs. The resulting products are called *Toxic Equivalents (TEQs)*, with units identical to that in which the concentrations of the individual congeners are expressed. The TEQ of the mixture is obtained by summing the individual TEQs.

While a number of toxicity rating schemes have been developed, the scheme that has been internationally adopted is that of NATO/CCMS (1988), under which the TEFs are termed *International TEFs*, or I-TEFs. The I-TEFs for the seventeen 2,3,7,8-positional congeners of PCDDs and PCDFs are presented in *Table 1.2b*: all other congeners that may be present in a sample are assigned a TEF value of 0.0. The summation of individual TEQs for a mixture of PCDDs and PCDFs, using the international system, is termed the *International Toxic Equivalent* or I-TEQ of the mixture.

Table 1.2b International Toxic Equivalent Factors (I-TEFs)

Congener	I-TEF
2,3,7,8-TCDD	1
1,2,3,7,8-PeCDD	0.5
1,2,3,4,7,8-HxCDD	0.1
1,2,3,7,8,9-HxCDD	0.1
1,2,3,6,7,8-HxCDD	0.1
1,2,3,4,6,7,8-HpCDD	0.01
OCDD	0.001
2,3,7,8-TCDF	0.1
2,3,4,7,8-PeCDF	0.5
1,2,3,7,8-PeCDF	0.05
1,2,3,4,7,8-HxCDF	0.1
1,2,3,7,8,9-HxCDF	0.1
1,2,3,6,7,8-HxCDF	0.1
2,3,4,6,7,8-HxCDF	0.1
1,2,3,4,6,7,8-HpCDF	0.01
1,2,3,4,7,8,9-HpCDF	0.01
OCDF	0.001

1.2.3 Nomenclature used in this Report

Throughout this report, "dioxins" are referred to in their more correct nomenclature, as PCDDs, PCDFs, or as PCDD/Fs if the sample contains both PCDDs and PCDFs.

Concentrations of PCDDs and PCDFs in stack gases and effluent samples are given as total or individual congeners and homologues of PCDD/Fs, or as I-TEQs, depending on the manner in which they are reported in the technical literature. Most emission and environmental data post-1988 are generally reported solely as I-TEQs.

1.3 Structure of this Report

The remainder of this report is structured as follows:

- *Section 2* briefly reviews the principles of immunoassay tests and some of the applications to environmental samples;

- *Section 3* discusses the development of immunoassay tests for PCDDs and PCDFs;

- *Section 4* examines the PCDD/F profiles in emissions to atmosphere from municipal waste incinerators, chemical waste incinerators and iron and steel works and effluent from the VCM manufacturing process, and discusses the applicability of immunoassay testing to screen for PCDD/Fs in the above releases;

- *Section 5* examines the practical aspects of immunoassay as applied to stack and effluent sampling, sample preparation, sample cleanup and quality assurance.

- *Section 6* presents the study summary and recommendations.

- *Section 7* lists the references quoted in the report.

- *Section 8* provides a brief glossary of some of the key terms used in this report.

The report is supported by three annexes:

- *Annex A*, which provides details of organisations involved in PCDD/F immunoassay research and development;

- *Annex B*, in which the dioxin concentrations in the relevant releases and the results of the statistical analysis are presented in graphical and tabular form.

- *Annex C*, which lists organic emissions from municipal and chemical waste incinerators.

1.4 Acknowledgements

ERM is grateful for the assistance of Mr Christoph Lau and Dr Heidi Fiedler of the University of Bayreuth in performing the cluster analysis reported in *Section 4*, and to Symonds Travers Morgan for the provision of data of PCDD/F emissions from thermal release sources.

2. IMMUNOASSAY TECHNIQUES IN ENVIRONMENTAL ANALYSIS

2.1 Introduction

Section 2 commences with a brief discussion of the general principles of immunoassay and of its application to trace chemical analysis in environmental samples. Following this overview, the specific issue of immunoassay testing of PCDD/Fs in environmental samples is addressed in *Section 3.*

2.2 The Principles of Immunoassay

The mammalian immune system develops a natural response to the presence of a foreign virus, protein or chemical in the body. B-type and T-type lymphocyte cells within the body mount a defence against the threat from these so-called *antigens* by producing proteins called *antibodies* which recognise, bind to and hence immobilise the molecules of the antigen. Thus, immunisation against diseases involves the introduction into the body of a benign form of the disease agent, inducing a targeted immunological response in the body. The antibodies generated circulate in the body and confer protection against infection from the foreign agent by seeking, binding and immobilising the agent.

In the technique of immunoassay, the antibodies generated as the result of an immune response to a target chemical are isolated and removed from the host mammal. The antibodies are then used in the laboratory as an external analytical agent for the detection and quantification of that target chemical in other media such as water, soil, foods, biota, etc. Early development of the technique as an analytical tool focused on applications in the clinical laboratory (for example, Yalow and Berson, 1959). Many of these successful immunoassay formats have subsequently been transferred directly from the clinical laboratory to environmental analysis (Vanderlaan *et al*, 1991). In a significant development by Catt and Tregear (1967), antibodies were made to adhere to a solid surface such as polystyrene or polypropylene at pH 9-10. This discovery helped to popularise immunoassay methods since the antibody reagent could be adsorbed onto preformed, mass produced test apparatus such as tubes and trays. This led to the commercial production and marketing of immunoassay reagents in the form of test kits, increasing the ease with which environmental analyses could be performed.

2.3 The Immunoassay Process

2.3.1 Developing a Target Antibody

Immunoassay techniques developed for the analysis of trace environmental chemicals are based on the production of antibodies that can target the chemical or class of chemicals of interest. A preparation of the chemical of interest is injected into mammals such as mice or sheep in doses that are sufficient, over a period of time, to stimulate the immune system into producing antibodies that respond to the target chemical. After a period of immunisation the antibody-secreting lymphocytes are removed by sacrificing or bleeding the animal: in the latter case the resulting serum is called *polyclonal antisera* and contains a broad population of antibodies. The lymphocytes are then grown in an appropriate culture medium in the laboratory, producing *antibody clones*. Conventional cultures contain a mixture of antibodies called *polyclonal antibodies* in which the desired antibody is diluted in a mixture of other antibodies essential for the general defence of the host mammal but irrelevant to the target chemical. Screening of the clones for antibodies specific to the target chemical and subsequent culture of the selected antibodies produces a single strain of cloned cells called *monoclonal antibodies*. Whereas the polyclonal mixture can vary over time in the test animal and can also vary from animal to animal, the monoclonal antibody is a uniform, invariant reagent that may be widely distributed, easily standardised and incorporated into regulated analytical methods (Vanderlaan *et al*, 1988). In the context of immunoassays for PCDD/Fs, monoclonal antibodies are generally preferred to polyclonal antibodies owing to the greater specificity and reproducibility of tests designed with the former type of antibodies.

However, a small molecule such as a PCDD or PCDF congener may not elicit an immunological response by itself. This type of chemical is called a *hapten*. An immune response to a PCDD/F congener is obtained by linking or "conjugating" an analogue of the hapten (ie a molecule structurally and chemically similar to the hapten) to a larger carrier protein molecule. An analogue molecule has to be synthesized which mimics the structure of the chemical to be analysed and which also contains an active site to which the carrier protein can be attached via a linkage. In the case of the 2,3,7,8-TCDD hapten a common analogue is 1-amino-3,7,8-TrCDD. Referring to *Figure 1.2a*, this analogue is characterised by chlorine atoms at the 3, 7 and 8 positions, and an amino (-NH) functional group at the 1 position. Typically, this analogue would then be conjugated to bovine, mouse or rabbit serum albumin carrier protein via a chemical linkage (for example, an adipamino linkage) attached to the nitrogen in the amino functional group.

The synthesized antigen is then introduced into the host mammal in order to elicit an immune response. Antibodies are produced in the host mammal that may recognise the hapten as well as the carrier protein, the linkage or sites representing both molecules (Harrison *et al*, 1991). These antibodies are carefully evaluated for specificity to the hapten. The selected antibodies are cloned in a culture medium to produce the necessary monoclonal antibodies.

2.3.2 Immunoassay Test Methods

The general principles of an immunoassay test method are as follows:

- The prepared antibody (usually monoclonal) is contacted with the test sample containing the antigen subject to analysis (ie the "analyte" - in the present case, single or multiple PCDD and/or PCDF congeners).

- The antibody is also contacted with a "labelled" antigen in a reactant solution. This label can be a radionuclide, a substance that can fluoresce, an enzyme that can be stimulated to produce a colour change, etc.

- The analyte in the test sample and the labelled antigen compete for binding sites on the antibody. The quantity of the labelled antigen that binds to the antibody is in inverse proportion to the quantity of analyte present in the test sample.

- After washing out the unbound analyte and labelled antigen, the bound antibody/analyte/labelled antigen complex is reacted with a second reagent that develops the properties of the label (ie radioactivity, fluorescence, luminescence, intensity of colour, etc). This response is measured.

- By calibrating against a series of test solutions, the intensity of the labelled response can be related to the quantity of analyte present in the sample. The response is inversely proportional to the amount of analyte in the sample.

A large number of immunoassay techniques have been developed, depending on the characteristics of the label attached to the antigen. Thus, a radioisotope label results in a *radioimmunoassay* (RIA) test, while other labels result in a *fluorescence immunoassay* (FIA), and a *luminescence immunoassay* (LIA). The test kits developed for PCDD/F analysis are based on a technique known as *enzyme immunoassay* (EIA) or *enzyme linked immunosorbent assay* (ELISA). Since this technique currently dominates the environmental immunoassay market, it will be described briefly in the following section.

2.3.3 Enzyme Linked Immunosorbent Assay (ELISA)

In ELISA, the analyte hapten (say, a PCDD congener) is linked to an enzyme such as horseradish peroxidase. The labelled hapten is then made up into a reagent solution (see *Figure 2.3a*).

In the most common immunoassay format (called *competition ELISA)*, a limited amount of antibody is immobilised in the form of a coating on the walls of a plastic tube or onto the walls of rows of wells mounted on a plate. The sample solution with the unlabelled analyte is then added, followed by the reagent solution. The unlabelled analyte in the test solution and the labelled hapten (ie the labelled analyte) in the reagent solution compete for the limited number of binding sites on the antibody coating. After a period of incubation and equilibration, the tube is washed free of unbound analyte, retaining the free and labelled analyte molecules which bind to the antibody.

In the final stage the amount of labelled hapten bound to the antibody is determined by introducing a solution of a "substrate" such as o-phenylenediamine, and then a solution of a "chromogen". The bound enzyme catalyses the reaction between the substrate and the chromogen to produce a colour. After a period of development, the reaction is stopped by introducing a dilute acid which destroys the enzyme. The intensity of the colour is determined by comparing the sample with a test card, or by using a spectrophotometer set at the appropriate wavelength. The intensity of the colour response from the test solution is inversely proportional to the amount of (unlabelled) analyte present in the sample solution.

The immunoassay test for a particular analyte is calibrated by taking prepared solutions containing different concentrations of the analyte and the labelled hapten through the test procedure. The resulting *competitive inhibition curve* is used to interpolate colour intensity readings for test sample. An example of a inhibition curve is given in ***Figure 2.3b***. At one extreme, the presence of a negligible amount of the free analyte in the test solution will result in the maximum binding of the labelled hapten to the immobilised antibody (ie 0% inhibition, or I_0). At the other extreme the presence of a large excess of the free analyte will result in a very low proportion of the labelled hapten to bind with the immobilised antibody (ie approaching 100% inhibition, or I_{100}). Because of the sigmoidal shape of the curve, the area in the region of 50% inhibition (ie I_{50}) is best suited to quantification of the free analyte in the test solution.

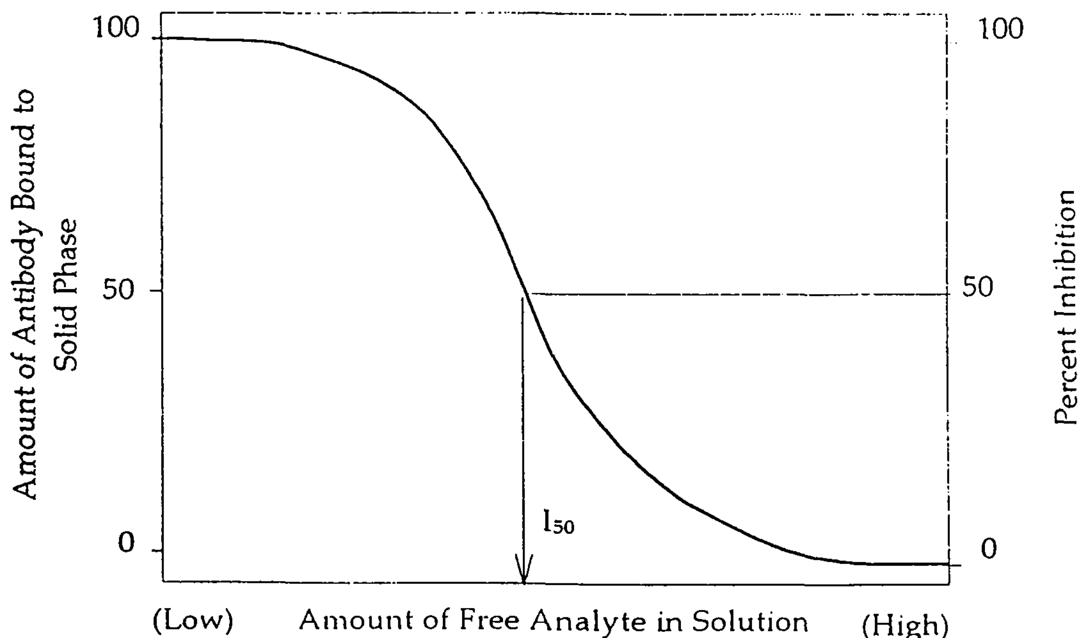


Figure 2.3b Percent Inhibition of Enzyme Binding to the Solid Phase Caused by the Presence of Free Analyte

2.4 Characteristics of Environmental Immunoassays

Some of the advantages and disadvantages of immunoassays are summarised in *Table 2.4a* (Sherry, 1995).

Table 2.4a Some Advantages and Disadvantages of Environmental Immunoassays

Advantages	Disadvantages
Sensitive and specific	Development can be costly
Rapid and easy to use	Hapten synthesis can be difficult
Cost-effective	Can be vulnerable to cross reactions with similar chemical species
Small sample size is generally adequate	Vulnerable to non-specific interferences from unrelated chemical species
Aqueous/soluble samples easy to handle	Non-aqueous media difficult to handle
Wide applicability	Requires independent confirmation
Potentially reduced sample preparation	Conservative attitudes towards new methods
Simultaneous analysis of multiple samples	Not suited to multi-residue determinations
Ideal for large numbers of samples	Not suited to small sample loads
Suitable for field use	

The key advantages of immunoassays have been recognised in the earliest environmental applications - the ability to handle large numbers of samples simultaneously, the relative simplicity of sample work-up and application of the assay, and the cost benefits that accrue from the above characteristics. However, it is important to appreciate the potential limitations of the assay, the most important of which are the following:

- *Sample screening*: If the analyst wishes to benefit from the cost and operational advantages of immunoassays, then the technique is best suited to screening of environmental samples for a particular analyte as opposed to a determination of an absolute concentration. We define a screening test as essentially a go/no-go test in which the absolute concentration of an analyte in a sample is of less importance than whether the concentration lies above or below a set point. Samples that test negative (ie the concentration of the analyte is below the set point) are usually not taken forward for more detailed analysis, save perhaps for a small proportion for the purpose of quality control. Samples that test positive in the screening test are all taken forward for detailed confirmatory analysis and quantification of an absolute concentration.

- *Cross reactivity*: While antibodies are designed so as to exhibit specificity towards the target hapten, the former also recognises chemicals with similar structures, shapes and functional groups. Thus, an anti-2,3,7,8-TCDD antibody may recognise (and therefore bind with) other 2,3,7,8-positional congeners, other halogenated organics such as PCBs and in particular could also recognise the analogue molecule (for example, 3,7,8-TrCDD). These interferences can be non-specific and not amenable to prior characterisation in an unknown sample.

- *External validation*: Analytical techniques such as gas chromatography/ mass spectroscopy (GCMS) can be validated by the introduction of internal standards at various stages of the sampling, clean-up and analytical procedure. For example in PCDD/F analysis, a ^{13}C PCDD/F tracer can be introduced on the filter of the stack sampler, and on the clean-up column when the sample is worked up in the laboratory. This tracer is carried through to the final analysis by GCMS and enables quantification of the sampling and clean-up efficiency, which can be corrected for in the final result. However, an immunoassay cannot differentiate between a ^{13}C PCDD/F tracer and a ^{12}C PCDD/F congener in the same sample, and therefore external validation and calibration is required before the test is applied to field samples.

- *Aqueous matrix*: In the context of PCDD/F analysis, an important issue is the matrix in which these chemicals are presented to the immunoassay test. Antibody-antigen interactions require an aqueous medium to effect the binding, whereas current PCDD/F stack sampling and sample preparation techniques employ organic solvents such as hexane or methylene chloride. This issue is discussed further in later sections.

The relevance of these issues to PCDD/F immunoassays is discussed in *Section 3*.

2.5 Commercial Applications of Immunoassay to Environmental Analysis

Immunoassay kits are available for a variety of environmental pollutants. Early development focused on pesticides, and this class of chemicals remains the most studied in terms of commercial applications, though the range of trace chemicals is being gradually extended. *Table 2.5a* lists a selection of the commercially available kits (Knopp, 1995). Because PCBs might contribute up to one third of the entire potential toxicity of dioxins and related compounds (US EPA, 1994) it should also be noted that an immunoassay has been developed for the most toxic of the PCB congeners (Carlson *et al*, 1995). The table *excludes* PCDD/F kit manufacturers; these kits are discussed in *Section 3*. Details of the manufacturers are given in *Annex A*.

It should be noted that Millipore's immunoassay division (Immunosystems Incorporated) was recently acquired by EnSys. The latter are currently evaluating the Millipore range of products, and specifically whether to assume rights to the Millipore dioxin test sold as part of their EnviroGard product line of environmental immunoassays. Since the acquisition of EnviroGard is as yet not widely known, we have retained the name of Millipore in *Table 2.5a*.

The main UK manufacturer of immunoassay kits is Guildhay Limited, based at the University of Surrey at Guildford. The company is currently marketing ELISA kits for the analysis of pesticides such as Atrazine and Isoproturon in water (Watts and Hegarty, 1995; Guildhay, 1996). Most other commercial UK-based immunoassay companies perform primarily sales and technical support functions for US organisations (EnSys, Biocode, etc).

Table 2.5a A Selection of Immunochemical Test Kits for Environmental Pollutants[a]

Compound(s) Detected	Manufacturer
Acetochlor	Millipore (P)
Alachlor	Baker (T), Millipore (P,T), Idetek (P)
Aldicarb	Baker (T), Millipore (P,T)
Altrazin (triazines)	Baker (T), Millipore (P[b],T), R Biopharm (P), Riedel de Haen (P), Idetek (P[b]), Guildhay (P)
Benomyl/MBC	Idetek (P)
Bioresmethrin	Millipore (P)
Captan	Baker (T)
Carbaryl	Baker (T)
Carbendazim	Baker (T)
Carbendazim/benomyl	Millipore (T)
Carbendazim/MBC	Millipore (P)
Carbofuran	Baker (T), Millipore (T)
Chlorodane	Millipore (T)
Chlorpyriphos	Baker (T)
Chlorpyriphos-ethyl	Millipore (P)
Chlorpyriphos-methyl	Millipore (P,T)
Chlorothalonil	Baker (T), Idetek (P)
Cyclodiene	Millipore (P,T)
2,4-D	Baker (T), Millipore (P,T)
DDT	Millipore (T)
Diazinon	Millipore (P)
Fenitrothion	Millipore (P,T)
Imazaquin	Idetek (P)
Imazapyr	Millipore (P)
Isoproturon	Millipore (P), Guildhay (P)
Metalaxyl	Millipore (P)
Methoprene	Millipore (P,T)
Metolachlor	Baker (T), Idetek (P)
Metsulfuron-methyl	Millipore (P)
Paraquat	Baker (T), Millipore (P)
Parathion/parathion-methyl	Millipore (P)
Primiphos-methyl	Millipore (P)
Procymidone	Millipore (P,T)
Toxaphene	Millipore (T)
Triasulfuron	Millipore (P)
Triclopyr	Baker (T)
Trifluralin	Idetek (P)
Urea herbicides	Millipore (P)

Compound(s) Detected	Manufacturer
BTEX	Baker (T), Millipore (P), Idetek (P)
Pentachlorophenol	Baker (T), Dräger, Ensys (T), Millipore (T)
Petroleum hydrocarbons	Dräger, Ensys (T), Millipore (T)
PCB	Baker (T), Dräger, Ensys (T), Millipore (T)
PAH	Baker (T), Dräger, Ensys (T), Millipore (T), Idetek (P)
TNT	Millipore (P,T), R-Biopharm (P)
Mercury	BioNebraska[b]

P=plate kit, T=tube kit.

[a] If not stated otherwise, test-kits are based on polyclonal antibodies. Adapted from Knopp, 1995. Millipore Immunosystems Inc has recently been acquired by EnSys.

[b] Monoclonal antibody based test kit available.

2.6 Regulatory and Industry Promotion of Immunoassay Techniques

There is increasing interest in promoting the acceptance and use of immunoassay techniques in environmental analysis. Four examples of initiatives in this area are given below.

2.6.1 Association of Official Analytical Chemists (AOAC)

The Association of Official Analytical Chemists is an international professional Association headquartered in the US, which approves and publishes in a quasi-regulatory capacity Methods of Analysis, principally for pesticide residue analysis. However, the Association has expressed an interest in environmental immunoassay and have recently validated their first pesticide immunoassay (developed by Ohmicron - see *Annex A*) for analysis of atrazine in water. The AOAC Research Institute was formed in 1993 to perform impartial evaluations of commercial immunoassay kit products. The AOACRI programme is presently focusing on mycotoxin immunoassays, but may begin evaluating tests for pesticides later in 1996. Tests for industrial wastes will probably not be considered in the near future.

2.6.2 Analytical Environmental Immunoassay Consortium (AEIC)

The AEIC is a consortium of about 30 industry and research groups with an interest in the advancement of immunochemical technology in the environmental field. The consortium does no research and has no regulatory authority, nor is it linked with professional associations or regulatory bodies. It is primarily a forum for exchange of information and for lobbying and educating regulators and users of the technology. Although the current secretary is based in the US, the AEIC has an international perspective and will be holding a meeting in Europe in 1997. The consortium has a strong role in identifying and publicising important issues in the environmental immunoassay field. The consortium holds regular technical meetings which cover a broad range of issues and research of current interest to consortium members and others in the environmental immunoassay community.

The AEIC is currently working with the US EPA to develop validation guidelines for environmental applications of immunochemical methods. The AEIC is also working towards establishing voluntary performance standards for immunoassay kits, and in the development of guidelines for quality control.

2.6.3 United States Environmental Protection Agency (US EPA)

The US EPA has commenced active investigation of the potential use of immunoassay technology for environmental monitoring, principally through the Methods Section of the Office of Solid Waste (OSW). As of mid-1995, OSW had completed validation of ten immunoassay methods utilising approximately 15 kits, and was in the final stages of validating several new methods and additional kits for existing methods (Lesnik, 1995). The validated kits cover immunoassays for the following chemicals: PCP, 2,4-D, PCBs, Total Petroleum Hydrocarbons, PAHs, Toxaphene, Chlordane, DDT, TNT and RDX explosives. Environmental media cover soil, water and oil. Each validated method has been assigned a formal Method number (eg Method 4020 for PCBs in soil and oil). A draft number, Method 4025, has been assigned to proposed PCDD/F immunoassay testing (Lesnik and Fordham, 1995).

2.6.4 The UK's Environment Agency (EA)

In addition to the present contract, several desk studies relating to immunoassay test kits have been conducted by the Environment Agency. The following information has been obtained from the project data sheets supplied by the Environment Agency:

- A nine month study, conducted in 1993, focused on the use and application of field test kits for analysis of pollutants in the aquatic environment such as pesticides, trace metals, BOD and ammonium (but excluding PCDD/Fs). The majority of these kits relied on simple colour chemistry followed by subsequent photometric determination, but immunoassay techniques were also considered. Details of suppliers (UK and worldwide), research activities and technology limitations were reported as well as their applicability to the needs of the regulatory body (NRA). Conclusions of the study indicated that the use of these kits should be expanded since they were a cheap means of testing providing that operatives knew which pollutant they were analysing for. Other types of instrumentation were also considered including portable instrumentation, on-line chemical analysers and portable GC-MS systems.

- A six month study (Feb-Aug 1996) was undertaken by the Robens Institute on behalf of the Environment Agency. The focus of the study was to investigate laboratory-based ELISA test kits commercially available in the UK, primarily for the detection of pesticides, and to assess immunoaffinity techniques as a means of sample preparation for subsequent chromatographic analysis. Over seventy compounds were identified as being capable of detection with suppliers of test kits including EnSys, Millipore, J T Baker (Ohmicron) and Guildhay. The study's conclusions indicated that test kits were rapid with little sample preparation and gave reasonable results with relatively clean matrices such as water. Costs were advantageous where a large number of samples were being analysed for relatively few analytes.

Immunoaffinity techniques, although not available commercially did provide good results in the laboratory and were useful as a means of sample preparation.

- A shorter project which lasted 2-3 months (1995-96) provided a review of capabilities and strategies for biosensors as laboratory based analytical tools. The main area of interest was in the determination of pesticides, biocides and herbicides in the aquatic environment. Objectives of the report were to provide the NRA with principles of analysis, product availability, research activities and future technological developments. Conclusions indicated there were few biosensors available for analysis of environmental pollutants with little interest being shown for the development of biosensors for the detection of dioxins. Research activity is mainly being conducted in the UK, Japan, Germany and the US.

3. IMMUNOASSAY FOR PCDDS AND PCDFS

3.1 Introduction

PCDD/Fs have been the subject of attempts to develop immunoassays for 20 years, thus far with relatively limited research and commercial success. This is primarily due to a combination of factors which set these chemicals apart from other environmental contaminants. Other analytes such as PCBs, which have been successfully analysed by commercial kit immunoassays for several years, present a different set of problems to the researcher trying to develop and apply an immunoassay. The most fundamental of these differentiating factors is an extremely low water solubility of 20 ng l^{-1} for congeners such as 2,3,7,8-TCDD (Shroy, 1985), which is approximately 1000-fold lower than values for PCBs and PAHs and 1,000,000-fold lower than values for many pesticides. This single factor has significant downstream effects on the development and application of immunoassays, which are generally performed in predominantly aqueous media. The set of factors deriving from this low solubility affects all aspects of the process in some fashion, including the chemistry of hapten/analogue synthesis, the extraction of samples, the introduction of prepared samples to the immunoassay, and the handling of standards.

In addition, PCDD/F testing presents other analytical problems not seen with most other analytes. The concentration of extracts needed to meet typical sensitivity requirements may in turn dictate significant sample cleanup to avoid concomitant amplification of matrix interferences. Water miscible solvents of intermediate polarity, such as the methanol used for PCB and PAH immunoassay analysis, are unlikely to be acceptable for extraction of low concentrations of PCDDs and PCDFs from solid samples such as the filter in a conventional stack sampler.

This section commences with a summary of developments in antibody design for PCDD/F immuonassay. There follows a general discussion on the application of PCDD/F immunoassays to environmental analysis. The two commercially available immunoassay kits are then described. Finally, some issues concerning the application of PCDD/F immunoassays in the field are discussed.

3.2 Development of Antibodies for PCDDs and PCDFs

Early work in this area has been summarised by Sherry (1992). To date there have been five research groups whose efforts at PCDD antibody development have been reported in the open literature. One group also developed an anti-2,3,7,8-PCDF antibody using closely related hapten chemistry. In addition, there are at least two projects currently under way to produce new anti-PCDD antibodies for immunoassay and related uses. The relevant publications are summarised in *Table 3.2a.*

Table 3.2a **Summary of Antibodies Developed for PCDDs and PCDFs**

Reference	Immunogen Chemistry (Reference)	Antibody Source(*)	Specificity or suitability for immunoassay
1. Albro *et al*, (1979); Albro *et al* (1980)	Chae *et al* (1977)	rabbit pAb	Several PCDDs and PCDFs
2. Kennel *et al* (1986)	Kennel *et al* (1986)	mouse mAb	Unsuitable for PCDD/F analysis
3. Luster *et al* (1980)	Luster *et al* (1980)	rabbit pAb	Several PCDFs
4. Stanker *et al* (1987); Vanderlaan *et al* (1989)	Chae *et al* (1977)	mouse mAb	Several PCDDs and PCDFs
5. Kerkhoven *et al* (1993)	Chae *et al* (1977)	recombinant mAb	Data too limited for conclusion
6. Langley *et al* (1992)	Langley *et al* (1992)	mouse mAb	Unsuitable for PCDD/F analysis
7. EnSys (1995)	unknown	mouse mAb	Nearly specific for 2,3,7,8-TCDD
8. Gilman *et al* (1995); Gee *et al* (1995)	various	pAb & mAb	Currently under development
9. ECOCHEM (1995)	unknown	mouse mAb	Currently under development

(*) mAb = monoclonal antibody pAb = polyclonal antibody

The specificity of two of the resulting PCDD antibodies (*Table 3.2a*, Nos 2 and 6) was primarily for the immunising hapten-analogue, preventing their use for the analysis of PCDDs. One other antibody (No.7) is highly specific for 2,3,7,8-TCDD and is therefore inappropriate for TEQ screening. Only one other toxic PCDD/F congener tested is significantly crossreactive - 2,3,7,8-TCDF at 4.6%, while 2,3,7-TrCDD gave 20% crossreactivity. The antibody targeted for PCDF analysis (*Table 3.2a*, No. 3) showed specificity for certain PCDFs, including strong recognition for two non-2,3,7,8- congeners: 56% crossreactivity for 2,3,8-TrCDF and 20% for 2,3,6,8-TCDF. Antibodies Nos 1 and 4 used (1-adipamino-3,7,8-TrCDD) as the immunising hapten and demonstrated recognition of both PCDDs and PCDFs, with a rough correspondence between recognition and congener toxicity. One group (*Table 3.2a*, No. 5) attempted to modify specificity by making recombinant antibodies using site-directed mutations of an existing hybridoma genome (Stanker *et al*, 1987; Kerkhoven *et al*, 1993). A hybridoma is a new cell type obtained from the fusion of other cells. This research group provided very limited specificity data, which were indistinguishable from the commercial immunoassay (Harrison and Carlson, 1996) using the monoclonal antibody from the original hybridoma.

Assays 1-3 of *Table 3.2a* were radioimmunoassays, typically using tritiated or iodinated PCDD in the routine execution of the assay. In contrast, all the other tests have used safer and simpler enzyme labels, which probably sacrifices some sensitivity.

It should also be noted that in all of the cases in *Table 3.2a* where sufficient data were presented, including the only highly specific assay (No. 7), significant recognition was observed for congeners which are considered "non-toxic" because they do not contain the 2,3,7,8 chlorination pattern. Most of these are not reported individually (if at all) in a typical GCMS analysis and could therefore cause difficulties in immunoassay calibration. This will be discussed in more detail in *Section 3.5.4.* In none of these cases was the number of PCDD and PCDF congeners employed in cross reactivity studies sufficient to allow prediction of the behaviour of real samples in an immunoassay.

However, such a prediction was made for an assay system using the DD3 antibody (Stanker *et al*, 1987) in a different format (Harrison and Carlson, 1996). In this study, cross reactivity data were obtained for 12 of the 17 toxic congeners. *Table 3.2b* and *Figure 3.2a* summarises the cross reactivity data. In *Figure 3.2a,* the EIA response is the cross reactivity relative to 2,3,7,8-TCDD, as shown in *Table 3.2b.* The congener labels reflect the actual location of the corresponding data points. The (X = Y) line represents a perfect correlation between EIA response and the I-TEF. The correlation between the response of the immunoassay and the I-TEQ of the soil sample is given in *Figure 3.2b,* indicating the possibility of predicting the I-TEQ of the sample from the immunoassay response. Values for individual congener concentrations from GCMS analysis were used to calculate I-TEQ values for each of 43 soil samples from three sources. Each mass concentration value was multiplied by the corresponding EIA cross reactivity value from *Table 3.2b* to obtain a predicted EIA response for that congener. The predicted EIA responses for the individual congeners were then summed for each sample to give the predicted EIA response for the sample. Samples from each of the three sources are grouped by symbol. The regression equation for predicted EIA response against I-TEQ was (Y = 0.99X - 0.53) with a correlation coefficient of 0.988. Several samples below 1 ppt were used for the regression calculation, but do not appear on the plot.

3.3 Applications of PCDD/F Immunoassay to Environmental Samples

Immunoassay analysis for PCDD/Fs in environmental samples has been limited because of the few successful antibody development attempts, as shown in *Table 3.2a.* Some of these immunoassay application efforts are summarised in *Table 3.3a.*

Table 3.2b Cross Reactivity of the DD3 Antibody Compared to I-TEFs (from Harrison and Carlson, 1996)

Congener	I-TEF	Cross Reactivity
2,3,7,8-TCDD	1	1
1,2,3,7,8-PeCDD	0.5	0.95
1,2,3,4,7,8-HxCDD	0.1	0.12
1,2,3,7,8,9-HxCDD	0.1	0.092
1,2,3,6,7,8-HxCDD	0.1	0.24
1,2,3,4,6,7,8-HpCDD	0.01	0.079
OCDD	0.001	< 0.001
2,3,7,8-TCDF	0.1	0.27
2,3,4,7,8-PeCDF	0.5	0.55
1,2,3,7,8-PeCDF	0.05	0.033
1,2,3,4,7,8-HxCDF	0.1	0.017
1,2,3,7,8,9-HxCDF	0.1	0.01 (estimated)
1,2,3,6,7,8-HxCDF	0.1	0.01 (estimated)
2,3,4,6,7,8-HxCDF	0.1	0.01 (estimated)
1,2,3,4,6,7,8-HpCDF	0.01	0.01 (estimated)
1,2,3,4,7,8,9-HpCDF	0.01	0.005 (estimated)
OCDF	0.001	< 0.001

Cross Reactivity with Some Non-2,3,7,8 Positional Congeners

Congener	I-TEF	Cross Reactivity
1-MCDD	< 0.001	< 0.001
2-MCDD	< 0.001	< 0.001
2,3-DCDD	< 0.001	0.021
2,7-DCDD	< 0.001	0.004
1,2,3-TrCDD	< 0.001	0.0013
1,7,8-TrCDD	< 0.001	< 0.02
2,3,7-TrCDD	< 0.001	0.14
1,2,3,4-TCDD	< 0.001	< 0.001
1,2,7,8-TCDD	< 0.001	0.14
1,2,3,4,6,7,9-HpCDD	< 0.001	< 0.001

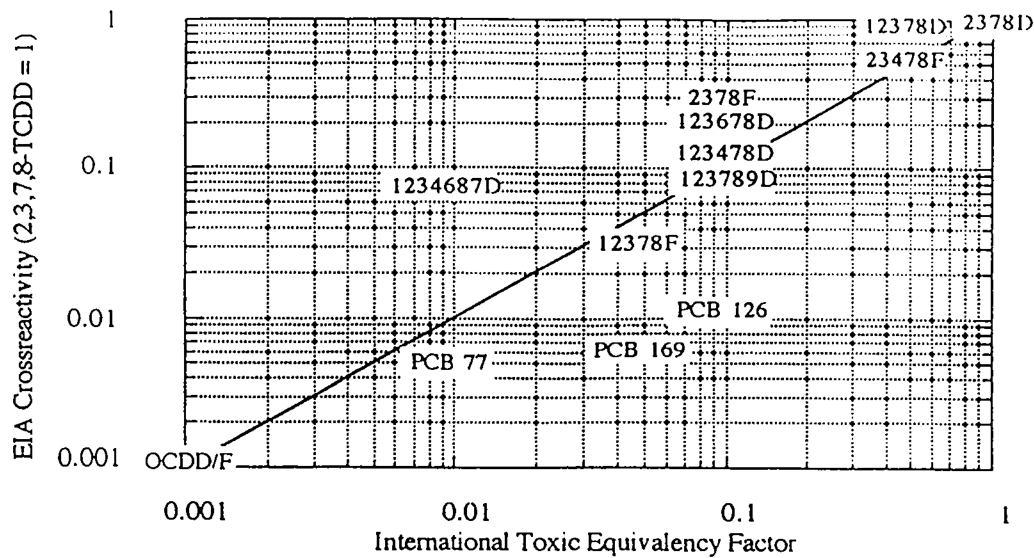


Figure 3.2a **Relationship between EIA response and I-TEF factors for the most toxic PCDD/F and PCB congeners (Harrison and Carlson, 1996).**

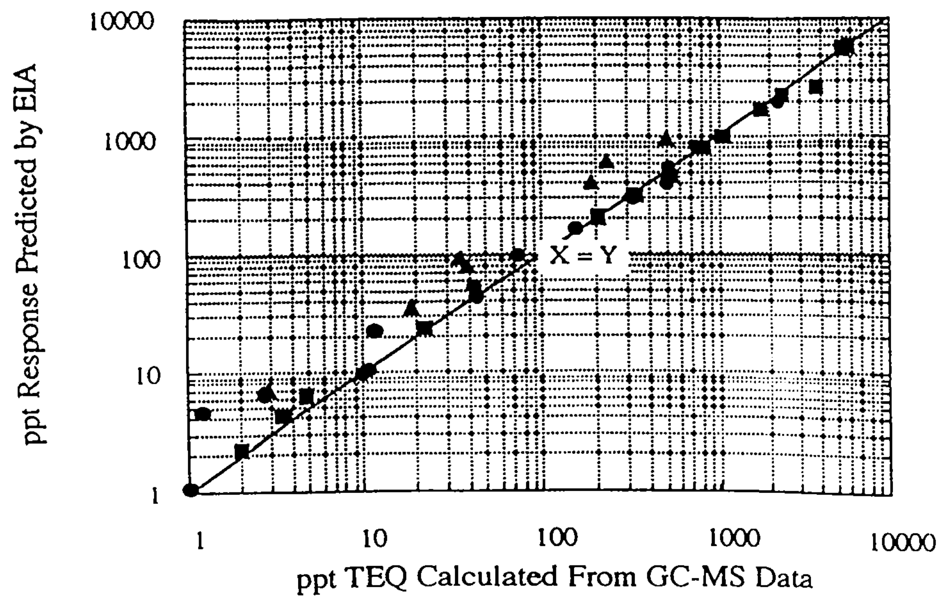


Figure 3.2b **Validation of the I-TEQ screening concept on 43 soil samples (Harrison and Carlson, 1996)**

Table 3.3a Applications of PCDD/F Immunoassays to Environmental Matrices

Research Group	Sample Matrix and Assay Type	Extraction, Solvent Clean-up and Sample Presentation
Sherry *et al* (1989); Albro *et al* (1979)	Fish tissue (RIA)	HCl/toluene; various combinations of chromatography steps; detergent or DMSO
Stanker *et al* (1987); Vanderlaan *et al* (1988)	Oil (EIA)	Dissolve in dichloromethane/cyclohexane; reactive and activated carbon chromatography; detergent
Stanker *et al* (1987); Vanderlaan *et al* (1988)	Still bottoms, fly ash, and soil (EIA)	Acid treat before hexane extraction; activated carbon and reactive chromatography; detergent
Stanker *et al* (1987); Vanderlaan *et al* (1995)	Still bottoms, fly ash, and soil (EIA)	Acid treat before toluene extraction; activated carbon and reactive chromatography; detergent

RIA = radio immunoassay, EIA = enzyme immunoassay

There is little practical difference in handling between the matrices of *Table 3.3a*. Because of the combination of matrix concentration and high sensitivity demands, immunoassay methods have not been able to escape the need for significant cleanup requirements. It is necessary for the analyst to balance sensitivity against cleanup needs, perhaps differently for each sample matrix or project. Sherry (1989) observed that the working range of the immunoassay is determined by the compromise between sample size and the degree of cleanup required. It is clear from prior work in the area (Sherry, 1989; Vanderlaan *et al*, 1988) that it may be possible to significantly reduce the need for sample cleanup in PCDD/F immunoassay. However, because of the variations among different sample matrices, different immunoassays, and different sensitivity levels even for one assay, this problem is likely to resist broad solutions for the immediate future.

Using simplified sample cleanup schemes, Sherry (1989) determined that residual hydrophobic substances reduced the assay sensitivity (false negative results). Harrison and Carlson (1996) demonstrated similar behaviour in the analysis of crude soil extracts by immunoassay. This problem highlights the distinction between biological lipids and hydrocarbons such as oils or PAHs. Most lipophilic material in a biological sample contains a polar moiety which can react during the acid treatment or reactive chromatography step of PCDD/F sample cleanup, allowing simple separation from the highly nonpolar PCDD/Fs. However, residual hydrocarbons lacking a polar group require the full range of chromatographic cleanup steps for complete removal (Smith *et al*, 1984; Vanderlaan *et al*, 1988; Vanderlaan *et al*, 1995).

3.4 Commercial PCDD/F Immunoassay Kits

Only two immunoassay kits for dioxin analysis have reached the market: the kits developed by EnSys and by Millipore respectively. These are described below. Both systems require further development in terms of simplified sample cleanup procedures and a robust validation study using conventional sample cleanup and analytical methods.

3.4.1 The EnSys Kit

The kit sold by EnSys is highly specific for 2,3,7,8-TCDD. However, the test has 4.6% cross reactivity for 2,3,7,8-TCDF, 20% for 2,3,7-TrCDD, and 2.2% for 2,3-DCDD. Cross reactivity data available for other toxic congeners indicate a high level of specificity which renders the kit unsuitable for toxicity based screening unless the samples are known to contain only 2,3,7,8-TCDD. The test is formatted as a rapid test using antibody-coated tubes and has similar equipment requirements and assay procedure to EnSys' other kits. The limit of detection is approximately 80 pg/tube (2,3,7,8-TCDD). No novel or rapid sample extraction or cleanup system has been described by EnSys. Analysts are directed to use approved sample preparation schemes, but there appears to be no published data comparing the kit to conventional methods and no specific matrices are supported. While actual kit sales are unknown, they appear to be very low.

In addition to being used in kits, the antibody is being offered for sale separately by Fitzgerald International Industries, Inc. of Concord MA, USA.

3.4.2 The Millipore Kit

The tube kit released by Millipore uses the DD3 antibody (Stanker *et al*, 1987) immobilized on polystyrene tubes. The test is formatted as a rapid tube test like the other kits in the EnviroGard product range, with very similar equipment requirements and assay procedure. The limit of detection is approximately 100 pg/tube (2,3,7,8-TCDD), which allows theoretical sensitivity of 1 ng g^{-1} in a solid sample with no extract concentration or 100 pg g^{-1} with a 10-fold concentration of the sample extract. This kit exhibits good correlation between cross reactivity and toxicity (see *Table 3.2b* and *Figure 3.2a* above) and is therefore appropriate for toxicity-based screening. The TEQ screening concept has been examined theoretically by using 43 soil samples to compare predicted immunoassay values to GCMS based TEQ values (Harrison and Carlson, 1996).

No novel or rapid sample extraction or cleanup system has been developed for use with the kit. However, preliminary data using crude acetone:hexane extracts of soil, exchanged to methanol, indicate that typical soils interfere significantly in the test (Harrison and Carlson, 1996). These results suggest that some sample cleanup will be needed to attain the theoretical sensitivity offered by the test. No data are available comparing the kit to GCMS using conventional sample preparation methods with field samples.

A microplate version of the test, also based on the DD3 antibody, has been prepared and partially characterised. Because full specificity data have not been developed for the microplate test, it is not possible to conclude whether it is as appropriate for TEQ screening as the tube kit. However, the improvement in sensitivity to 25 pg/well could offer significant advantages over the tube test (Harrison and Carson, 1996). This system theoretically would allow detection of 250 pg g^{-1} in a solid sample with no extract concentration or 25 pg g^{-1} with a 10-fold concentration of extract.

The Millipore kits are now under evaluation by EnSys following their purchase of the EnviroGard product line. The test is subject to two relevant licences from FMS and ECOCHEM (see *Annex A*).

3.5 Characteristics of Immunoassay for PCDD/Fs

3.5.1 Introduction

A summary of the strengths and limitations of immunoassay screening for PCDD/Fs is given in *Table 3.5a.*

Table 3.5a Strengths and Limitations of Immunoassay Screening for PCDD/Fs

Strengths	Limitations
Sensitivity	Sensitivity not fully competitive with GC-MS methods. Sensitivity may vary according to environmental medium/matrix.
Speed	Speed is reduced if significant sample cleanup is required.
Simplicity	Probable use of incompatible solvents for sample extraction. Probable need for solvent exchange matrix concentration may be required for adequate sensitivity.
Low cost	Cost is increased if sample cleanup is required.
Parallel processing	Sample preparation less likely to offer parallel processing.
Group recognition of toxic congeners	Recognition of nontoxic congeners ignored by GCMS method. Inability to define level for any specific congener. Need for partial GCMS confirmation to support interpretation.
Potential for TEQ screening	Requires validation by comparison to GCMS-analysed samples. Requires calibration for correct interpretation of results.
Possible use of GCMS extract	Potential conflict with GCMS internal standard protocol.

The commonly recognised ***general strengths*** of immunoassays, such as speed, simplicity, low cost, and parallel processing of many samples, can be applied to any PCDD/F immunoassay if it is correctly configured. The commercial kits described above may not necessarily be designed to maximise these advantages. Further, these advantages may be partially neutralised if the sample processing requirements are too extreme.

The most commonly observed ***limitation*** of immunoassay is that the compound signal is comprised of responses to all of the individual responding analytes. This is discussed in more detail in *Section 3.5.3*. Another general limitation is the requirement for validation against an appropriate set of field samples before screening use can proceed with confidence. Some level of confirmation by conventional methodology will also be required as part of the ongoing quality assurance program. These issues are discussed in more detail in *Section 3.5.4*.

3.5.2 Sample Preparation

In general, current conventional PCDD/F analysis utilises exhaustive extraction with toluene, hexane, hexane:acetone, or a similarly nonpolar solvent (Clement, 1991; Firestone, 1991). The resulting extract, regardless of original sample matrix or extraction method, is then subjected to some variation of multi-column reactive chromatography cleanup (Smith *et al*, 1984) prior to GCMS analysis. One or more solvent exchanges are generally required during sample preparation because of the different solvent requirements at different stages in the procedure.

Conventional methods of PCDD/F analysis demonstrate significant variation among sample matrices due to the very different physical and chemical natures of these matrices. The tendency of PCDD/Fs to bind very tightly to surfaces and organic material requires careful design of extraction and clean-up techniques. The extreme chemical stability of PCDD/Fs allows the use of acid treatments or reactive chromatography for destruction and removal of interfering materials. Matrices such as fly ash, may require acid digestion to disrupt the physical structure of the sample before extraction. This is necessary for complete extraction of PCDD/Fs which may have been physically entrapped by particle aggregation during the ash formation process.

The generally simple sample preparation for established environmental immunoassays is responsible for a large part of their appeal. The typical brief extraction of a solid sample with methanol is rarely expanded by anything more complex than the addition of sodium sulfate for drying wet samples. The extraction solvent may be methanol, isopropanol, alkaline methanol, or even methanol/water in some cases, but the processing is approximately the same for all common analytes and kit manufacturers. The crude extract is filtered and added directly to the immunoassay (with dilutions in the appropriate solvent as needed). Current kits typically tolerate 5% to 20% of methanol or other solvent added directly to the test. Water analysis is even simpler for most analytes. Petroleum fuels, pentachlorophenol and most pesticides require no sample treatment and are analysed by adding the water sample directly to the immunoassay. Less polar analytes such as PCB or PAH, which adsorb more strongly to suspended solids in the water sample, can still be analysed directly after dilution of the water sample with methanol to cause analyte desorption.

3.5.3 Sensitivity, Sample Preparation and Matrix Interferences

These three factors will be treated together because they are not completely independent in practice. While sensitivity is usually an important advantage of environmental immunoassays, this is not the case for PCDD/Fs because of the lack of antibodies of superior affinity. The ability of a PCDD/F immunoassay to capture the advantages described above is contingent on the system having adequate sensitivity to meet the analytical goals. Presently available antibodies may not meet those goals if sensitivity requirements are too aggressive. Sherry (1995) observed that during their development of sample preparation methods for use with a PCDD immunoassay, the analytical requirements were extended to beyond the capability of the immunoassay. Thus, it is critical at the beginning of a project to clearly define the sensitivity goals.

The only current commercial system capable of TEQ screening (Harrison and Carlson, 1996) can detect 100 pg/tube. As noted in *Section 3.4.2*, in a soil matrix this allowed a theoretical sensitivity of 1 ng g^{-1} in the original solid sample with no extract concentration or 100 pg g^{-1} with a 10-fold concentration of the sample extract. The potential improvement in sensitivity offered by the microplate version of the assay would allow detection of
25 pg g^{-1} with additional sample preparation. While lower detection limits than this may be found in the literature, these values are typically from procedures which are not suitable for routine monitoring programs, due to impracticalities such as the use of radiolabels.

In some environmental immunoassays such as Ohmicron's PCB test (sold in Europe by J T Baker) and Millipore's PAH test, matrix interferences are minimised by the extract dilution that is allowed by a very sensitive antibody. In the case of PCDD/F immunoassay, because of the low levels of analyte typically present in a stack gas or effluent sample, dilution is unlikely to present a practical solution, and matrix effects can be expected even when using a very sensitive antibody. Once the required method sensitivity is established, the limit of detection observed for the immunoassay can be used to determine the matrix concentration factor required. Only when this concentration factor is known can the matrix interferences be evaluated, as they vary according to the concentration factor.

Because of the fundamentally different operating principles of GCMS and immunoassay, the interferences of one method may be irrelevant in the other method. The literature addressing this issue is virtually non-existent. In developing sample preparation methods specifically for immunoassay use, it is crucial to appreciate this principle and to understand which factors which may uniquely affect the immunoassay method. Immunoassays for other environmental chemicals such as PAH and PCB, have avoided this issue by extracting with a polar solvent such as methanol, followed by direct analysis of the extract. The ability to deal effectively with both analytical systems independently will be critical to any successful attempt to develop an immunoassay specific rapid sample preparation system.

3.5.4 Specificity

Cross reactivity patterns of the available PCDD/F immunoassays have been discussed briefly in *Section 3.2* and are described in detail by Albro *et al* (1979), Luster *et al* (1980), Stanker *et al* (1987), EnSys (1995) and Harrison and Carlson (1996). In agreement with observations from other environmental immunoassays absolute specificity for one of a group of structurally related compounds is very unlikely (Sherry, 1992). Only one of the antibodies listed in *Table 3.2a* is close to absolute specificity (EnSys, 1995), and this has 20% crossreactivity for 2,3,7-TrCDD, the trichlorodioxin most closely related to 2,3,7,8-TCDD. This lack of absolute specificity means that any result is likely to be the compound sum of signals from individual congeners. The test designer must, either directly or indirectly through the user, determine a calibration procedure which takes this fact into account. This is now discussed further.

Cross reactivity of the antibody with other chlorinated aromatics can limit immunoassay utility if these compounds are present in sufficiently high concentrations in the samples. However, this has not been adequately addressed in the PCDD/F immunoassay literature. Most immunoassay developers have demonstrated that recognition of compounds such as chlorophenols is minor, relative to the primary targets. However, none have shown that realistic environmental levels of mixtures of these compounds do not interfere in the immunoassay. This would require experimental demonstration that cross reactivity to individual chlorobenzenes or chlorophenols is well below the 0.1% level.

3.5.5 Validation Requirements, Calibration and Interpretation of Results

These three factors also will be treated together because they are not completely independent in practice. Immunoassays which give a single measurable response to a group of structurally similar compounds must be calibrated against an appropriate alternative analytical result to give results which are meaningful to the user. Because of the inherent group recognition of even very specific assays, it is necessary to validate the relationship between the immunoassay responses and the results of the conventional method. If the analytical goal for a PCDD/F immunoassay is toxicity-based screening, the simplest way to address this issue is to use a system in which test response correlates with toxicity. If that is not possible, then a set of correlation data would be required, typically consisting of results from both methods for a statistically significant number (in the order of 20-40) of field samples.

Assuming the observed correlation to be acceptable, then the slope of the regression line would be calculated. This slope would then define a calibration adjustment factor to be applied to the raw immunoassay result. This adjustment would typically be transparent to the kit user, but would allow meaningful results, such as I-TEQ, to be read directly from the test. This calibration adjustment factor may vary significantly for PCDD/F samples from different sources, depending on their variability of congener composition. Proper quality assurance in immunoassay kit use would include ongoing examination of this calibration adjustment factor relative to actual sample composition.

3.6 Developments in Immunoassays for PCDD/Fs

There are four possible strategies to address the current limitations of PCDD/F immunoassay that would allow full exploitation of the advantages of the technique. These are discussed more fully in *Sections 5* and *6*, but it is pertinent to close *Section 3* with a brief overview:

- The PCDD/F-hydrocarbon separation could be performed in a simpler, faster, and potentially fieldable sample preparation system;

- The tolerance of the immunoassay for such interferences could be improved through the different use of solvents and detergents;

- The immunoassay could be performed in organic solvent.

- If an antibody of acceptable specificity offered 10-fold better sensitivity than the best published dioxin immunoassay value, it would overcome many of the problems that have plagued the field.

Though the conventional view is that immunoassays require aqueous media, this is not always true. Bignami (1993) has attempted to perform a DD3-based immunoassay in hexane and toluene. The results showed that the DD3 antibody tolerates water saturated organic solvents, but requires the medium to be predominantly water to drive the hydrophobic attractions between 2,3,7,8-TCDD and the antibody which allow binding. Thus, it is possible that in the future a new antibody could be selected based on binding to the analyte in organic solvent. Though this example is outside the scope of this project, it serves to illustrate the methods that are being explored within the field to extend the performance of the current immunoassay systems.

It is also pertinent to comment on other potential bioassay PCDD/F screening methods. In general, these methods utilise the aryl hydrocarbon (AH) receptor, either in isolated form or expressed in a specially developed cell line. This receptor is capable of binding most tightly to PCDDs and PCDFs, but also to PAHs, certain PCBs, and other unidentified compounds known to occur in some environmental samples. The interaction of the sample with the receptor system is measured, giving an estimate of the dioxin-like toxicity of the sample. The most important advantage of this method is that the recognition of PCDD/F congeners by the test should parallel the toxicity of those congeners because the receptor which is used for the analysis mediates the toxicity in vivo. However, the major drawback of this type of screening test is the high frequency of positive responses attributable to PAHs and other non-PCDD/F compounds which occur in samples containing low levels of these compounds. In situations having minimal interferences, such as from PAHs, these tests may be practical. However, this is not likely to be the case in monitoring combustion derived sources.

Some of the researchers in this field include Dr Nigel Bunce (University of Guelph), Dr Tim Zacharewski (University of Western Ontario), Dr Mike Denison (University of California at Davis), Paracelsian, Inc. (Ithaca NY), Dow Chemical (Midland MI), and Hybrizyme, Inc. (Research Triangle Park NC). Paracelsian Inc has developed a receptor-based immunoassay method for commercial sale and has licensed the method to Dow.

The specificity of the test derives from the AH receptor, but the detection of the dioxin-receptor complex occurs via immunoassay. Execution of the analytical method (exclusive of sample preparation) is significantly more complex than typical environmental immunoassays.

4. STATISTICAL ANALYSIS OF PCDD/F DATA

4.1 Introduction

A statistical analysis was performed on PCDD/F concentrations in stack emissions from municipal waste incinerators (MWIs), chemical waste incinerators and iron and steel works, and effluent from the manufacture of vinyl chloride monomer (VCM). The statistical analysis served two purposes:

(1) to examine the full congener profile of the samples, drawing out similarities and dissimilarities between PCDD/F releases from the four sources, and to characterise any systematic trends or groupings in the profiles;

(2) to identify a 2,3,7,8-positional PCDD/F congener which might serve as a "marker" or target analyte for an immunoassay screening test.

Section 4 commences with a description of the data compiled on PCDD/F emissions from municipal waste incinerators, chemical waste incinerators, iron and steel works and effluent discharges from VCM manufacture. This is followed by a brief summary of the statistical techniques employed. The PCDD/F release data is then analysed and discussed. The section concludes with a summary of the main findings. Supporting data and graphical representations are provided in *Annex B.*

4.2 PCDD/F Release Data

4.2.1 Data Sources

PCDD/F release data were obtained from a variety of sources:

- The Environment Agency's monitoring database, supplementing total I-TEQ data available on the public register;
- For the VCM process, a submission to HMIP from ICI, put into the public domain in 1993 in relation to a public inquiry in North West England (Environment Agency, 1997);
- Data from specific UK sites, provided by Symonds Travers Morgan and by plant management;
- Data provided by the University of Bayreuth, on PCDD/F emissions from a German MWI;
- Data on PCDD/F emissions from German and Dutch MWIs, obtained by ERM from the open literature.

For reasons of confidentiality, site details have not been presented in this report. A summary of the data (sample identification number and total PCDD/F concentration in units of I-TEQ) is provided in *Table B1* in *Annex B*. The complete congener profiles are provided in *Table B2*. Sampling and analytical details were not available for reasons of confidentiality, and therefore issues such as measurement uncertainty could not be addressed. Each data set is discussed briefly below.

4.2.2 Releases to Atmosphere from Iron and Steel Works

The data are identified as samples 1-16 in *Table B1*, and derive from plants in the UK, covering six sites. The data have been normalised to standard emission conditions.

4.2.3 Releases to Atmosphere from Municipal Waste Incinerators

All data are reported under standard emission conditions. The data are identified as samples 17-67 in *Table B1*. Samples 17-28 have been obtained from the Environment Agency's database and relate to 10 individual incinerators. Samples 29-59 have been obtained from individual UK sites: as the I-TEQ data indicates, these plants exhibit a wide range of PCDD/F emission concentrations, from 1 ng I-TEQ m^{-3} to 170 ng I-TEQ m^{-3}. All plants are of "old" designs, save for one modern facility. Samples 60-67 relate to 4 German incinerators, with and without abatement for PCDD/F emissions. These data have been obtained from Kaune *et al* (1991).

Two further partial data sets (not reported in *Table B1*) were obtained for the purposes of identifying a marker congener. Slob *et al* (1993) present information in graphical from on PCDD/F congener emissions from 14 Dutch MWIs, coupled with a table containing total I-TEQ emission data. The total data set comprises 47 samples. Fifteen data points relating to releases from a single German MWI were provided by the University of Bayreuth, again limited to a few specific congeners and to total I-TEQ concentrations. The interest in this data set is that the samples span a period of three years during which the plant was upgraded to meet more stringent PCDD/F emission standards. Earlier samples therefore relate to the unmodified plant, while later samples relate to the retrofitted plant. Further discussion of the Dutch and German data is provided in *Section 4.4* below.

4.2.4 Emissions to Atmosphere from Chemical Waste Incinerators

All data are reported under standard emission conditions. The data are identified as samples 68-109 in *Table B1*. Samples 68-77 have been obtained from individual sites. Samples 78-109 have been obtained from the Environment Agency's database and relate to a range of incineration operations covering chemical waste (merchant and in-house operations), pharmaceutical waste, solvent waste, and refinery waste. The total I-TEQ emission concentrations are generally very low, and accordingly many of the PCDD/F congeners have been reported as being below the analytical limit of detection.

4.2.5 Effluent from VCM Manufacture

Inquiries were made in the UK, US and Europe for data relating to PCDD/F congener concentrations in effluent from VCM manufacture. The data made available from non-UK plants was incomplete in that the majority of PCDD/F congeners were reported as being below the analytical limit of detection (see for example, Carroll *et al*, 1996). The sole data set with sufficient information for the purposes of the present study was that of ICI (see *Section 4.2.1* above). However, the latter submission to HMIP contains data for a number of other processes, some of which are inter-related to the manufacture of VCM. The aqueous effluent from the VCM process combines with aqueous effluent from the manufacture of perchloroethylene and trichloroethylene (using a side stream from the manufacture of VCM as the starting material) and enters a settlement and balancing lagoon prior to discharge into the Weston Canal. From the perspective of the present study, the streams of relevance are the aqueous effluent from the VCM process, the combined influent to the lagoon, and the effluent from the lagoon. The influent to the lagoon and the effluent to the Weston Canal are uniformly low in PCDD/F content (see samples 128-130, *Table B1*) relative to the in-plant streams: the latter are not discharged from the site and are therefore not strictly relevant. However, for completeness we have retained all the samples reported in the submission to HMIP, together with their identity.

4.3 Statistical Analysis of Congener Profiles

4.3.1 Methodology

Cluster Analysis (CA) was used to examine the complete data set for trends or groupings among the 2,3,7,8-PCDD/F congener profiles. A "profile" is the pattern produced by the relative concentrations of the PCDD/F homologues or 2,3,7,8-positional congeners in a sample. A profile is best visualised through a graphical representation of the homologue or congener concentrations. Homologue or congener profiles have been used extensively with CA and other statistical methods to characterise sources of contamination (for example Pitea *et al*, 1989; Edgerton *et al*, 1991; Kjeller *et al*, 1995; Kjeller *et al*, 1996; Fiedler *et al*, 1996).

In CA the variance between the profiles in the sample data set is analysed by the use of hierarchical statistical algorithms. The average profile for the data set is computed and the algorithms calculate the variances between this average and the actual profiles of the samples. The Ward Method is one of the more commonly used algorithms (Backhaus *et al*, 1994; Fiedler *et al*, 1996). The individual samples within the data set are then organised into groups or clusters to show both similarities and (graded) differences between the homologue profiles. When the statistical analysis is undertaken without an *a priori* organisation of the clusters (ie the clusters are permitted to develop according to the natural variability within the data set) the technique is known as *unsupervised analysis*. The resulting clusters can be represented in a *dendrogram* in which clusters are connected by branches or dendrites, the lengths of the branches indicating the variance (ie the degree of similarity) between clusters.

An example dendrogram is shown in *Figure 4.3a*, in which 10 hypothetical samples are clustered by unsupervised analysis according to the similarity or otherwise of their congener profiles.

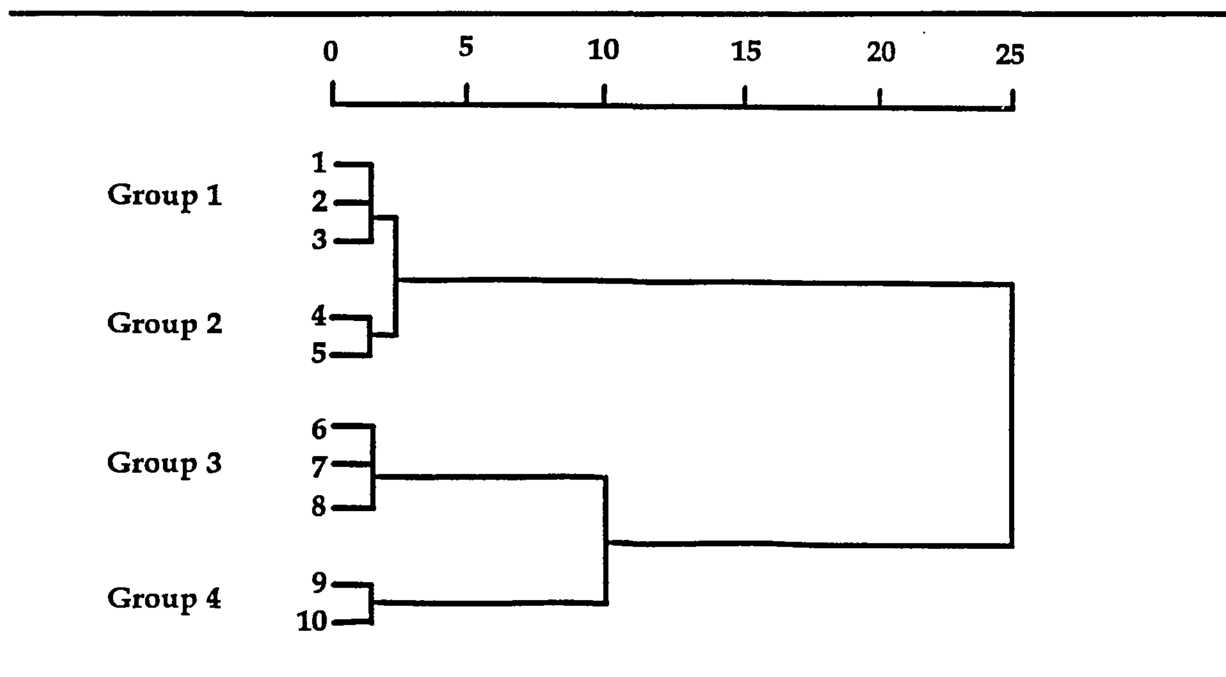


Figure 4.3a A Hypothetical Example of Cluster Analysis

The variance between the samples and their clusters is represented by their separation on an arbitrary scale on the horizontal axis, from 0 (= high similarity) to 25 (= low similarity). If the vertical line combining two clusters is at a low value (for example, < 5 on the relative scale) there is a high similarity between the congener profiles of the constituent samples in the connected clusters, while a high value of > 20 reflects low relative similarity between the profiles of the samples. In the figure, samples (1, 2, 3); (4, 5); (6, 7, 8) and (9, 10) form four groups, each containing samples with similar congener profiles. (Group 1 and Group 2) samples are more similar than (Group 3 and Group 4) samples, as demonstrated by branches joining these group pairings at distances of 2.5 and 10 respectively. Further, there is little similarity between Groups 1/2 and Groups 3/4 since the branch connecting these groups is at a relative distance of 25.

4.3.2 Results

Cluster analysis was used to elicit detail from the complete data set. CA in unsupervised mode was applied separately to the three stack emission sources and to releases from the VCM process, and in addition to the total data set for the four release sources. The data sets were normalised by dividing each congener concentration, expressed in units of I-TEQ, by the total I-TEQ of the sample. This latter method of data transformation has been found to be more sensitive in CA than, say, normalising congener concentrations against the total PCDD/F homologue concentration or by the absolute total concentration of the seventeen 2,3,7,8-positional congeners (Fiedler *et al*, 1996).

The resulting sample dendrograms are presented in *Annex B* as follows:

- *Figure B1*: Dendrogram of emissions from iron and steel works
- *Figure B2*: Dendrogram of emissions from municipal waste incinerators
- *Figure B3*: Dendrogram of emissions from chemical waste incinerators
- *Figure B4*: Dendrogram of effluent from VCM manufacture
- *Figure B5*: Dendrogram of the unsupervised analysis of the total data set

The dendrogram for releases from iron and steel works reveals that the PCDD/F congener profile for sample 14 is distinct from the remaining 15 samples. Further, samples 15 and 16 also form a distinct set. Samples 1-14 derive from several iron and steel plants, but collected and analysed by a single laboratory. Samples 15 and 16 are from a single iron and steel works, and have been collected and analysed by a different laboratory.

The dendrograms for releases from MWIs and from chemical waste incinerators indicate several distinct clusters within these release sources, but examination of the data indicates that there is no clear grouping according to the incinerator(s) tested nor according to the test laboratories involved.

The dendrogram for effluents from VCM and other manufacturing processes yields interesting information. Samples 113-115 and 122-124 are clustered together, indicating a similar congener profile. These samples derive from two related process effluent streams: samples 113-115 represent the VCM sidestream starting material for perchloroethylene and trichloroethylene, while samples 112-124 derive from the fractionated products. Sample 128 is identified as the "lagoon inlet", and is seen to be in a different cluster to that of samples 129 and 130, representing the "lagoon outlet" (cluster separation distance = 23). Inspection of the total I-TEQ concentration in these samples (see *Table B1*) shows that they are of a similar order, and therefore the large relative difference in their congener profiles is due to physical processes occurring within the settlement lagoon.

The data for releases from VCM and related manufacturing processes are clustered together, and are markedly different from the remaining samples which derive from combustion sources. The latter data are grouped into smaller clusters, but these clusters are composed of a mixture of combustion sources. There is no clear differentiation between the congener profiles of the various combustion sources.

4.4 Identification of a Marker Congener

4.4.1 Methodology

Data reduction for the purpose of cluster analysis indicated that 2,3,4,7,8-PeCDF was generally the dominant contributor to the total congener concentration of the samples when measured in units of I-TEQ. *Figures B6-B10* represent in the form of spectral plots the contribution of the 17 congeners to the total I-TEQ of the samples in each data set. For all the thermal sources the dominance of the 2,3,4,7,8-PeCDF congener in the total I-TEQ is clearly evident.

For VCM effluent *Figure B9b* indicates a strong bias of the PCDD/F congener profile towards OCDF, with the OCDF and the 1,2,3,4,7,8-HxCDF congeners contributing about 30% each to the total I-TEQ. There was therefore *prime facia* evidence to suggest that 2,3,4,7,8-PeCDF was a potential candidate for a target analyte, from which a total I-TEQ value might be derived, even in the case of VCM effluent. The data sets were tested for a correlation between the concentration of this congener and the corresponding total I-TEQ of the sample. A linear regression equation was fitted to the data, which minimised the sum of the squared vertical distance of all data points. The general regression equation took the form:

I-TEQ = b (2,3,4,7,8-PeCDF)

where ***b*** is the intercept of the best-fit line. Since negative I-TEQ values have no physical meaning, the intercept of the linear regression equation was set to zero.

The equation was applied to the data presented in *Table B1*, and in addition to the Dutch PCDD/F emission data presented by Slob *et al* (1993) and to the German MWI emission data provided by the University of Bayreuth.

4.4.2 Results

The results are shown in *Figures B11-B17* in *Annex B*. Values of ***b*** and of the correlation coefficient R^2 are summarised in *Table 4.4a*.

Table 4.4a Calculated Regression Parameters for the Equation [I-TEQ = b (2,3,4,7,8-PeCDF)] for the four PCDD/F Release Sources

Data Set (Number of Samples)	b	Standard Deviation	Correlation Coefficient R^2
Iron and Steel, excluding outlier sample 15 (N = 15)	0.873	0.165	0.961
UK and German MWIs, excluding outlier samples 44, 46 and 47 (N = 48)	1.747	2.621	0.991
Chemical waste incinerators (N = 42)	1.217	0.620	0.941
VCM industry, excluding outlier sample 120 (N = 20)	2.927	11.363	0.740
All data in *Table B1*, excluding the above outliers	1.859	6.995	0.897
Additional Data			
Dutch MWIs (N = 47)	1.704	11.038	0.905
German MWI (N = 15)	1.503	0.235	0.979

The iron and steel data set contained one poorly correlated data point which was treated as an outlier. In the MWI data set, three sample points (44, 46 and 48) were treated as outliers. These data relate to one plant, sampled on the same day. It is interesting to note that samples 44 and 46 form a discrete cluster in *Figure B2*. In the VCM data set one data point, sample 120, was omitted from the correlation. Due to the non-availability of data on analytical methods or sampling conditions, it was not possible to explore further the significance of the outliers. Their omission from the correlations may not be justified, but in the absence of supporting information this decision was felt to be defensible.

Inspection of *Figures B11-B17* and the corresponding correlation coefficients indicates a generally excellent correlation between the concentration of 2,3,4,7,8-PeCDF and the total I-TEQ of the sample. Discounting the notional outliers, the correlation coefficients for releases from iron and steel works, UK, Dutch and German MWIs and chemical waste incinerators are consistently above 0.9. The VCM data are less well correlated ($R^2 = 0.74$ with a standard deviation of 11.4) but as was noted in *Sections 4.2.1* and *4.2.5* the samples derive from multiple sources and the higher PCDFs dominate the congener profile. The excellent correlation for municipal and chemical waste incinerators is of interest for the following reasons:

- the data for MWIs include plants of differing designs and ages, with concentrations of PCDD/F emissions ranging over two orders of magnitude;

- the value of ***b*** in the regression equation is similar for the three MWI data sets (UK, Dutch and German plants). The correlation coefficient is also the highest for this data set;

- the data for MWIs include plants which have limited pollution abatement equipment, and those with specific PCDD/F abatement technologies. The data for the German MWI, which covers pre- and post-retrofit operating conditions, is especially well correlated (see *Figure B17*);

- the data for chemical waste incinerators cover a wide range of feedstock, also combusted in furnaces of different designs.

The correlation coefficient obtained by collectively fitting all the data in *Table B1* also approaches 0.9. Removal of the VCM data and inclusion of the Dutch and German MWI data sets (ie confining the correlation to the thermal emission sources) would improve the correlation, raising the correlation coefficient to 0.96.

The 2,3,4,7,8-PeCDF congener is therefore identified as a potential marker for the total I-TEQ of the emission and effluent samples from the release processes discussed in this report. A recent study by Mohr *et al* (1996) confirms this observation for MWIs: their regression plot comparing measured and predicted emission I-TEQ values based on the concentration of 2,3,4,7,8-PeCDF measured in two German MWIs is reproduced in *Figure B18* in *Annex B*. This group has also developed simplified sampling and analytical techniques exploiting the use of 2,3,4,7,8-PeCDF as a marker congener. These aspects are discussed in *Section 5*.

4.5 Implications for Immunoassay

There are two issues to be considered in the light of the analysis presented above:

- the implications for "broad spectrum" immunoassay in the form of I-TEQ screening of the emission/effluent sample using an existing antibody such as DD3 (see *Section 3.2*);
- whether the alternative route (ie development of a new antibody specific to 2,3,4,7,8-PeCDF followed by correlation of the immunoassay response with total I-TEQ) is the preferred option.

Given that a newly developed antibody targeted to 2,3,4,7,8-PeCDF will also exhibit cross reactivity towards other PCDD/F congeners (at this point, an unknown factor) it is of interest to examine in the first instance the likely performance of the DD3 antibody on the emission and effluent samples before recommending an alternative route. Referring to *Table 3.2b*, the cross reactivity of DD3 with 2,3,4,7,8-PeCDF, as reported by Harrison and Carlson (1996), is of the same order as the I-TEF for this congener, and after 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD, is the most strongly recognised congener.

In this regard an alternative set of cross reactivities, derived from the data of Stanker *et al* (1987) and shown in *Table 4.5a*, is of interest. The cross reactivities have been obtained by dividing the quantity of 2,3,7,8-TCDD required for 50% inhibition (see *Figure 2.3b*) by an equivalent quantity required for other congeners.

Table 4.5a Cross reactivity of the DD3 antibody as characterised by Stanker et al (1987)

Congener	Cross reactivity	Congener	Cross reactivity
1-MCDD	< 0.01	1,2,3,7,8-PeCDD	3.2
2,7-DCDD	2.5	1,2,3,4,7,8-HxCDD	0.13
1,2,4-TrCDD	< 0.01	1,2,3,6(7),7(8),8(9)-HxCDD	< 0.01
1,2,3,4-TCDD	< 0.01	OCDD	< 0.01
2,3,7,8-TCDD	1.0	2,8-DCDF	0.01
1,3,7,8-TCDD	5.6	2,3,7,8-TCDF	3.6
1,2,3,7(8)-TCDD	1.3	2,3,4,7,8-PeCDF	8.3
1,2,6(8),7(9)-TCDD	0.25	1,2,3,4,8,9-HxCDF	< 0.01
1,3,6(7),8(9)-TCDD	3.6	OCDF	< 0.01
1,2,3,4,7-PeCDD	0.78		

The data of Stanker *et al* (1987) shows strikingly different cross reactivity to that of Harrison and Carlson (1996) for the same DD3 antibody. Specifically, the Stanker data suggests that DD3 has the greatest affinity for the marker congener 2,3,4,7,8-PeCDF than for 2,3,7,8-TCDD. The reasons for this discrepancy are not clear, but most likely relate to differences in assay procedure. The Stanker *et al* (1987) assay was undertaken with an immobilised, labelled analyte, with the antibody and unlabelled analyte in the liquid phase. In contrast, Harrison and Carlson (1996) contact an immobilised antibody with labelled and unlabelled analyte in the liquid phase. Of perhaps greater significance, in the Stanker *et al* (1987) assay the analyte-antibody reaction was taken to equilibrium by adopting an incubation period of several hours. In the Harrison and Carlson (1996) assay the contact time was limited to 15 minutes because the purpose of the assay was as a rapid field test.

There is insufficient information in the open literature to explore this issue further. However, the tentative conclusion is drawn that the specificity of an existing anti-dioxin antibody, DD3, may be adapted by modification of the assay conditions to serve as an I-TEQ screen or as an antibody with high specificity for 2,3,4,7,8-PeCDF. In either case it will be noted that DD3 exhibits cross reactivity with other PCDD/F congeners, and therefore further experimentation is required to develop an assay procedure and quality assurance regime that optimises the performance of this antibody for the particular application of interest. Assuming for illustrative purposes the assay procedure of Harrison and Carlson (1996) (ie using the assay as a measure of the I-TEQ of the sample) we have applied their calculated I-TEFs given in *Table 3.2b* to the 2,3,7,8-positional congener concentrations listed in *Table B2* and have plotted the resulting theoretical immunoassay response against the GCMS measured I-TEQ concentration in *Figure 4.5a* - the plot is similar to *Figure 3.2b* for soil samples. For all four PCDD/F release sources, including VCM manufacture, there is an excellent correlation with the I-TEQ concentration measured by GCMS, as is shown by the fit with the (X = Y) line.

As with the soil samples, the correlation has to be experimentally validated. It will be noted, for example, that DD3 cross reactivity with PCDD/F congeners not reported in a GCMS analysis may well raise the immunoassay response above the value of the I-TEQ measured by GCMS. Most current GCMS reports do not discriminate between 1,2,3,4-TCDD, which is not recognised by the DD3 antibody, and 1,2,7,8-TCDD, which is more cross reactive than several 2,3,,8-positional congeners including 1,2,3,7,8-PeCDF and 1,2,3,7,8,9-HxCDD. Owing to the regulatory focus on 2,3,7,8-positional congeners, current analytical methods do not report DCDDs or TrCDDs, limiting the amount of information available in the literature on occurrence of these congeners in samples.

Both the highly specific EnSys monoclonal antibody (see *Section 3.4.1*) and the broadly specific DD3 antibody strongly recognise 2,3,7-TrCDD. Limited information (Tsuji *et al*, 1987; Safe *et al*, 1987; Theisen *et al*, 1989; Schwind, 1991) suggests that the TrCDD homologue (containing 14 congeners) and the TrCDF homologue (containing 28 congeners) would be expected to be present in stack gas samples from municipal and chemical waste incinerators at about 50% of the concentration of the TCDD and TCDF homologues respectively. It is therefore likely that the mass of 2,3,7-TrCDD in the sample will be at about the same absolute level as 2,3,7,8-TCDD.

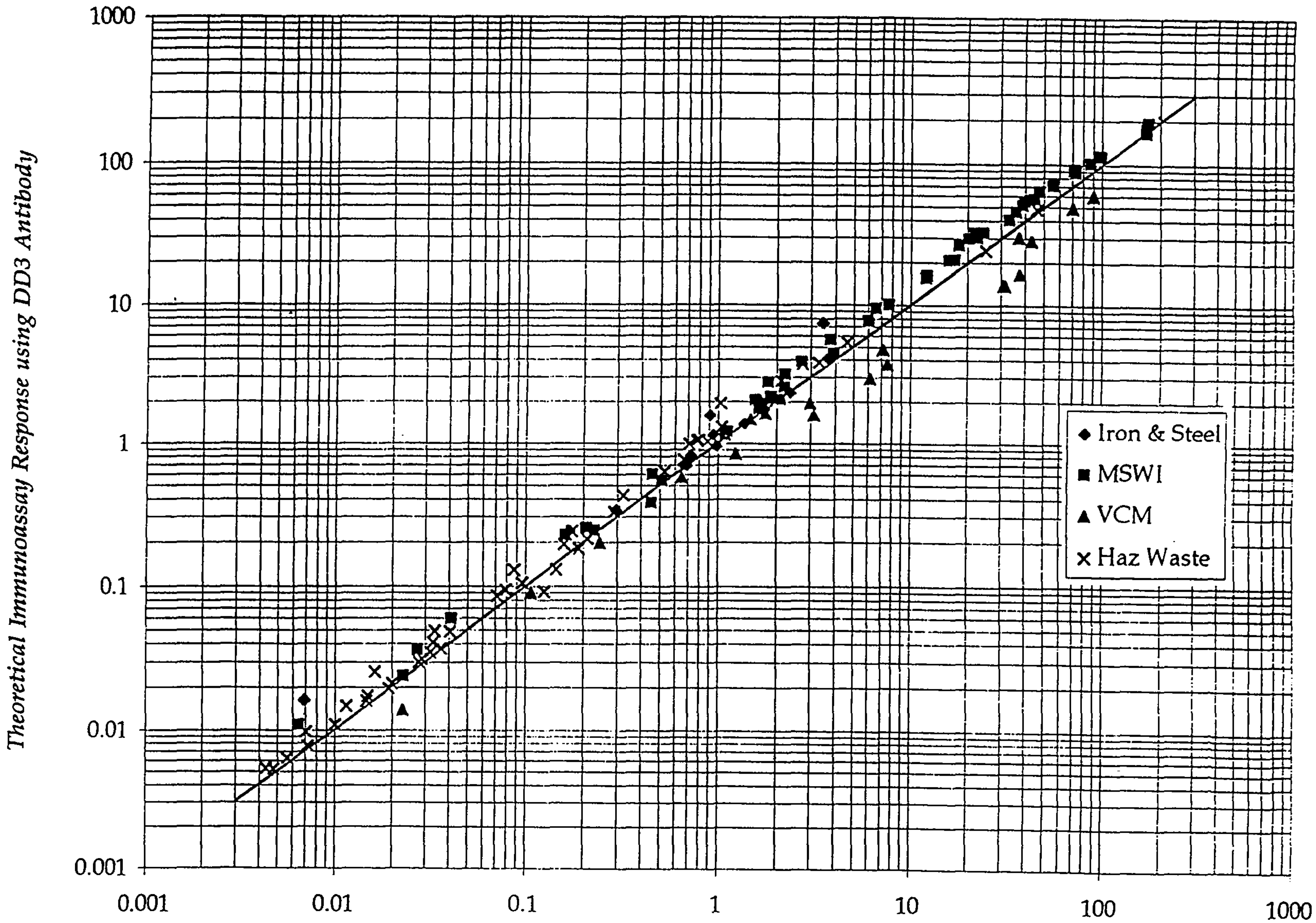


Figure 4.5a **Theoretical Immunoassay Response against total I-TEQ vales Measured by GCMS for all Samples, using Cross Reactivity Data in Table 3.2b (Axes in ng m $^{-3}$ or ng kg $^{-1}$)**

Given that the maximum antibody cross reactivity with 2,3,7-TrCDD is 20% that of 2,3,7,8-TCDD and that the latter tends not to be a major constituent of emissions from thermal processes, it seems unlikely that the presence of 2,3,7-TrCDD will add significantly to the total immunoassay response of the samples from the processes under consideration. However, this conclusion requires experimental verification. No information is available on the levels of 2,3,7-TrCDD or the TrCDD homologue in VCM effluent.

4.6 Summary

Statistical analysis using cluster analysis has failed to identify sufficiently distinctive profiles of the 2,3,7,8-positional congeners which could be attributed to individual thermal release sources. The VCM data exhibits a pattern distinct from that of the thermal release sources, but since the samples derive from a number of related process and effluent streams it is not possible to allocate an unequivocal congener profile specific to VCM manufacture. Changes in the congener profile of the outflow from the settlement lagoon relative to the effluent entering the lagoon at ICI Runcorn also make it difficult to allocate a representative congener profile relevant to the VCM process.

For the thermal release sources, the congener 2,3,4,7,8-PeCDF makes the greatest contribution to the total I-TEQ concentration of the sample. The concentration of this congener was therefore plotted against the total I-TEQ of each sample (including VCM effluent), and the resulting correlation tested for linearity. For the three thermal sources excellent linear correlations have been obtained. The correlation is less satisfactory for the VCM data set, but the samples derive from a number of different process and effluent streams, albeit related to the manufacture of VCM and other products, and the congener profile in VCM effluent is markedly different from that of the thermal sources. The parameter b differs for each release source, suggesting that the source of a sample would have to be known in order to select the appropriate regression equation to estimate the total I-TEQ of the sample from the concentration of 2,3,4,7,8-PeCDF.

5. PRACTICAL IMPLICATIONS OF IMMUNOASSAY TESTING

5.1 Introduction

In the previous section we reviewed PCDD/F congener profiles in emissions from municipal and chemical waste incinerators and iron and steel plants, and in effluent discharges from VCM manufacture and associated processes. We deduced from an examination of the available data on PCDD/F immunoassays that an existing monoclonal anti-dioxin antibody, DD3, has the potential to be applied both in an I-TEQ screen or in an assay that is highly selective towards 2,3,4,7,8-PeCDF, depending on the assay conditions. In *Section 5* we examine the practical implications of applying immunoassay techniques to the analysis of PCDD/Fs in samples of stack gas and VCM effluent.

In seeking to introduce immunoassay screening alongside established sampling and analytical practices, it is helpful to view the former technique in the following context:

- The information obtained from the test should be compatible with that required for regulatory purposes. For stack sampling, two emission concentrations are of relevance:

  (1) 1 ng I-TEQ m^3 (the release limit specified in the UK Process Guidance Notes);

  (2) 0.1 ng I-TEQ m^3 (the guide value in the UK Process Guidance Notes, and the proposed release limit in the EU Incineration Directive);

A concentration or mass release limit for PCDD/Fs in VCM effluent has not been set by the Environment Agency.

- It is preferable in the first instance to maintain compatibility with existing sampling protocols which currently are accepted (or indeed defined in the case of the US EPA's Method 23 or the proposed CEN method for stack sampling) for regulatory purposes. If a sample fails an immunoassay screening test, then the same extract can be subjected to a conventional GCMS analysis without the need to re-sample. The immunoassay must be sensitive enough to preserve sufficient extract for possible subsequent GCMS analysis, whether before or after sample clean up. Increases in immunoassay sensitivity will simplify sample handling at this point. Decreasing the share of the extract required by the immunoassay will greatly expand the options available in an immunoassay screening programme.

- Quality control on the sampling and analytical procedures should be robust.

- There should be more than a marginal saving in cost and/or time when applying the immunoassay technique, such that a clear advantage over conventional test methods is demonstrated. This is particularly important for a screening test, which by definition is designed to complement but not supplant conventional sampling techniques and GCMS analysis for PCDD/Fs.

These issues are explored below under the following headings: sampling and sample preparation; sample clean up and quality control. Current non-immunoassay sampling and analytical practice is first discussed, followed by an assessment of the implications for immunoassay. Finally, the section will conclude with a discussion on the costs associated with the application of immunoassay techniques to stack and effluent sampling.

5.2 Current Sampling and Analytical Methodologies

5.2.1 Stack Sampling Methods

There are a number of stack sampling techniques that are currently in use. Many regulatory agencies have developed and specified sampling methods for use on stationary combustion sources; for example the US EPA's Method 23, the French national guideline NFX 43-313, the Italian national guideline Unichem Method 825, and four methods described under the German Ordnance VDI 3499. Of these, three basic types are perhaps most frequently applied: the filter method typified by US EPA's Method 23, the cooled probe method developed by Marklund (1990), and the dilution method developed in Germany at the University of Tubingen (Hagenmaier *et al*, 1986). These are described briefly below. These three basic types are included as options in the draft European Standard currently being developed by the European Committee for Standardization (CEN).

The US EPA's *Method 23* is illustrated in *Figure 5.2a(i)*, and is similar to the German method VDI 3499/2. The sampling apparatus consists of a heated probe lined with borosilicate glass or quartz, leading to a quartz fibre filter held in a heated box to prevent condensation. The gases then enter a glass water cooled condenser followed by an adsorbent trap containing XAD-2 resin, which traps organic micropollutants such as PCDDs and PCDFs. The condensate is collected in a flash beneath the vertically aligned condenser/resin trap, while the gases are dried by passing through silica gel traps held in an ice bath. Finally, the dried gases pass to the gas control unit comprising a pump, a gas meter and an orifice plate flowmeter. The typical gas flow rate is 1-1.5 $m^3 h^{-1}$. The *Nordic Method* (Marklund, 1990) is similar to Method 23 except that the liquid trapped in the condenser is removed from the system prior to passing through the resin trap.

The *cooled probe method*, equivalent to the German method VDI 3499/3, is illustrated in *Figure 5.2a(ii)*. The sampling train consists of a glass lined probe which is provided with a water jacket, cooling the gas sample to below 20°C in seconds. The condensate is collected in an ice cooled trap, followed by a train of impingers filled with ethoxy ethanol. In a variant design, thecondensate trap is followed by a polyurethane foam plug and a back-up charcoal trap.

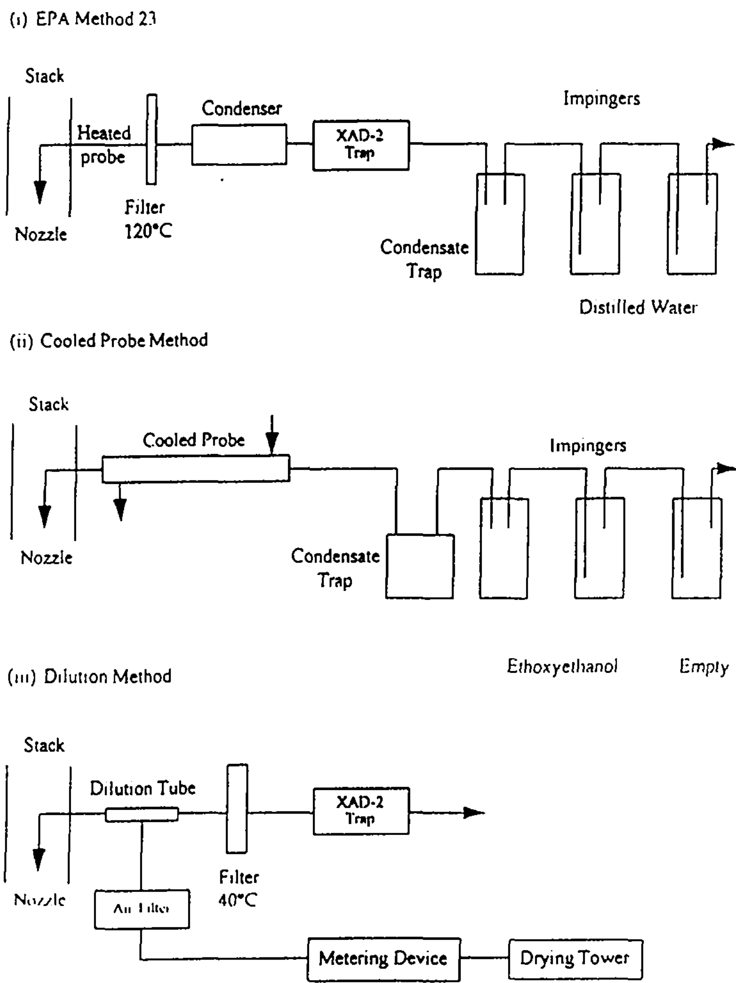


Figure 5.2a **Block Diagrams of Sampling Trains According to USA EPA Method 23, Cooled Probe and Dilution Methods**

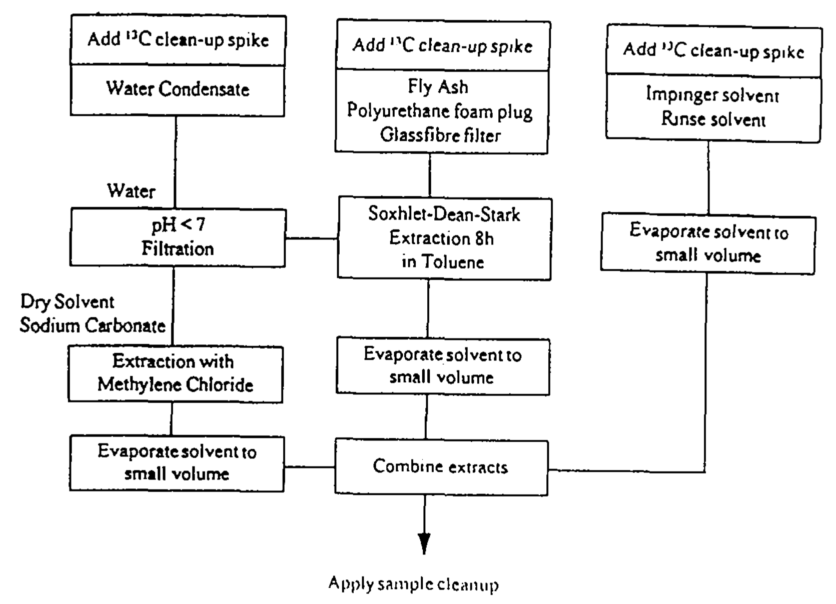


Figure 5.2b **Typical extraction Procedure for Incineration Samples (Markland, 1990)**

The *dilution method*, equivalent to the German method VDI 3499/1, is illustrated in *Figure 5.2a(iii)*. The flue gas is withdrawn through a heated glass lined probe and immediately mixed with clean and dry dilution air. The dilution air flow is regulated such that the temperature at the downstream quartz fibre filter is less than 50°C. The filter is impregnated with paraffin. A back-up trap of absorbent (charcoal, florisil, polyurethane foam, etc) completes the sampling train.

These systems have been compared in parallel sampling studies (Hagenmaier *et al*, 1986; Marklund, 1990; Marklund *et al*, 1992; Wallbaum *et al*, 1995; Hagenmaier *et al*, 1996). The results have been inconclusive. Early tests indicated that comparable results were obtained between the three systems for the total PCDD/F concentration in the stack gas and the overall homologue or congener profile (Hagenmaier *et al*, 1986; Marklund, 1990) whereas the results of the CEN validation trials indicated an order of magnitude difference between the results of the trial participants, possibly as a result of differences in cleanup and analytical procedures (Hagenmaier *et al*, 1996). These latter workers have developed an alternative sampling system which seeks to address some of the problems encountered in the CEN trials. The system comprises an air cooled sampling probe followed directly by a cartridge filled with a polyethylene adsorbent. A glass sintered filter plate captures particulate matter present in the gas stream, while the centre of the cartridge holds an adsorbent layer spiked with the labelled PCDD/F recovery standards.

5.2.2 Effluent Sampling Methods

Effluent sampling techniques for the purpose of PCDD/F analysis are similar to other standard sampling methods in relation to preparation of sample containers, representative sampling, collection of solids, etc. Typically, sample sizes of 1-2 litres are collected.

5.2.3 Sample Preparation

Conventional sample preparation methods can differ according to the laboratory undertaking the testing, but the general principles are common to all variants. Some regulatory guidance is available: for example, the US EPA's Method 1613 for the analysis of PCDD/Fs in wastewaters. A typical sample preparation protocol for stack samples is illustrated in *Figure 5.2b* (Marklund, 1990). The key stages are as follows:

- *particulates*, either from the heated filter in the case of Method 23 and the dilution method, or separated from the condensate in the case of the cooled probe method, are subjected to exhaustive extraction with the aid of an organic solvent such as toluene. In the case of the composite system of Hagenmaier *et al* (1996) the entire cartridge is inserted into the extraction apparatus;

- *adsorbents* such as polyurethane foam, polyethylene, charcoal and XAD-2 resin are also extracted with an organic solvent;

- *aqueous and condensate phases* are also exchanged with an organic solvent, which is evaporated to a small volume.

Extraction of aqueous effluent samples follows these principles: the low aqueous solubility of PCDD/Fs and the tenacity with which these chemicals adsorb onto solid surfaces requires aggressive extraction methods during sample preparation. Hence solvents such as xylene, toluene, hexane and methylene chloride are commonly used as extractants and carrier solvents for the ensuing cleanup prior to presentation of the sample to the GCMS.

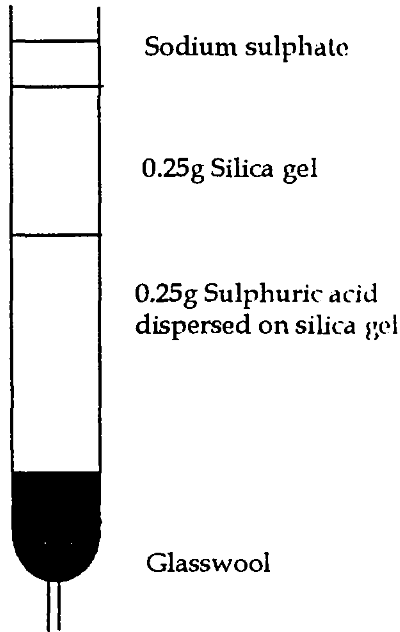
5.2.4 Cleanup Methods

Sample cleanup is standard practice for conventional analysis of PCDD/Fs by GCMS, since many organic species (alkanes; phenols; polychlorinated diphenyl ethers (PCDEs); PCBs; polychlorinated benzenes, naphthalenes (PCNs), terphenyls (PCTs) and quadphenyls (PCQs); PAHs; etc) can potentially interfere in the GCMS analysis. A typical sample cleanup protocol is presented in *Figure 5.2c* (Marklund *et al*, 1986). As with sample collection and sample preparation, differences between laboratories are often encountered. The principles are common to all methods. The collected sample (stack gas or effluent) is extracted with an organic solvent and concentrated by reducing the volume. The concentrated sample is then applied to a series of adsorbent matrices, typically consisting of a basic alumina column, and an acidic silica gel column. In a variant, a third column typically consisting of activated carbon can be used as a final cleanup stage. Finally, the chemical components in the sample are separated by the addition of solvents in different combinations of volume and polarity. Referring to *Figure 5.2c*, hexane added to Column A will elute chlorobenzenes and aliphatics, which are discarded. Eluting Column B with a limited quantity of hexane removes PCBs, PCTs, PCNs, PCDEs, etc. Further elution of Column B with a more polar mixture of 50:50 hexane/methylene chloride removes the PCDD/F fraction which is collected, concentrated, and analysed.

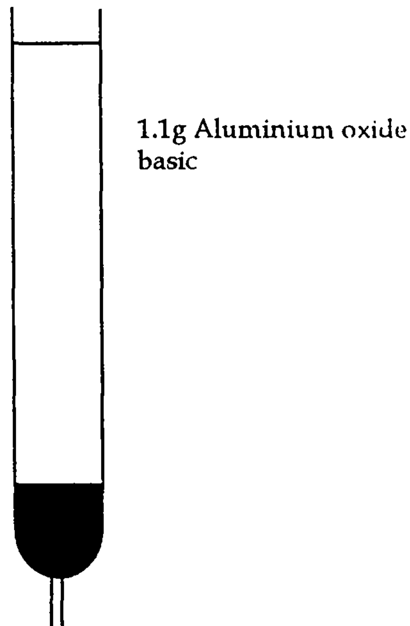
5.2.5 Quality Control

Quality control is a vital aspect of sampling and analysis for PCDD/Fs. With conventional GCMS analysis this is typically undertaken by spiking the sampler and/or the sample with ^{13}C and ^{37}Cl labelled PCDD/F congeners. The spikes serve as quality control standards, and are sufficiently similar to the analytes as to mimic their collection within the sampler, transfer to the analytical solvent and passage through sample cleanup, but yet can be identified as distinct chemical species in the mass fragmentograms. Each stage of the analysis can therefore be monitored, and corrections can be applied as required to allow for losses and inefficiencies. *Table 5.2a* lists the labelled congeners stipulated in Method 23 and the european draft CEN method for use in quality control during the sampling and GCMS analysis of PCDD/Fs in stack gas. Referring to Method 23 as an example, measured quantities of the ^{13}C *surrogate standards* are introduced as a spike into the resin trap of the sampler, prior to sampling. Measured quantities of the corresponding *internal standards* are added to the resin trap in the Soxhlet apparatus after sampling but before extraction. The difference between the two quantities provides an indication of sampling losses. The ^{37}Cl *surrogate standard* is added to the extracted sample prior to clean-up. The ^{13}C *recovery standards* are added to the extracted and cleaned sample prior to GCMS analysis. The difference between the quantities of the *recovery standards* and the *surrogate standards* provides an estimate of losses/efficiency of the extraction and cleanup procedure. In practice, only a selection of the standards listed in *Table 5.2a* is used for quality control purposes.

Column A



Column B



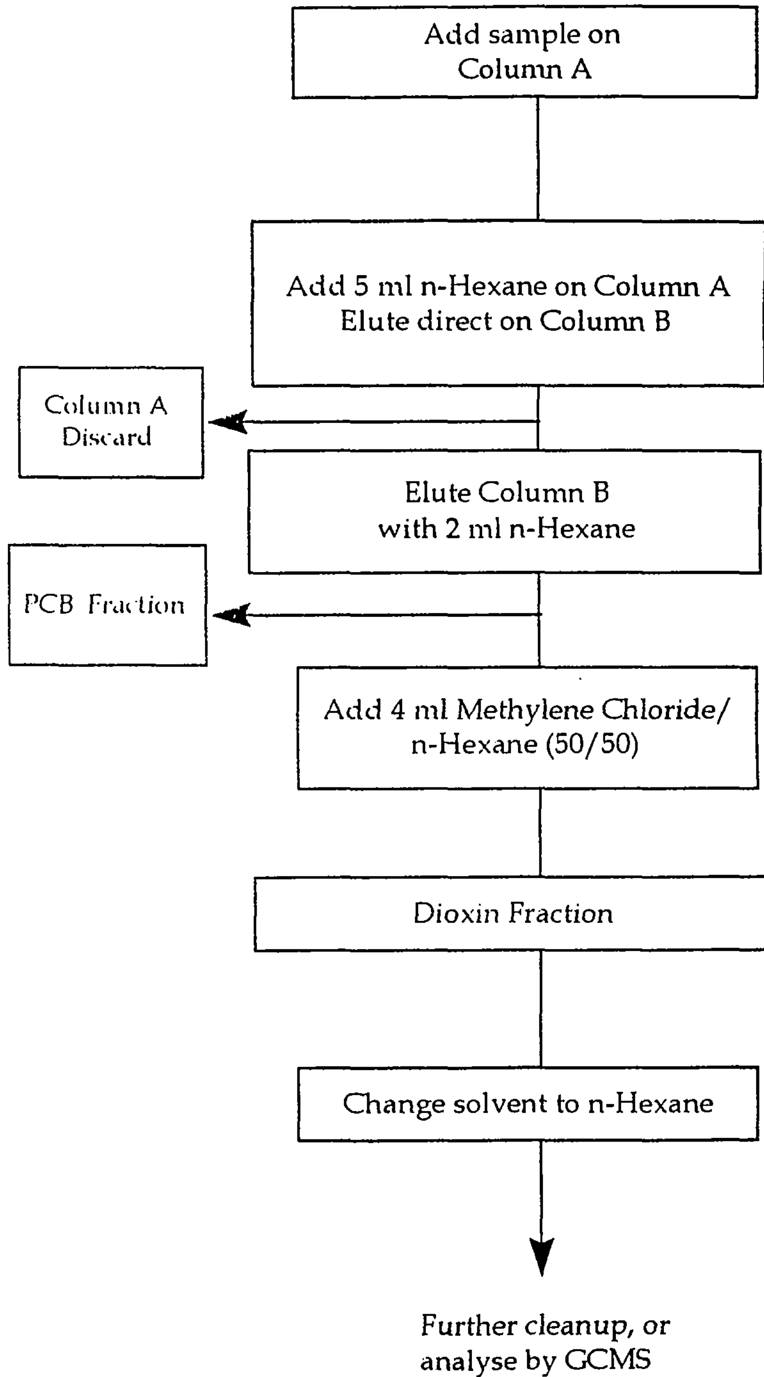


Figure 5.2c Example Cleanup for Incineration Samples (Markland et al, 1986)

Table 5.2a PCDD and PCDF Congeners used in GCMS Quality Control

Method 23 (a)	CEN (b)
Internal Standards	*Sampling Standards*
^{13}C-2,3,7,8-TCDD	^{13}C-1,2,3,7,8-PeCDF
^{13}C-1,2,3,7,8-PeCDD	^{13}C-1,2,3,7,8,9-HxCDF
^{13}C-1,2,3,6,7,8-HxCDD	^{13}C-1,2,3,4,7,8,9-HpCDF
^{13}C-1,2,3,4,6,7,8-HpCDD	
^{13}C-2,3,7,8-TCDF	*Extraction Standards*
^{13}C-1,2,3,7,8-PeCDF	^{13}C-2,3,7,8-TCDD
^{13}C-1,2,3,6,7,8-HxCDD	^{13}C-1,2,3,7,8-PeCDD
^{13}C-1,2,3,4,6,7,8-HpCDF	^{13}C-1,2,3,4,7,8-HxCDD
	^{13}C-1,2,3,6,7,8-HxCDD
Surrogate Standards	^{13}C-1,2,3,4,6,7,8-HpCDD
^{37}Cl-2,3,7,8-TCDD	^{13}C-OCDD
^{13}C-1,2,3,4,7,8-HxCDD	^{13}C-2,3,7,8-TCDF
^{13}C-2,3,4,7,8-PeCDF	^{13}C-2,3,4,7,8-PeCDF
^{13}C-1,2,3,4,7,8-HxCDF	^{13}C-1,2,3,4,7,8-HxCDF
^{13}C-1,2,3,4,7,8,9-HpCDF	^{13}C-1,2,3,6,7,8-HxCDF
	^{13}C-2,3,4,6,7,8-HxCDF
Recovery Standards	^{13}C-1,2,3,4,6,7,8-HpCDF
^{13}C-1,2,3,4-TCDD	^{13}C-OCDF
^{13}C-1,2,3,7,8,9-HxCDD	
	Recovery Standards
	^{13}C-1,2,3,4-TCDD
	^{13}C-1,2,3,7,8,9-HxCDD

(a) Most laboratories use a selection (3-4) of these congeners.

(b) Most laboratories use a selection (3-4) of these congeners. Other European methods (for example NFX 43-313) share the same set of standards as proposed by CEN.

5.3 Issues Concerning Immunoassay Tests

5.3.1 Stack and Effluent Sampling

Because of the need for complete extraction of PCDD/Fs from stack gases, aqueous effluents and their entrained particulates, there appears to be very limited scope for modification of existing sampling procedures for either stack sampling or effluent sampling. Efficient capture and extraction of particulates is critical to both sample types because of the strong affinity of PCDD/Fs for solid surfaces. However, of the stack sampling methods described in *Section 5.2.1* the relatively simple cooled probe/condensate method would be especially favourable for immunoassay testing provided sample preparation could be accomplished on the aqueous condensate.

Mohr *et al* (1996) have tested this sampling procedure on two German MWIs, and have also developed a high pressure liquid chromatography (HPLC) sample preparation protocol that obviates the need to partition the condensate with a aromatic solvent such as toluene. However, there might still be a need to concentrate up the cleaned sample in order to meet the sensitivity requirements of an immunoassay relative to a GCMS (see below).

Regarding sampling of VCM effluent, the effluent before settlement contains significant quantities of PCDD/Fs, especially the HpCDD/Fs and OCDD/Fs. After settlement the clarified effluent is substantially reduced in PCDD/Fs, signifying that collection and extraction of solids is a key element of sample preparation.

5.3.2 Sample Preparation

Sample preparation is generally required for two purposes: (1) to improve the sensitivity of the analysis by concentrating up the sample, and (2) to remove potential interfering chemicals present in the sample along with the PCDD/Fs. The latter is addressed in *Section 5.3.3* below.

For stack gas monitoring, sample collection times and sample sizes vary from test to test, but typically approximately 5 m^3 of gas is collected over a 4-5 hour period, sampling at about 1 m^3 h^{-1}. Exceptions can be found in the literature; for example, Hagenmaier et al (1986) collected 20 m^3 of gas over a 5 hour sampling period. If the requirement is to measure PCDD/F emission concentrations to below 0.1 ng I-TEQ m^{-3}, say with a detection limit equivalent to 0.02 ng I-TEQ m^{-3}, then a typical sample of 5 m^3 volume would contain (0.02 x 5 = 0.1) ng I-TEQ of PCDD/Fs. Assuming for the purpose of discussion that 2,3,4,7,8-PeCDF is adopted as a marker in an immunoassay test, then using the information obtained in *Section 4*, approximately 40% of this value would comprise 2,3,4,7,8-PeCDF (ie 0.08 ng or 80 pg, correcting for an I-TEF of 0.5). The total liquid from the sampler (including rinses) typically amounts to about 200 ml. Assuming an (immunoassay) analytical sensitivity of, say, 100 pg/tube in units of I-TEQ or for the marker congener (see *Section 3.4.2*) then even reduction of the sample to dryness will be insufficient to detect 80 pg of 2,3,4,7,8-PeCDD. Assuming that as a minimum the quantity of congener presented to the immunoassay test should be at least three times the detection limit for a valid analytical result, then detection of 300 pg/tube of the marker congener will be equivalent to a PCDD/F detection limit in the flue gas of approximately 0.08 ng I-TEQ m^{-3}.

The implication of this calculation is that in common with GCMS analysis, significant sample reduction will be required for the immunoassay test to approach the analytical sensitivity required to recognise PCDD/Fs at the 0.1 ng I-TEQ m^{-3} limit for PCDD/Fs in stack gas. In practice immunoassay screening is likely to be more effective at the 1 ng I-TEQ m^{-3} level to reduce the problem of false positive and false negative readings. The development of an immunoassay with increased sensitivity was noted in *Section 3.4.2*, and is discussed further in *Section 6* below.

For effluents, a sample of 2 litres is typically collected, filtered and extracted with an organic solvent as described above. Following the calculation procedure for stack samples, an (immunoassay) analytical sensitivity of 100 pg/tube implies a theoretical capability of detecting PCDD/F concentrations in the region of 50-100 pg I-TEQ l^{-1} of effluent, with a practical working limit of perhaps 150-300 pg I-TEQ l^{-1}. The PCDD/F concentrations in effluent from VCM manufacture ranged from approximately 0.1 ng I-TEQ l^{-1} for effluent outfall to approximately 40 ng I-TEQ l^{-1} for the aqueous effluent prior to entry into the settlement lagoon. Increasing the sample size will result in a corresponding lowering of the detection limit of the immunoassay.

Sample preparation in conventional GCMS analysis is generally conducted with organic solvents such as hexane or methylene chloride. Given the desire to maintain a solvent medium compatible with the immunoassay test (see *Section 3.6*) it is of interest to briefly review studies devoted to sample extraction: the focus of these studies has been on extraction of PCDD/Fs from flyash, since about 40% of the PCDD/Fs collected in the stack gas sample is typically found on particulate material (Liberti *et al*, 1982; Hagenmaier *et al*, 1986). Lustenhouwer *et al* (1980) and Kooke *et al* (1981) examined the efficacy of various extraction techniques; relative to Soxhlet extraction with toluene, other methods were less effective. Acid treatment improved the effectiveness of the extraction. Clement *et al* (1984) studied the extraction of PCDD/Fs using a range of solvent types. Aromatic solvents such as toluene and benzene were found to be far more effective than chlorinated hydrocarbons, cyclohexane and methanol. Other studies on solvents and extraction techniques (ultrasonic probe, refluxing, shaking, etc) again confirm the efficacy of Soxhlet extraction with hexane/acetone, toluene, xylene or similar solvent (Stieglitz *et al*, 1986; Liberti *et al*, 1982). A further advantage of employing a solvent such as hexane or toluene is that the final sample reduction (in the case of GCMS, typically to about 10-50 μl) can be achieved by the simple expedient of drying in a gentle stream of air or nitrogen, minimising potential loss of the less volatile analyte.

In both stack samples and VCM effluent samples, a substantial proportion of the PCDD/Fs is likely to be present on the particulate phase, and therefore thorough extraction of the sample is as important in the case of immunoassay analysis as for GCMS analysis. The use of organic solvents such as hexane or toluene for extraction and sample work-up has hitherto appeared unavoidable, as has been recognised in studies on immunoassay testing of environmental samples. However, it is possible to undertake a solvent exchange after evaporation of the organic solvent to dryness in the final stage of the sample work-up. Thus, Watkins *et al* (1989) proceed with conventional soil preparation and cleanup techniques using solvents such as hexane, toluene and methylene chlorine, and then exchange with "assay buffer" after evaporating the organic solvent to dryness. Harrison and Carlson (1996) exchange toluene used in the conventional cleanup of soil with methanol. The latter workers also add the detergent Triton X100 in a 100 ppb solution to the reconstituted sample to facilitate the recovery of the analyte from the walls of the tube.

An alternative approach integrating sample collection with sample cleanup has been reported by Mohr *et al* (1996). These workers used the cooled probe/condensate method to collect stack samples from two German MWIs and then subjected the condensate to sample preparation and cleanup using HPLC.

The method was specifically designed to obtain a fraction rich in 2,3,4,7,8-PeCDF, which was subsequently analysed by GCMS. Applying an appropriate conversion factor Mohr *et al* (1996) then obtained an estimate of the I-TEQ of the emission sample (see *Section 4.4.2*). Elution was accomplished with methanol, a solvent which is compatible with an immunoassay. However as noted above, further concentration of the sample may be required to cater for the lower sensitivity of current immunoassay techniques relative to GCMS.

New solid phase methods for extraction from aqueous matrices are becoming more common and should be investigated for extraction of VCM effluents. Aprotic solvents such as dimethylformamide (DMF), dimethylsulphoxide (DMSO) and dimethylacetamide (DMA) should be capable of adequately extracting PCDD/Fs from solid matrices for immunoassay analysis, but this approach would require development work before routine use. This is discussed further in *Section 6*.

5.3.3 Sample Cleanup

There is a general recognition of the need for sample cleanup with respect to immunoassay analysis of PCDD/Fs in environmental media such as soils. Thus, Watkins *et al* (1989) state that "immunoassays for dioxins, the one described [in their paper] as well as others, cannot be performed on crude matrices and dioxins must be extracted and cleaned up to remove interfering substances." Harrison and Carlson (1996) demonstrated that significant false positives resulted from analysis of crude sample extracts of soil.

Most current sample cleanups for the purpose of immunoassay employ some variation of the standard GCMS cleanup described in *Section 5.2.4* above. The interference most likely to confound immunoassays is total hydrocarbon load. Samples containing high hydrocarbon levels typically cause reduced sensitivity to the analyte due to micelle or droplet formation under some conditions. In some cases, decreasing the polarity of the incubation mixture may simply reduce the partitioning coefficient and thereby the sensitivity to the analyte. Analyses of stack gas samples for the full range of organic micropollutants are very scarce. Two such analyses, for a municipal waste incinerator (Jay and Stieglitz, 1995) and a chemical waste incinerator (Wienecke *et al*, 1995) are listed in *Annex C*. The concentrations of the various organic groups of relevance to an immunoassay are summarised in *Table 5.3a*, in addition to the reported PCDD/F concentrations.

While the specificity of an immunoassay should theoretically allow the direct analysis of crude mixtures, it is clear from the data in *Table 5.3a* that many of the groups of organic compounds are present at concentrations from 1,000-fold to 1,000,000-fold higher than the I-TEQ values.

This overwhelming abundance would be expected to cause problems for any analytical method, and immunoassay is no exception. Direct immunoassay analysis of crude extracts represented by either of the samples represented in *Table 5.3a* would not be likely to succeed for any immunoassay described in the current literature.

Table 5.3a Concentrations of PCDD/Fs and Several Groups of Potential Interferences from Municipal and Chemical Waste Incinerators

Chemical Species	Concentration ($\mu g\ m^{-3}$)
Municipal Waste Incinerator	
PCDD/Fs	0.020 (I-TEQ = 0.0003)
Miscellaneous solvents (Boiling Point ≤ toluene)	> 80
Miscellaneous solvents (Boiling Point ≥ toluene)	> 10
Benzoic and ethylbenzoic acids	≈ 136
Long chain aliphatic acids	> 50
PCBs	0.7
Total identified aliphatic and alicyclic hydrocarbons	≈ 26
Total identified PAHs and related hydrocarbon species	≈ 9
Chemical Waste Incinerator	
PCDD/Fs	0.012 (I-TEQ = 0.0002)
Chlorophenols	> 30
Phthalate esters	> 1300
Nitroaromatics	> 200
PCBs	> 1.2
PAHs	> 65

The critical steps in an immunoassay cleanup would probably remove the high levels of polar compounds such as phthalates, phenols and carboxylic acids, as well as most of the high boiling solvents and hydrocarbons. The major goal should be the removal of bulk organics which would otherwise be capable of overwhelming the aqueous medium, thereby disrupting the interaction of the antibody and the analyte. Residual PAHs, PCBs and chlorobenzenes present in much lower concentrations should be of lesser importance to an immunoassay for PCDD/Fs than to a GCMS method because of the selectivity of the antibody.

A methanol-based HPLC sample cleanup technique specifically designed to enrich the 2,3,4,7,8-PeCDF congener has been described by Mohr *et al* (1996). It is also important to appreciate that any new immunoassay with dramatically improved sensitivity for PCDD/Fs should also reduce the complications from interferences. This will typically be due to the ability to dilute the matrix further or to add a smaller sample size while still detecting the target analyte.

5.3.4 Quality Assurance

Quality assurance (QA) in the field of environmental immunoassay has not been developed to the same extent as with conventional techniques such as GCMS. Ohmicron provided a guide to QA for users of their immunoassay kits but this is of limited utility in the environmental field and includes primarily general QA information available in the open clinical immunoassay literature. Third party vendors have yet to market QA materials for any environmental immunoassay kit.

Prior to the acquisition of Millipore by EnSys, the former company was in the process of designing spiked soil samples for QA use with the three largest selling hazardous waste kits in the EnviroGard range (PCB, TPH and PAH). It is not known whether this programme is being continued by EnSys. The 2,3,7,8-TCDD immunoassay kit marketed by EnSys may prove to be a useful immunoassay QA model for I-TEQ screening, but the difference between a highly specific assay relative to a broad spectrum assay may throw up new problems that can only be identified and addressed through a carefully structured laboratory programme.

Existing PCDD/F QA methods rely on stable isotope labelled internal standards which are quantified by GCMS (see *Section 5.2.5*). In the case of immunoassay tests, spiking with a radiolabelled PCDD or PCDF congener does not provide the necessary quality control since the assay cannot differentiate between ^{12}C and ^{13}C isotopes. An internal standard in the form of a ^{13}C-PCDD or PCDF congener would interfere with the reaction between the ^{12}C analyte and the antibody, resulting in overprediction of the PCDD/F concentration in the sample. The presence of a large signal due to added isotope labelled internal standards would obscure immunoassay responses near the detection limit and severely undermine the utility of the test.

Another consideration is whether a sample collected for the purposes of an immunoassay test can, in the event that the sample fails the screening analysis, be taken forward for GCMS analysis, possibly after further cleanup. It would clearly be advantageous to introduce labelled congeners into the sampler or sample prior to the cleanup for the immunoassay test. Harrison and Carlson (1996) have addressed this issue by proposing a suite of ^{13}C labelled PCDD and PCDF congeners which exhibit low cross reactivity with the DD3 antibody (see *Table 3.2b*) and could be calibrated to correct for residual cross reactivity. The suggested labelled congeners are listed in *Table 5.3b*.

Table 5.3b Suggested ^{13}C PCDD/F Congeners in Immunoassay that are Compatible with Subsequent GCMS Analysis

^{13}C PCDD/F Congeners (Harrison and Carlson, 1996)
^{13}C-1,2,3,4-TCDD
^{13}C-OCDD
^{13}C-1,2,3,4-TCDF
^{13}C-1,2,3,7,8-PeCDF
^{13}C-1,2,3,4,7,8-HxCDF
^{13}C-1,2,3,6,7,8-HxCDF
^{13}C-1,2,3,7,8,9-HxCDF
^{13}C-1,2,3,4,6,7,8-HpCDF
^{13}C-1,2,3,4,7,8,9-HpCDF
^{13}C-OCDF

This suggested procedure has yet to be tested and full developed in the laboratory, and in any event does not in itself address the issue of QA within the immunoassay.

Some workers, including Vanderlaan *et al* (1988) have used radiolabelled PCDD/F congeners as an internal standard. This approach offers advantages only for laboratories which can quantify radioisotopes (ie laboratories with a scintillation counter). This method has the disadvantage of generating radioactive PCDD/F mixed waste, the disposal of which will require care. The method is not known to be used significantly outside of biochemically oriented research laboratories. Vanderlaan *et al* (1988, 1995) have also described the use of the dye Fat Blue B (1,4-bis(butylamino)-9,10-anthraquinone) for tracking the PCDD/F fraction of their samples through cleanup procedures. This dye travels with the PCDD/F fraction through the alumina elution of the column cleanup (see *Section 5.2.4*) and separates during the carbon column cleanup. The dye is commercially available as Solvent Blue 35. Use of such marker dyes for this purpose is not widespread in the PCDD/F literature. It may be possible to quantify recovery through part of the extraction and cleanup for immunoassay by spectrophotometric tracking of the dye.

Another possible QA approach which has been used extensively in pesticide residue analysis is the split/spike technique for tracking recovery. A sample is split into two subsamples, one of which is spiked with the analyte of interest. Both samples are then prepared and analysed in parallel. The measured difference in analyte concentration is compared to the known spike level and the fractional recovery is calculated. This recovery value is then applied as a correction factor to the unspiked sample to estimate the recovery-corrected residue level. A disadvantage of this method is that the recovery is assumed to be the same for both spike analyte and incurred residue, and this may not be the case. Another disadvantage is that some of the sample preparation and analytical costs are doubled. Nonetheless this approach may be viable for PCDD/F immunoassay because of the compatibility with the stable isotope labelled internal standards which are written into most PCDD/F QA methods.

5.4 Costs

It is difficult to determine with certainty what the cost advantage of an immunoassay screening programme would be before knowing the structure of the programme. However, in an attempt to define boundaries and evaluate the relative importance of different cost factors, a simple screening model based on Sherry (1992) will be employed. It is assumed that all samples are first screened by immunoassay. All positives in this screen will be confirmed by GCMS, as will some proportion of the negative samples. Because of the structure of this screening programme, the actual cost per sample depends on the following factors:

- the cost of conventional analysis;
- the cost of the immunoassay;
- the frequency of positive samples which are confirmed by GCMS;
- the proportion of the negative samples which are selected for GCMS confirmation.

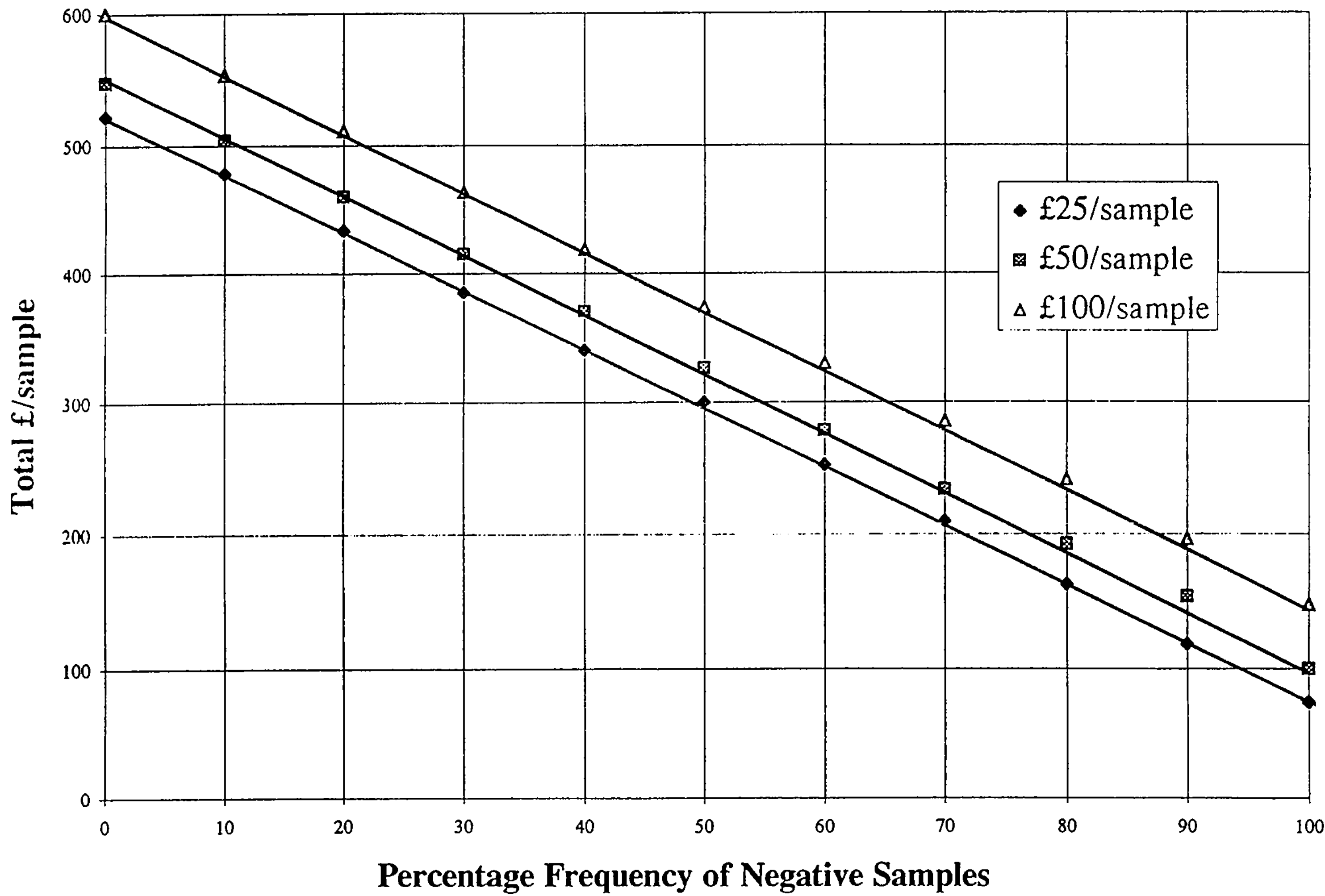


Figure 5.4a Cost Reduction by Immunoassay Screening: Total £/sample as a function of Negative Sample Frequency

Table 5.4a Calculated Cost Savings of Immunoassay Screening for PCDD/Fs

Condition	Cost of Sample Preparation and Analysis (£/sample) (1, 2)		% of Negative samples Confirmed by GCMS (3)	Total Cost (£/sample) at Negative Rates of:				Negative Sample Rate Required to Break Even
				0%	30%	70%	90%	
Completely separate	50	500	20	550	430	270	190	13%
sample preparation for	50	500	10	550	415	235	145	11%
both methods	50	500	5	550	408	218	123	10%
Immunoassay analysis	217	334	20	550	470	363	310	19%
of crude conventional	217	334	10	550	460	340	280	17%
extract in GCMS queue (4)	217	334	5	550	455	328	233	16%
Immunoassay analysis	384	167	20	550	510	457	430	33%
of cleaned conventional	384	167	10	550	505	445	415	33%
extract in GCMS queue (5)	384	167	5	550	503	440	408	32%

Notes

(1) Immunoassay cost of £50 per sample. This includes sample preparation and (inexpensive) cleanup.

(2) GCMS cost is £500 per sample, consisting of three equal components for (a) sample preparation and extraction; (b) Extract cleanup; (c) analysis and data reduction.

(3) All immunoassay positives are analysed by GCMS using the same extract as for immunoassay. Immunoassay negatives are confirmed by GCMS at 5%, 10% or 20% frequency as noted.

(4) Immunoassay cost includes 1/3 or GCMS cost (sample preparation and extraction).

(5) Immunoassay cost includes 2/3 of GCMS cost (sample preparation, extraction and cleanup)

The cost of sample collection has not been considered in this analysis since it is assumed to be a common factor irrespective of the method of analysis.

Therefore the total cost per sample is given by the following relationship:

$$C_t = I_c + (GCMS_c \times F_{pos} \times F_{neg} \times F_{con})$$

where:

C_t = total cost per sample
I_c = immunoassay cost per sample
$GCMS_c$ = GCMS cost per sample
F_{pos} = frequency of immunoassay positive samples
F_{neg} = frequency of immunoassay negative samples
F_{con} = frequency of GCMS confirmation of immunoassay negative samples

It is important to note that the frequency of immunoassay positive samples is outside the control of the analyst and significantly affects two of the three terms in the above equation. *Figure 5.4a* illustrates the cost reduction using three different immunoassay analytical costs, and positive sample rates from 0% to 100%. *Table 5.4a* shows the same type of data for an example set of costs, including the economic break-even point for several different conditions. The cost assumptions are shown on the table: clearly they influence the conclusions to be drawn on cost benefit, and while they generally reflect current market rates, they are presented in this report solely for illustrative purposes.

Bearing in mind the caveat regarding costs, the key points arising from *Table 5.4a* and *Figure 5.4a* are as follows:

- the economic value of a PCDD/F immunoassay is greatest when the frequency of positive samples is lowest;

- the frequency of positive samples has a greater effect on the economic value of a PCDD/F immunoassay than the cost of the immunoassay (within reasonable limits) or the confirmation rate of negative samples;

- the most important factor over which the analyst has some control is the point of divergence of the immunoassay sample preparation from the GCMS sample preparation. Cost reduction would be maximised by having either no sample cleanup or an inexpensive sample cleanup distinct from the conventional sample cleanup.

With a sufficiently high number of negative assays, cost savings could be realised even if the immunoassay were used for screening conventionally extracted and cleaned samples. However, in general the conclusion can be drawn that immunoassay will be most attractive if the test can support a limited, less costly sample cleanup procedure. We have discussed one such approach (Mohr *et al*, 1996) in previous sections.

6. SUMMARY, INFORMATION GAPS AND RECOMMENDATIONS

6.1 Summary

6.1.1 Sampling and Sample Preparation

Because of the need for complete extraction of PCDD/Fs from stack gases, aqueous effluents and their entrained particulates, efficient capture of the sample is essential. The tight binding of PCDD/Fs to solids, either trap adsorbent or sample matrix, indicates that extraction of particulates is critical to both sample types.

It is likely that the immediate future of PCDD/F immunoassay will rely upon conventional sample capture and extraction. While this may not ultimately be the best or least costly method of implementing an immunoassay programme, in the short term such a compromise seems necessary to reduce the number of analytical variables. However, research groups such as that of Mohr *et al* (1996) are developing promising alternative integrated sampling and cleanup techniques which are in principle compatible with the requirements of immunoassay. Successful validation of an immunoassay using conventionally prepared and cleaned extracts would then allow research to progress confidently to the next step of using these alternative sampling, extraction and/or cleanup methods.

In *Section 5.3.2* it was suggested that water miscible aprotic solvents such as DMF, DMSO or DMA could be used for extraction of PCDD/Fs from samples. These polar solvents are not perceived as being acceptable for routine GC use, but they have the potential of going directly into immunoassay without further sample cleanup. However, since this group of solvents typically have high boiling points, this is not feasible unless the sensitivity of the available immunoassay is sufficient for direct analysis of extracts (ie no concentration by solvent evaporation). This would require sensitivity of at least 20 pg per immunoassay sample (20 μl of extract containing 1 pg I-TEQ μl^{-1}). While this is significantly better than the 100 pg/tube of the tube-based I-TEQ screening immunoassay described by Harrison and Carlson (1996), it approaches the range of the less than fully characterised microplate test described by the same authors (25 pg/well). Improvement in sensitivity to better than 10 pg per immunoassay sample would justify examination of this group of solvents for direct extraction and analysis.

6.1.1 Sample Cleanup

Successful analysis of crude sample extracts is unlikely to be achievable by immunoassays except for very clean samples. However, the specificity intrinsic to immunoassays suggests that sample extracts do not need to be cleaned to the same degree as for GCMS. Thus, an adequate partial cleanup of conventionally prepared extracts should exist somewhere between these two extremes. Significant work has been devoted recently to the development of solid phase extraction (SPE) methods for aqueous matrices (Turner *et al*, 1992; Price, 1995). This system has the advantage of potentially coupling directly to the extract cleanup system to provide a one-step extraction and cleanup.

By introducing the necessary sample concentration factor at the extraction stage, it may be possible to elute an SPE disk or column with a polar solvent such as DMF or isopropanol, which could then be analysed directly by immunoassay.

The HPLC method of Mohr *et al* (1996) has been shown to be effective for the separation of 2,3,4,7,8-PeCDF from a stack emission sample. This type of cleanup is also amenable to automation, and merits further consideration.

6.1.2 Antibodies and Test Formats

We deduced from a statistical analysis of PCDD/F release data that 2,3,4,7,8-PeCDF has the potential to serve as a marker for the total I-TEQ of a sample: this has also been confirmed by Mohr *et al* (1996). However, information in the technical literature indicates that under different assay conditions a commercially available anti-dioxin antibody, DD3, either recognises PCDD/F congeners in the same proportion as their I-TEFs, or exhibits high specificity for the 2,3,4,7,8-PeCDF congener. This suggests that the DD3 antibody, although primarily developed for the recognition of 2,3,7,8-TCDD, should also be successful in an immunoassay for 2,3,4,7,8-PeCDF provided the assay conditions were tailored accordingly. The precise assay conditions and quality control measures need to be elucidated through careful experimentation.

Analytical sensitivity of immunoassays and the effects of potential interferences on those assays are determined primarily, but not completely, by the antibodies used. Since antibodies are large and complex molecules, it follows that considerable variability exists among immunoassays, even those directed against the same analyte. For example, observe the difference in specificity between the anti-dioxin monoclonal antibodies described by EnSys (1995) and Harrison and Carlson (1996). In addition the format or other aspects of the immunoassay can significantly affect any performance characteristic of the assay. For example, observe the difference in specificity of the DD3 monoclonal antibody cited by groups using two different test formats (Stanker *et al*, 1987; Harrison and Carlson, 1996). Though these demonstrated differences are in antibody specificity, variability can also be expected in other factors such as tolerance of solvents or other interferences.

Thus it is difficult to make generalisations about many aspects of immunoassays. Solvents which are inappropriate for one assay may be helpful in another and should not be rejected *a priori*. For example, in *Section 3.6* it was noted that Bigami (1993) has shown that some antibodies can tolerate neat organic solvents. This work stands in contrast to much of the early conventional wisdom from clinical immunochemistry, where most matrices are aqueous. These examples are provided to emphasise that extensive room exists for exploration of improved immunoassay performance through the innovative use of organic solvents, format changes or other devices.

The statistical value of 2,3,4,7,8-PeCDF as a marker for total I-TEQ of the sample has been identified in *Section 4* as a theoretically viable approach to immunoassay screening. However, a decision to pursue a new antibody with specificity for 2,3,4,7,8-PeCDF should be tempered with an understanding of the effort required and the lack of assurance that the resulting specificity will be limited to the single marker congener.

The theoretical effectiveness of this single congener strategy asymptotically approaches but can never exceed the effectiveness of a sound I-TEQ screen. The reason for this is that correlation of a single congener with I-TEQ will never be perfect because of the inevitable variability of composition among samples. If this approach were undertaken, it would first require hapten/analogue design and synthesis, the simplest approach to which requires three steps from commonly available starting materials, the reactions typically giving low yields of products which are difficult to handle and purify. Once the immunogen has been synthesised, the major concern becomes the likelihood of obtaining specificity for the marker congener. It should be noted that to date there have been only two successful attempts at development of monoclonal anti-dioxin antibodies in two decades. One of these (DD3) is broadly specific, providing I-TEQ screening capability in one configuration. The other (EnSys, 1995) is more specific than most other anti-dioxin antibodies, but still has significant recognition for at least one 2,3,7,8-positional congener and two non-toxic congeners. Thus, the likelihood of absolute single congener specificity appears to be low, based on the available history in the field.

To balance these comments, it should be stated that new PCDD/F antibodies are currently in development. As has been described previously in *Table 3.2a*, two groups in the US are pursuing new monoclonal antibodies against PCDD/Fs using novel hapten chemistry (Gee *et al,* 1995; ECOCHEM, 1995). ECOCHEM reports that hybridoma fusions have been performed and monoclonal antibodies are currently being screened. Preliminary indications are for significantly improved sensitivity in response towards sample I-TEQ (in the order of 10 pg/tube) and further data on sensitivity and specificity will be released in due course.

6.2 Information Gaps and Recommendations for Developmental Work

There are a number of information gaps relating to the use of immunoassay for the applications under consideration. These have been discussed in individual sections above, and will not be repeated save to note that all stages of the assay, from sample preparation, through sample cleanup and to the assay format, require further elucidation and perhaps more importantly, standardisation through an appropriate QA scheme. Our recommendations are directed towards addressing these shortcomings. We have grouped our recommendations into five discrete areas, commencing with antibody development and then addressing each key stage of an assay. These are listed below.

6.2.1 Antibody Developmental Work

Based on the arguments presented in *Section 6.1.3*, research effort can proceed along two fronts.

- Firstly, antibodies capable of direct I-TEQ screening should be further characterised and assessed for their potential to be incorporated into an appropriate immunoassay test format. These antibodies include the commercially available DD3 antibody, and antibodies with greatly improved sensitivity currently being developed by ECOCHEM.

- Secondly, since 2,3,4,7,8-PeCDF shows promise as a marker congener, there is merit in considering the development of an antibody for this congener together with the necessary cleanup methods, test procedures and quality assurance.

For the reasons given in *Section 6.1.3*, it is recommended that initial effort be directed towards a fuller evaluation of existing antibodies to directly measure the I-TEQ of a sample, before proceeding with the greater resource requirements of developing a new anti-2,3,4,7,8-PeCDF antibody. However, in the event that research and commercial organisations might independently be developing anti-2,3,4,7,8-PeCDF antibodies, we recommend that the Environment Agency maintains contact with EnSys, ECOCHEM and the research group at the University of California at Davis to receive regular updates, both on this issue and on progress towards an anti-dioxin antibody of improved sensitivity.

6.2.2 Sample Extraction

The discussion in *Section 5.3.4* and *Section 6.1.1* identifies three areas which should be investigated further. These include the use of aprotic solvents such as DMF, DMSO and DMA for solid sample extraction, the use of SPE adsorbents for aqueous sample extraction, and the further development and characterisation of HPLC systems using elution solvents which are compatible with the requirements of immunoassay. It should be noted that the history of PCDD/F analytical method development is intimately tied to GCMS, and the historic avoidance of polar solvents for GCMS analysis has prevented the exploration of the latter solvents in PCDD/F cleanups. Aprotic solvents have the advantage of being excellent solvents for PCDD/Fs but mediocre solvents for the aliphatic hydrocarbons which frequently accompany PCDD/Fs in far higher concentrations. The utility of such methods would be low unless immunoassay sensitivity were improved more than ten-fold from the present best. However, this level of improvement is entirely possible (see *Section 6.1.3*) so radically different extraction methods should not be discounted.

6.2.3 Extract Cleanup

The discussion of costs in *Section 5.4* demonstrates the need for simplified sample cleanup specifically designed for use with immunoassay in order to maximise the utility of a screening test. Work in this area should be preceded by identification of an immunoassay capable of providing the necessary screening capability. Subsequently, work should be directed toward simplification of the current cleanup in a manner that can be packaged more conveniently, but will still remove the interferences discussed in *Section 5.3.3*. HPLC offers one such route and as Mohr *et al* (1996) have shown, the marker congener 2,3,4,7,8-PeCDF can be successfully isolated using this procedure.

6.2.4 Quality Assurance

It is essential to accommodate adequate QA into an immunoassay which will ultimately be used to inform decisions relating to regulatory control of processes. It is therefore critical to determine an acceptable QA approach as a priority. The QA options presented in *Section 5.3.4* should be discussed with active PCDD/F analysts and pursued to pave the way for an appropriately designed validation project.

As noted in *Section 4.5*, the format of an immunoassay test can influence the performance of the antibody to a significant extent. An important goal of QA will be to clarify and to standardise as far as is possible an assay format relevant to the application of interest. The format should cover sample preparation, sample cleanup, quality control, assay procedure and data analysis.

6.2.5 Pilot Study

No field validation data have been generated for the sole I-TEQ screening approach described in the literature (Harrison and Carlson, 1996) and currently marketed by EnSys. On the basis of the discussion in *Section 4*, this approach appears to have potential, and there is a need to undertake a pilot study for validation of I-TEQ screening using field samples which have been conventionally extracted and cleaned. This can be coupled with the simultaneous development of simplified/alternative sample cleanup which can be applied to conventionally prepared extracts prior to immunoassay analysis, followed by validation of the immunoassay I-TEQ screening test. In the first instance the pilot study can be undertaken with commercially available kits (both from EnSys), but potential method improvements and changes can be implemented as more sensitive immunoassays become available.

The Environment Agency could explore the potential for collaboration with academic and other research institutions demonstrating capabilities in the development of antibodies, sample preparation techniques, and development of immunoassay test procedures.

6.2.6 Coordination of Agency Programmes

In order to assist in the coordination of present and future projects in the field of immunoassay (see *Section 2.6.4*) it is recommended that the Environment Agency explores means by which the exchange of information on research findings, antibody development, analytical procedures and field validation studies can be facilitated.

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8. GLOSSARY

Analogue: A molecule structurally and chemically similar to the chemical to be analysed.

Analyte: The chemical to be analysed.

Antibody: Proteins produced by the body as an immune response to the presence of a foreign chemical (antigen). *Monoclonal* antibodies are a single strain of antibodies specific to a particular chemical, while *polyclonal* antibodies are a mixture of different types.

Antigen: A chemical or other foreign agent (virus, bacterium, etc) which enters the body. In the case of the present study, PCDDs and PCDFs are classed as antigens.

Antisera: Serum from the blood of an immunised animal, containing a broad population of antibodies.

Aprotic Solvents: Solvents that are "neutral" in the sense that they neither accept nor donate hydrogen ions via interactions with the analyte.

Assay: An analytical test procedure.

Chromogen: A chemical introduced in the later stages of the immunoassay to produce a colour change proportional to the amount of analyte in the test solution.

Cluster Analysis: A statistical technique by which the PCDD/F congener profiles of the samples are grouped or "clustered" according to the degree of similarity.

Congener: One of the 210 individual chemicals making up the family of dioxins and dibenzofurans.

Congener Profile: The pattern prodused by the relative concentrations of the PCDD/F homologues or the seventeen 2,3,4,7,8-positional congeners in a sample.

Conjugate: A chemical formed by linking an analogue of the hapten molecule to a larger carrier protein molecule.

Cross reactivity: Recognition by the antibody of chemicals with similar structures, shapes and functional groups as the target analyte.

Dendrogram: A graphical representation of the results of a cluster analysis, in which the clusters are joined by branches or dendrites, the lengths of which indicate the degree of similarity.

ELISA: Enzyme linked immunosorbent assay. The test response is obtained through the catalytic action of the enzyme, causing a chromogen to produce a colour, the intensity of which is proportional to the amount of analyte in the test solution.

Fusion: Causing two cells to join in a culture medium.

GCMS: Gas chromatography-mass spectroscopy. A combination of instrumental techniques that is currently the standard method of analysis for PCDDs and PCDFs.

Genome: The set of chromosomes found in each nucleus of a given species

Hapten: A small molecule (antigen) that may not elicit an immune response by itself, but may do so when conjugated to a larger protein molecule.

Homologue: Groups of PCDD or PCDF congeners containing the same number of chlorine atoms.

HPLC: High Pressure Liquid Chromatography. A sample preparation and cleanup method in which the components are separated on a column through which the liquid sample is forced under pressure.

Hybridoma: A new cell type obtained from the fusion of other cells.

Immunoassay: Antibodies generated as an immune response to a target chemical are removed from the host mammal, and then used in the laboratory as an external analytical agent for the detection and quantification of that target chemical in other environmental media.

Inhibition: Suppression of the binding of the labelled analyte to the antibody due to the presence of the unlabelled analyte in the test solution.

Labelled hapten: A hapten to which an enzyme, radionuclide, etc is attached. The attached molecule provides the test response which is measured, in the case of an enzyme label, via the intensity of colour.

Mass fragmentogram: The output from a mass spectrometer which presents the type and abundance of the molecular fragments generated by the chemicals in the test solution.

Matrix: The medium in which the analyte is present (soil, water, air, etc).

Sample preparation: Various physical or chemical processes performed on the sample to convert it into a form that is compatible with the analytical technique to be used.

Screening: A go/no-go test in which the concentration of a chemical in a sample is expressed as being above or below a set point rather than as an absolute concentration.

Soxhlet extraction: Extraction of a chemical from a solid sample by contacting it with a continuous stream of boiling solvent in a specially designed "Soxhlet" apparatus.

Specificity: The degree of cross reactivity exhibited by the antibody with chemicals other than the target analyte.

Substrate: A solution added to the imunoassay test in the later stages, followed by the addition of a chromogen. The enzyme attached to the labelled hapten catalyses the reaction between the substrate and the chromogen to produce a colour.

Surrogate: A chemical (not necessarily related to the target analyte) that can be measured or controlled instead of the analyte, and whose concentration varies proportionately with that of the analyte.

Annex A

Immunoassay Research Activities

ORGANISATIONS ACTIVE IN DIOXIN (OR OTHER) ENVIRONMENTAL IMMUNOASSAY RESEARCH

A1. ACADEMIC GROUPS

A1.1 University of California at Davis

The research group at the University of California is led by Professor Bruce Hammock, who is generally regarded as the founder of the field of environmental immunoassay. The scope of research effort is very broad, with some current effort on PCDD/Fs (Gilman *et al*, 1995; Gee *et al*, 1995). Current emphasis is on pesticides, but the group has sufficient expertise and facilities to cover other types of environmental pollutants. The group collaborates with the hybridoma facility at University of California at Berkeley, headed by Dr Alex Karu.

A1.2 Institute of Hydrochemistry, Technical University of Munich

The group is headed by Professor Reinhard Niessner, with Dr Dietmar Knopp a significant colleague. The Technical University of Munich is the main academic location in Europe for environmental immunoassay, but there is no past or present activity with PCDD/Fs. The group is presently engaged in the development of immunoassays for pesticides, PAHs, nitro-PAHs, BTX, etc. Activities include immunogen preparation, immunisation of rabbits (development of polyclonal antibodies), test format development and validation of the assays by conventional analytical methods.

A1.3 University of Guelph (Ontario)

The group, headed by Professor Christopher Hall, has published extensively in the field of pesticide immunoassay, and is considered to be one of the most prominent academic laboratories in Canada in this field. The group has not addressed the issue of PCDD/Fs, but current research interests in the field of engineered antibody technology may benefit the development of PCDD/F immunoassays.

A1.4 Robens Institute, University of Surrey

The Analytical Centre is headed by Dr Derek Stevenson. The group is active in the general immunoassay field, and is currently involved in a project for the Environment Agency, examining the use of immunoassay techniques in environmental analysis. The focus of activity is on the use of immunoasay techniques in occupational health monitoring. The group has successfully developed antibodies for chlortoluron and isoproturon. The group is

currently active in the development of portable ELISA kits and immunoaffinity columns. While not involved in dioxin research, the group has expressed a strong interest to be involved in future work.

A2. GOVERNMENT GROUPS

A2.1 Health and Welfare Canada (Ottawa, Ontario)

The group, headed by Dr Harvey Newsome, has considerable expertise in the development and application of immunoassays in environmental analysis, for example developing a radioimmunoassay for PCBs (Newstone and Shields, 1981). The group has not researched immunoassay applications in the field of PCDD/Fs.

A2.2 Environment Canada, Canadian Center for Inland Waters (Burlington, Ontario)

The group is headed by Dr Jim Sherry, whose extensive knowledge of the environmental immunoassay area is reflected by his definitive review (Sherry, 1992) as well as an update (Sherry, 1995). The group's experience includes significant research on PCDD/F immunoassays (Sherry *et al*, 1989), but it is not currently active in the area. However, the group represents a valuable base of experience in the application of dioxin immunoassays to field samples.

A2.3 US EPA Environmental Monitoring Systems Laboratory (Las Vegas NV)

The group is headed by Dr Jeanette Van Emon, whose previous experience in PCDD/F immunoassays has included a postdoctoral placement at Lawrence Livermore Laboratory, contributing to the latter laboratory's PCDD/F immunoassay work. The group has collaborated with MRI-California on several projects on immunoassay and novel sample preparation methods, especially supercritical fluid extraction. The group has participated in the Superfund Innovative Technology Evaluation (SITE) program by coordinating evaluations of commercial kits in field situations. The group also reviewed field portable immunoassays, including those evaluated under the SITE program (Van Emon and Gerlach, 1995). Dr Van Emon co-sponsors the annual "Immunochemistry Summit" meetings.

A2.4 Institute of Food Research (Norwich)

The group is headed by Dr Mike Morgan, and has been involved in environmental immunoassays for several years. The focus is generally on pesticides in foods. The outcome

A2.4 Institute of Food Research (Norwich)

The group is headed by Dr Mike Morgan, and has been involved in environmental immunoassays for several years. The focus is generally on pesticides in foods. The outcome of a MAFF funded project on the development of immunoaffinity columns for PCDD/F analysis was published in 1992 (Langley *et al*, 1992), but no further work on PCDD/Fs has been undertaken. The group has the capability to contribute technically in the future, if called upon.

A2.5 Central Science Laboratory

Currently located at Slough, Berkshire but the dioxin laboratory is due to move shortly to York with other parts of the organisation due for relocation at Norwich. The laboratory was formed four years ago from the restructuring of ADAS where its staffing were principally associated with the pest infestation laboratory. Activities are primarily associated with MAFF requests for research and surveillance in relation to foods with the conventional use of GC/MS techniques for analysis. They have not been involved in the development of antibodies for dioxin immunoassays but are currently evaluating a dioxin immunoassay test kit from Millipore. This is driven by continued interest in the field from MAFF who assess new technologies and products as and when these become available. The Central Science Laboratory have been involved with some work in the field of immunoassays working with the University of East Anglia and AFRC, examining immunoaffinity and abstraction technologies.

A2.6 Central Veterinary Laboratory, Slough

The group, headed by Dr Roy Jackman, has been active in the field of immunoassays and antibody development for veterinary medicines. The group has developed over 50 antibodies for different haptens related to the veterinary and biochemical field, and has also developed ELISA kits for the detection of drugs and other chemicals in foods and meat. The group has currently expanded to include related areas such as ovine monoclonal and recombitant antibodies, commercial antisera production and studies on the immune response to hapten immunogens in sheep. The group has expressed a strong interest, and has the capability to contribute to antibody design and method development for dioxin immunoassay in the future.

A2.7 Centres for Disease Control (Atlanta GA)

Dr Don Patterson and Dr Wayman Turner of CDC have been active in the dioxin immunoassay area in recent years. The CDC laboratory has an interest in developing applications for PCDD/F immunoassay in biological samples, in collaboration with both (ex) Millipore and FMS.

A2.8 US Department of Agriculture (College Station TX)

The group is headed by Dr Larry Stanker who has maintained an interest in the PCDD/F immunoassay area since departing Lawrence Livermore Laboratory. However, PCDD/Fs are not part of the group's current immunoassay programme. Dr Stanker has contributed significantly to the field of PCDD/F immunoassays (Stanker *et al* 1987; Vanderlaan *et al,* 1988; Vanderlaan *et al,* 1989; Vanderlaan *et al,* 1995), and has continued collaboration with key academic research groups, for example at the University of California at Davis (Gilman *et al,* 1995; Gee *et al,* 1995).

A3. INDUSTRY GROUPS

A3.1 J T Baker

This company is one of a number of distributors for Ohmicron products throughout Europe (see below).

A3.2 Bionebraska (Lincoln, NE)

Most efforts by this company have been unrelated to the field of environmental immunoassay and there are no future plans for advancing this area. A subgroup of 10-11 technical staff are involved with development of metals immunoassay and to date the company's only commercial product is an immunoassay kit for the detection of mercury. A lead immunoassay test kit is expected to be on the market within six months and further enhancement of the mercury test to include tissue applications is also under development.

A3.3 Dräger, Lubeck

The Dräger organisation consists of a German parent company, Dräger Werk AG, with approximately 100 relatively independent daughter companies. Involvement with the dioxin field has primarily been in the marketing of EnSys registered test kits for approximately the past two years under the EnviCheck trade name. These are available in most parts of Europe except the UK where EnSys themselves are active. Dräger's expertise has been traditionally in the areas of air and fluids whereas the EnviCheck test kits aim to analyse soils, other solids and oils. Other than marketing the EnSys product line, the company is not involved in the research or development of antibodies or immunoassay technologies and has no interest in developing this sector.

A3.4 Envirologix (Westbrook, ME)

Envirologix is a relatively new company consisting of Bruce Ferguson, original founder of ImmunoSystems, and two other staff from who were also formally employed by ImmunoSystems. The emphasis is the development and marketing of immunoassay kits for biological markers relating to chemical exposure in humans. The company does not have significant interest or expertise in the dioxin field and are not looking to develop this market in the future.

A3.5 Biocode Inc (Cambridge, MA)

The key technical contact is Dr Jim Rittenburg, who previously worked with Quantix until their sale to Idetek. The company's corporate headquarters are at Cambridge (MA), with a second office located in Pennsylvania. Biocode has a R&D capability in the UK (York) comprising of 16 members who are aiming to develop product marker systems using a variety of dyes, coupled to immunoassay detection systems. Prior to 1995 the company had a series of products for the detection of mycotoxin detection, which was then sold to Rhone-Poulenc. Some preliminary effort was directed to the development of dioxin immunoassays. The experience gained in developing technologies to detect marker compounds in lipophilic matrices could be relevant to dioxin sample preparation.

A3.6 Ensys (Research Triangle Park, NC)

Current leader in the environmental immunoassay marketplace after purchasing the EnviroGard product line of environmental immunoassays from Millipore. The company is currently evaluating whether to assume rights to the Millipore dioxin test. The company has a UK presence at Four Marks, Hampshire. In July 1996 EnSys announced the signing of a non-binding letter of intent to merge with Strategic Diagnostics Inc (see A3.16).

A3.7 Ohmicron (Newtown, PA)

Ohmicron sell immunoassay kits for PCB, PAH, pentachlorophenol, petroleum fuels, and many pesticides, but not currently for dioxin. Much development work has been done with Dr James Fleeker of North Dakota State University.

Ohmicron is currently developing an immunoassay test kit (capable of processing 50 samples per hour) using polyclonal and monoclonal antibody systems targeted at the 17 2,3,7,8 PCDD/F congeners. The company has not targeted one specific marker congener, but proposes to estimate an I-TEQ figure through the use of calibration curves. The test can be run in quantitative or semi-quantitative modes using non-dioxin standards which mimic dioxins but do not have the associated implications of health and safety and cost. The Ohmicron system for pesticides uses magnetic particles for the support of antibodies rather

than the walls of a test tube. Removal of antibodies from the free anylate is performed by the application of a magnetic field with results being comparable with tube systems.

Ohmicron immunoassay kits are marketed in Europe by J T Baker.

A3.8 Ecochem Research Inc. (Chaska, MN)

Dr Robert Carlson has participated in the development of several EnviroGard immunoassays by Millipore (Carlson, 1995), including the EnviroGard dioxin test (Harrison and Carlson, 1996), all of which are now under evaluation by EnSys following their purchase of the product line. ECOCHEM also has obtained a Small Business Innovative Research grant from NIH for the production of anti-dioxin antibodies (ECOCHEM, 1995) and has developed a PCB toxic congener specific immunoassay (Carlson *et al*, 1995).

A3.9 FMS (Fluid Management Systems Inc., Watertown MA)

Hamid Shirkhan is the president and technical specialist for FMS, which makes automated sample clean-up systems for conventional dioxin analysis. FMS also currently maintains the rights to the Lawrence Livermore anti-dioxin antibodies (Stanker *et al*, 1987) and has been developing a market for dioxin immunoassay in collaboration with Millipore. Millipore had an agreement to sublicense one of these antibodies from FMS prior to the sale of the EnviroGard product line to EnSys.

A3.10 Guildhay Limited (Guildford, Surrey)

Guildhay Limited was formed in 1976 as a joint venture between the University of Surrey and the National Health Service. In 1992 the company was acquired by private investors. The company specialises in the development of antisera and monoclonal antibodies for a range of biological and pharmaceutical chemicals. In the environmental field, Guildhay has developed antisera for a range of pesticides and herbicides, and is also marketing ELISA test kits for the determination of atrazine and isoproturon in drinking water under the trade name of AQUASCREEN. The company has recently obtained a grant from the European Union to develop immunoassay test kits for a further twelve pesticides in drinking water. It is believed that EnSys has marketed a selection of Guildhay's products.

A3.11 Clifmar Associates, University of Surrey, Guildford

The company was formed in 1985 as an offshoot of the University of Surrey, and is headed by Dr Peter Kwasowski. The group is involved in the development of antibodies and in ELISA applications. While the current focus is on immunoassay techniques for analysis of drugs, the group is moving into the environmental field and has expressed a strong interest to be involved in future developmental work on dioxin immunoassays.

A3.12 Idetek (Sunnyvale, CA)

Idetek's primary interest is in food analysis for antibiotics and similar residues. In 1994 Idelek bought Quantix, which consisted primarily of tests for PAH, petroleum fuels, and several pesticides. The company has the technical capability to develop dioxin immunoassay systems, although they do not currently do so.

A3.13 MRI-California (Midwest Research Institute, Mountain View CA)

This group has participated at some level in environmental immunoassay for several years, primarily by contracts given to outside groups or by investigating interfaces between immunoassays and novel sample preparation methods. The California Operations Director, Dr. Viorica Lopez-Avila, has undertaken significant collaborations with the US EPA. The Institute's key technical expertise is in related areas such as novel and conventional analytical methods and novel sample preparation methods. The MRI facility in Kansas City has a dioxin analytical capability and has limited experience in dioxin immunoassay, but is not currently active in the area.

A3.14 R Biopharm, Darmstadt, Germany

R Biopharm has a range of approximately 40 tests for a variety of haptens including several mycotoxins, other food toxins, hormones, vitamins and veterinary drugs. The company has only two tests for environmental pollutants; these are concerned with the assay of trazine based pesticides and TNT explosives. The company has no plans to expand further into the examination of industrial wastes.

A3.15 Riedel de Haen, Seelze, Germeny

The company has a similar focus to R Biopharm (ie bio-medical immunoassay tests) with only one other system for the detection of triazine pesticides.

A3.16 SDI (Strategic Diagenostics, Inc, Newark DE)

The company expressed an interest in dioxin immunoassay in 1991, but this does not appear to have resulted in either product, publications, or presentations. The company appears to have a long term commitment of support from Merck and EM Science, and also undertakes developmental work for Biocode Inc.

Table3.1x List of Commercial Contacts

#	Group/Person	Location	Phone/Fax Number
1	BioNebraska	Lincoln NE	402-470-2100/402-470-2345
2	Dräger	Lübeck FRG	451-882-4012/451-882-3152
3	EnviroLogix	Westbrook ME	207-854-3600/
4	BioCode	PA/MA/UK	215-795-0930/215-795-0940
5	EnSys	Research Triangle Park NC	919-941-5509/919-941-5519
6	Ohmicron	Newtown PA	215-860-5115/215-860-5213
7	ECOCHEM	Chaska MN	612-448-4337/612-448-1651
8	FMS	Watertown MA	617-926-1521/617-923-2168
9	Guildhay	Guildford UK	01483 573727
10	ClifMar Associates	Guildford, UK	01483-259708
11	Idetek	Sunnyvale CA	408-745-0544/408-745-0243
12	MRI	Mountain View CA	415-694-7700/415-691-6844
13	R Biopharm	Darmstadt FRG	06151-8102-0/06151-8102-20
13	Riedel de Hähn	Seelze FRG	5137-999-557/5137-999-135
14	SDI	Newark DE	302-456-6789/302-456-6770

List of Contacts

#	Group/Person	Location	Phone Number/Fax Number
1	University of California	Davis CA	916-752-7519/916-752-1537
2	Technical University of Munich	Munich FEG	8161-713396/8161-714403
3	University of Guelph	Guelph ONT	519-824-4120/519-837-0442
4	Robens Institute	Guildford UK	01483-259220/01483-503517
5	Health and Welfare Canada	Ottawa ONT	613-957-0947/613-941-4775
6	Environment Canada	Burlington ONT	905-336-4813/905-336-4989
7	US EPA	Washington DC	703-308-0476/
8	US EPA	Las Vegas NV	702-798-2154/702-798-2243
9	Institute of Food Research	Norwich UK	06-03-255-000/06-03-507-723
10	Central Science Laboratory	Slough, UK	
11	Central Veterinary Laboratory	Surrey, UK	01932 341111/01932 347046
12	Centres for Disease Control	Atlanta GA	404-488-4176/404-488-4609
13	US Department of Agriculture	College Station TX	409-260-9306/409-260-9332
14	AOAC	Gaithersberg MD	301-924-7077/301-924-7089
15	AIEC	Midland MI (Pat Nugent - Dow Chemicals)	517-636-5181/517-638-6856

Table A1 Organizations currently involved in dioxin immunoassay

Group	Category	Reference	Nature of Activity
EnSys	Commercial	EnSys (1995); Harrison and Carlson (1996)	Sales of previously developed kit(s)
FMS	Commercial	Harrison *et al* (1995); Turner *et al* (1992)	Development of systems using DD3 antibody. Interface of kits with automated sample preparation system.
ECOCHEM	Commercial	ECOCHEM (1995); Harrison and Carlson (1996); Carlson (1995)	Novel hapten design and synthesis. Antibody development.
Hammock (University of California)	Academic	Gilman *et al* (1995); Gee *et al* (1995)	Novel hapten design and synthesis. Antibody development. New assay formats.
Stanker (US Department of Ariculture)	Government	Gilman *et al* (1995); Gee *et al* (1995)	Molecular modelling, hapten design.

Annex B

Dioxin Congener Profiles and Statistical Analysis

Table B1 **Numbers and identification of the samples used for statistical analysis (Additional data for Dutch and German waste incinerators are not included)**

Sample No.	Sample ID	I-TEQ (ng m^{-3})	Sample No.	Sample ID	I-TEQ (ng/m^3)
Iron and Steel Works (Nos 1 to 16)			34	MWI 18	24.32
1	I&S 1	0.17	35	MWI 19	40.18
2	I&S 2	0.72	36	MWI 20	22.70
3	I&S 3	0.95	37	MWI 21	20.46
4	I&S 4	0.30	38	MWI 22	2.69
5	I&S 5	1.92	39	MWI 23	17.15
6	I&S 6	0.69	40	MWI 24	22.01
7	I&S 7	0.66	41	MWI 25	3.79
8	I&S 8	1.72	42	MWI 26	18.16
9	I&S 9	3.66	43	MWI 27	42.53
10	I&S 10	2.35	44	MWI 28	170.10
11	I&S 11	1.37	45	MWI 29	73.25
12	I&S 12	0.99	46	MWI 30	170.90
13	I&S 13	1.69	47	MWI 31	173.68
14	I&S 14	0.01	48	MWI 32	55.81
15	I&S 15	3.43	49	MWI 33	39.05
16	I&S 16	0.91	50	MWI 34	36.19
UK Municipal Waste Incinerators (Nos 17 to 59)			51	MWI 35	16.10
17	MWI 1	71.69	52	MWI 36	44.83
18	MWI 2	2.19	53	MWI 37	33.45
19	MWI 3	7.69	54	MWI 38	3.95
20	MWI 4	1.56	55	MWI 39	1.87
21	MWI 5	12.14	56	MWI 40	1.09
22	MWI 6	0.46	57	MWI 41	1.62
23	MWI 7	2.20	58	MWI 42	1.63
24	MWI 8	0.21	59	MWI 43	1.12
25	MWI 9	0.51	**German Municipal Waste Incinerators (Nos 60-67)[a]**		
26	MWI 10	6.01	60	MWI 44	6.60
27	MWI 11	0.23	61	MWI 45	0.16
28	MWI 12	0.03	62	MWI 46	1.81
29	MWI 13	12.26	63	MWI 47	0.04
30	MWI 14	99.17	64	MWI 48	0.45
31	MWI 15	96.72	65	MWI 49	0.02
32	MWI 16	87.61	66	MWI 50	0.05
33	MWI 17	47.29	67	MWI 51	0.001

[a] Samples 60,62,64 and 66 relate to pre-retrofits on 4 German MWIs respectively. Samples 61,63,65 and 67 relate to post-retrofit emissions for the respective MWIs.

Table B1 **Numbers and identification of the samples used for statistical analysis (Additional data for Dutch and German waste incinerators are not included) continued**

Sample No.	Sample ID	I-TEQ (ng/m^3)	Sample No.	Sample ID	I-TEQ (ng m^{-3})
Chemical Waste Incinerators (Nos 68 to 109)			100	CWI 33	0.03
68	CWI 1	0.27	101	CWI 34	0.04
69	CWI 2	0.09	102	CWI 35	0.01
70	CWI 3	0.03	103	CWI 36	0.01
71	CWI 4	0.89	104	CWI 37	0.03
72	CWI 5	0.21	105	CWI 38	0.01
73	CWI 6	0.19	106	CWI 39	0.08
74	CWI 7	0.12	107	CWI 40	2.11
75	CWI 8	0.14	108	CWI 41	0.17
76	CWI 9	0.07	109	CWI 42	0.16
77	CWI 10	0.29	**VCM industry (Nos 110 to 130)[a]**		
78	CWI 11	0.04	110	Aqu Effluent, sample 1	37.22
79	CWI 12	0.03	111	Aqu Effluent, sample 2	1.74
80	CWI 13	1.05	112	Lights	0.02
81	CWI 14	0.78	113	Heavies - 1	7.56
82	CWI 15	2.72	114	Heavies - 2	6.13
83	CWI 16	4.62	115	Heavies - 3	3.10
84	CWI 17	0.32	116	Aqu - sample 1	2.97
85	CWI 18	0.53	117	Aqu - sample 2	1.24
86	CWI 19	0.71	118	Aqu HCI - sample 1	7.23
87	CWI 20	0.80	119	Aqu HCI - sample 2	7.15
88	CWI 21	0.03	120	Reactor Cat	1523.17
89	CWI 22	0.02	121	Cyclon Car	1.47
90	CWI 23	0.67	122	LEWA - sample 1	31.00
91	CWI 24	0.10	123	LEWA - sample 2	37.67
92	CWI 25	0.02	124	LEWA - sample 3	31.59
93	CWI 26	0.00	125	DOP - sample 1	70.39
94	CWI 27	0.01	126	DOP - sample 2	90.08
95	CWI 28	0.01	127	DOP - sample 3	43.11
96	CWI 29	0.00	128	Lagon Inlet - sample 1	0.24
97	CWI 30	0.01	129	Lagoon Outlet, sample 1	0.65
98	CWI 31	0.01	130	Lagoon Outlet, sample 2	0.11
99	CWI 32	0.02			

[a] I-TEQ results are in units of ng kg^{-1}

Table B2 Detailed congener data for complete data set

Iron and Steel Works (Samples I&S 1 - 16)																
Concentrations (ng/Nm-3)																
Sample #	I&S 1	I&S 2	I&S 3	I&S 4	I&S 5	I&S 6	I&S 7	I&S 8	I&S 9	I&S 10	I&S 11	I&S 12	I&S 13	I&S 14	I&S 15	I&S 16
2378D	0.0000	0.0577	0.0542	0.0251	0.0417	0.0183	0.0149	0.0338	0.0000	0.0321	0.0000	0.0404	0.0677	0.0000	0.1700	0.0210
12378D	0.0596	0.1298	0.1527	0.0503	0.1607	0.0642	0.0498	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2280	0.0730
123678D	0.1225	0.1058	0.3153	0.0553	0.4048	0.0872	0.0796	0.1435	0.5936	0.1357	0.1224	0.1061	0.1927	0.0000	0.0620	0.0350
123478D	0.0596	0.0769	0.1724	0.0352	0.2381	0.0596	0.0498	0.0928	0.3927	0.1000	0.0884	0.1465	0.1719	0.0000	0.2000	0.1080
123789D	0.0828	0.0817	0.2808	0.0352	0.3274	0.0688	0.0647	0.1646	0.4018	0.1000	0.0952	0.0657	0.1354	0.0000	0.0770	0.0730
1234678D	0.3311	0.3029	2.7586	0.1608	3.9286	0.3807	0.5473	1.6878	7.3059	0.8214	1.5646	0.3788	1.1458	0.1355	0.2730	0.2250
OCDD	0.2318	0.4375	4.7783	0.1759	7.7381	0.3853	0.9453	3.6709	15.5251	1.9643	4.2857	0.7071	1.8750	1.4486	0.3730	0.2840
2378F	0.0927	0.5288	0.4581	0.2060	0.9524	0.3807	0.4229	1.0970	2.4658	1.1786	0.8844	0.6566	1.2500	0.0000	22.8250	4.0410
12378F	0.0430	0.3173	0.2956	0.1307	0.8333	0.3211	0.3085	0.9283	1.7352	1.0714	0.6803	0.6061	0.9896	0.0000	0.9600	0.4210
23478F	0.1457	0.7212	0.7389	0.2864	1.8452	0.7339	0.7463	2.0253	4.2466	3.1071	1.5646	1.0606	1.8229	0.0000	1.3520	0.6070
123478F	0.0629	0.3942	0.4926	0.1658	1.2500	0.5046	0.4129	1.1814	2.9224	1.5357	1.0204	0.8081	1.3542	0.0000	0.3940	0.3480
123678F	0.0530	0.3221	0.4433	0.1307	1.1310	0.4266	0.3383	0.9705	2.1005	1.1429	0.8163	0.6061	1.0417	0.0000	0.3660	0.3430
234678F	0.0795	0.4375	1.1330	0.1859	2.3214	0.6422	0.5970	1.5612	2.6027	1.8571	1.4966	0.9091	1.5104	0.0000	0.2660	0.2260
123789F	0.0331	0.1875	0.3645	0.0905	0.7143	0.2431	0.2040	0.4641	1.2329	0.6071	0.4422	0.3434	0.5156	0.0000	0.0110	0.0080
1234678F	0.1490	0.5769	2.4631	0.2915	4.4048	1.1009	0.8955	3.3755	6.3927	3.2857	3.2653	1.5152	2.7604	0.1869	0.2650	0.3590
1234789F	0.0364	0.1010	0.7882	0.0553	0.8333	0.1560	0.1642	0.2743	1.4612	0.4286	0.5374	0.2374	0.4219	0.0000	0.0220	0.0320
OCDF	0.1391	0.3221	3.1034	0.1156	4.1071	0.3670	0.5970	2.3629	12.3288	1.1786	2.3810	0.4293	1.1458	2.2430	0.0430	0.1280
I-TEQ																
Sample #	I&S 1	I&S 2	I&S 3	I&S 4	I&S 5	I&S 6	I&S 7	I&S 8	I&S 9	I&S 10	I&S 11	I&S 12	I&S 13	I&S 14	I&S 15	I&S 16
2378D	0.0000	0.0577	0.0542	0.0251	0.0417	0.0183	0.0149	0.0338	0.0000	0.0321	0.0000	0.0404	0.0677	0.0000	0.1700	0.0210
12378D	0.0298	0.0649	0.0764	0.0251	0.0804	0.0321	0.0249	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1140	0.0365
123678D	0.0123	0.0106	0.0315	0.0055	0.0405	0.0087	0.0080	0.0143	0.0594	0.0136	0.0122	0.0106	0.0193	0.0000	0.0062	0.0035
123478D	0.0060	0.0077	0.0172	0.0035	0.0238	0.0060	0.0050	0.0093	0.0393	0.0100	0.0088	0.0146	0.0172	0.0000	0.0200	0.0108
123789D	0.0083	0.0082	0.0281	0.0035	0.0327	0.0069	0.0065	0.0165	0.0402	0.0100	0.0095	0.0066	0.0135	0.0000	0.0077	0.0073
1234678D	0.0033	0.0030	0.0276	0.0016	0.0393	0.0038	0.0055	0.0169	0.0731	0.0082	0.0156	0.0038	0.0115	0.0014	0.0027	0.0023
OCDD	0.0002	0.0004	0.0048	0.0002	0.0077	0.0004	0.0009	0.0037	0.0155	0.0020	0.0043	0.0007	0.0019	0.0014	0.0004	0.0003
2378F	0.0093	0.0529	0.0458	0.0206	0.0952	0.0381	0.0423	0.1097	0.2466	0.1179	0.0884	0.0657	0.1250	0.0000	2.2825	0.4041
12378F	0.0022	0.0159	0.0148	0.0065	0.0417	0.0161	0.0154	0.0464	0.0868	0.0536	0.0340	0.0303	0.0495	0.0000	0.0480	0.0211
23478F	0.0728	0.3606	0.3695	0.1432	0.9226	0.3670	0.3731	1.0127	2.1233	1.5536	0.7823	0.5303	0.9115	0.0000	0.6760	0.3035
123478F	0.0063	0.0394	0.0493	0.0166	0.1250	0.0505	0.0413	0.1181	0.2922	0.1536	0.1020	0.0808	0.1354	0.0000	0.0394	0.0348
123678F	0.0053	0.0322	0.0443	0.0131	0.1131	0.0427	0.0338	0.0970	0.2100	0.1143	0.0816	0.0606	0.1042	0.0000	0.0366	0.0343
234678F	0.0079	0.0438	0.1133	0.0186	0.2321	0.0642	0.0597	0.1561	0.2603	0.1857	0.1497	0.0909	0.1510	0.0000	0.0266	0.0226
123789F	0.0033	0.0188	0.0365	0.0090	0.0714	0.0243	0.0204	0.0464	0.1233	0.0607	0.0442	0.0343	0.0516	0.0000	0.0011	0.0008
1234678F	0.0015	0.0058	0.0246	0.0029	0.0440	0.0110	0.0090	0.0338	0.0639	0.0329	0.0327	0.0152	0.0276	0.0019	0.0027	0.0036
1234789F	0.0004	0.0010	0.0079	0.0006	0.0083	0.0016	0.0016	0.0027	0.0146	0.0043	0.0054	0.0024	0.0042	0.0000	0.0002	0.0003
OCDF	0.0001	0.0003	0.0031	0.0001	0.0041	0.0004	0.0006	0.0024	0.0123	0.0012	0.0024	0.0004	0.0011	0.0022	0.0000	0.0001
															0.0000	0.0000
Total	0.1689	0.7231	0.9488	0.2958	1.9238	0.6919	0.6629	1.7197	3.6607	2.3535	1.3733	0.9876	1.6921	0.0069	3.4341	0.9068

UK Municipal Waste Incinerators (Samples 17-59)

Concentration (mg m-^{3})

Sample #	MWI 1	MWI 2	MWI 3	MWI 4	MWI 5	MWI 6	MWI 7	MWI 8	MWI 9	MWI 10	MWI 11	MWI12	MWI 13	MWI 14
2378D	3.9735	0.1400	0.1045	0.0363	0.5326	0.0200	0.2411	0.0368	0.1729	1.0067	0.0381	0.0048	1.4810	2.8360
12378D	26.4898	0.4000	1.9741	0.2124	2.8824	0.1200	0.5098	0.0946	0.0000	3.6353	0.0000	0.0144	1.9250	18.4080
123478D	28.6973	0.4000	2.3806	0.6475	4.6996	0.1100	1.1856	0.0210	0.0229	0.6152	0.0381	0.0000	1.6410	19.9630
123678D	35.3197	0.4800	4.7031	1.3986	7.5194	0.2800	2.2328	0.0315	0.0280	0.6711	0.0634	0.0048	3.6990	43.4330
123789D	35.3197	0.6300	3.0773	0.8288	5.0129	0.2100	1.5294	0.0210	0.0229	0.6991	0.0507	0.0000	3.1550	40.2640
1234678D	198.6733	2.5400	34.8377	5.4391	56.3952	1.5500	11.8440	0.0210	0.0229	0.5034	0.0465	0.0024	29.5330	251.4440
OCDD	286.9726	4.0400	63.8692	9.3241	119.0566	3.4500	22.9173	0.0000	0.0000	0.0000	0.0000	0.0000	68.7080	299.7820
2378F	10.8167	0.9200	0.6968	0.2849	0.7833	0.1500	0.8689	0.0210	0.0941	0.4195	0.0296	0.0024	15.8320	52.6160
12378F	24.2823	0.8100	1.3935	0.4662	2.6004	0.2000	0.9089	0.0158	0.0356	0.3356	0.0169	0.0024	5.9520	27.6040
23478F	41.9421	2.2400	4.8773	1.3209	6.2661	0.3000	0.4347	0.1839	0.5848	5.0336	0.2960	0.0288	7.2880	58.6820
123478F	41.9421	1.2100	4.8192	0.7252	7.5194	0.3500	1.8574	0.0578	0.0890	1.2864	0.0846	0.0000	8.3180	92.5850
123678F	41.9421	0.6600	4.7031	0.8288	10.0258	0.3100	2.1696	0.0473	0.0839	1.0626	0.0550	0.0000	9.4570	96.5260
234678F	64.0170	1.5400	11.0320	1.3468	15.9786	0.3400	1.7744	0.0736	0.0915	1.3702	0.0846	0.0000	9.4740	146.6310
123789F	20.5296	0.2800	2.2645	0.2849	4.0730	0.1000	0.5493	0.0210	0.0178	0.3076	0.0000	0.0000	0.3960	3.8310
1234678F	156.7312	4.4100	26.1283	3.3670	53.2621	1.2700	7.5087	0.0263	0.0229	0.4754	0.0338	0.0024	26.3920	310.7300
1234789F	44.1496	0.2400	2.9612	0.3626	8.4593	0.1600	0.8694	0.0053	0.0051	0.0503	0.0042	0.0024	3.0790	53.1920
OCDF	147.9013	2.0600	14.5157	1.2691	40.7299	1.2700	3.6714	0.0000	92.9931	191.2917	138.8985	46.2945	18.9860	365.7140

I-TEQ

Sample #	MWI 1	MWI 2	MWI 3	MWI 4	MWI 5	MWI 6	MWI 7	MWI 8	MWI 9	MWI 10	MWI 11	MWI12	MWI 13	MWI 14
2378D	3.9735	0.1400	0.1045	0.0363	0.5326	0.0200	0.2411	0.0368	0.1729	1.0067	0.0381	0.0048	1.4810	2.8360
12378D	13.2449	0.2000	0.9871	0.1062	1.4412	0.0600	0.2549	0.0473	0.0000	1.8177	0.0000	0.0072	0.9625	9.2040
123478D	2.8697	0.0400	0.2381	0.0648	0.4700	0.0110	0.1186	0.0021	0.0023	0.0615	0.0038	0.0000	0.1641	1.9963
123678D	3.5320	0.0480	0.4703	0.1399	0.7519	0.0280	0.2233	0.0032	0.0028	0.0671	0.0063	0.0005	0.3699	4.3433
123789D	3.5320	0.0630	0.3077	0.0829	0.5013	0.0210	0.1529	0.0021	0.0023	0.0699	0.0051	0.0000	0.3155	4.0264
1234678D	1.9867	0.0254	0.3484	0.0544	0.5640	0.0155	0.1184	0.0002	0.0002	0.0050	0.0005	0.0000	0.2953	2.5144
OCDD	0.2870	0.0040	0.0639	0.0093	0.1191	0.0035	0.0229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0687	0.2998
2378F	1.0817	0.0920	0.0697	0.0285	0.0783	0.0150	0.0869	0.0021	0.0094	0.0419	0.0030	0.0002	1.5832	5.2616
12378F	1.2141	0.0405	0.0697	0.0233	0.1300	0.0100	0.0454	0.0008	0.0018	0.0168	0.0008	0.0001	0.2976	1.3802
23478F	20.9711	1.1200	2.4387	0.6605	3.1331	0.1500	0.2174	0.0920	0.2924	2.5168	0.1480	0.0144	3.6440	29.3410
123478F	4.1942	0.1210	0.4819	0.0725	0.7519	0.0350	0.1857	0.0058	0.0089	0.1286	0.0085	0.0000	0.8318	9.2585
123678F	4.1942	0.0660	0.4703	0.0829	1.0026	0.0310	0.2170	0.0047	0.0084	0.1063	0.0055	0.0000	0.9457	9.6526
234678F	6.4017	0.1540	1.1032	0.1347	1.5979	0.0340	0.1774	0.0074	0.0092	0.1370	0.0085	0.0000	0.9474	14.6631
123789F	2.0530	0.0280	0.2265	0.0285	0.4073	0.0100	0.0549	0.0021	0.0018	0.0308	0.0000	0.0000	0.0396	0.3831
1234678F	1.5673	0.0441	0.2613	0.0337	0.5326	0.0127	0.0751	0.0003	0.0002	0.0048	0.0003	0.0000	0.2639	3.1073
1234789F	0.4415	0.0024	0.0296	0.0036	0.0846	0.0016	0.0087	0.0001	0.0001	0.0005	0.0000	0.0000	0.0308	0.5319
OCDF	0.1479	0.0021	0.0145	0.0013	0.0407	0.0013	0.0037	0.0000	0.0930	0.1913	0.1389	0.0463	0.0190	0.3657
Total I-TEQ	71.6924	2.1905	7.6852	1.5631	12.1390	0.4595	2.2043	0.2068	0.6056	6.2027	0.3672	0.0736	12.2600	99.1653

German Municipal Waste Incinerators (Samples 60-67)

Concentration (mg m-^{3})

Sample #	MWI 44	MWI 45	MWI 46	MWI 47	MWI 48	MWI 49	MWI 50	MWI 51
2378D	0.5300	0.0090	0.1700	0.0000	0.0000	0.0000	0.0000	0.0000
12378D	1.4100	0.0400	0.4700	0.0050	0.0390	0.0000	0.0000	0.0000
123478D	1.4300	0.0300	0.5800	0.0110	0.0390	0.0000	0.0070	0.0040
123678D	1.8100	0.0400	1.2400	0.0360	0.1840	0.0020	0.0080	0.0060
123789D	1.3000	0.0300	0.6800	0.0250	0.1230	0.0020	0.0060	0.0000
1234678D	31.9800	0.8200	9.6700	0.2500	0.8780	0.0480	0.0290	0.0510
OCDD	136.2000	4.0300	8.3000	0.4100	1.2000	0.0600	0.1130	0.0310
2378F	3.7300	0.0700	0.6300	0.0180	0.1060	0.0130	0.0120	0.0060
12378F	4.7700	0.1500	1.5300	0.0350	0.1670	0.0330	0.0520	0.0070
23478F	4.2800	0.1000	1.1700	0.0230	0.2330	0.0240	0.0380	0.0050
123478F	4.4600	0.1300	0.9700	0.0370	0.4950	0.0140	0.0730	0.0050
123678F	4.5100	0.1300	1.0900	0.0410	0.8000	0.0300	0.0890	0.0050
234678F	3.1500	0.1000	0.7300	0.0300	0.9530	0.0130	0.0530	0.0030
123789F	0.2600	0.0000	0.0600	0.0040	0.0380	0.0030	0.0000	0.0000
1234678F	41.4500	0.9300	3.9600	0.2100	1.8200	0.0900	0.2040	0.0130
1234789F	2.6700	0.0800	0.1300	0.0060	0.3700	0.0080	0.0160	0.0000
OCDF	22.1000	0.4400	0.5000	0.1000	0.6900	0.0000	0.0410	0.0000

I-TEQ

Sample #	MWI 44	MWI 45	MWI 46	MWI 47	MWI 48	MWI 49	MWI 50	MWI 51
2378D	0.5300	0.0090	0.1700	0.0000	0.0000	0.0000	0.0000	0.0000
12378D	0.7050	0.0200	0.2350	0.0025	0.0195	0.0000	0.0000	0.0000
123478D	0.1430	0.0030	0.0580	0.0011	0.0039	0.0000	0.0007	0.0004
123678D	0.1810	0.0040	0.1240	0.0036	0.0184	0.0002	0.0008	0.0006
123789D	0.1300	0.0030	0.0680	0.0025	0.0123	0.0002	0.0006	0.0000
1234678D	0.3198	0.0082	0.0967	0.0025	0.0088	0.0005	0.0003	0.0005
OCDD	0.1362	0.0040	0.0083	0.0004	0.0012	0.0001	0.0001	0.0000
2378F	0.3730	0.0070	0.0630	0.0018	0.0106	0.0013	0.0012	0.0006
12378F	0.2385	0.0075	0.0765	0.0018	0.0084	0.0017	0.0026	0.0004
23478F	2.1400	0.0500	0.5850	0.0115	0.1165	0.0120	0.0190	0.0025
123478F	0.4460	0.0130	0.0970	0.0037	0.0495	0.0014	0.0073	0.0005
123678F	0.4510	0.0130	0.1090	0.0041	0.0800	0.0030	0.0089	0.0005
234678F	0.3150	0.0100	0.0730	0.0030	0.0953	0.0013	0.0053	0.0003
123789F	0.0260	0.0000	0.0060	0.0004	0.0038	0.0003	0.0000	0.0000
1234678F	0.4145	0.0093	0.0396	0.0021	0.0182	0.0009	0.0020	0.0001
1234789F	0.0267	0.0008	0.0013	0.0001	0.0037	0.0001	0.0002	0.0000
OCDF	0.0221	0.0004	0.0005	0.0001	0.0007	0.0000	0.0000	0.0000
I-TEQ	**6.5978**	**0.1623**	**1.8109**	**0.0411**	**0.4507**	**0.0229**	**0.0490**	**0.0064**

Chemical Waste Incinerators (Samples 68 - 109)

Concentration (mg m^{-3})

Sample #	CWI 1	CWI 2	CWI 3	CWI 4	CWI 5	CWI 6	CWI 7	CWI 8	CWI 9	CWI 10	CWI 11	CWI 12	CWI 13	CWI 14	CWI 15	CWI 16	CWI 17	CWI 18	CWI 19	CWI 20
2378D	0.0110	0.0020	0.0050	0.0200	0.0090	0.0090	0.0080	0.0090	0.0080	0.0290	0.0030	0.0030	0.0169	0.0082	0.2430	0.4310	0.0160	0.0620	0.0160	0.0180
12378D	0.0440	0.0200	0.0110	0.1700	0.0100	0.0130	0.0130	0.0130	0.0110	0.0580	0.0050	0.0050	0.0254	0.0572	0.4110	0.8620	0.1440	0.1930	0.0240	0.0270
123478D	0.0590	0.0130	0.0030	0.1500	0.0130	0.0130	0.0130	0.0130	0.0110	0.0110	0.0050	0.0050	0.0254	0.0122	0.2430	0.1910	0.1410	0.2730	0.0240	0.0270
123678D	0.1930	0.0410	0.0190	0.4000	0.0130	0.0130	0.0130	0.0130	0.0110	0.0110	0.0050	0.0050	0.0254	0.0122	0.6170	0.7180	0.1980	0.2740	0.0240	0.0270
123789D	0.1100	0.0500	0.0090	0.2500	0.0130	0.0130	0.0130	0.0130	0.0110	0.2190	0.0050	0.0050	0.0254	0.0122	0.4480	0.6700	0.1740	0.1630	0.0240	0.0270
1234678D	1.3310	0.4570	0.1220	2.4700	0.2490	0.3410	0.5320	0.3940	0.5800	0.4470	0.1000	0.0800	0.2032	0.1839	3.3700	2.1100	0.4720	0.7830	1.4500	1.1600
OCDD	1.7860	1.4230	0.4480	2.2400	1.2980	1.1900	2.5920	1.9350	1.5450	1.4170	0.4000	0.2900	0.6901	0.8580	12.9000	7.2800	0.8350	2.1260	2.4400	0.1820
2378F	0.2850	0.0890	0.0270	0.1800	0.0860	0.0350	0.0083	0.0086	0.0080	0.0800	0.0030	0.0030	1.6639	1.4954	5.0480	4.5480	0.1330	0.1090	0.9270	1.3100
12378F	0.0680	0.0220	[illegible]	0.3100	0.3150	0.2625	0.0083	0.1284	0.0080	0.2630	0.0860	0.0800	0.8722	0.4903	4.8610	5.6010	0.2250	0.3040	1.8200	1.4200
23478F	0.0900	0.0420	0.0160	0.7400	0.2230	0.1749	0.0083	0.0942	0.0080	0.2560	0.0220	0.0170	1.3464	0.9724	1.7950	3.8290	0.1800	0.2670	0.9110	0.8890
123478F	0.2300	0.0740	0.0290	0.5400	0.1830	0.2099	0.2658	0.1712	0.0830	0.1610	0.0320	0.0210	0.5504	0.3432	1.5890	3.4950	0.1970	0.3210	0.0160	0.5080
123678F	0.2720	0.0650	0.0160	0.6700	0.1490	0.1837	0.2409	0.1627	0.1130	0.1320	0.0290	0.0170	0.3302	0.1961	1.0840	1.9150	0.1980	0.3680	0.0160	0.3450
234678F	0.4500	0.1000	0.0020	1.1400	0.1430	0.1662	0.3738	0.2483	0.1730	0.1680	0.0380	0.0210	0.2794	0.1798	1.0840	2.1060	0.1850	0.3800	0.0160	0.0180
123789F	0.0040	0.0020	0.0220	0.1500	0.0130	0.0131	0.0125	0.0128	0.0110	0.0110	0.0050	0.0050	0.0254	0.0122	0.2990	1.0530	0.0250	0.1360	0.0240	0.0270
1234678F	1.2860	0.3240	0.0120	1.9500	0.2260	0.2975	0.2658	0.3852	0.2860	0.2630	0.0640	0.0620	0.6054	0.3759	2.3370	4.3080	0.1310	0.2610	1.0600	0.7800
1234789F	0.1210	0.0430	0.0110	0.1700	0.0860	0.0875	0.0831	0.0856	0.0750	0.0730	0.0500	0.0400	0.1694	0.0858	0.7900	1.6800	0.1640	0.3510	0.1600	0.1800
OCDF	0.9840	0.6130	0.1760	1.3000	0.1720	0.1749	0.1661	0.1712	0.1510	0.1460	0.8900	0.5900	0.3387	0.3400	8.2300	8.6200	0.3280	3.9490	0.3300	0.3600

I-TEQ

Sample #	CWI 1	CWI 2	CWI 3	CWI 4	CWI 5	CWI 6	CWI 7	CWI 8	CWI 9	CWI 10	CWI 11	CWI 12	CWI 13	CWI 14	CWI 15	CWI 16	CWI 17	CWI 18	CWI 19	CWI 20
2378D	0.0110	0.0020	0.0050	0.0200	0.0090	0.0090	0.0080	0.0090	0.0080	0.0290	0.0030	0.0030	0.0169	0.0082	0.2430	0.4310	0.0160	0.0620	0.0160	0.0180
12378D	0.0220	0.0100	0.0055	0.0850	0.0050	0.0065	0.0065	0.0065	0.0055	0.0290	0.0025	0.0025	0.0127	0.0286	0.2055	0.4310	0.0720	0.0965	0.0120	0.0135
123478D	0.0059	0.0013	0.0003	0.0150	0.0013	0.0013	0.0013	0.0013	0.0011	0.0011	0.0005	0.0005	0.0025	0.0012	0.0243	0.0191	0.0141	0.0273	0.0024	0.0027
123678D	0.0193	0.0041	0.0019	0.0400	0.0013	0.0013	0.0013	0.0013	0.0011	0.0011	0.0005	0.0005	0.0025	0.0012	0.0617	0.0718	0.0198	0.0274	0.0024	0.0027
123789D	0.0110	0.0050	0.0009	0.0250	0.0013	0.0013	0.0013	0.0013	0.0011	0.0219	0.0005	0.0005	0.0025	0.0012	0.0448	0.0670	0.0174	0.0163	0.0024	0.0027
1234678D	0.0133	0.0046	0.0012	0.0247	0.0025	0.0034	0.0053	0.0039	0.0058	0.0045	0.0010	0.0008	0.0020	0.0018	0.0337	0.0211	0.0047	0.0078	0.0145	0.0116
OCDD	0.0018	0.0014	0.0004	0.0022	0.0013	0.0012	0.0026	0.0019	0.0015	0.0014	0.0004	0.0003	0.0007	0.0009	0.0129	0.0073	0.0008	0.0021	0.0024	0.0002
2378F	0.0285	0.0089	0.0027	0.0180	0.0086	0.0035	0.0008	0.0009	0.0008	0.0080	0.0003	0.0003	0.1664	0.1495	0.5048	0.4548	0.0133	0.0109	0.0927	0.1310
12378F	0.0034	0.0011	0.0004	0.0155	0.0158	0.0131	0.0004	0.0064	0.0004	0.0132	0.0043	0.0040	0.0436	0.0245	0.2431	0.2801	0.0113	0.0152	0.0910	0.0710
23478F	0.0450	0.0210	0.0080	0.3700	0.1115	0.0875	0.0042	0.0471	0.0040	0.1280	0.0110	0.0085	0.6732	0.4862	0.8975	1.9145	0.0900	0.1335	0.4555	0.4445
123478F	0.0230	0.0074	0.0029	0.0540	0.0183	0.0210	0.0266	0.0171	0.0083	0.0161	0.0032	0.0021	0.0550	0.0343	0.1589	0.3495	0.0197	0.0321	0.0016	0.0508
123678F	0.0272	0.0065	0.0016	0.0670	0.0149	0.0184	0.0241	0.0163	0.0113	0.0132	0.0029	0.0017	0.0330	0.0196	0.1084	0.1915	0.0198	0.0368	0.0016	0.0345
234678F	0.0450	0.0100	0.0002	0.1140	0.0143	0.0166	0.0374	0.0248	0.0173	0.0168	0.0038	0.0021	0.0279	0.0180	0.1084	0.2106	0.0185	0.0380	0.0016	0.0018
123789F	0.0004	0.0002	0.0022	0.0150	0.0013	0.0013	0.0013	0.0013	0.0011	0.0011	0.0005	0.0005	0.0025	0.0012	0.0299	0.1053	0.0025	0.0136	0.0024	0.0027
1234678F	0.0129	0.0032	0.0001	0.0195	0.0023	0.0030	0.0027	0.0039	0.0029	0.0026	0.0006	0.0006	0.0061	0.0038	0.0234	0.0431	0.0013	0.0026	0.0106	0.0078
1234789F	0.0012	0.0004	0.0001	0.0017	0.0009	0.0009	0.0008	0.0009	0.0008	0.0007	0.0005	0.0004	0.0017	0.0009	0.0079	0.0168	0.0016	0.0035	0.0016	0.0018
OCDF	0.0010	0.0006	0.0002	0.0013	0.0002	0.0002	0.0002	0.0002	0.0002	0.0001	0.0009	0.0006	0.0003	0.0003	0.0082	0.0086	0.0003	0.0039	[illegible]	0.0004
I-TEQ	0.2719	0.0878	0.0337	0.8879	0.2096	0.1894	0.1247	0.1440	0.0711	0.2878	0.0364	0.0289	1.0498	0.7815	2.7164	4.6230	0.3232	0.5296	0.7111	0.7976

VCM Industry (Samples - 110 -130)

Concentration (mg m-^{3})

Sample #	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130
2378D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.35	0.40	0.00	0.00	0	0
12378D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.89	1.10	0.00	0.00	0	0
123478D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	78.80	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.01	0	0
123678D	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	84.80	0.00	0.00	0.30	0.00	0.00	0.00	0.00	0.01	0	0
123789D	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	131.00	0.00	0.20	0.00	0.00	1.90	0.00	0.00	0.01	0	0
1234678D	6.80	0.00	0.00	1.00		0.50	1.90	0.60	3.60	3.60	990.00	0.34	4.00	4.50	4.20	22.00	29.20	20.80	0.14	0	0
OCDD	87.80	1.60	0.00	2.70	2.20	1.10	10.40	5.10	26.00	28.90	9480.00	1300.00	10.00	12.30	9.80	184.00	240.00	164.00	1.60	0.9	0.19
2378F	0.10	0.00	0.00	1.20	0.40	0.30	0.60	0.30	1.50	1.40	131.00	0.28	3.00	3.20	2.90	17.50	21.40	8.20	0.17	0	0.01
12378F	1.10	0.00	0.00	4.30	3.30	1.60	1.90	0.30	2.00	2.60	446.00	0.16	17.20	19.70	19.70	29.00	34.70	14.30	0.06	0	0.01
23478F	0.32	0.00	0.00	0.51	0.30	0.20	0.70	0.20	2.00	2.20	228.00	0.07	2.80	3.50	2.70	26.00	31.80	13.00	0.04	0	0.01
123478F	55.80	0.80	0.00	36.60	28.80	13.90	9.30	4.20	23.10	22.90	3420.00	0.33	155.00	186.00	161.00	230.00	317.00	153.00	0.68	0.5	0.11
123678F	12.60	0.27	0.00	8.00	6.30	3.10	2.50	0.90	5.50	4.90	1000.00	0.00	42.90	49.20	41.50	49.10	64.90	28.10	0.15	0.2	0.04
234678F	2.80	0.00	0.10	1.60	1.20	0.60	1.80	0.50	4.50	4.10	516.00	0.38	8.00	9.50	7.30	32.40	44.80	17.50	0.10	0.1	0.05
123789F	2.40	0.00	0.00	1.30	1.00	0.60	0.80	0.00	2.00	1.60	137.00	0.00	0.60	7.70	5.30	3.90	8.80	4.40	0.04	0	0.01
1234678F	1040.00	20.00	0.80	75.50	77.50	40.10	39.30	16.30	102.00	89.40	14950.00	1.10	425.00	501.00	417.00	703.00	986.00	568.00	2.57	10.6	1.5
1234789F	286.00	5.60	0.00	16.30	14.90	8.90	9.20	3.90	24.70	23.70	3630.00	0.17	78.80	100.00	69.70	150.00	198.00	126.00	0.62	2.8	0.22
OCDF	16200.00	1380.00	4.80	1290.00	1120.00	577.00	510.00	317.00	1140.00	1230.00	631830.00	10.90	2660.00	3280.00	2540.00	12730.00	13430.00	7460.00	67.31	435	61.4

I-TEQ

Sample #	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130
2378D	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3500	0.4000	0.0000	0.0000	0.0000	0.0000
12378D	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.4450	0.5500	0.0000	0.0000	0.0000	0.0000
123478D	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	7.8800	0.0000	0.0200	0.0000	0.0000	0.0000	0.0000	0.0000	0.0008	0.0000	0.0000
123678D	0.0110	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	8.4800	0.0000	0.0000	0.0300	0.0000	0.0000	0.0000	0.0000	0.0008	0.0000	0.0000
123789D	0.0080	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	13.1000	0.0000	0.0200	0.0000	0.0000	0.1900	0.0000	0.0000	0.0009	0.0000	0.0000
1234678D	0.0680	0.0000	0.0000	0.0100	0.0000	0.0050	0.0190	0.0060	0.0360	0.0360	9.9000	0.0034	0.0400	0.0450	0.0420	0.2200	0.2920	0.2080	0.0014	0.0000	0.0000
OCDD	0.0878	0.0016	0.0000	0.0027	0.0022	0.0011	0.0104	0.0051	0.0260	0.0289	9.4800	1.3000	0.0100	0.0123	0.0098	0.1840	0.2400	0.1640	0.0016	0.0009	0.0002
2378F	0.0100	0.0000	0.0000	0.1200	0.0400	0.0300	0.0600	0.0300	0.1500	0.1400	13.1000	0.0280	0.3000	0.3200	0.2900	1.7500	2.1400	0.8200	0.0168	0.0000	0.0010
12378F	0.0550	0.0000	0.0000	0.2150	0.1650	0.0800	0.0950	0.0150	0.1000	0.1300	22.3000	0.0080	0.8600	0.9850	0.9850	1.4500	1.7350	0.7150	0.0032	0.0000	0.0005
23478F	0.1600	0.0000	0.0000	0.2550	0.1500	0.1000	0.3500	0.1000	1.0000	1.1000	114.0000	0.0350	1.4000	1.7500	1.3500	13.0000	15.9000	6.5000	0.0210	0.0000	0.0050
123478F	5.5800	0.0800	0.0000	3.6600	2.8800	1.3900	0.9300	0.4200	2.3100	2.2900	342.0000	0.0330	15.5000	18.6000	16.1000	23.0000	31.7000	15.3000	0.0683	0.0500	0.0110
123678F	1.2600	0.0270	0.0000	0.8000	0.6300	0.3100	0.2500	0.0900	0.5500	0.4900	100.0000	0.0000	4.2900	4.9200	4.1500	4.9100	6.4900	2.8100	0.0145	0.0200	0.0040
234678F	0.2800	0.0000	0.0100	0.1600	0.1200	0.0600	0.1800	0.0500	0.4500	0.4100	51.6000	0.0380	0.8000	0.9500	0.7300	3.2400	4.4800	1.7500	0.0099	0.0100	0.0050
123789F	0.2400	0.0000	0.0000	0.1300	0.1000	0.0600	0.0800	0.0000	0.2000	0.1600	13.7000	0.0000	0.0600	0.7700	0.5300	0.3900	0.8800	0.4400	0.0042	0.0000	0.0010
1234678F	10.4000	0.2000	0.0080	0.7550	0.7750	0.4010	0.3930	0.1630	1.0200	0.8940	149.5000	0.0110	4.2500	5.0100	4.1700	7.0300	9.8600	5.6800	0.0257	0.1060	0.0150
1234789F	2.8600	0.0560	0.0000	0.1630	0.1490	0.0890	0.0920	0.0390	0.2470	0.2370	36.3000	0.0017	0.7880	1.0000	0.6970	1.5000	1.9800	1.2600	0.0062	0.0280	0.0022
OCDF	16.2000	1.3800	0.0048	1.2900	1.1200	0.5770	0.5100	0.3170	1.1400	1.2300	631.8300	0.0109	2.6600	3.2800	2.5400	12.7300	13.4300	7.4600	0.0673	0.4350	0.0614
I-TEQ	37.2198	1.7446	0.0228	7.5607	6.1312	3.1031	2.9694	1.2351	7.2290	7.1459	1523.1700	1.4690	30.9980	37.6723	31.5938	70.3890	90.0770	43.1070	0.2426	0.6499	0.1063

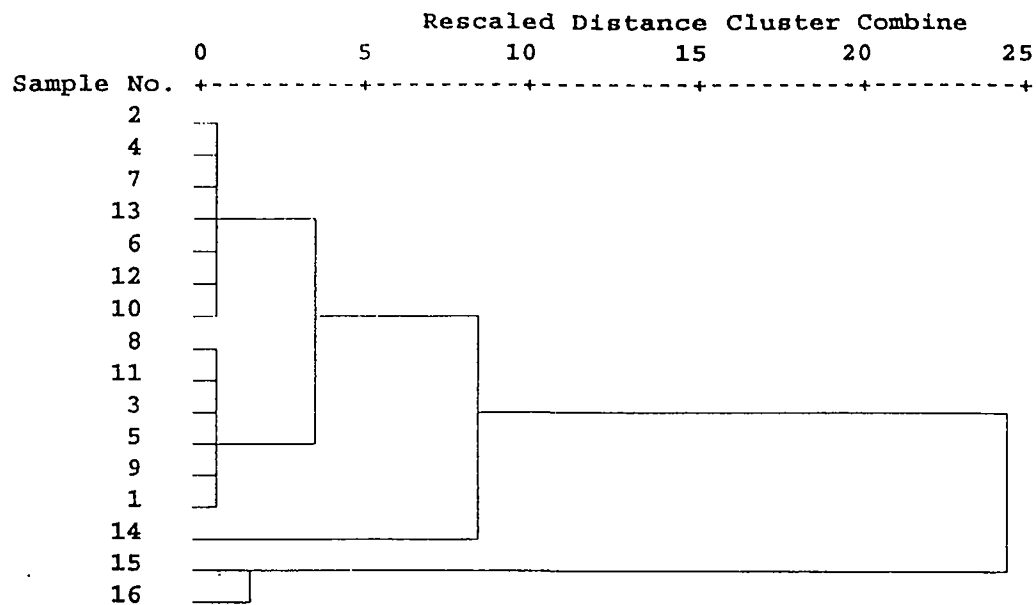

Figure B1 **Denogram of Emissions from Iron and Steel Works**

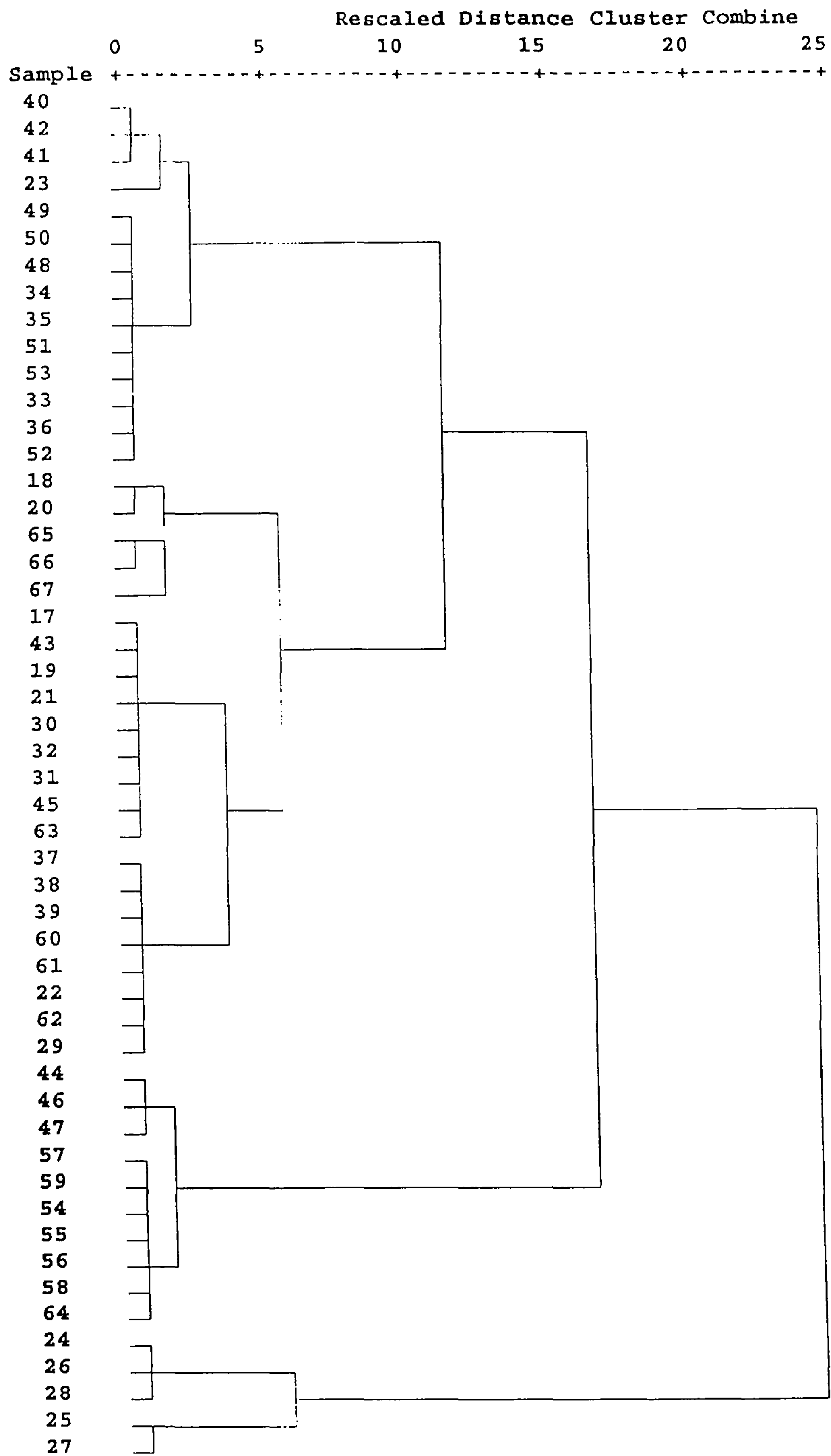

Figure B2 Dendogram of Emissions from Municipal Waste Incinerators

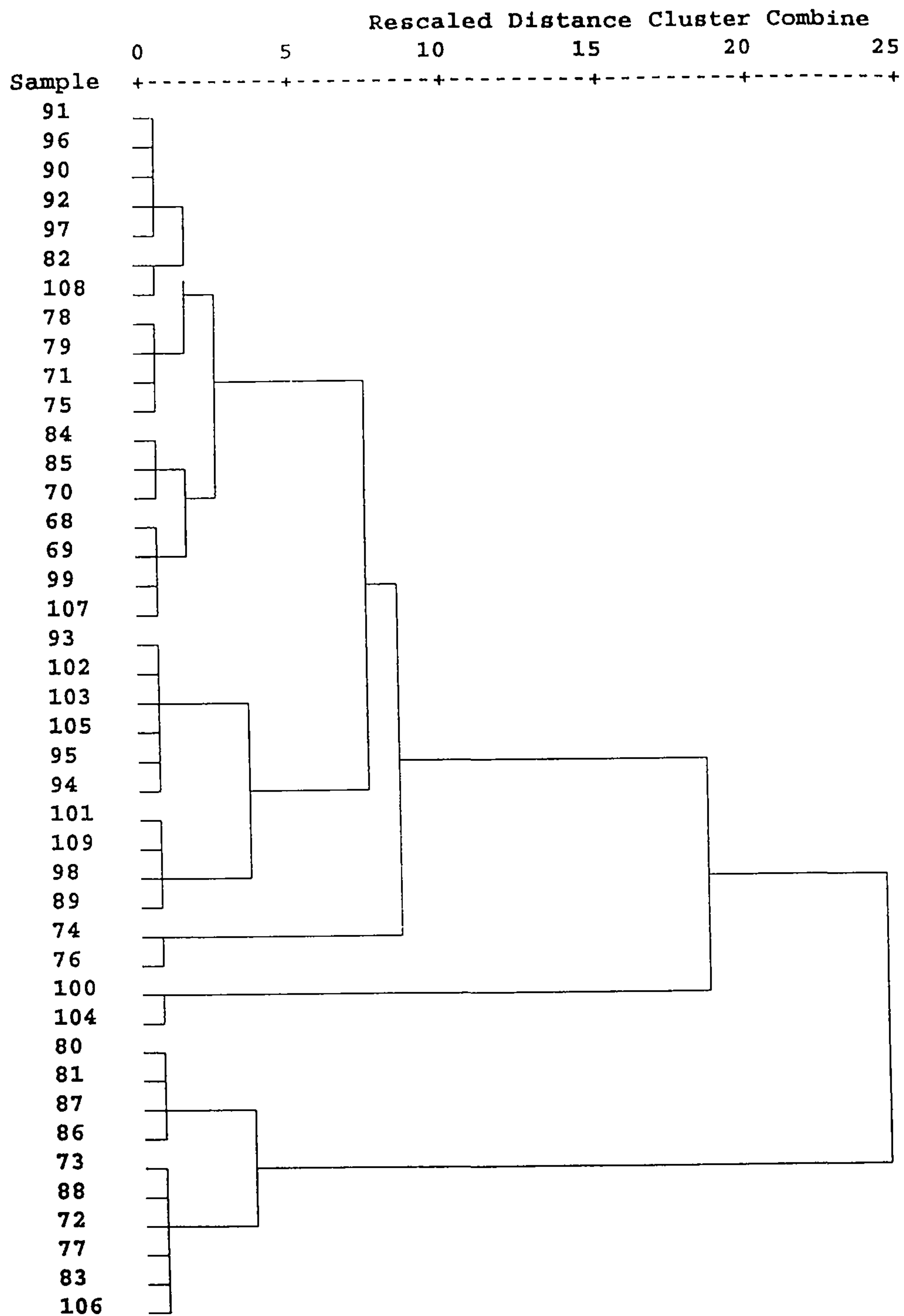

Figure B3 Dendogram of Emissions from Chemical Waste Incinerators

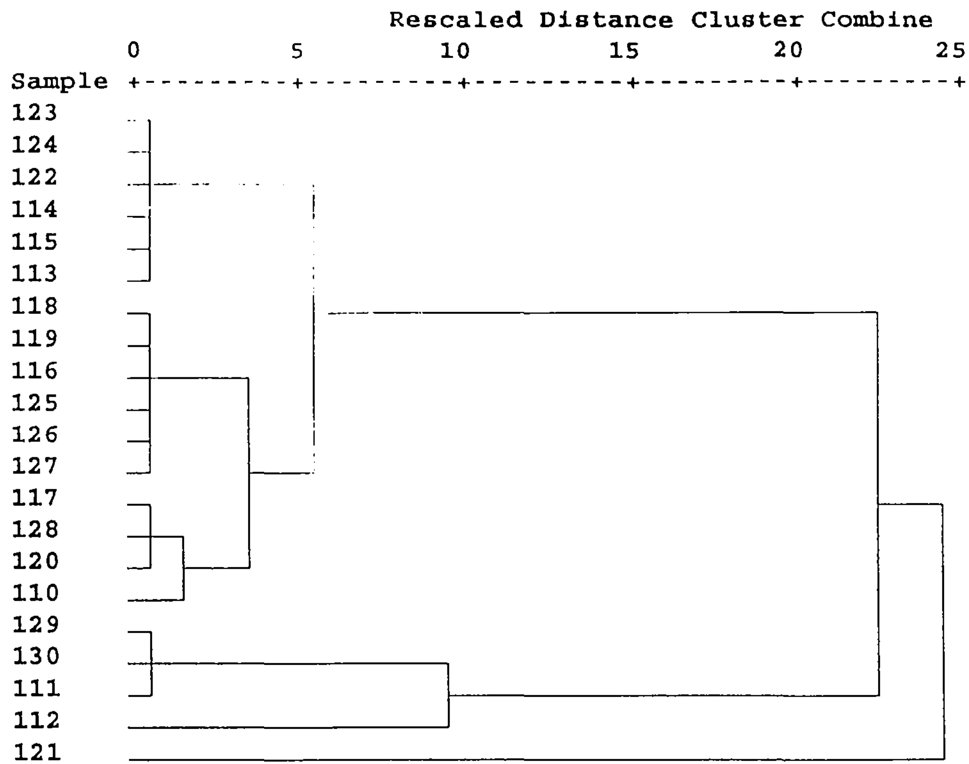

Figure B4 **Denogram of Effluent from VCM Manufacture**

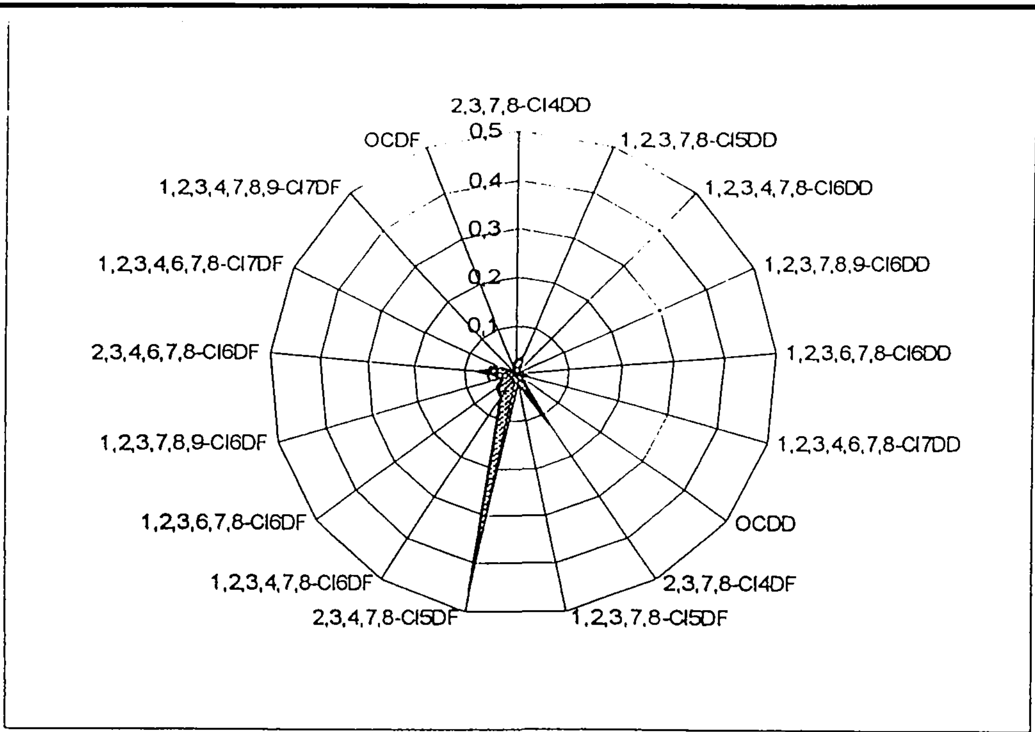

Figure B6a Spectra Plot of Mean I-TEQ Ratio of Iron and Steel Samples (n=16)

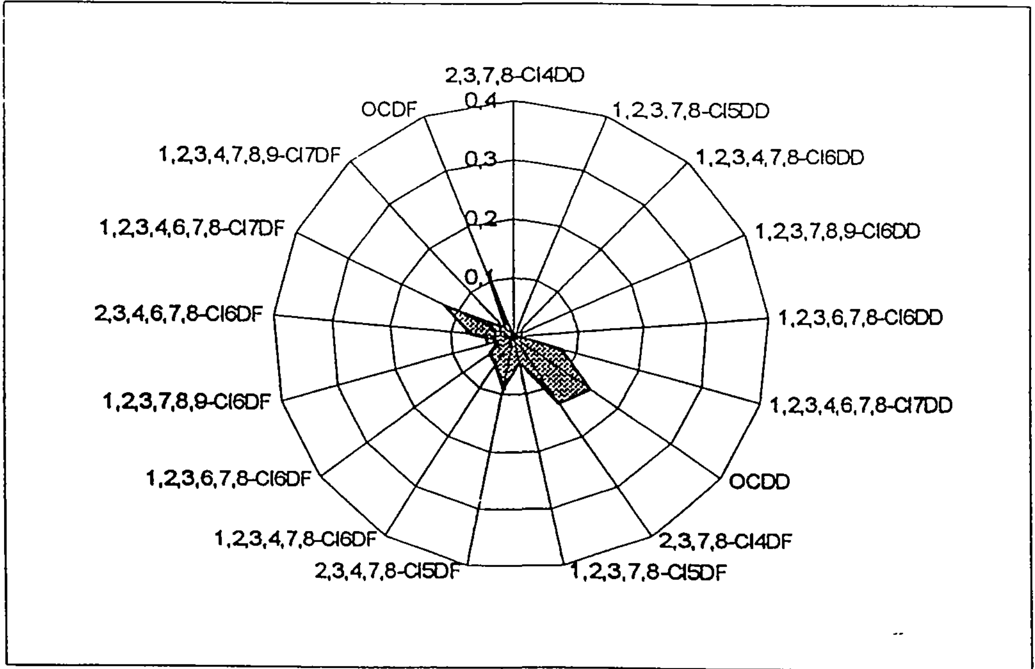

Figure B6b Spectra Plot of Mean Congener-Ratio of Iron and steel Samples (n=16)

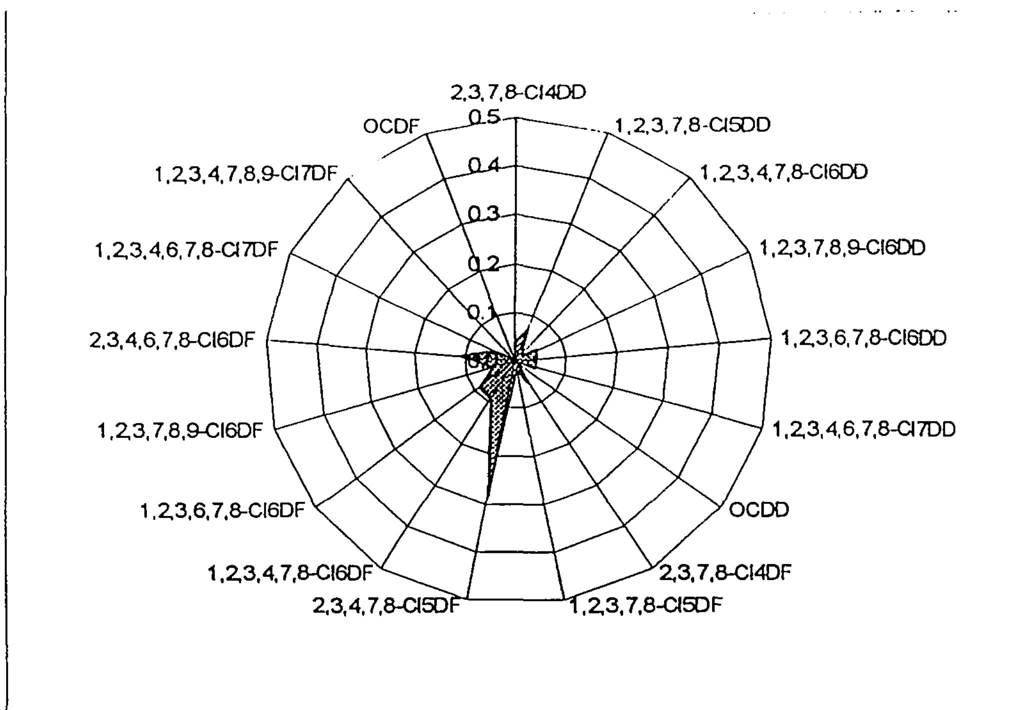

Figure B7a Spectra Plot of Mean I-TEQ Ratio of Municipal Waste Incinerators (n-51)

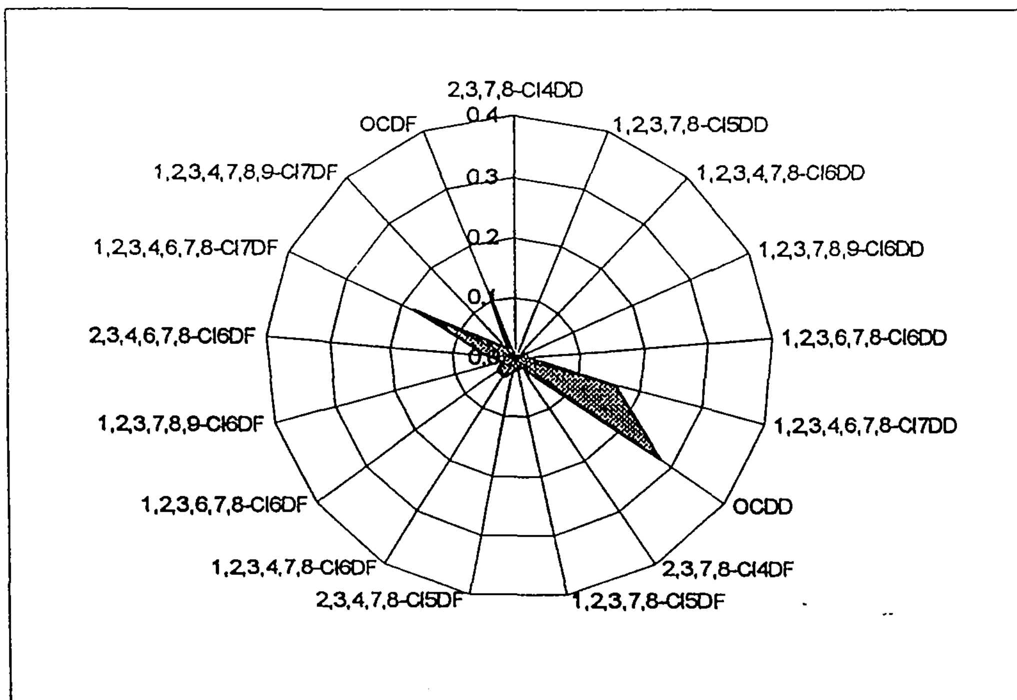

Figure B7b Spectra Plot of Menu Congener Ratio of Municipal Waste Incinerators (n=51)

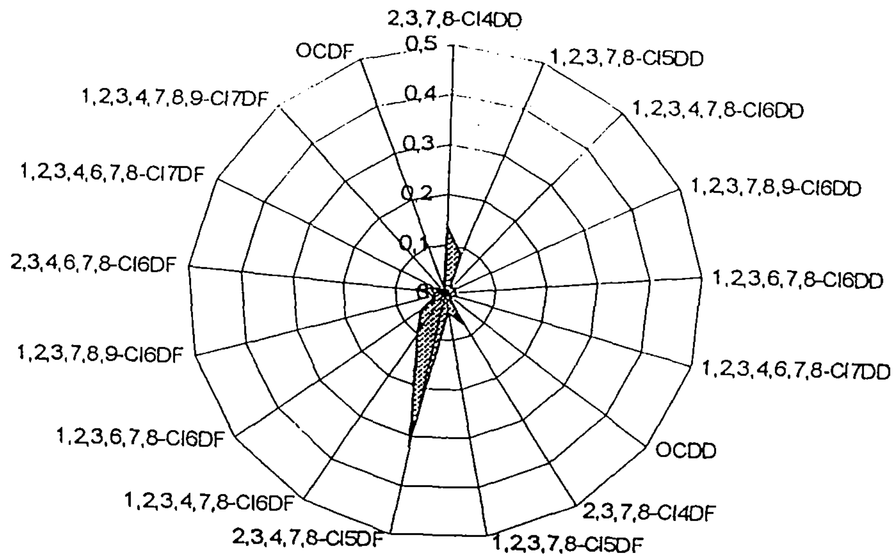

Figure B8a **Spectra Plot of Mean I-TEQ Ratio of Chemical Waste Incinerators (n=42)**

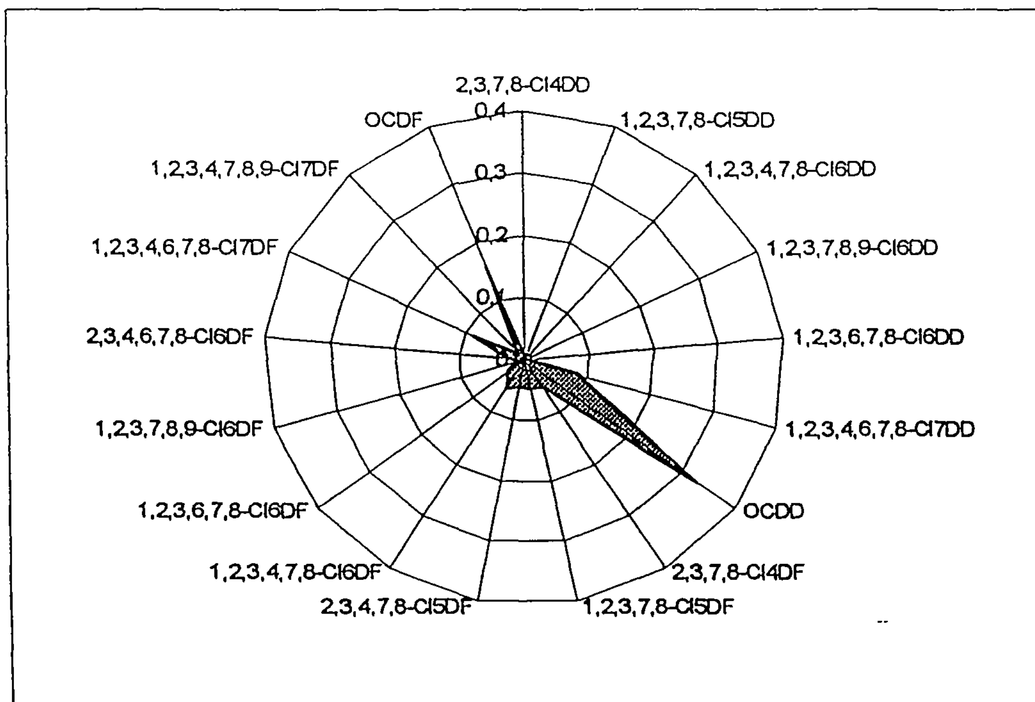

Figure B8b **Spectra Plot of Menu Congener Ratio for Chemical Waste Incinerators (n=42)**

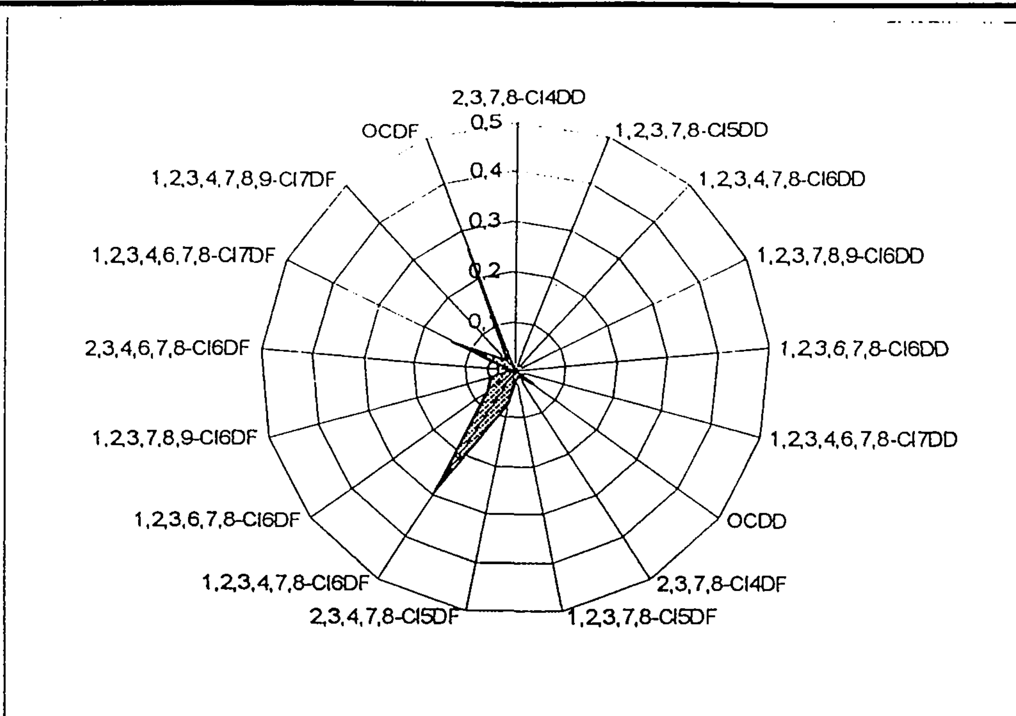

Figure B9a Spectra Plot of Mean I-TEQ Ratio for VCM Manufacture (n=21)

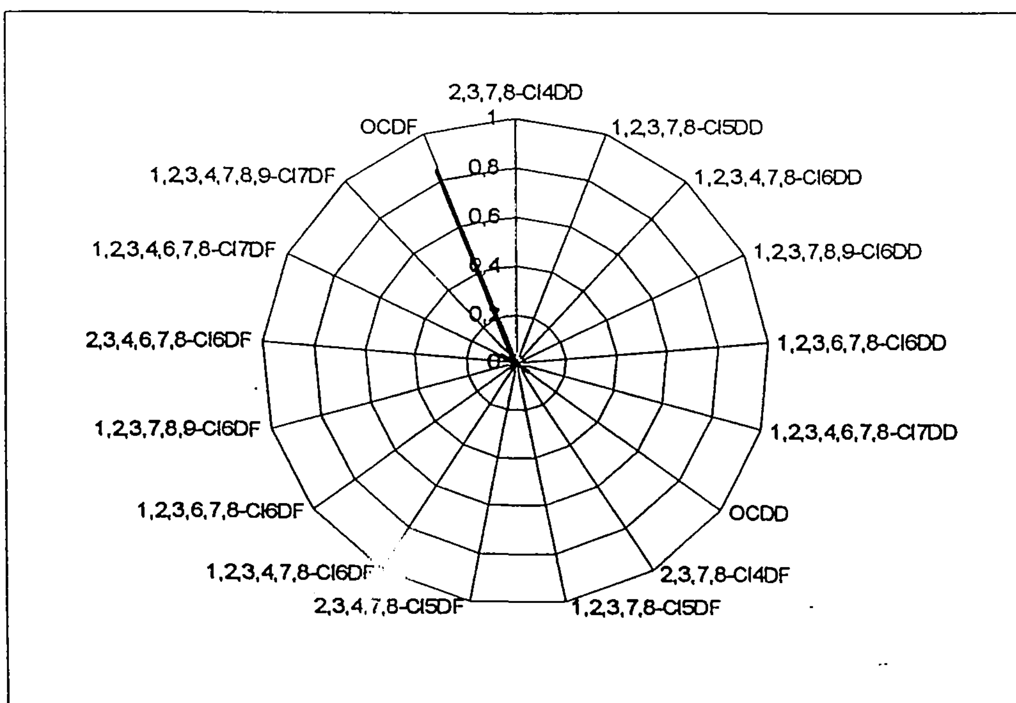

Figure B9b Spectra Plot of Mean I-TEQ Ratio for VCM Manufacture (n=21)

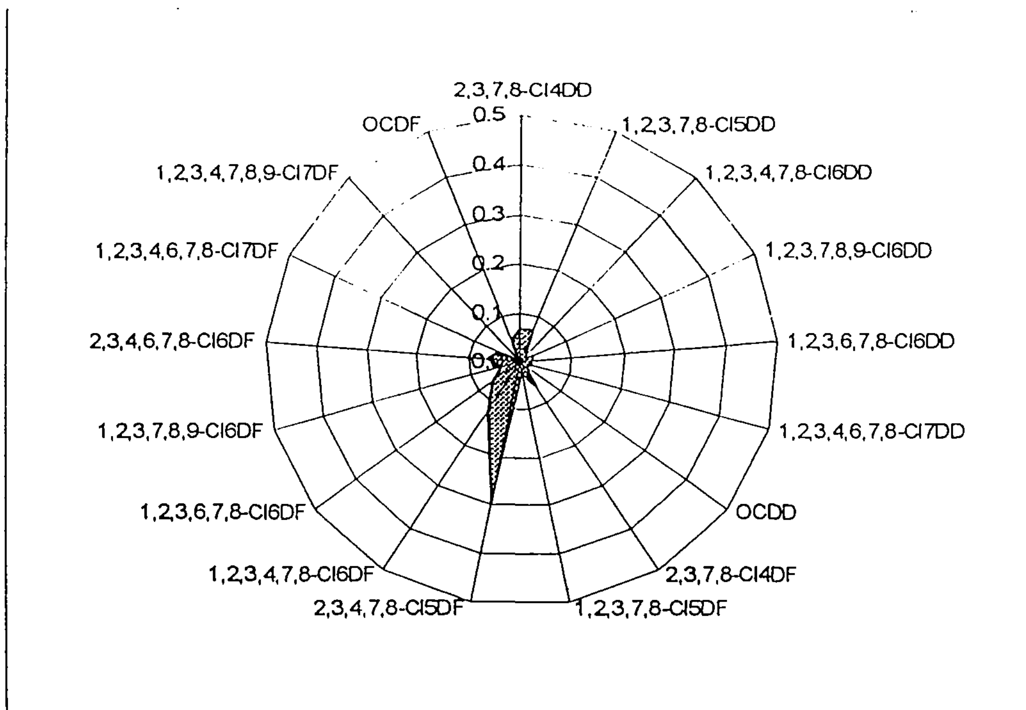

Figure B10a Spectra Plot of Mean I-TEQ Ratio for All Samples (n=130)

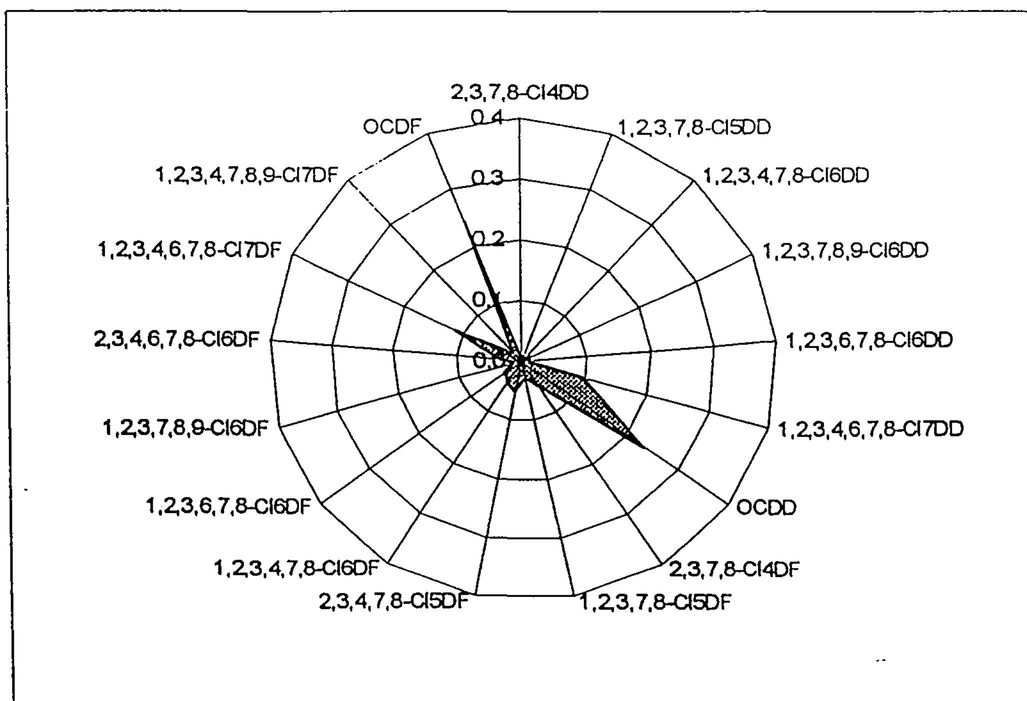

Figure B10b Spectra Plot of Mean Congener Ratio for All Samples (n=130)

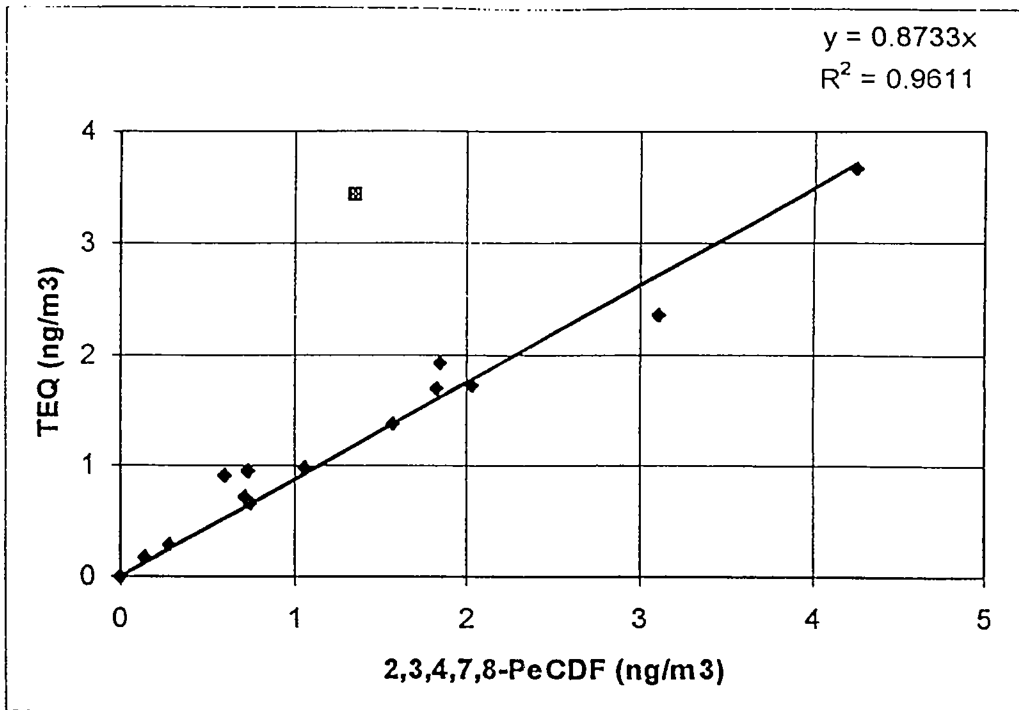

Figure B11 **Relationship Between Concentration of 2,3,4,7,8-PeCDF and I-TEQ for Iron and Steel Works (n=15)**

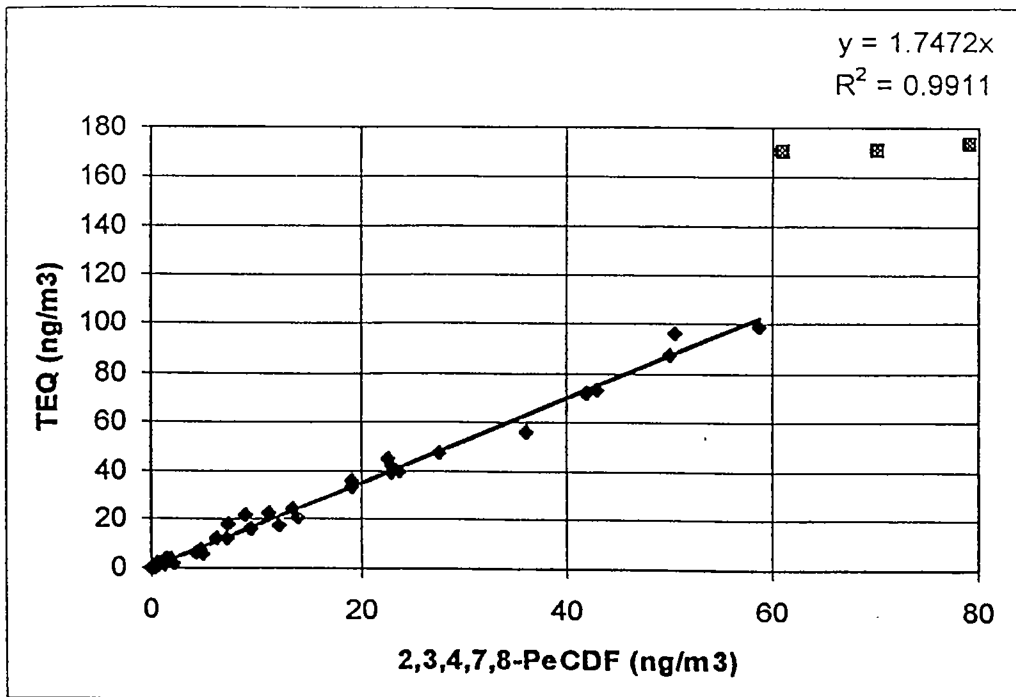

Figure B12 **Relationship Between Concentration of 2,3,4,7,8-PeCDF and I-TEQ for UK and German MWIs Samples (n=48)**

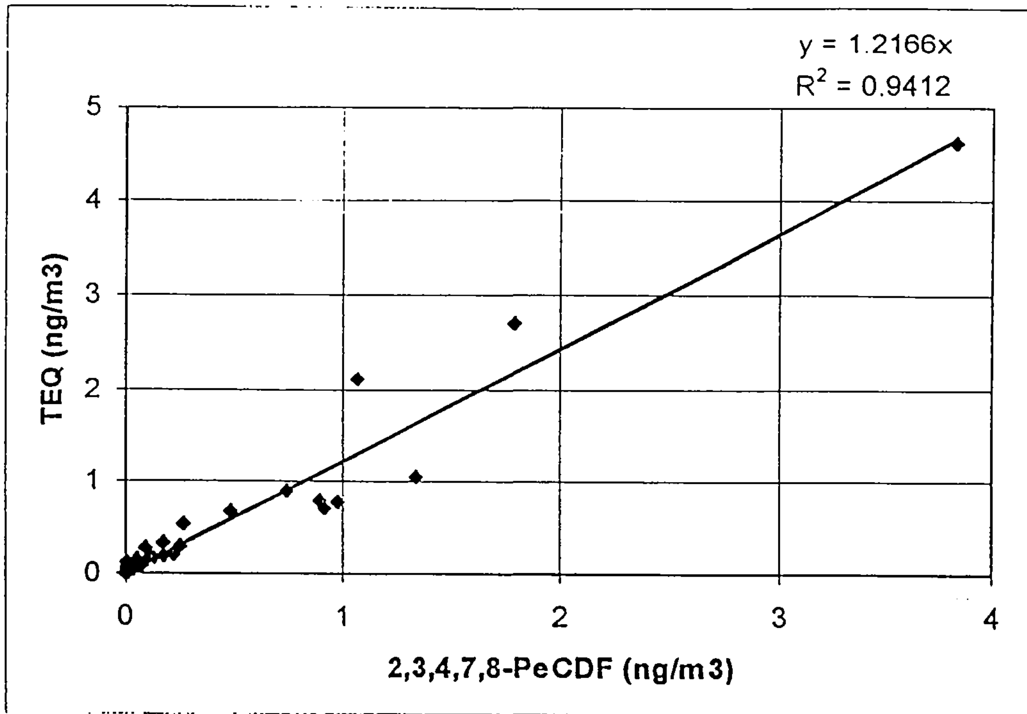

Figure B13 **Relationship Between Concentration of 2,3,4,7,8-PeCDF and I-TEQ for Chemical Waste Incinerators (n=42)**

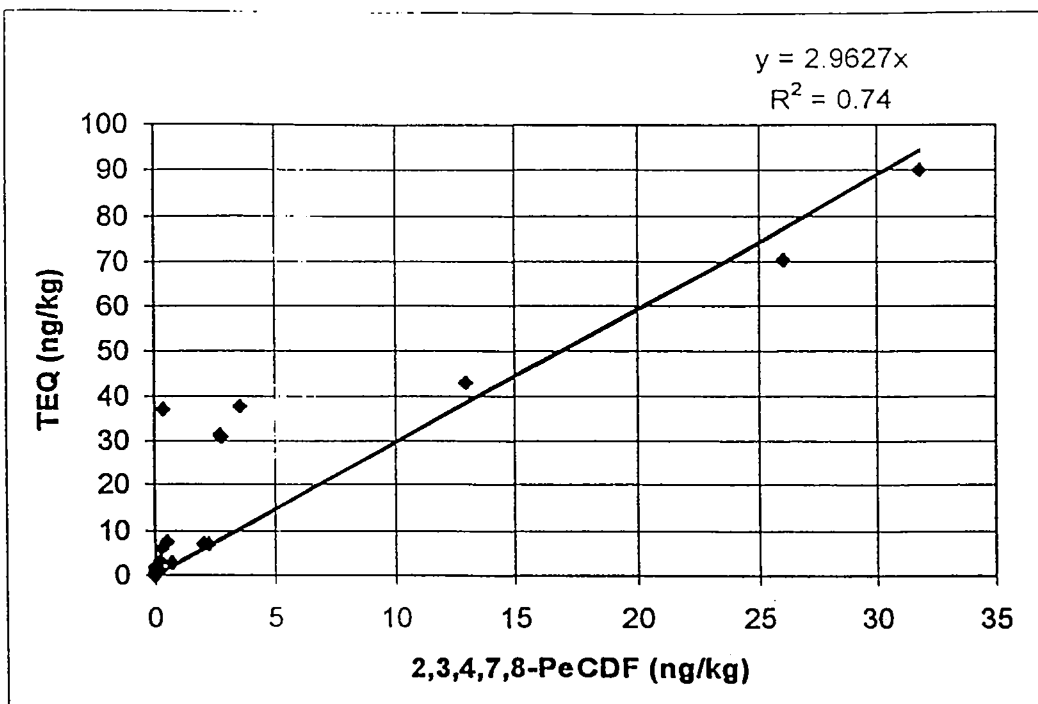

Figure B14 **Relationship Between Concentration of 2,3,4,7,8-PeCDF and I-TEQ for VCM Industry (n=20)**

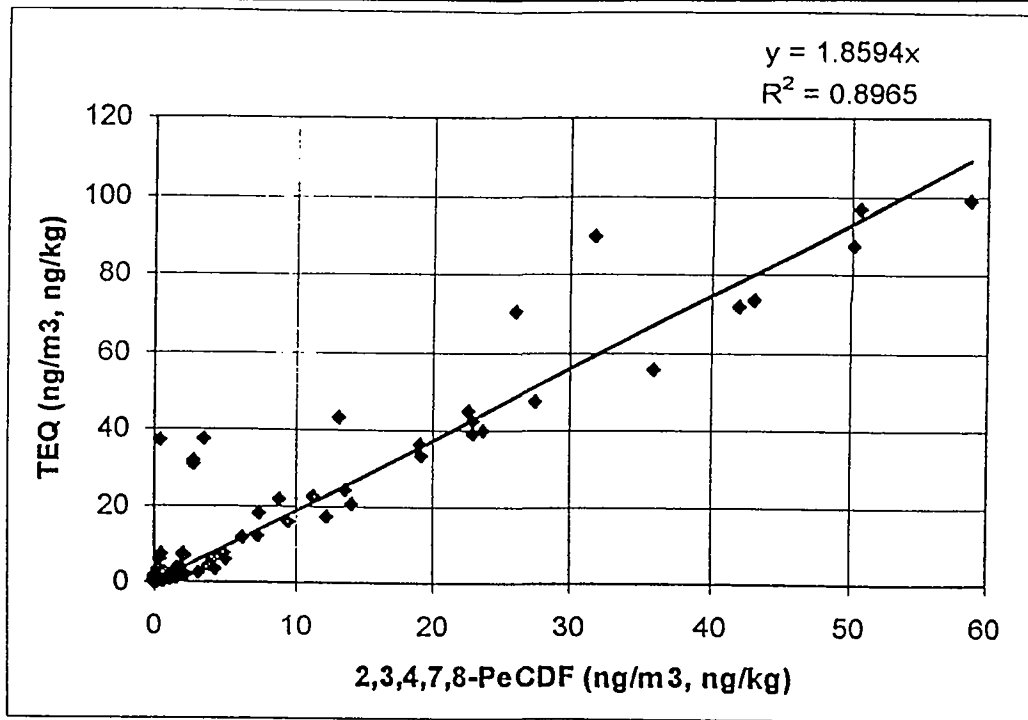

Figure B15 Relationship Between Concentration of 2,3,4,7,8-PeCDF and I-TEQ for All Data Excluding Outliner Samples

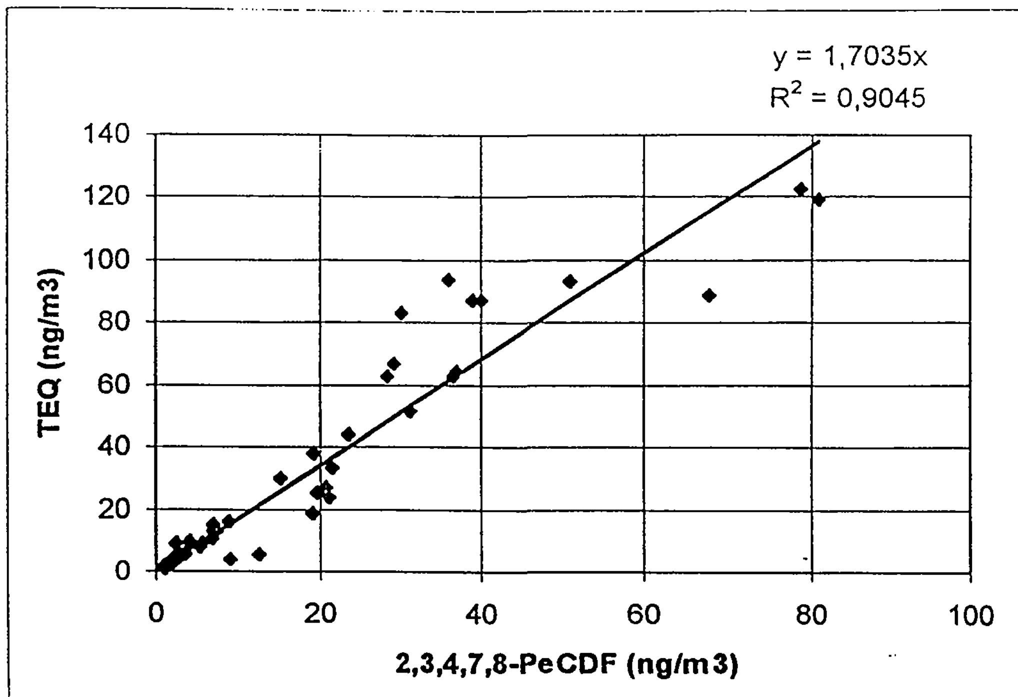

Figure B16 Additional Data Dutch MWIs (n=47)

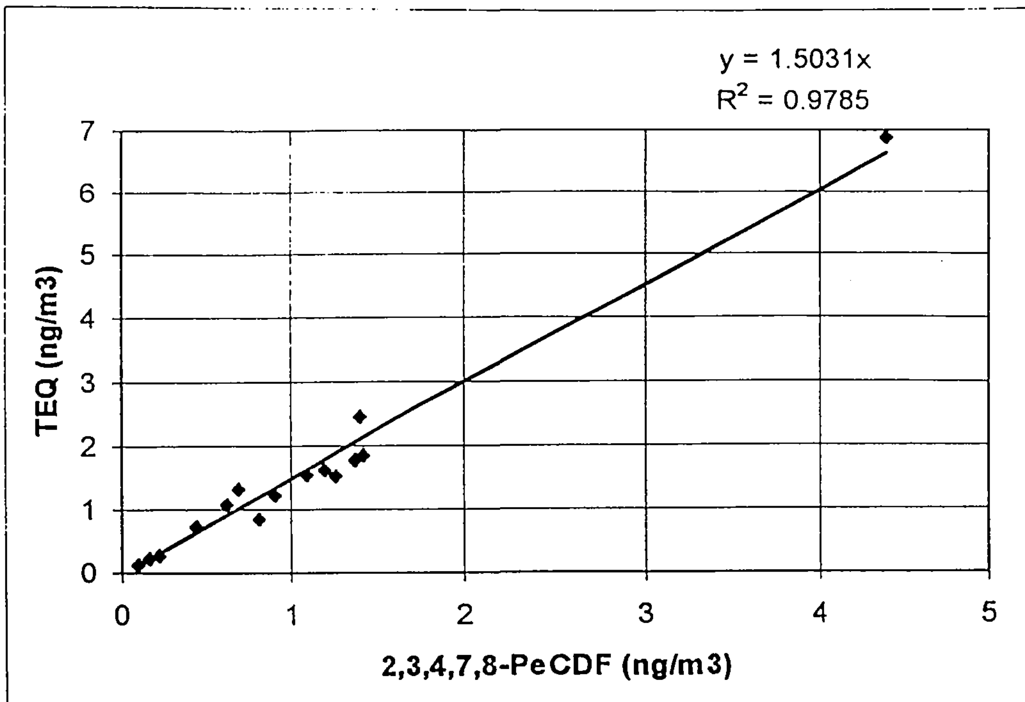

Figure B17 Additional Data German MWIs (n=15)

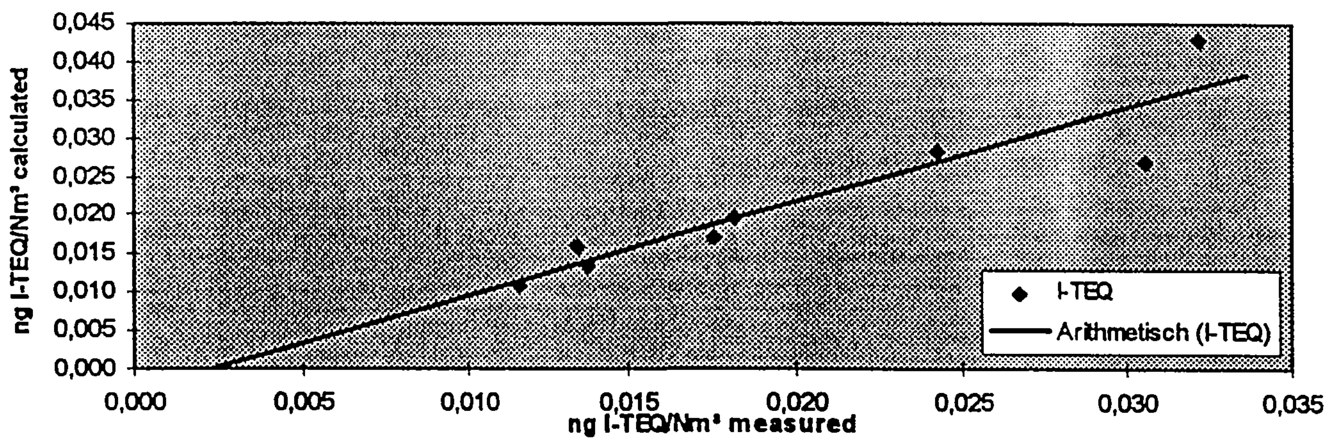

Figure B18 Identification of 2,3,4,7,8-PeCDF as a Marker Congener for I-TEQ Screening in MWI Stack Emissions (Reproduced from Mohr et al, 1996)

Annex C

Published Data on Organic Emissions from Municipal and Chemical Waste Incinerator

Table C1 Concentration (µg/m³) of Indivdual Compounds in the Emissions of a Municipal Waste Incineration Plant [1]

Substance	µg/m³	Substance	µg/m³
Pentane	1.00	Ethylcyclohexane	n.q.
Trichlorofluoromethane	2.00	2-Methyloctane	0.12
Acetonitrile	13.70	Dimethyldioxane	n.q.
Acetone	17.60	2-Furanecarboxaldehyde	0.18
Iodomethane	0.50	Chlorobenzene	2.11
Dichloromethane	20.00	Methyl hexanol	0.20
2-Methyl-2-propanol	n.q.	Trimethylcyclohexane	0.05
2-Methylpentane	3.20	Ethyl benzene	2.56
Chloroform	2.00	Formic acid	n.q.
Ethyl acetate	4.80	Xylene	7.77
2,2-Dimethyl-3-pentanol	7.00	Acetic acid	n.q.
Cyclohexane	1.70	Aliphatic carbonyl	0.19
Benzene	15.00	Ethylmethylcyclohexane	0.07
2-Methylhexane	3.60	*Xylene*	1.79
3-Methylhexane	2.70	2-Heptanone	n.q.
1,3-Dimethylcyclopentane	1.00	2-Butoxyethanol	0.23
1,2-Dimethylcyclopentane	1.00	Nonane	0.57
Trichloroethane	4.00	Isopropyl benzene	0.11
Heptane	4.70	Propylcyclohexane	0.16
Methylcyclohexane	4.70	Dimethyloctane	0.07
Ethylcyclopentane	1.00	$C_{10}H_{22}HC$	0.27
2-Hexanone	1.60	$C_{10}H_{22}HC$	0.18
Toluene	34.00	Pentanecarboxylic acid	1.31
1,2-Dimethylcyclohexane	0.03	Propyl benzene	0.28
2-Methylpropyl acetate	0.23	$C_6H_{12}OHC$	0.11
3-Methyleneheptane	n.q.	Benzaldehyde	1.32
Paraldehyde	n.q.	5-Methyl-2-furane carboxaldehyde	0.15
Octane	n.q.	1-Ethyl-2-methylbenzene	1.26
Tetrachloroethylene	0.16	1,3,5-Trimethylbenzene	0.34
Butanoic acid ethyl ester	0.08	$C_{12}H_{26}O$ alcohol	0.13
Butyl acetate	5.72	Trimethylbenzene	0.42
Benzotrile	0.30	Ethyl benzaldehyde	1.81
Methylpropylcyclohexane	0.02	2,4-Dichlorophenol	2.39
Methylpropylcyclohexane	0.24	1,2,4-Trichlorobenzene	0.55
2-Chlorophenol	0.53	Napthalene	1.51

Notes: [1] n.q. = not quantified

Substance	μg/m³	Substance	μg/m³
1,2,4-Trimethylbenzene	1.17	Cyclopentasiloxanedecamthyl	0.06
Phenol	1.40	Methyl acetophenone	0.76
$C_{10}H_{20}HC$	0.04	C_9H_8O aromatic compound	0.11
1,3-Dichlorobenzene	0.21	Ethanol-1-(-2-butoxyethoxy)	0.08
1,4-Dichlorobenzene	0.51	4-Chlorophenol	1.25
Decane	0.94	Benzathiazole	0.31
Hexanecarboxylic acid	1.07	Benzoic acid	100.92
1-Ethyl-4-methylbenzene	0.22	Octanoic acid	0.28
2-Methylisopropylbenzene	2.19	2-Bromo-4-chlorophenol	0.47
Cyclotetrasiloxaneoctamethyl	n.q.	1,2,5-Trichlorobenzene	0.12
1,2-Dichlorobenzene	0.02	Dodecane	0.14
Benzyl alcohol	3.97	Bromochlorophenol	0.23
Trimethylbenzene	n.q.	2,4-Dichloro-6-methylphenol	0.16
1-Methyl-3-propylbenzene	0.10	Dichloromethylphenol	0.26
2-Ethyl-1,4-dimethylbenzene	0.10	*Dichloromethylphenol*	0.15
$C_6H_{10}O_2$ aliphatic carbonyl	0.26	Hydroxybenzonitrile	0.08
2-Methylbenzaldehyde	1.87	Tetrachlorobenzene	0.28
1-Methyl-2-propylbenzene	0.12	Methylbenzoic acid	4.23
Methyl decane	0.06	Nitrogen compound, m.w. 269	2.04
$C_{12}H_{26}HC$	0.05	$C_9H_{18}O_3$ aliphatic compound	0.30
4-Methylbenzaldehyde	0.96	Trichlorophenol	0.18
$C_{13}H_{28}HC$	0.34	2-(Hydroxymethyl) benzoic acid	0.50
1-Ethyl-3,5-dimethylbenzene	n.q.	2-Ethylnaphtalene-1,2,3,4-tetrahydro	0.08
1-Methyl-(-1-propenyl)benzene	0.21	2,4,6-Trichlorophenol	9.55
Bromochlorobenzene	n.q.	4-Ethylacetophenone	0.59
4-Methylphenol	0.43	2,3,5-Trichlorophenol	0.49
Benzoic acid methyl ester	0.12	4-Chlorobenzoic acid	1.16
2-Chloro-6-methylphenol	0.10	2,3,4-Trichlorophenol	0.48
Ethyldimethylbenzene	0.00	1,2,3,5-Tetrachlorobenzene	0.30
Undecane	0.67	1,1Biphenyl (2-ethenyl-napthalene)	0.64
Heptanecarboxylic acid	0.50	3,4,5-Trichlorophenol	0.04
$C_8H_{14}O$ cyclohexane derivative	0.16	Chlorobenzoic acid	0.91
1-(-Chloromethyl)-4-methylbenzene	0.28	2-Hydroxy-3,5-dichlorobenzaldehyde	0.03
1,3-Diethylbenzene	0.15	2-Methylbiphenyl	0.20

Substance	μg/m³	Substance	μg/m³
1,2,3-Trichlorobenzene	0.07	2-Nitrostyrene(2-nitroethenyl benzene)	0.76
C_4 alkylbenzene	0.05	Decanecarboxylic acid	1.44
C_5 alkylbenzene	0.03	Hydroxymethanoxybenzalde hyde	0.12
4-Methylbenzyl alcohol	0.95	Hydroxychloroacetophenone	0.07
Ethylhexanoic acid	0.60	Ethylbenzoic acid	35.31
C_9H_8O aromatic compound	0.05	Tetradecanecarboxylic acid	15.08
2,6-Dichloro-4-nitrophenol	0.05	Octadecane	0.91
Sulphonic acid, m.w 192	0.58	Phthalic ester	0.17
4-Bromo-2,5-dichlorophenol	1.20	N-bearing aromatic compound m.w.405	2.08
2-Ethylbiphenyl	0.17	Tetradecanoic acid isopropyl ester	0.20
Bromodichlorophenol	0.33	Caffeine	0.14
1(3H)-Isobenzofuranone-5-methyl	0.11	12-Methyltetradecanecarboxylic acid	2.71
Dimethylphthalate	0.32	Phthalic ester + C_{15} acid	1.06
$C_8H_5O_2N$	0.12	Pentadecanecarboxylic acid	1.41
Si organic compound	0.24	Methylphenanthrene	0.06
2,6-Di-tertiary-butyl-p-benzoquinone	0.20	N-bearing aromatic compound, m.w. 405	0.58
3,4,6-Trichloro-1-methyl-phenol	1.50	Nondecane	0.48
2-Tertiary-butyl-4-methoxyphenol	1.23	9-Hexadecene carboxylic acid	1.92
2,2′-Dimethylbuphenyl	0.78	Anthraquinone	0.14
2,3′-Dimethylbiphenyl	1.72	Dibutylphtalate	7.66
Pentachlorobenzene	0.42	Hexadecanoic acid	36.78
Bibenzyl	0.82	Elemental sulphur, S_8	1.87
2,4-Di-tertiary-butylphenol	1.77	Eicosane	0.28
2,4′-Dimethylbiphenyl	0.67	Methylhexadecanoic acid	1.97
$C_8H_5BrCl_3$,aromatic compound,m.w.284	0.24	Fluoroanthene	0.19
1-Methyl-2-phenylmethylbenzene	0.68	Pentachlorobiphenyl	0.21
Benzoic acid phenyl ester	0.35	Aliphatic alcohol?	0.88
2,3,4,6-Tetrachlorophenol	4.42	Heptadecanecarboxylic acid	n.q.
Tetrachlorobenzofurane	0.26	Pyrene	0.25
Fluorene	0.01	Heneicosane	0.31
Phthalic ester	0.05	Octadecadienecarboxylic acid	0.00
Dodecanecarboxylic acid	0.94	Octadecadienal	1.67

Substance	$\mu g/m^3$	Substance	$\mu g/m^3$
3-3'-Dimethylbiphenyl	0.72	Pentalchlorobiphenyl	0.16
3,4-Dimethylbiphenyl	1.01	Aliphatic amide	0.74
Dimethylbiphenyl	0.14	Octadecanecarboxylic acid	2.23
Hexadecane	0.50	Hexadecane amide	1.20
Benzophenone	1.16	Docosane	0.35
$C_{11}H_{15}O_2N$ aromatic compound	0.53	Hexachlorobiphenyl	0.30
Tridecanoic acid	0.14	Benzylbutylphthalate	2.80
Hexachlorobenzene	0.11	Aliphatic amide	26.67
Heptadecane	0.36	Diisooctylphthalate	11.23
Fluorenone	1.69	Hexadecanoic acid hexadecyl ester	0.48
Dibenzothiophene	0.19	Cholesterol	0.70
Pentachlorophenol	1.92		
Sulphonic acid, m.w. 224	0.27		
Phenanthrene	1.09	Total ($\mu g/m^3$)	525.48

Source: K Jay, L Steiglitz (1995) **Identification and Quantification of Volatile Organic Components in Emissions of Waste Incineration Plants** Chemosphere

Table C2 Compounds detected in the emissions and identified by GC-MS in the samples taken at the flue gas stack of the hazardous waste incinerater of Biebesheim;

Organic Compounds	Sample 1	Sample 2
Chlorobenzenes		
Dichlorobenzene	1.29	0.65
Trichlorobenzene	0.64	0.28
Tetrachlorobenzene	74.70	48.00
Pentachlorobenzene	6.44	3.66
Hexachlorobenzene	2.06	1.38
Chlorophenols		
Dichlorophenol	0.09	0.13
Trichlorophenol	4.63	5.53
Tetrachlorophenol	24.47	20.74
Pentachlorophenol	0.68	0.34
2-Chloro, 4-bromophenol	1.55	0.23
Chlorobiphenyls		
Pentachlorobiphenyl	0.52	n.d.
Hexachlorobiphenyl	0.20	0.06
Heptachlorobiphenyl	n.d.	0.98
Dichlorodiphenylethane	0.10	0.16
Chloronapthalenes		
Monochloronapthalene	0.21	0.22
Dichloronapthalene	n.d.	0.32
Trichloronapthalene	n.d.	0.56

Organic Compounds	Sample 1	Sample 2
Nitroaromatic compounds		
Nitronapthalene	93.04	69.02
Nitroacenapthalene	6.44	4.23
Nitrosopyrrolidone	14.17	12.85
4-Nitrobiphenyl	3.35	1.79
Dinitrobenzene	106.95	71.51
Other organohalogenes		
Chloromethylphenol	1.29	0.16
Chloroanthracene	0.08	0.26
Chlorothiophenol	8.35	11.23
Bromothiophenol	17.25	18.71
Chlorotoluene	19.45	13.67
Dichlorobenzoylchloride	0.39	0.42
Dichlorobenzylalcohol	1.67	2.70
Dichloroquinoline	0.53	0.42
Bromo, chlorothiophene	0.44	0.34
Tetrachloroaniline	0.17	n.d.
Tetrachlorothiophene	0.39	0.20
PAH's		
Napthalene	53.24	30.91
Biphenyl	1.91	1.22
Anthracene	10.02	7.97
Fluoroanthene	0.50	0.30
Pyrene	0.93	0.42
Chrysene	0.27	0.09
Phthalate esters		
Dibutylphthalate	388.97	313.60
Butylbenzylphthalate	19.09	17.46
Diisooctylphthalate	454.41	371.93
Diisonoylphthalate	462.41	344.25
Phosphoric acid ester		
Tributylphosphate	80.00	79.00
Acrylonitrile (dimer)	20.90	16.40

Notes:

n.d.= not detectable, the concentration is below the detection limit or the identification is uncertain

Source: J Wienecke et al (1995) **Organic Compounds in the Flue Gas of a Hazardous Waste Incinerator** Chemosphere.

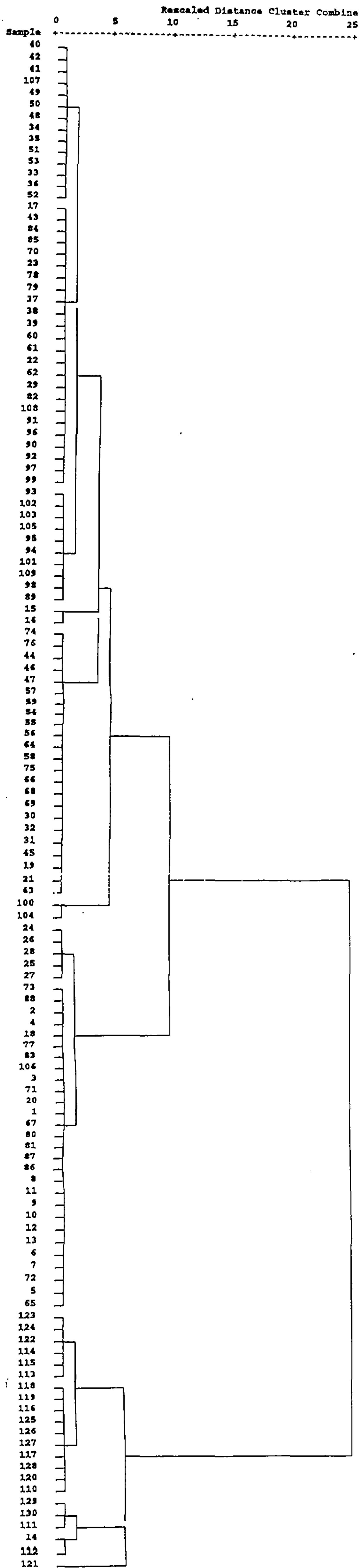

Figure B5 Denogram of All Data