Novel Methods for the Detection of Historical Biocidal Residues in Heritage Environments

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A thesis submitted to the University of Strathclyde in part fulfilment of the regulations for the degree of Doctor of Philosophy.

2016

Dedicated with love and pride to my Grandma, who taught me how to grit my teeth.

Elizabeth C. Park

1920 - 2015

Declaration of original research

This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

Signed: 

Date: 05 Dec 2016

Abstract

Work in this thesis is comprised of three inter-related projects to develop methods for detection of biocide residues in heritage environments. In the first phase of the work gas- or liquid-chromatographic methods of analyses were developed to detect 10 target analytes (aldrin, camphor, chloronaphthalene, dichlorodiphenyltrichloroethane, dichlorvos, dieldrin, endrin, hexachlorocyclohexane, naphthalene and thymol). The results obtained were summarised in a flowchart that could be easily interpreted by potential end-users (non-scientific personnel in heritage environments). The utility of the methods was demonstrated by performing a number of case studies in U.K. based heritage institutions. A number of the target analytes (camphor, chloronaphthalene, hexachlorocyclohexane, naphthalene) were successfully detected in the vapour-phase across several institutions and a tentative identification of dichlorvos was made after swabbing a case study object. The sampling technique for the developed methods was performed by museum staff after sending samplers to institutions *via* Royal Mail, highlighting the ease with which sample collection for these techniques can be conducted. The analysis showed that the concentrations of the substances detected in air were not likely to pose a significant hazard to human health, demonstrating the potential of the developed methods for use as a means of determining the safety of those working with the contaminated objects.

In the second phase of work, an agar sensor was loaded with an immobilised enzyme, acetylcholinesterase, to develop a sensor for the detection of organophosphates in swab or vapour-phase samples. The presence of a model organophosphate (dichlorvos) inhibited a colour change reaction in which the enzyme substrate, indoxyl acetate, was oxidised to indigo after undergoing an enzyme-catalysed hydrolysis. This sensor potentially has significant advantages over current methods of detection for organophosphate pesticides in heritage institutions, as it was shown to be simple, cheap, easy to use and did not require specialist training or equipment to perform the analysis or determine the result. The developed method had a detection limit of 13 mg dm^{-3} dichlorvos in solution, and masses of as low as 20 μg were shown to provide positive results when collected using a swab. Furthermore, the gels developed have a shelf life of several weeks and are thus easily transported for use within heritage

institutions. Significantly, the developed organophosphate sensor was also demonstrated to work in the vapour-phase, possibly allowing objects considered too fragile for contact sampling to be tested for the presence of organophosphate pesticides.

Finally, preliminary work was conducted to develop a method for the determination of mercury in heritage environments. Silica xerogel or agar hydrogel supports were used to immobilise diphenylcarbazone (DPC), a colorimetric reagent used for the detection of mercury, in order to address an unmet need for simple and cost-effective detection of organic biocides. Agar gel was considered to be the most suitable matrix and was used to form the basis of a cheap, easy to use, selective and sensitive method for the detection of mercury chloride in heritage environments. This work was concluded by spiking simulated objects with mercury chloride solution, following detection with the newly developed sensor. The sensor was shown to produce a colour change that was readily observable using the naked eye down to concentrations of 10 mg dm^{-3} without the need for instrumentation, offering a significant cost advantage over established techniques for the detection of mercury in heritage institutions such as x-ray fluorescence spectrometry. DPC gels were also shown to be effective when dried. This significantly extended the shelf life of the DPC reagent, which, according to literature studies, previously required preparation immediately before use.

The phases of work presented here have developed several novel methods for the detection of both organic and inorganic biocides in heritage institutions. The work was specifically focussed on making the developed methods suitable for use by untrained personnel from a non-scientific background without the need for specialist training or equipment.

Acknowledgements

I would like to extend my deepest thanks to Dr Lorraine Gibson for the opportunity to work on this project and for her support and guidance throughout this work. Acknowledgement is also due to the project partners: Dr Catherine Higgitt at the British Museum and Dr Matija Strlič at University College London. I wish to thank Dr Jim Tate, formerly of National Museums of Scotland; Dr Linda Ramsay and Saho Arikawa, National Records of Scotland; and Dr David Thickett, English Heritage. Funding was gratefully received from the Science and Heritage Program of the AHRC and EPSRC (AH/H032630/1).

Particular thanks are due to Arizona State Museum for accepting me as an international scholar, where I developed the mercury sol-gel work. Thanks are due to the staff and students at the museum for making me feel most welcome. My profound gratitude is offered to Prof. Nancy Odegaard, and Drs Teresa Moreno and Sheila Hoban not only for making me so welcome during my time there, but also for giving up their own time for me and for their generous support and friendship when I was in need. I would very much like to take this opportunity to remember the University of Arizona's Dr Werner Zimmt, who sadly passed away unexpectedly on 12th September 2014 at the age of almost 93. I found discussions with him both personally and professionally valuable, and was very much inspired by seeing a man for whom a quiet, sedentary retirement meant nothing of the sort.

The staff and students of the analytical group at Strathclyde have been a pleasure to work with, and made the difficulties and stresses of completing a PhD easier by offering help and friendship. Thanks also to Dr Margaret Smith and Ms Jenna Vint for their input into the HPLC method development and organophosphate sensor work respectively.

I would like to thank Mum, Dad, Gordon and Emma for their unwavering belief, support, guidance and love. Finally, I want to thank my partner and best friend, Dr Natalie Monks for her enduring support and encouragement, and for making our home a place of fun instead of stress and doubt.

List of publications from this thesis

1. I. D. Rushworth, C. Higgitt, M. Smith and L. T. Gibson, Non-invasive multiresidue screening methods for the determination of pesticides in heritage collections, *Heritage Science*, 2014, **2**.

2. M. Hacke, J. Willey, G. Mitchell, I. D. Rushworth, C. Higgitt and L. T. Gibson, Investigation of long term storage solutions for rubber garments, *Journal of the Institute of Conservation*, 2014, **37**, 179-196.

List of chemicals and safety information

All experiments in this thesis were performed in a safe and controlled manner and were subject to risk assessments prior to beginning the work. A laboratory coat, safety glasses and nitrile gloves were worn at all times when undertaking experimental work. Substances were handled according to their associated hazards, with volatile substances being used in a fume cupboard and stored in appropriately vented locations. The substances used in this thesis, and their associated hazards are presented in **Table i**.

Table i: Grades and suppliers of substances used throughout this thesis

Substance	Grade	Supplier	Associated hazards
1-chloronaphthalene	Technical grade	Sigma-Aldrich, Gillingham	Harmful Causes skin/eye irritation Very toxic to aquatic life
4,4'-DDT	98%	Sigma-Aldrich, Gillingham	Toxic if swallowed Toxic in contact with skin May cause cancer Causes damage to organs through repeated or prolonged exposure Very toxic to aquatic life with long-lasting effects
5,5'-dithiobis (2-nitrobenzoic acid)	≥98% BioReagent	Sigma-Aldrich, Gillingham	Causes skin/eye irritation

Substance	Grade	Supplier	Associated hazards
Acetylcholinesterase	Type VI-S, lyophilized powder	Sigma-Aldrich, Gillingham	-
Acetylthiocholine chloride	≥99% (TLC)	Sigma-Aldrich, Gillingham	Causes serious eye damage
Agar agar powder	Laboratory reagent	Fisher Scientific, Loughborough	-
Aldrin	Pestanal analytical standard	Sigma-Aldrich, Gillingham	Fatal if swallowed Fatal in contact with skin Suspected of causing cancer Causes damage to organs through repeated or prolonged exposure Very toxic to aquatic life with long-lasting effects
Bovine serum albumin	Lyophilized powder, >96%	Sigma-Aldrich, Gillingham	-

Substance	Grade	Supplier	Associated hazards
Camphor	Purum, ≥95.0%	Sigma-Aldrich, Gillingham	Flammable solid Harmful if swallowed Causes skin irritation Causes serious eye damage
Dichlorvos	Pestanal analytical standard	Sigma-Aldrich, Gillingham	Fatal if swallowed Fatal in contact with skin Very toxic to aquatic life May cause an allergic skin reaction
Dieldrin	Pestanal analytical standard	Sigma-Aldrich, Gillingham	Toxic if swallowed Fatal in contact with skin Suspected of causing cancer Causes damage to organs through prolonged or repeated exposure Very toxic to aquatic life with long-lasting effects
Diphenylcarbazone	Mixture with diphenylcarbazide ~60%	Sigma-Aldrich, Gillingham	-

Substance	Grade	Supplier	Associated hazards
Endrin	Pestanal analytical standard	Sigma-Aldrich, Gillingham	Fatal if swallowed Toxic in contact with skin
Ethanol	Absolute, HPLC grade	Fisher Scientific, Loughborough	Highly flammable liquid and vapour Causes serious eye irritation
Glycerol	≥99%	Sigma-Aldrich, Gillingham	-
HCH (1:1:1:1 α:β:γ:σ isomers)	Pestanal analytical standard	Sigma-Aldrich, Gillingham	Toxic if swallowed Harmful in contact with skin Suspected of causing cancer Harmful to breast-fed children May cause damage to organs through prolonged or repeated exposure Very toxic to aquatic life with long-lasting effects
Hexane	95% n-Hexane	Fisher Scientific, Loughborough	Highly flammable liquid and vapour May be fatal if swallowed and enters airways Causes skin irritation

Substance	Grade	Supplier	Associated hazards
			May cause drowsiness or dizziness Suspected of damaging fertility May cause damage to organs through prolonged or repeated exposure Toxic to aquatic life with long-lasting effects
Hydrochloric acid	10 N Certified	Fisher Scientific, Loughborough	May be corrosive to metals Causes severe skin burns and eye damage May cause respiratory irritation
Indoxyl acetate	>95% (TLC)	Sigma-Aldrich, Gillingham	Harmful if swallowed Causes skin irritation Causes serious eye irritation May cause respiratory irritation
Isopropyl alcohol	HPLC grade	Rathburn, Walkerburn	Highly flammable liquid and vapour Causes serious eye irritation May cause drowsiness or dizziness

Substance	Grade	Supplier	Associated hazards
Mercury chloride	ACS reagent, ≥99.5%	Sigma-Aldrich, Gillingham	Fatal if swallowed Fatal in contact with skin Causes severe skin burns and eye damage Suspected of causing genetic defects Suspected of damaging fertility Causes damage to organs through prolonged or repeated exposure Very toxic to aquatic life with long-lasting effects
Naphthalene	Scintillation grade, ≥99.0%	Sigma-Aldrich, Gillingham	Flammable solid Harmful if swallowed Suspected of causing cancer Very toxic to aquatic life with long-lasting effects
Phosphate buffered saline tablets	-	Sigma-Aldrich, Gillingham	-
Potassium dichromate	ACS reagent, ≥99.0%	Sigma-Aldrich, Gillingham	May intensify fire; oxidizer Toxic if swallowed Fatal in contact with skin

Substance	Grade	Supplier	Associated hazards
			Fatal if inhaled Causes severe skin burns and eye damage May cause an allergic skin reaction May cause allergy or asthma symptoms or breathing difficulties if inhaled May cause genetic defects May cause cancer Suspected of damaging fertility Causes damage to organs through prolonged or repeated exposure Suspected of damaging the unborn child Very toxic to aquatic life with long-lasting effects
Potassium hydroxide	Certified ACS	Fisher Scientific, Loughborough	May be corrosive to metals Harmful if swallowed Causes severe skin burns and eye damage
Tenax TA sorbent tubes	-	Markes International, Llantrisant	-

Substance	Grade	Supplier	Associated hazards
Tetraethyl orthosilicate	≥99.0% (GC)	Sigma-Aldrich, Gillingham	Flammable liquid and vapour Causes serious eye irritation Harmful if inhaled May cause respiratory irritation
Thymol	≥99.0%	Sigma-Aldrich, Gillingham	Harmful if swallowed Causes severe skin burns and eye damage Toxic to aquatic life with long-lasting effects

Contents

1. Introduction

1.1. Pesticides and heritage collections

Pesticides are substances which can be used to protect heritage collections by preventing, repelling or killing pests that would otherwise damage objects. The application of these hazardous chemicals to natural history, ethnographic and botanical material in heritage institutions to protect against insect and fungal attack is generally accepted as having been widespread. Before the use of interventive treatments like these, collections containing organic material were often lost to decay and infestation.[1] Application methods for pesticides are varied and include spraying, dipping, sublimation, painting, brushing and dusting techniques, depending on the substance being applied.[2]

Biocide preparations were often applied to objects following the instructions of larger museums such as the American Museum of National History and the Smithsonian Institution.[3] These activities were typically considered part of the general care of the collection and were rarely documented. As Briggs *et al.*[4] noted with toxic mercury compounds used for this purpose: "it is not [the Cambridge Herbarium's] custom to indicate… that specimens have been treated with mercuric chloride". As such, records of biocidal treatment are often incomplete, if they exist at all.

Pesticides, and other chemical hazards, used as interventive pest treatments may still be present in the form of residues and pose a significant health risk to staff and visitors to the collections. To develop methods for the determination of pesticides in heritage collections, it is important to fully understand the scale of the problem facing analytical chemists performing this task. Objects within a collection may have been exposed to more than one substance as part of continual routine disinfestation.[5] The scope of the challenge has been discussed by Pool *et al.*[6] who detailed a list of nearly 100 pesticides known to have been used in heritage institutions. This list described the dates of first synthesis, date of federal registration as a pesticide, the legislative status of the substance (cancelled, restricted, or continued use), method of application, physical

appearance, target pests, field half-life and environmental persistence. This list highlighted the wide use of different pesticides and that the choice of pesticide use was not always recorded.

1.1.1. Usage trends in the 19th Century

In the 19th century, arsenic soaps and mercuric salts were widely used in conservation treatments for objects held in ethnographic or natural science collections.[1] The early 19th century also saw an increase in exploratory expeditions, such as those made by Lewis and Clark, and Henry Morton Stanley, in which explorers were ordered to create maps and collect natural samples, which needed to be preserved.[1] The expeditions themselves are discussed at length in literature.[7-11] The preferred method of preservation of the collected samples was an application of mercury and/or arsenic compounds, as described by Baird[12] and later Goldberg.[2] The chemicals were applied to the materials in a variety of ways, for example, skins were buried in sand saturated with mercuric chloride ($HgCl_2$) solutions, or materials were rubbed with the chemical treatment solution. As Goldberg noted, arsenic and mercury compounds were in regular use at the Smithsonian until the late 1800s. It is therefore expected that other institutions adopted similar treatment methods. Hough[13] also described a treatment method that used a solution of $HgCl_2$ in 50% alcohol, with a small amount of naphtha added to prevent recrystallisation of the mercury salt.

While these treatments were not commonly applied to ethnographic collections after the late 1800s, their use continued in natural history and taxidermy collections into the latter half of the 20th century.[1] As a result, various publications reported the use of $HgCl_2$ (also known as corrosive sublimate) as an insecticide in ethnographic and herbarium collections.[2, 5, 14-20] Arsenic trioxide (As_2O_3), in the form of arsenical powders, soaps or solutions, were also discussed as treatment methods and therefore were a later source of potential contamination in ethnographic collections.[21] The extent of treatment methods involving the use of arsenic salts was, as determined by Sirois,[21] more excessive than for mercuric chloride heritage collections.

The use of natural biocides was also common at this time. Camphor was used frequently in natural history and ethnographic collections as it could be easily, and cheaply, steam distilled from the wood and leaves of the camphor laurel *Cinamomum*

camphora.[1, 2, 22] Camphor would have been used in a similar manner as mothballs, where small containers of the material would be placed in display cases and allowed to volatilise into the environment.

1.1.2. Usage trends in the early-mid 20th Century (1900 – 1950s)

Throughout the early half of the 20th Century, metal-based pesticides were commonly used. By the 1940s, the agricultural and domestic use of arsenic trioxide (As_2O_3) in the United States of America was approximately 40 000 tonnes per annum. In addition, several tonnes of other arsenic compounds, such as Paris green, a copper acetoarsenite pesticide, were also used.[1]

This time period also saw the introduction of organochloride pesticides which were used as treatment methods for fumigation. Paradichlorobenzene (PDB), a substituted aromatic compound, was widely used as it readily volatilises at room temperature. Together with naphthalene, PDB is still one of the major constituents of mothball formulations.[15, 23] The popularity of organochloride pesticides grew in the agricultural industry and a greater range of compounds was developed to include organochloride functionality. In 1939, the insecticidal properties of dichlorodiphenyltrichloroethane (DDT) were discovered by Swiss chemist Paul Müller, for which he won the Nobel Prize for Medicine in 1948.[24] During World War II, DDT was successfully used against mosquitos to provide troops with protection from malaria when fighting in the jungle and was not available for use outside the military.[25-27]

1.1.3. Usage trends in the mid-late 20th Century (1950s – 1990s)

After World War II, DDT was made available for civilian use as an agricultural and household pesticide and its production and use increased greatly. **Figure 1.1** shows an image from National Geographic magazine published in 1996 of DDT being sprayed from a TIFA (Todd Insecticidal Fog Applicator) at Jones Beach in New York.[28] At the same beach, **Figure 1.2** illustrates a public safety image for DDT where the substance was sprayed around Kay Heffernon to demonstrate that it would not contaminate her food.[29] Soon after, environmental concerns were raised about DDT in the early 60s by campaigners such as Rachel Carson,[30] and its popularity decreased. More importantly, its usage as a pesticidal treatment was outlawed in over 170 countries due to

environmental concerns, as well as the evidence of carcinogenicity seen in experimental animals.[31-34]

Figure 1.1: DDT being sprayed at Jones Beach, New York, 1953[28]

One of the main environmental concerns with the use of organochloride pesticides is that they are persistent pollutants due to their hydrophobic nature and are easily biomagnified. This means that as DDT, and its metabolites such as dichlorodiphenyldichloroethylene (DDE), move up through the food chain the toxic effect of DDT increases, as the result of consuming contaminated food on lower trophic levels provides organisms higher in the food chain with larger cumulative doses than that of animals on lower levels. The deleterious effects of organochloride pesticides also include the thinning of eggshells of larger birds of prey such as peregrine falcons leading to a sharp decrease in their numbers.[35]

Figure 1.2: Kay Heffernon pictured in a fog of DDT to demonstrate its safe use[29]

Organophosphate (OP) pesticides, such as dichlorvos (2,2-dichlorovinyl-dimethylphosphate or DDVP, also known as Vapona™), were also developed at this time. As an extension of G- and V-class nerve agents developed by the British armed forces at Porton Down, dichlorvos acts on the parasympathetic nervous system to increase the level and duration of action for the neurotransmitter acetylcholine by inhibiting the action of acetylcholinesterase (AChE).[36] Under normal action, a neuron fires when acetylcholine binds to receptors on the cell, leading to muscle contraction. After this, AChE degrades acetylcholine into acetic acid and choline. However, dichlorvos molecules phosphorylate the AChE necessary for the relaxation of the neuron, permanently deactivating the enzyme.[37] This renders the muscle or organ unable to relax the contractions, leading to paralysis and death. Exposure to dichlorvos and other volatile OPs can occur though inhalation or via dermal absorption. In terms of persistence, OPs are not regarded to be as long lasting as organochloride pesticides, but this is countered by their greater acute toxicity. Smaller quantities of OPs were therefore applied to kill a target organism and as OP pesticides were not as stringently controlled as organochloride pesticides, their usage was favoured during this time period.

1.1.4. Usage trends in the late 20th and early 21st Centuries (1990s – present)

Modern pest control methods in museums avoid the use of potentially hazardous chemicals wherever possible. Current disinfestation treatments involve either low temperature processes, where the object is frozen to kill pests, or methods use carbon dioxide or nitrogen as asphyxiant gasses.[5] However both methods require significant space to set up and are unsuited to the treatment of large objects. Some modern pesticides feature a toxophore based on pyrethrum, a natural insecticide derived from plants of the genus *Chrysanthemum*, specifically *C. cinerariifolium* and *C. coccineum*.[38] These substances, called pyrethroids, have also found use in heritage environments due to their conversion to non-toxic metabolites in mammals. [39-41]

1.2. Health effects of selected pesticides used in heritage collections

A set of 10 chemical hazards was selected for study in this thesis after consultation with a number of heritage institutions and professionals across the UK. Thes institutions and professionals were asked (to the best of their knowledge) which pesticides either were or had potentially been used in their collections. The biocides were selected based on how commonly they were used, with consideration of their harmful health effects also being made. The biocides selected for study were: aldrin, camphor, chloronaphthalene, dichlorvos, DDT, dieldrin, endrin, hexachlorocyclohexane (HCH), naphthalene and thymol. This list comprised six organochlorides, two simple aromatics, one organophosphate and one terpenoid substance. Inorganic biocides were represented by investigating colorimetric methods for the determination of $HgCl_2$. Vapour densities of each of the biocides of interest to this project may be calculated from vapour pressures at a given temperature as per **equation 1.1**, where d is the saturated vapour density, R is the gas constant, p is the vapour pressure of the substance, M is the molecular weight and T is the absolute temperature.

$$d = \frac{pM}{RT}$$

Equation 1.1

Physical and toxicological properties of the selected pesticides are presented below in **Table 1.1** in order of descending saturation vapour density. The table shows the vapour pressure at a given temperature, as well as the calculated saturation vapour density in μg dm^{-3} at that temperature. LD_{50} values for substances where available, along with their route of administration. Specific hazard information for each substance may be found in **Table i** at the start of this thesis.

Table 1.1: Physical and toxicological properties of selected heritage biocides

Compound	**Temperature (°C)**	**Vapour pressure (mPa)**	**Saturation vapour density (μg dm^{-3})**	**LD_{50} (mg kg^{-1}), exposure route, animal**
Camphor[42]	20	20 000	1246.69	1310, oral, mouse
Naphthalene[43]	20	6500	341.20	490, oral, rat
Thymol[43]	20	5370	330.38	980, oral, rat
Chloronaphthalene[44]	25	3857	252.54	1540, oral, rat
Dichlorvos[43]	20	290	26.25	25, oral, rat
Mercury[45, 46]	20	171.3	14.07	Data not available*
$HgCl_2$[43]	35	18.6	1.97	41, dermal, rat
Aldrin[47]	25	8.56	1.01	39, oral, rat

Compound	Temperature (°C)	Vapour pressure (mPa)	Saturation vapour density (μg dm^{-3})	LD_{50} (mg kg^{-1}), exposure route, animal
Lindane[43]	20	5.6	0.67	88, oral, rat
Dieldrin[47]	25	0.4	0.05	38, oral, rat
Endrin[48]	25	0.036	4.27×10^{-3}	3, oral, rat
DDT[43]	20	0.02527	3.67×10^{-3}	87, oral, rat

* LC_{50} (concentration required to kill half the dosed population) for inhalation by rats[46] over 2 h was <27 mg m^{-3}

1.2.1. Properties of aldrin and dieldrin

Aldrin and dieldrin (see chemical structures in **Figure 1.3**) are structurally related organochloride pesticides formed via a Diels-Alder reaction of hexachlorocyclopentadiene with norbornadiene.[49] A further epoxidation of aldrin yields dieldrin.[50] Both compounds were used as insecticides and agricultural seed treatments for the control of termites, grasshoppers, wood borers, beetles, and textile pests.[51] Both compounds are practically insoluble in water and moderately to highly soluble in alkanes, aromatic and chlorinated solvents. The vapour pressure of aldrin is 8.56 mPa, and dieldrin is 0.4 mPa at 25 °C.[51]

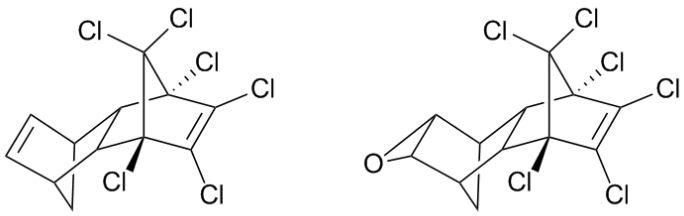

Figure 1.3: Chemical structures of aldrin (L) and dieldrin (R)

The lowest reported dose in man with a fatal outcome was 10 mg kg^{-1} body weight.[51] Oral LD_{50} values for rats (the dose required to kill half of the dosed population) are

low at 39 and 38 mg kg^{-1}, indicating high acute toxicity. The effects of acute toxicity include tremors, convulsions and hyperexcitability, as the substance affects the central nervous system (CNS). Long term carcinogenicity studies were carried out with aldrin and dieldrin, in which all mice were found to develop both benign and malignant tumours as a result.[51]

1.2.2. Properties of camphor

Camphor (see **Figure 1.4**) is a terpenoid compound found in the leaves and wood of the camphor laurel, *Cinnamomum camphora*.[52] Camphor is a flammable waxy solid with a distinctive, penetrating odour and is soluble in ethanol, ether and chloroform. Camphor is known to cause skin and eye irritation.[52] The main risks from exposure to camphor are to the CNS and kidneys. Convulsions followed by depression and renal damage have been reported after exposure to relatively small amounts of the substance with toxic effects being observed after ingestion of approximately 2 g, and an LD_{50} of approximately 1310 mg kg^{-1} in rats.[42, 53] Camphor is highly volatile, with a vapour pressure at 20 °C of 20 000 mPa – the highest of the 10 pesticides selected for study in this work.[42]

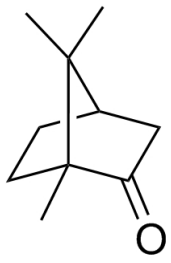

Figure 1.4: Chemical structure of camphor

1.2.3. Properties of chloronaphthalene

Chloronaphthalene (**Figure 1.5**) is a chlorinated compound structurally related to naphthalene (**Figure 1.10**) in that the 1 position of the ring system has been substituted, replacing H with Cl.

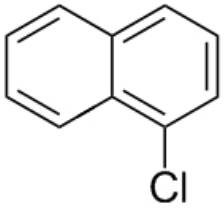

Figure 1.5: Chemical structure of chloronaphthalene

It is a colourless oily liquid with a vapour pressure of 3900 mPa at standard temperature.[44] The substance causes skin irritation and is harmful if swallowed, as well as being very toxic to marine life.[54]

1.2.4. Properties of dichlorvos

Dichlorvos (**Figure 1.6**) is an acutely toxic organophosphate compound that kills the target organism by inhibiting the action of AChE (refer to **Section 1.1.3**). Dichlorvos is an oily colourless-yellow liquid at room temperature with a sharp odour. The substance is toxic through inhalation, dermal absorption and ingestion.[43] The vapour pressure of dichlorvos at 20 °C is 290 mPa.[43] The LD_{50} (oral, rat) is reported at 28 mg kg^{-1}.[55] The maximum level stated by one study, causing no significant toxicological effect in man is 0.01 mg kg^{-1} day^{-1} by inhalation.[55]

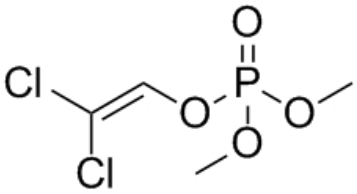

Figure 1.6: Chemical structure of dichlorvos

1.2.5. Properties of DDT

DDT (**Figure 1.7**) is an organochloride pesticide with the appearance of white crystals, and is practically insoluble in water, but readily soluble in non-polar organic solvents. DDT has a vapour pressure of 0.0235 mPa at 20 °C[56] and is a CNS stimulant acting on the cerebellum and motor cortex, inducing hyperexcitability, tremor and muscle weakness. Convulsions and myocardial sensitivity may also occur.[57] DDT has a high

fat/water partition coefficient leading to higher concentrations of the substance being stored in adipose tissues than elsewhere in the body.[56] As such, DDT is eliminated from the body slowly, at a rate of approximately 1% of stored DDT per day.[58] DDT has limited toxicity through dermal absorption and the low volatility reduces the risk of poisoning by inhalation of vapour. As such, the most likely route of exposure is through accidental ingestion of the substance.

In humans, DDT is considered likely to be a carcinogen and is classified as moderately hazardous by the World Health Organisation based on the rat oral LD_{50} of 87 mg kg^{-1}.[59] As an endocrine disruptor, DDT has also shown to disrupt normal pregnancy cycles, and adversely affect menstruation cycles and semen quality.[60]

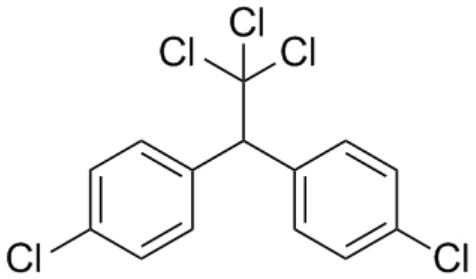

Figure 1.7: Chemical structure of DDT

1.2.6. Properties of endrin

Endrin is an organochloride pesticide that shares some structural similarities with other cyclodiene pesticides such as dieldrin and aldrin. Endrin is sysnthesised by condensing hexachlorocyclopentadiene with acetylene at 150-175 °C at 13 700-27 500 kPa. The product is condensed with cyclopentadiene at 50-90 °C at ambient pressure and epoxidised by peracetic acid.[50] The structure of endrin is shown in **Figure 1.8**. Properties in common with aldrin and dieldrin include low solubility in water but high solubility in lipids and a low vapour pressure of 0.036 mPa at 25 °C.[43]

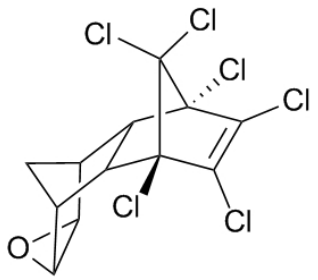

Figure 1.8: Chemical structure of endrin

The oral toxicity of endrin to rats is as low at 8 mg kg^{-1}, making it the most toxic of the organochloride pesticides studied here.[61] The molecular mechanism of endrin's toxicity is not known. Unlike aldrin and dieldrin, bioaccumulation is relatively limited due to the rapid biodegradation of the substance.

1.2.7. Properties of HCH

HCH is a name used collectively for the isomers of 1,2,3,4,5,6-hexachlorocyclohexane, formed *via* the photochlorination of benzene. The isomers differ in axial-equatorial substitution pattern on the ring, with each isomer being denoted by the Greek letters α, β, γ, δ, ε, η, and θ, as shown in **Figure 1.9**. It should also be noted that α-HCH has two enantiomeric forms. The γ-HCH isomer, also known as lindane, has the highest pesticidal activity.[62] The physical and chemical properties of these substances are summarised in **Table 1.2**.[62] The table also lists a bioconcentration factor (BCF) for each substance, defined as the ratio of concentrations of the substance in a selected tissue and water.

According to Willett *et al.*[62] toxicological mechanisms for HCH are largely unknown, however the substances primarily affect the CNS as well as renal and hepatic functions. Lindane poisoning causes ataxia, tremors, convulsions, and stimulated respiration. At very high doses, lindane causes CNS damage resulting in convulsions and death. Lower doses are quickly metabolised, however renal, hepatic and reproductive problems have been reported.

Table 1.2: Selected physical and chemical properties of selected HCH isomers[62]

Property	**α**	**β**	**γ**	**δ**	**ε**
Melting point (°C)	159−160	309−310	112−113	138−139	219−220
Vapour pressure (mPa, at 20 or 25 °C)	16 ± 0.9	0.042 ±0.003	5.3 ± 1.4	2.1	No data
log K_{ow}	3.9 ± 0.2	3.9 ± 0.1	3.7 ± 0.5	4.1 ± 0.02	No data
BCF in human fat	20 ± 8	527 ± 140	19 ± 9	8.5	No data
BCF in aquatic animals	2.6 ± 0.5	2.9 ± 0.3	2.5 ± 0.4	No data	No data

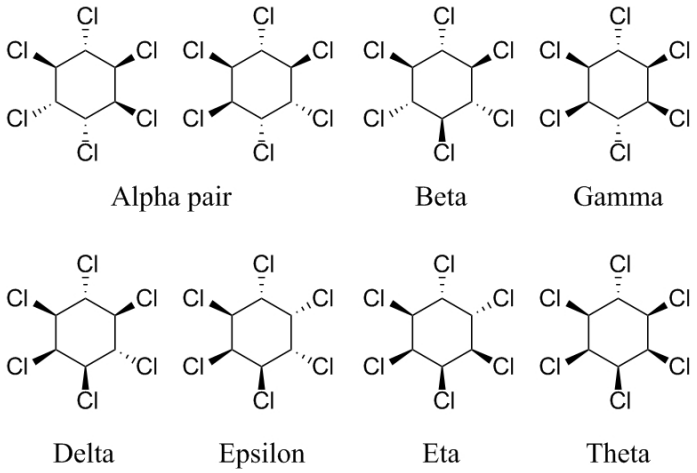

Figure 1.9: Structures of α-, β-, γ-, δ-, ε-, η-, and θ-HCH (top L – bottom R)

1.2.8. Properties of naphthalene

The chemical structure of naphthalene is shown in **Figure 1.10**. Naphthalene is a bicyclic aromatic compound formed by fusing 2 benzene rings, with the physical appearance of white solid crystals or flakes with a strong characteristic odour which Amoore and Hautala[63] claim is detectable in air by humans at concentrations as low as 0.08 ppm v/v, equivalent to 0.42 mg m^{-3}.

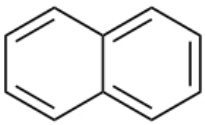

Figure 1.10: Chemical structure of naphthalene

Naphthalene is soluble in alcohols and ethers, and has a vapour pressure of 6500 mPa at 20 °C.[43] Exposure routes for naphthalene are predominantly oral and by inhalation. Naphthalene- and dichlorobenzene-containing mothballs have also been reported to have been abused by inhalation.[64] Dermal exposure to naphthalene can cause erythema and dermatitis as hypersensitivity reactions. Systemic reactions, such as jaundice and haemolysis have occurred after dressing infants in clothing stored with naphthalene mothballs suggesting that percutaneous absorption may occur.[65] Ingestion of naphthalene results in the formation of an epoxide metabolite responsible haemolysis and hepatic necrosis.[65] Naphthalene exposure has been linked to laryngeal and intestinal carcinoma.[65] Oral LD_{50} values for naphthalene in rats are between 1760 and 2400 mg kg^{-1}.[65]

1.2.9. Properties of thymol

Thymol (**Figure 1.11**) is a monocyclic terpene that provides the characteristic flavour and strong odour of common thyme (*Thymus vulgaris*), comprising 45-47% of the composition of thyme essential oil.[66] Thymol is a colourless crystalline solid with a pungent aroma of thyme.

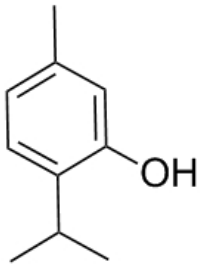

Figure 1.11: Chemical structure of thymol

The vapour pressure of thymol is 5370 mPa at 20 °C.[43] Thymol is a corrosive substance and causes severe skin burns as well as unscheduled DNA synthesis, morphological

transformation and sister chromatid exchange in hamster embryos.[67] The oral LD_{50} is 980 mg kg^{-1} in rats.[67]

1.2.10. Properties of mercury (II) chloride

Mercury(II) chloride, $HgCl_2$, is an inorganic compound with the appearance of white crystals or powder. The substance is known to cause severe burns on contact with skin, and was previously known as corrosive sublimate. The vapour pressure of $HgCl_2$ is 18.6 mPa at 35 °C making it less volatile than elemental mercury.[43, 45] The dermal LD_{50} for rats is 41 mg kg^{-1}.[68]

1.3. Current methods for the measurement of volatile pesticides in heritage collections

Current methods for the analysis of pesticides in museum environments predominantly rely on contact sampling. This typically involves swabbing objects with solvents, before analysing the swab. In a method previously used by the British Museum, a swab wetted with hexane was applied to an object in order to test for the presence of organochloride pesticides. The swab was extracted in 2:1 hexane/acetone using a Soxhlet process. The extraction solvent prior was evaporated to dryness and reconstituted in 5 cm^3 hexane and analysis by gas chromatography (GC) with electron capture detection (ECD).[69] In the same reference, correspondence from the laboratory conducting the analysis stated: “levels of DDT were very high and we therefore had to recalibrate our instruments accordingly”, indicating that the concentrations detected were significantly higher than anticipated. The report aimed to determine the presence of a number of organochloride pesticides: aldrin, chlordane, DDT and metabolites, dieldrin, endosulfan, endrin, HCH (all isomers), heptachlor and hexachlorobenzene. Of this list only dieldrin, HCH and DDT were detected. A summary of the results is presented in **Table 1.3**.

Table 1.3: Summary of results from 1996 pesticides investigation[69]

Sample type	Number of 10 cm^2 areas swabbed	Residues / µg $area^{-1}$
Box lid (pre 1970)	1	HCH 0.11, DDT 2.8
India clay and skin drum	1	DDT 0.14
Sumatra shield	1	DDT 0.13
East African basket	1	DDT 0.28
East African oxhide	1	HCH 0.06, DDT 1.71
Eskimo model boat	2	DDT 0.19
Oc. Melanesia paddle	2	DDT 0.18
Melanesia mat	1	DDT 0.17
Black pottery vessel	1	DDT 0.15, dieldrin 0.06
North American bow	3	DDT 0.13

While the Soxhlet extraction method used by the museum was successful in determining the presence of DDT, HCH and dieldrin, collection of samples by swabbing is generally not encouraged in heritage environments and is often only acceptable under special circumstances, where it is absolutely necessary. This is because the swabbing of often delicate or brittle objects with solvents poses a significant risk of damage to the object, which conservation staff are obviously keen to avoid. As a further limitation of this method, the Soxhlet extraction process is time consuming and complicated, making the preparation of even a modest number of samples for analysis very laborious.

Glastrup[70] reported the results of a survey of the Danish National Museum's Ethnographic Department, whereby samples were collected from objects or cases directly as either powders or surface scrapes taken with a scalpel. Samples were extracted using carbon disulfide and analysed by GC using flame ionisation detection. The author reported the detection of aldrin, DDT, dichlorobenzene, dieldrin, lindane, methoxychlor and naphthalene as per **Table 1.4**. The author does not describe if the

increased instances of DDT detection in the samples was attributed to the low volatility of the substance causing high persistence, or if the prevalence was explained by the previous extensive application of the substance in the museum.

Table 1.4: Summary of pesticide usage in the Danish National Museum[70]

Analyte	**Number of samples tested**	**Number of positive results (percentage of total)**
Aldrin	102	1 (1)
DDT	102	50 (49)
Dichlorobenzene	96	26 (27)
Dieldrin	102	1 (1)
Lindane	102	2 (2)
Methoxychlor	102	28 (27)
Naphthalene	96	26 (27)

The method used by Glastrup was destructive, involving microsampling of objects by taking scrapings and solvent extracting them using carbon disulfide. This manner of sample collection is unlikely to be acceptable for many institutions for both the destructive nature of the sampling and the use of carbon disulfide, a toxic and highly flammable solvent. Further, the author recommends an 18 h extraction period which is likely to cause long sample processing times.

Mayer *et al.*[71] reported both destructive and non-destructive techniques for the evaluation of organochloride pesticides in simulated wooden art objects. Destructive sampling was typically the milling of wood samples into sawdust, from which pesticides were extracted using accelerated solvent extraction with dichloromethane at 100 °C, and a pressure of 100 bar, before analysing the extractant by GC with mass spectrometry (MS). While the authors report successful detection of the target pesticides using this extraction technique, the technique in question is destructive and

is thus affected by the same problems as other destructive techniques discussed previously.

The same report also detailed a procedure for the determination of organochlorides in air. The authors reported the use of a 23 dm^3 test chamber loaded with uncovered wooden blocks previously soaked in pesticide solutions and approximately 5 dm^3 of the chamber air was collected onto Tenax TA (hereafter just Tenax) sorbent tubes at a rate of 100 cm^3 min^{-1}. Sorbent tubes were thermally desorbed and the effluent was passed into a GC-MS instrument for analysis. The authors report detection of 228 µg m^{-3} dichlorobenzene, 1.2 µg m^{-3} lindane and 0.13 µg m^{-3} DDT with lower concentrations being detected when fewer samples were placed in the chambers. Samples were also collected while exposing the sample chamber to a constant flow of air, generating one air exchange per h. Under these conditions, the concentrations of the biocides detected was lower than measuring the chamber without constant flow, at 119 µg m^{-3} dichlorobenzene, 0.56 µg m^{-3} lindane and 0.10 µg m^{-3} DDT.

While dichlorobenzene and lindane showed lower concentrations, DDT was detected at approximately the same level. The use of an exposure chamber offers preconcentration of the analytes present, potentially increasing the concentrations to detectable levels, despite the authors describing the transport of the objects to the chamber as being prohibitively expensive and therefore likely to be of limited utility to heritage users.

A second air sampling method was described by Mayer and co-workers[71] using Gerstel Twister™ bars, stir bars coated with poly(dimethyl siloxane) strips, as passive samplers. These devices are typically used in stir bar sorptive extraction of non-polar analytes from aqueous matrices. In the described work, the stir bar was placed on a watch glass next to wooden blocks soaked in pesticide solutions inside the sampling chamber described by Mayer above. After 1, 2, 4 or 7 d, the stir bars were removed and analysed by thermal desorption GC-MS (TD-GC-MS). Dichlorobenzene, lindane and DDT were detected on the bars in masses up to 1060 ng (dichlorobenzene), 156 ng (lindane) or 18 ng (DDT) with higher masses being trapped after longer exposure times. However, as data were reported as masses it was difficult to determine the air

flow over the surface of a stir bar, making quantification unlikely using this method and decreasing its overall usefulness to museum collection workers.

In 2005, Schieweck *et al.*[72] reported a study detailing organic and inorganic pollutants in a German museum. In the study air was sampled, for formaldehyde and organic acids, by pumping air at 2 dm^3 min^{-1} through distilled water and 0.1 mol dm^{-3} NaOH. The formaldehyde sample was derivatised with 2,4-pentanedione and ammonium acetate before spectrophotometric analysis at 412 nm. Ion exclusion chromatography was used for the determination of acids in the NaOH solution. Volatile organic compounds were sampled by pumping air through stainless steel sorbent tubes packed with Tenax at a rate of 150 cm^3 min^{-1} for 40 min before analysing by TD-GC-MS.

Photo-acoustic spectroscopy was also applied for continuous monitoring of total VOC (TVOC) values as a non-specific metric for indoor air quality.[73] Dust was also collected from surfaces and digested in nitric acid before analysis by inductively-coupled plasma–atomic emission spectrometry for the determination of arsenic, lead, cadmium, copper, chromium and nickel, while mercury was analysed by cold vapour atomic absorption spectrometry. The authors found formaldehyde concentrations ranging from 25-110 µg m^{-3}, which was in good agreement with previous studies.[74] Formic acid was detected in concentrations ranging from less than 10 µg m^{-3} to 100 µg m^{-3}, and acetic acid was detected in ranges of less than 5 µg m^{-3} to 481 µg m^{-3}, again in agreement with previous studies.[74] Volatile biocides dichlorobenzene and camphor were detected at concentrations of 40 µg m^{-3} and 110 µg m^{-3} indicating previous use of these compounds, which was confirmed by a conservator. Dichlorobenzene was used in the institution sampled between 1956 and 1959, with synthetic camphor being used between 1956 and 2000 as prevention against insects and fungi. Naphthalene was also detected at concentrations between 2 and 9 µg m^{-3}. Crucially, the authors also noted that HCH was detectable in the indoor environment at a concentration of 0.16 µg m^{-3}. As the area sampled was fitted with local ventilation, the authors noted that it was likely that the concentrations would be significantly higher under normal conditions. The potential cost of photo-acoustic spectroscopy combined with the need for specialised training in data interpretation potentially reduces the likelihood this technique can be adopted in many heritage institutions.

Dust analysis revealed that there were significant concentrations of biocides present that would not have been detectable using the air sampling method, due to the low volatility of the analytes. Concentrations of Eulan® (permethrin) were detected at 2760 and 110 µg m^{-2}, indicating significant contamination. Chloronaphthalene and pentachlorophenol were also present, which were part of the formulation of Xylamon®. Non-contact samples may also be collected using solvent extracts of dust that settled inside display cases, storage areas or on other surfaces and has been reported in literature a number of times.[75-77] This would also apply to any the analysis of any fragments that had dropped from the main body of the artefact and were not able to be repaired. As with previous solvent extraction analyses, the process detailed here is time consuming and laborious. While the technique offers the advantage that it is non-destructive, it is also difficult to determine which object the dust, and therefore the contamination, belongs to.

Schieweck *et al.*[78] followed this work by measuring of biocidal contamination in museums. As before, Tenax sorbent tubes were used to collect air from the sampling environment in both active (pumped) sampling mode, and in passive (diffusive sampling) mode, before analysis by TD-GC-MS. Extraction of residues from dust was again used to determine the quantity of non-volatile and semi-volatile pesticides. Concentrations of chlorinated naphthalenes were measured at between 10 and 100 mg kg^{-1} of dust, with pentachlorophenol (PCP), lindane and DDT being measured at 10-50 mg kg^{-1}. Pentachlorophenol concentrations in particular, were noted to be at the same order of magnitude as those detected by Krooß and Stolz[79], who quoted ranges of up to 400 mg kg^{-1} in collected dust. Krooß and Stolz[79] also performed air analysis and measured concentrations in the range of <0.03-0.50 and 0.22-2.40 µg m^{-3} for PCP and lindane. Analysis of dust resulted in measured DDT concentrations of 5-82 mg kg^{-1}. As a reference, a median of 0.279 mg kg^{-1} and a maximum of 9.61 mg kg^{-1} DDT in house dust was previously determined by Rudel *et al.*[80] In a separate study, a DDT concentration of 4.2 mg kg^{-1} measured by Butte and Heinzow[81] for sieved house dust in Germany. This would indicate that the concentrations detected by Schieweck and co-workers were considered relatively low.

1.4. Methods used to determine low volatility and inorganic pesticides in heritage collections

A number of simple colorimetric tests exist for the determination of a wide range of analytes. The tests are often referred to as spot tests and typically involve swabbing a small area on an object before adding a reagent to the swab and observing a colour change, of which the intensity is typically proportional to the concentrations of analyte present. Many of these tests are also available for the detection of chemical hazards in museums, with the work of Odegaard and co-workers[22] being significant in the heritage science sector; see **Table 1.5**. Odegaard *et al.*[22] used commercially available test kits, reducing the need for museum staff to prepare regents themselves, thus reducing the complexity of the analyses. However, as the formulation of the test kits and papers is proprietary, information on the chemical nature of the tests has not been disclosed. As a result, the author of this thesis has provided suggestions for the likely colorimetric chemistry involved in these reactions and has referenced primary literature appropriately. More than half of the target pesticides discussed by Odegaard were metals, or contained metals, indicating that contamination with these substances is a significant problem for heritage institutions.

An alternative to spot testing for metals is available through the use of X-ray fluorescence (XRF) spectrometry as it is able to detect a wide range of elements quickly and non-destructively.[14, 15, 82-91] Typically, XRF instruments are expensive and require specialist training due to legislation pertaining to sources of ionising radiation, and only measure discrete points on an object where the beam focusses. Dussubieux *et al.*[88] described the capability of XRF as "semi-quantitative at best" and noted that the technique did not take matrix or surface variation into account. The matrix emission intensity of each element under study is a function of the concentration of the element within the sample matrix, but also of the sample matrix itself.[92]

Table 1.5: Spot tests for the presumptive determination of chemical hazards in heritage environments[22]

Target hazard	Method of sample collection	Reaction and colour change	Notes
Arsenic compounds	Swab	Yellow → brown 1) $As_2O_3 + 12H^+ \rightarrow 2AsH_3 + 3H_2O$ 2) $AsH_3 + 3HgBr_2 \rightarrow As(HgBr)_3 + 3HBr$	Test papers (Merkoquant®, Macherey-Nagel, Düren) are toxic. Hydrogen must be generated *in situ* using zinc and corrosive HCl. Arsine and $HgBr_2$ are extremely toxic.
Borate compounds	Swab	1) Boric acid + test paper (diferulolylmethane) → red (rosocyanine) 2) red paper + alkali → blue	Requires addition of concentrated HCl and ammonia. Both are toxic and corrosive.

Table 1.5: Spot tests for the presumptive determination of chemical hazards in heritage environments[22]

Target hazard	Method of sample collection	Reaction and colour change	Notes
Carbamates	Swab	Colourless → blue For negative reaction. Presence of carbamates inhibit reaction and no colour develops	Analysis used InQuest OP/Carbamate Screen Kit (Strategic Diagnostics, Newark). Chemistry involved is proprietary but indicated to be enzyme-based. Solutions have no health ratings.
Copper	Swab	Cu^{2+} + test paper → pink-purple complex	Reagent in test papers (Cuprotesmo®, Macherey-Nagel, Düren) not stated, but suspected to be dithiooxamide based.[94, 95]

Table 1.5: Spot tests for the presumptive determination of chemical hazards in heritage environments[22]

Target hazard	Method of sample collection	Reaction and colour change	Notes
Lead	Swab	Forms pink complex	Reagent in test papers (Plumbtesmo®, Macherey-Nagel, Düren) not stated, but suspected to be sodium rhodizonate.[96]
Mercury vapour	Passive vapour	Grey-white → yellow $2CuI + Hg \rightarrow HgI_2 + 2Cu$	Applied as a slurry to a microscope slide, exposed passively over 7 days.
Organophosphates	Swab	Colourless → blue For negative reaction. Presence of carbamates inhibit reaction and no colour develops	Analysis used InQuest OP/Carbamate Screen Kit (Strategic Diagnostics, Newark). Chemistry involved is proprietary but indicated to be enzyme-based. Solutions have no health ratings.

Table 1.5: Spot tests for the presumptive determination of chemical hazards in heritage environments[22]

Target hazard	Method of sample collection	Reaction and colour change	Notes
Sulfate compounds	Swab	Red → yellow	Barium perchlorate and thorin for a red complex, which changes to yellow in the presence of sulfate ions.
Zinc	Swab	Forms pink/red colour	Requires 32% sodium hydroxide, which is corrosive. Reagent in test kit (Quantofix®, Macherey-Nagel, Düren) not stated, but suspected to be sodium dithizone.[95]

Odegaard *et al.*[85] reported the use of a handheld XRF spectrometer to determine the presence of heavy metal based pesticides on museum objects. Estimation of the total amount of heavy metal present on the sample was determined by multiplying the object area by the mean amount detected on the object.

Ireland *et al.*[93] used XRF to determine inorganic compounds by taking measurements at 40 keV for 60 s, before manually assigning peaks to elements using the XRF instrument software. Other than a container specifically labelled as containing calomel dust ($HgCl_2$), the authors did not detect any other metal pesticides using XRF. While the results were not quantified, the authors reported that the technique provided reliable and quick results but noted that as there is a lack of instrument availability outside a specialist laboratory, institutions may need to consider the costs of external analysis if dealing with historic pesticide contamination.

Other techniques for the determination of metal-based pesticides in heritage environments include the use of proton (or particle) induced X-ray emission spectroscopy, or PIXE spectroscopy.[97] The benefits of this technique are such that it is able to detect a number of elements very rapidly, and it is non-destructive. However, instrumentation is very expensive for the use of PIXE, which may be prohibitive for some institutions.[98] As a further consideration, it may not be possible to perform PIXE analysis *in situ*, potentially limiting the applicability to more fragile objects which cannot be moved.[99]

1.5. Pesticide measurement in other industries

Anastassiades *et al.*[100] published a method in 2003 for the determination of pesticides in agricultural products, and coined the acronym QuEChERS – Quick Easy, Cheap, Effective, Rugged and Safe. Traditional methods of analysis in the food industry were reliant on multi-stage procedures and extensive clean-up steps. With the adoption of the QuEChERS method, many of these steps were omitted. As such, the method is popular as it not only helps avoid these costly and time consuming steps, but also extracts several analytes at each step, making it a multi-class and multiresidue process. While current procedures used in laboratories have been modified, the overall process is similar.[101] In contrast to methods used in the heritage sector, there is no advantage

to be gained from implementing new, non-invasive, technologies over the comparatively cheap QuEChERS-and-chromatography process, as sample destruction is not an issue in this industry.

Carbamate pesticides and their conjugated structures have also been determined by high performance liquid chromatography (HPLC) with fluorescence.[102-104] GC has also been used with flame photometric detectors (FPD) for organophosphorus compounds,[105, 106] ECD for electronegative compounds including organochlorides[107-110] and nitrogen-phosphorus detectors (NPD) for organophosphorus,[111] carbamates,[112, 113] triazoles,[114] and triazines.[115]

Whilst studies involving the application of UV-vis and fluorescence techniques for pesticide analyses are still being published, the last decade has seen a rise in publications using mass spectrometry as a method of determination for pesticides.[105, 109, 116-125] This is not surprising given the decreased cost and size of MS instruments over recent years has made them an increasingly attractive prospect for analysts. Liquid chromatography-tandem MS (LC-MS/MS) instruments are being more used for the analysis of pesticides.[126-128] In Alder *et al.*[124] ion trap MS was compared to a quadrupole-based instrument, and the relative merits of GC-MS and LC-MS/MS were discussed with electron impact (EI) and electrospray ionisation (ESI) techniques for GC and LC. The article reported 500 "high priority pesticides" over a range of substance classes including organochloride, organophosphorus, nitrogen-based, and pyrethroids amongst others. The frequency of detection, applicability and sensitivity for GC-EI-MS and LC-ESI-MS/MS for the pesticides were compared. Alder *et al.*[124] concluded that GC was generally out-performed by LC-MS with the exception of the analysis of organochlorides, and that in all other instances LC-MS/MS offered wider scope and sensitivity. GC was used in this work as it was considered to be more widely available and typically has lower instrument costs than an equivalent LC-MS system.

1.6. Project aims and objectives

Historical biocide residues in heritage environments present significant health risks to persons handling contaminated objects or accessing areas used for storage or display of such objects. The contamination of these objects is compounded by previous poor

record keeping on behalf of many heritage institutions with respect to which treatments were applied to particular objects, meaning that a broad range of pesticides could potentially have been applied to a single object over the course of its lifetime within that collection. Identification of contaminated objects necessitates the use of analytical techniques which are able to detect a wide range of pesticides, ideally with non-contact, *in situ* sampling which can be performed cheaply by institutions which may be unable to invest in expensive instrumentation. As such, the work previously undertaken in this field of study (discussed *vide supra*) does not meet these criteria.

Current methods often employ swabbing techniques which may be potentially damaging to objects and often require complex solvent extractions to be able to provide a sample ready for analysis, rendering them unsuitable for use. Non-contact techniques such as X-ray fluorescence spectroscopy require specialised knowledge and training to use and the instruments are often expensive to purchase, leading to its adoption only in comparatively affluent institutions. Where swabbing of objects is permitted in order to identify legacy biocidal residues, the chemistry involved in performing these presumptive spot tests is often complex, and beyond the ability or confidence of staff who are not familiar with handling potentially hazardous chemicals.

This work aims to describe approaches that allow conservation staff to select an analytical technique or techniques for the detection of organic and inorganic pesticidal residues in museums based on the individual needs of their collections. It is believed that the methods developed herein address an unmet need for analytical methods allowing the detection of these chemical hazards, while remaining economical to implement and requiring a minimum of technical skill on behalf of the museum staff to perform.

In **Chapter 3**, the development of methods suitable for the determination of pesticides in museums has been discussed and is presented with an emission survey of UK heritage institutions. The development of two methods is discussed and presented with a flow chart used to aid the selection of the most appropriate sampling procedure and analytical technique for the collection. Once selected, a sampling protocol for the technique is followed, allowing the detection of a number of chemical hazards

commonly found in heritage institutions. Methods for passive and active air sampling are described, as well as analysis by GC-MS, GC-GC-FID and LC-UV. A number of case studies were conducted using the developed methods in order to determine the background levels of volatile organic compounds (VOCs) typically encountered in contaminated collections. Critical evaluation of the chromatograms collected allowed identification of a number of common VOCs present across all sampling locations as well as establishing a link between these VOCs and emission sources within the sampling environment. This work was continued in a number of case studies which were conducted using the non-invasive methods developed in this thesis. The VOC profiles of a number of objects in several collections across the UK were sampled in order to determine if pesticides were present in the collections and to quantify the results. The objects sampled varied in age, physical size, and sampling location within the collection. Critical examination of these chromatograms allowed identification of complex VOC profiles as well as identification of biocides preciously applied to the objects. The use of non-contact vapour-phase sampling in this work allowed detection of a wide range of volatile substances including biocidal agents without the need for potentially damaging swabbing techniques. The sample collection process required minimal technical knowledge to implement and allowed multiple heritage sites across the UK to conduct the sampling themselves. In addition, the use a single off-site laboratory to analyse the samples reduces expense and fulfils the low-cost requirement of the analysis.

Chapter 4 describes an enzyme immobilisation technique used to develop a vapour-phase test for the detection of organophosphate and carbamate pesticides. Reactivity of the test gels towards dichlorvos was demonstrated as working in both solution and vapour-phase, as well as being able to recover dichlorvos from spiked filter papers offering functional flexibility to how the test is performed. The use of indoxyl acetate as the chromogenic reagent offers a significantly more easily differentiated colour change than is currently available through the widely used Ellman's reagent.

The preliminary work undertaken in this chapter proposes the use of an organophosphate sensor which is quick, economical, and easy to use, thus overcoming the need for long sampling times required for the determination of biocides in air.

Further, the proposed sensor also gives a clear easily interpreted colour change reaction in the absence of organophosphate and other cholinesterase inhibitors that can be determined without specialist training. As the testing kit is self-contained and ready to use, it meets one of the key requirements of the end users in that it does not require mixing of chemicals or preparation of solutions at the point of use. This significantly simplifies the procedures used in current enzyme-based organophosphate detection assays.

Current investigation into inorganic pesticides relies heavily on the use of X-ray technology. However, there is a need to develop sensors for the selective determination of substances, such as mercury, which will be sensitive and cheaper. **Chapter 5** addresses this need by using a simple colorimetric reagent immobilised in a gel matrix. This immobilised reagent offered selective and sensitive determination of mercury in the presence of other metal ions (chromium) while granting the reagent a substantial increase in shelf life compared to previously reported literature values. This allows formation of the gels to be performed well in advance of sampling, negating the need for on-site preparation of reagents and the need for open solutions of chemicals to be present at sampling. The prepared mercury sensor allows determination of mercury in heritage collections without the need for the preparation of solutions at the point of use. It is proposed that the immobilised reagent may be used as a means to cheaply and rapidly determine the presence of mercury in collections where the preparation of chemical solutions or expensive instrumentation is not possible.

Finally, **Chapter 6** discusses overall conclusions and present suggestions for future work.

1.7. References

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2. Theory of instrumental techniques

2.1. Air sampling

Many commonly used air sampling techniques rely on the use of sorbent media to trap vapour-phase analytes from the sampling environment. Air sampling in this project has used solid sorbents as trapping media. The sampling of vapour-phase analytes from an environment typically involves one of two processes: active or passive air sampling.

2.1.1. Active sampling

Active sampling methods are used to sample a known volume of air which is pumped though a sorbent tube at a fixed rate for a set length of time. After collection, the analytes are identified and quantified using chromatographic techniques, allowing the mass of volatiles adsorbed by the sorbent to be expressed as a mass per unit volume of air. Sorbents typically used in sampling tubes include silica gels, carbonaceous substances such as activated charcoal and graphitised carbon blacks, and porous polymeric substances such as polyphenylene oxides.[1]

The airflow through the sample tube is low, usually around 100 cm^3 min^{-1}, to allow a known volume of air to be drawn though the sorbent bed at a fixed rate with minimal back pressure. Sampling times are typically 8 h in duration giving a short-term time-weighted average concentration of the volatiles collected from the sampled environment. Accuracy of measured concentrations depends on the air sampling rate and the sampling pump used must be calibrated before use.

It should be noted that active sampling is not always appropriate. Pump noise is an important consideration for sampling domestic or heritage environments where people are likely to be disturbed by constant noise over several hours. Air inside a small museum drawer or cabinet can be sampled by active sampling, however care needs to be taken not to remove more than 20% of the volume of the container as dilution effects then become significant. In such instances, passive sampling is a better option.

2.1.2. Passive sampling

Operation of the sampling tube in passive, or diffusion mode, relies upon the formation of a linear concentration gradient of each analyte, from the open end of the tube to the sorbent, with the concentration of analyte in the air at the sorbent-air interface assumed to be zero. It is further assumed that the diffusion gradient is linear, and that the principles of Fick's first law of diffusion, **Equation 2.1**, can be used to obtain the concentration of pollutant present in the atmosphere.

$$W_1 = -D_{1,2} A \frac{dc}{dx}$$

Equation 2.1

In this equation, W_1 is the rate of mass transfer of gas$_1$ (µg m^{-2} s^{-1}), $D_{1,2}$ is the diffusion coefficient of gas$_1$ in gas$_2$ (m^2 s^{-1}), A is the cross-sectional area of the diffusion path (m^2) and dc/dx is the instantaneous rate of change in concentration over the diffusion path, where x is the position in the diffusion path.

If the change in concentration ($C_1 - C_0$) along the diffusion path length in metres (L) is considered, then **Equation 2.2** is used:

$$W = D \frac{A}{L} (C_1 - C_0)$$

Equation 2.2

C_1 is the concentration (µg m^{-3}) of gas in the exterior atmosphere and C_0 is the concentration (µg m^{-3}) of gas in the air directly above the trapping reagent.

As mentioned, the sorbent is assumed to be 100% efficient and the concentration of pollutant at the surface of the sorbent will be zero. By rearranging **Equation 2.2** and multiplying by time, t (s), **Equation 2.3** is obtained.

$$C_1 = \frac{ML}{DAt}$$

Equation 2.3

Where M is the total mass (ng) of the contaminant taken up by the trapping reagent, t is the exposure time (s) of the sampling period and D was the diffusion coefficient. Since L, D, and A can be measured for each end of the tube, and the mass of pollutant adsorbed is determined experimentally for a period of exposure (t), the concentration (ng m^{-3}) of each pollutant can be determined for trapping at the sorbent-air interface of the tube and expressed in units more appropriate for sampling large volumes of air – typically µg m^{-3}.

2.1.3. Sorbent choice for air sampling

The ability of a substance to trap analytes from air being sampled obviously dictates its utility as a sorbent. The sorbent ability of a given substance is determined by a number of physical and chemical factors.

The surface area of sorbent particles gives an indication as to the amount of an analyte that may be adsorbed by the material. Higher surface areas indicate there may be a higher number of active sites present on the surface of the sorbent and therefore it can trap a larger number of analyte molecules.

While the surface area of a sorbent dictates the amount of substance that can be adsorbed, it is the polarity of the sorbent that influences the type of analytes it is able to "trap". The mechanism of this interaction is determined by physisorption. The process of physisorption is one in which intermolecular forces are used, as opposed to valence forces used in chemical bonding (chemisorption).

As an analyte molecule approaches the sorbent, the electrons in the sorbent and analyte repel each other, forming instantaneous dipoles. The dipoles attract each other, with larger atoms forming forces with greater attractive power. The formation of these dipoles mean that as an analyte approaches a sorbent and the distance between them decreases, a low-energy well forms before strong repulsive forces arising from the overlap of electron density causes significant increase in the energy of interaction. It

is in this well that the energy of interaction between the sorbent and analyte is lowest, and the analyte is adsorbed to the sorbent. The result of physisorption is that the interaction of the analyte with the sorbent does not perturb the electronic structure of either, so that the absorbed species are identical to those that remain untrapped. As such, the elementary step in the physisorption process does not involve an activation energy and the adsorption process occurs at ambient temperatures.

As a result, physisorbed particles are still relatively far from the surface of the sorbent and are highly mobile due to the low binding energy. The low interaction energy also gives rise to low desorption temperatures. Physisorbed particles may also serve as precursors to chemisorptive processes within chemical reactions.

Table 2.1 below summarises the properties of common solid sorbent materials. The table shows that polar inorganic sorbents, such as those based on silicon or aluminium oxides, are more suited to the sampling of polar analytes and have a wide range of surface areas. Carbon based sorbents can have very large surface areas enabling them to trap low boiling species, but lack the ability to trap more polar analytes. Porous polymers are used for typically non-polar analytes, but lack the extreme thermal stability offered by carbonaceous sorbents and offer relatively small surface areas. It is therefore vitally important to select an appropriate sorbent for the target analytes being trapped.

Table 2.1: Properties of selected solid sorbents[1]

Type	Structure	Surface area (m^2 g^{-1})	Products	Desorption method	Compounds tested (b.p. range)	Polarity	Thermal stability	Water affinity
Inorganic	Silica gels	1-30	Volasphere, Florisil	Solvent	Pesticides, chlorinated biphenyls	High	~400 °C	High
	Molecular sieves	500-800		Solvent	Permanent gasses	High	<400 °C	High
	Aluminium oxides	~300	Alumina F1	Solvent	Hydrocarbons	High	300 °C	High
Carbon based	Activated charcoal	800-1200		Solvent	Non-polar and slightly polar VOCs (>50 °C)	Medium	>400 °C	High
	Carbon molecular sieves	400-1200	Carboseive, Ambersorb, Spherocarb Carboxen	Solvent	Non-polar and slightly polar VOCs (>-80 °C)	Low	>400 °C	Low-medium
	Graphitised carbon blacks	12-100	Carbotrap, Carbopak, Carbograph	Thermal/ Solvent	Non-polar VOCs (>60 °C)	Low	>400 °C	Low
Porous polymers	Styrene, divinylbenzene, polyvinylpyrrolidone polymers	300-800	Porapak Q/N, Chromosorb 106/102	Thermal/ Solvent	Non-polar and moderately polar VOCs (450 °C)	Variable	<250 °C	Low
	Phenylphenylene oxide polymers	20-35	Tenax	Thermal	Non-polar VOCs (>60 °C)	Low	<350 °C	Low
	Polyurethane foams			Solvent	Pesticides	Low	<200 °C	Low

2.2. Thermal desorption (TD)

TD is a process by which analytes adsorbed onto a sorbent can be introduced to a chromatographic instrument for analysis. After an initial desorption period from the sorbent sampler, the compounds of interest are transferred from the sampling tube to the focusing trap, which is then thermally desorbed itself, carrying analytes into the chromatographic instrument in a tight band.

In this study, thermal desorption of analytes from a Tenax sampling tube was used as the method of sample introduction into a gas chromatograph. In general, a thermal desorption process occurs in several stages.

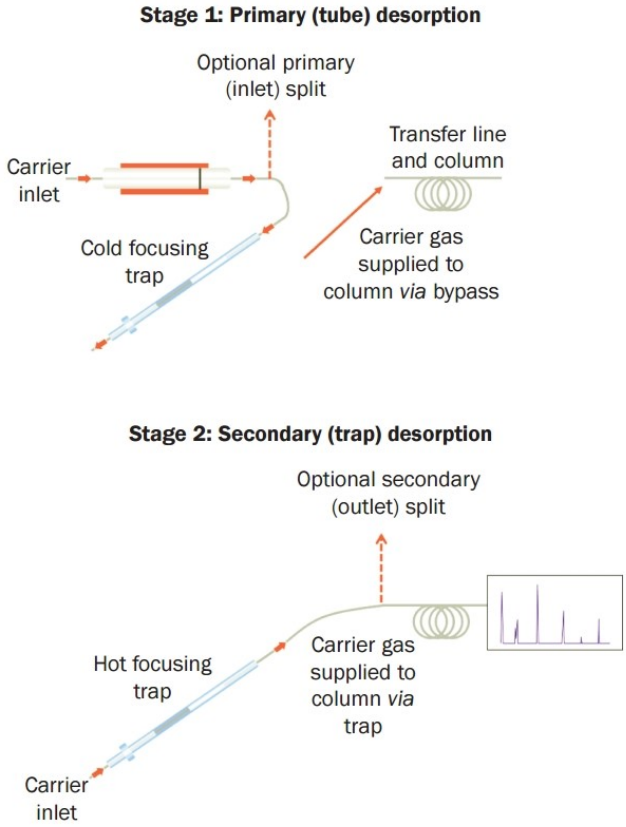

Figure 2.1: Illustration of two-stage thermal desorption process[2]

Sampling tubes made of stainless steel or glass are used to introduce the trapped analytes to a thermal desorption unit. The tube is placed on a heating block and connected to a pressurised inert gas line. The gas used will typically be the same as that used as the carrier gas in the analyser, such as helium, and at increased flowrates

(30-50 mL min^{-1}).[3] The thermal desorption unit heats the sampled sorbent tubes while backflushing with the carrier gas used in the gas chromatography (GC) instrument in the opposite direction to the air flow during sampling. In this way, organic vapours are transferred from the sampling tube onto a Peltier-cooled focussing trap maintained at sub-ambient temperature, usually -30 °C. This allows preconcentration of the sample, and allows for 100% transfer of the analytes desorbed from the sampler to be introduced to the analyser. The temperature required for the primary desorption of the analytes from the sampler must be sufficiently high that analytes are desorbed, but not so high that either they or the sorbent thermally degrade.

The focussing trap is rapidly heated (heating rates of approximately 100 °C min^{-1} are reported by manufacturers) to 300 °C in a stream of carrier gas, carrying analytes into the GC instrument in a tight "band" of vapour, as expected from a standard injection onto a GC column.

Thermal desorption units may have an optional split line, allowing either split or splitless "injection" of the cryofocussed sample onto the GC column. By employing multiple splits (splitting before and after the cold trap) analysis of milligram-level analytes may be undertaken, while splitless modes permit analysis of nanogram- and picogram-level contaminants.[2]

The benefits of thermal desorption are that the technique is faster and more environmentally friendly than solvent desorption techniques such as Soxhlet extraction, and the technique can be automated. Crucially, the technique offers preconcentration for analytes with almost full recovery.

The main disadvantage to using the technique is that the analysis is considered "single shot" as once desorbed the sample typically cannot be recovered. While some instrument manufacturers are offering instruments with outlet splits capable of re-trapping the outflow from a split valve, and thus partially recovering a sample, this is unsuitable for trace level analysis as the mass of analyte trapped is too low to allow sample splitting.

2.3. Gas Chromatography (GC)

GC is a technique used to resolve complex mixtures of analytes into their component parts for analysis on an instrument called a gas chromatograph. To be suitable for analysis, a compound must be thermally stable, and have appropriate volatility that it may be analysed in the vapour-phase.

Liquid or gas samples are introduced to the chromatographic system by injection through a heated port, flash-vaporising the sample. High purity gasses such as He, N or H, called carrier gasses, carry the vaporised sample through a heated column for separation, and then into the detector. The physical properties of the substance determine the rate at which the analytes travel through the column, as analytes experiencing stronger interaction with the column migrate more slowly. As such, analytes move through the column at different speeds, with faster moving, unretained compounds exiting (eluting) into the detector first.

Separation is performed as the analytes move through the column, the internal surface of the column is coated with a liquid known as a stationary phase. The stationary phase is bonded to the column wall which is typically made of fused silica. The choice of stationary phase is dictated by the principle “like dissolves like”, and it is important to select a stationary phase with intermolecular bonding similar to that of the analyte. For example, the stationary phase for the separation of alcohols should be able to undergo hydrogen bonding with the –OH group; non-polar analytes should be resolved on stationary phases able to interact via van der Waal’s forces, such as 5% diphenyl/95% dimethyl siloxane; stereoisomers can be separated on stationary phases containing a single enantiomer, which displays a higher affinity for one analyte enantiomer over the other.

When a sample is introduced to the chromatographic system, it is flash-vapourised in the heated injection port and partitions between the mobile phase (carrier gas) and the stationary phase (column coating) forming an equilibrium. When the analyte is interacting with the stationary phase, it is immobile, thus interaction with the stationary phase slows the passage of the analyte through the column resulting in separation of the mixture. The length of time it takes a substance to elute from the column is known

as a retention time, and should be specific to that analyte under the given chromatographic conditions of the method in order for it to be resolved from the mixture.

A retention factor *k*, is defined as the ratio of time an analyte (*a*) spends being retained by the stationary phase to being advanced by the mobile phase (**Equation 2.4**). In **Equation 2.4**, t_r represents the retention time of the analyte and t_m is the void time, or the time it takes for an un-retained analyte to elute. For k_a of less than one, analytes will pass through the chromatographic system too quickly, and values greater than 20 indicate the analytes interacts too strongly with the stationary phase, taking a very long time to elute. The ideal range for an analyte is typically 1-5. Selectivity between species is expressed by the selectivity factor α, defined as the ratio of retention factors between the two species.

$$k_a = \frac{t_r - t_m}{t_m}$$

Equation 2.4

Column efficiency is measured in theoretical plates. This is a mathematical concept used to develop the theoretical plate model of chromatography. The number of theoretical plates is determined by **Equation 2.5**, where *N* is the number of theoretical plates, t_r is the retention time and w_h is the peak width at half height (in units of time).

$$N = 5.545 \left(\frac{t_r}{w_h}\right)^2$$

Equation 2.5

Columns with a higher number of plates are considered to be more efficient, giving sharper peaks (caused by analytes migrating along the column as a tighter "band") than columns with a lower *N* value. The height equivalent to one theoretical plate, or simply plate height (H), is calculated by dividing the column length by the number of plates. H values are often quoted in mm, and a shorter plate height translates into a larger number of plates per column, and thus higher column efficiency.

Ideal chromatography is a hypothetical case where no band-broadening effects operate, implying an infinite plate number. In real, non-ideal systems, the analyte band experiences broadening as it migrates through the chromatographic system. Band broadening is defined by a number of factors. In capillary columns, 4 processes contribute to broadening, thus total plate height:

- H_{diff} – longitudinal diffusion
- H_{con} – convective mixing
- $H_{x,m}$ – kinetics of mass exchange from mobile phase to interface between mobile and stationary phase
- $H_{x,s}$ – kinetics of mass exchange from stationary phase

The contribution of longitudinal diffusion, H_{diff}, is caused by the presence of the higher concentration in the analyte band forming a concentration gradient with the surrounding mobile phase. Analytes diffuse along this gradient into less concentrated zones. Analytes with large diffusion coefficients, D_m, and longer retention times within the chromatographic system experience a larger contribution from longitudinal diffusion, leading to a loss of efficiency. This effect varies inversely with mobile phase velocity, v. The contribution of longitudinal diffusion is more important in GC than in HPLC, as the diffusion coefficient of analytes in gas is approximately 10^4 times greater than in liquids.[4]

$$H_{diff} = \frac{2D_m}{v}$$

Equation 2.6

Flow through an open tube, such as a capillary, introduces band broadening through convective mixing. The shape of the band front can be considered as curving forward in the direction of migration, as the velocity of the analytes is less at the walls of the tube compared to the centre of the tube with radius r and the carrier gas velocity is represented by v.

$$H_{con} = \frac{r^2}{24D_m} v$$

Equation 2.7

Resistance to mass transfer describes the band broadening caused by transporting analytes between the phases via diffusion and convection. In an open tubular column, this is described as:

$$H_{xm} = \frac{c_m d_c^2}{D_m} v$$

Equation 2.8

Where d_c is the diameter of the column and c_m is defined:

$$c_m = \frac{(1 + 6k + 11k^2)}{96(1 + k)^2}$$

Equation 2.9

This describes the finite rate of mass transfer from the inner part of the mobile phase to the interface with the stationary phase. At the front of the band, the analyte concentration is higher than at equilibrium between the mobile and stationary phase, and at the rear of the band the analyte concentration is lower, leading to broadening of the band.

In the stationary phase, where d_f is the film thickness of the stationary phase and D_s is the diffusion constant:

$$H_{xs} = \frac{2kd_f^2}{3(1 + k)^2 D_s} v$$

Equation 2.10

With films of less than 0.25 μm, this term can be neglected as it is very small.

Combining these terms gives the Golay equation:

$$H = \frac{2D_m}{v} + \frac{(1 + 6k + 11k^2)r^2}{(1 + k)^2 24D_m}v + \frac{2kd_f^2}{3(1 + k)^2 D_s}v$$

Equation 2.11

From the combined equation, several points are obvious. Firstly, decreasing the r^2 term (column radius) gives higher efficiency separations. The *D* terms for mobile and stationary phase also allow higher efficiency if they are larger, thus stationary phase films with high diffusivity are considered more efficient. Carrier gasses with high diffusivity, such as hydrogen, are more efficient for the same reason.

Equation 2.11 can be simplified into **Equation 2.12**:

$$H = \frac{B}{v} + (C_m + C_s)v$$

Equation 2.12

Where *B* is the longitudinal diffusion and C_m and C_s are the mass transfer terms in the mobile and stationary phase. It is clear from the equation that mobile phase velocity plays an important part in influencing band dispersion, with the *B* term varying inversely, and the *C* term proportionally.

This is similar to the van Deemter equation discussed in **Section 2.5**. However, **Equation 2.12** does not contain an eddy diffusion term as this is not applicable to open tubes such as capillary columns. This will be discussed in **Section 2.5**.

Another factor crucial to separation on GC systems is temperature, and fine control of temperature parameters is necessary for reproducible analyses. Raising the temperature of the chromatographic system increases elution speed by increasing the vapour pressure of the analytes, and decreases the time taken for the analyte equilibrium between mobile phase and stationary phase to be established. It is therefore important to optimise this condition in order to achieve timely separation of analytes.

GC analyses can be conducted using a single set temperature throughout the analysis (isothermal) or can change the temperature of the oven as the analysis proceeds, forming a temperature gradient to progressively elute less volatile substances. It should be noted that if the GC instrument is operating at constant pressure mode (where the column head pressure is fixed throughout the analysis), increasing the temperature of the system will increase the viscosity of the carrier gas which will in turn decrease the linear velocity of the mobile phase. As seen from the Golay equation above (**Equation 2.12**) this can lead to changes in peak elution patterns. GC instruments capable of operating in constant flow mode (where the gas flow is fixed throughout the run) can minimise this effect.

The lower temperature value of the analysis is determined by the properties of the analyte. If the analytes are injected at too low a temperature, they can condense on the column head and do not migrate through the column. If the column is warm, but still too cool for analysis, then the mass transfer between the stationary phase and mobile phase is slow, resulting in band broadening as described in the Golay equation.

The highest temperature used in the separation is determined not only by the thermal stability of the analyte, but also by the thermal stability and vapour pressure of the liquid stationary phase. At increased temperatures the stationary phase itself can be vaporised or thermally degraded, leading to these substances eluting as part of the chromatogram. This is a process known as “column bleed”, and is particularly noticeable in GC-mass spectrometric analysis, where a characteristic mass of 207 is usually indicative of column bleed. Therefore in mass spectrometry, low-bleed stationary phases which have a lower tendency to degrade are recommended. Column bleed will, over time, reduce the efficiency of the column and can also foul detectors, making them less sensitive.

2.4. Mass spectrometry (MS)

Mass spectrometry is an analytical technique where analytes are ionised by, collisions with an ionising gas (such as methane or ammonia as is the case for chemical ionisation (CI)), or bombardment with electrons in the case of electron impact (EI) mode. Further

ionisation techniques are available, but tend to be application-specific and will not be discussed here; instead this section will focus on EI mode ionisation.

Mass spectrometers are typically coupled to gas chromatographs, and when working in EI mode comprise several sections: the interface and ion source, the quadrupole mass filter, and the electron multiplier tube detector. All of these components are contained under low pressure conditions (approximately 10^{-5} torr). The low pressure within the system is required to prevent ions deviating from their trajectories through collisions with background molecules. This is established by housing a turbomolecular pump on the analyser body to establish the "high" vacuum, and a second foreline pump (also known as a roughing pump), external to the instrument to generate a "rough" vacuum and to remove the exhaust form the high vacuum pump. The ion source and quadrupole are heated to 230 and 150 °C respectively.

Analytes are introduced to the source via the GC interface, which allows the outflow from the GC column to be transferred to the MS system without compromising the vacuum in the MS. The interface typically consists of a heated sleeve through which the column passes, with the column protruding 1 to 2 mm into the ionisation chamber. Analytes are introduced directly into the chamber to be ionised. Direct introduction interfaces are perhaps the most common design, as capillary columns significantly reduce the volumetric gas flow exiting a column. As such, the need to split analytes from the carrier gas flow is no longer present.

Analytes elute from the column into the ionisation chamber of the source held at 230 °C. They are subjected to bombardment by electrons generated by thermionic emission from a heated metal filament perpendicular to the column outflow. The generated electrons are concentrated into a beam using a trap cathode opposite the filament, allowing the analytes to be directed into the flow of fast moving electrons. The electrons interact with the analytes inducing fragmentation and ionisation, giving product radical cations (**Scheme 2.1**). The unstable radicals fragment further into smaller fragments and cations. A repeller plate electrode provides a repulsive force for the cations towards the quadrupole and detector.

$$M + e^- \rightarrow M^{\cdot +} + 2e^-$$

Scheme 2.1: Formation of ions by electron impact

The fragmentation of analytes typically follows predictable paths. The energy used to ionise the substances of interest is usually 70 eV as standard. As such, EI is often referred to as a "hard" ionisation technique, as it provides a greater number of fragments than "soft" ionisation techniques such as electrospray ionisation, or CI. Subsequently, EI is the preferred method of analysing smaller molecules such as volatile organic compounds (VOCs) as opposed to large polymers, biomolecules or drug substances.

After ionisation, cations are repelled into the quadrupole where they are filtered according to their mass/charge ratios. The quadrupole consists of four parallel rods with direct current voltages applied across opposite pairs, and a radio-frequency voltage superimposed on the DC voltage.

When ions are introduced to the quadrupole, the radio frequency voltages across opposite pairs of rods cause the ions to adopt helical trajectories along the length of the quadrupole. By altering the voltages applied to the rods it is possible to destabilise the trajectory of ions with a specific mass/charge (m/z) ratio, causing them to collide with the quadrupole and preventing them from reaching the detector.

Quadrupole voltages can be manipulated to select ions of a specific m/z value, or can be operated in scan mode, which allows selection of a range of m/z values to be filtered.

Once selected ions have travelled through the quadrupole, they are detected using an electron multiplier tube. This is a vacuum tube containing a set of discrete dynodes, each held at a higher potential than the previous, before a final anode. As incident electrons strike the first dynode, secondary emission causes more electrons to be emitted by the dynode. The emitted electrons are accelerated towards the second dynode, with each electron striking the dynode producing yet more electrons. This process continues along the remaining dynode stages producing an "avalanche" effect, greatly increasing the number of electrons that strike the final anode.

As the avalanche of electrons strikes the final anode, the sharp increase in current is recorded by data capture software for integration and interrogation.

2.5. High performance liquid chromatography – UV-detection

High performance liquid chromatography, also known as HPLC, is a separation technique similar to GC. The main differences between the two techniques are:

- in HPLC, the mobile phase is a liquid,
- the HPLC column is packed with functionalised silica particles as opposed to a fluid film coated on the inside of a capillary,
- the separation occurs at or close to ambient temperatures and relies on interaction between the analyte and stationary phase considerably more than GC.

HPLC systems are modular and at their most simple, consist of a pump, 6-port rotary injection valve, sample loop, column and detector.

The pump is used to deliver a continuous flow of the liquid mobile phase (eluent) through the system. Pumps are often described according to the number of solvent inlet lines as unitary, binary, ternary or quaternary for 1-4 solvent lines respectively. In unitary pumps, the eluent has been pre-mixed, and can only be pumped with the eluent components in that fixed ratio (isocratic flow). Binary, ternary and quaternary pumps are also used to deliver gradient flow eluents of 2, 3, and 4 components respectively. In gradient systems, a more strongly eluting mobile phase component (usually organic) is gradually introduced to another mobile phase component (usually aqueous) to progressively elute more strongly retained analytes from the column. Pumps for chromatographic systems typically use reciprocating pump heads, to deliver solvent at a constant pressure, as opposed to a single pump head causing a pressure spike as it pushes eluent out of the pump head onto the column.

The advantage of using systems with multiple solvent inlets is that they are able to generate precise gradients to separate a wider range of mixtures than unitary isocratic pumps. The systems are more complex however, and can be more expensive to purchase and maintain.

The pump feeds eluent to the injector, typically a 6-port system known as a 6-port rotary valve. A schematic is shown below in **Figure 2.2**. The rotary valve allows injection of a precise volume of sample into the pressurised eluent system in a reproducible manner without loss of flow.

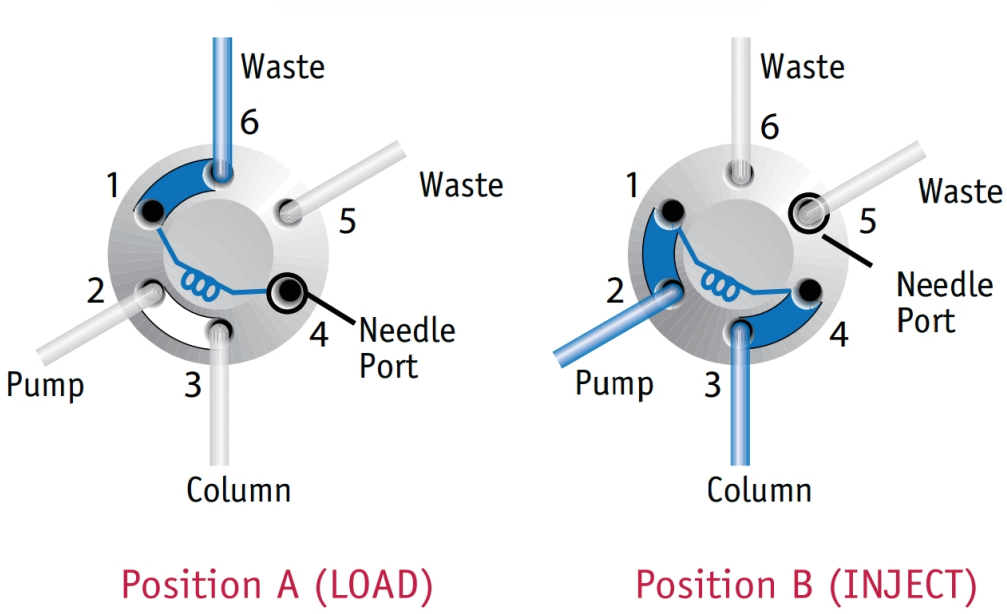

Figure 2.2: Schematic of Rheodyne valve showing flow in both load and inject positions[5]

With the valve in the "load" position, a sample is injected in port 4, filling the fixed volume sample loop, which connects to port 1. Any overfilling of the loop causes excess to be carried to waste by design.

When the valve is turned to the "inject" position, the connections to the eluent and waste lines change position and the sample loop is backflushed with solvent from the pump (port 2) as the vale rotates, carrying the sample in the loop out of port 3 and onto the column for separation (as shown by the blue line). This also cleans the sample loop in preparation for the next sample.

The use of interchangeable fixed volume sample loops mean a very precise volume of sample is carried onto the column, given that the loop is completely filled. Loops are typically long and narrow as opposed to short and wide, in order to quickly flush out the sample and minimise band broadening effects.

As with GC systems, it is interaction of the analyte with a stationary phase that causes separation of the sample components into individual analytes. In HPLC systems, packed rather than capillary columns are used. The stationary phase within the column is no longer a viscous liquid bonded to the inner column wall, but monodisperse micron-scale particles of chemically-modified silica. Where the Golay equation (**Equation 2.12**) describes an open tube system, the van Deemter equation (**Equation 2.13**) below describes flow through a packed column.

$$H = A + \frac{B}{v} + (C_m + C_s)v$$

Equation 2.13

The difference in the two equations is the inclusion of a third term in the van Deemter equation, alongside the longitudinal diffusion term, B, and C_m and C_s representing the mass transfer terms in the mobile and stationary phase as discussed in Section 2.3. The A term in the van Deemter equation represents band broadening caused by eddy diffusion of the analyte through the packed stationary phase.

As analytes are introduced to the packed column, there is no "straightforward" path through the packed stationary phase particles, thus analyte molecules each take separate paths around the silica particles in their way. In addition to this, silica particles have a secondary internal porosity, allowing analytes to take paths travelling through silica particles as well as around them. As a result, some path lengths are longer than others, resulting in band broadening.

The key factor in the separation of mixtures by HPLC is the polarity of the analytes and stationary phase. Unmodified silica has an active hydrophilic surface as a result of polar, acidic silanol groups. The activity of these silanol groups may be modified

through bonding to less polar groups, typically cyanopropyl, *n*-octasilyl-, and *n*-octadecylsilyl. These are often referred to as CN, C8 and C18 columns respectively.

HPLC was initially developed on unmodified (polar) silica using a non-polar mobile phase, retaining polar compounds most strongly through interaction with surface silanol groups. This is known as normal-phase chromatography. In order to separate the larger number of non-polar analytes (compared to non-polar analytes), the polarities of the stationary phase and mobile phase were reversed, receiving the name reversed-phase chromatography. In reversed-phase chromatography, a polar aqueous mobile phase carries analytes through non-polar stationary phases, with non-polar analytes being retained more strongly by the stationary phase.

One of the most common HPLC detectors is the ultraviolet detector. Ultraviolet detectors consist of a deuterium lamp to provide the ultraviolet (UV) wavelength light, which is made monochromatic by passing the incident beam through a diffraction grating. Half of the light from the monochromatic beam passes through a half-mirrored surface to pass through the flow cell containing the eluate from the column, and half the remaining light being reflected into a photodiode acting as a reference beam as shown in **Figure 2.3** below.

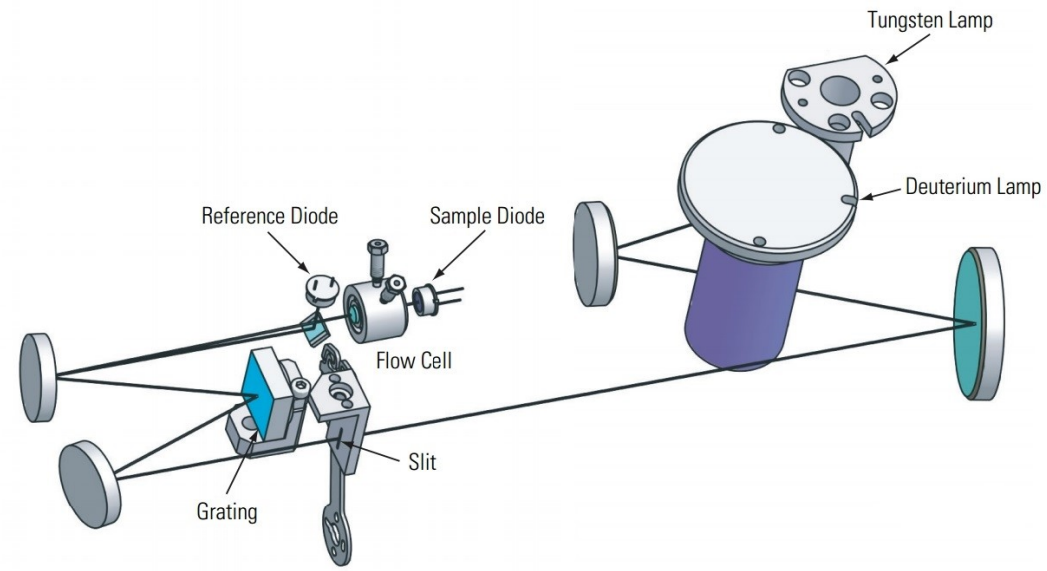

Figure 2.3: Diagram of Dionex variable wavelength UV detector for LC[6]

The photons passing through the sample in the flowcell are absorbed depending on the chromophores present in the sample, and the photons passing through the sample are captured by a detector. An absorption value for the solution is calculated according to the Beer-Lambert Law **Equation 2.14**, where I is the intensity of the transmitted light, I_0 is the intensity of the incident light, ε is the molar absorptivity of the analyte, l is the path length of the photons through the cell and c is the molar concentration of the absorbing substance.

$$A = -\log_{10}\left(\frac{I}{I_0}\right)$$

$$A = \varepsilon l c$$

Equation 2.14

The signals from the photodetectors are recorded by data capture software, producing the chromatogram for interpretation.

In the detectors, light is typically generated by standard tungsten incandescent lamps in combination with deuterium arc lamps. Deuterium lamps have a continuous emission spectrum over the range of 180 to 360 nm and when used in conjunction with

a tungsten lamp, wavelengths can cover a range of approximately 180 to 900 nm. This covers the UV, visible and near infrared range.

The light in deuterium lamps is produced through arcing. The arc lamp consists of a quartz glass envelope containing deuterium gas and the filament/anode assembly. A tungsten filament is placed opposite an anode. The filament is heated for a short period before a high 'striking' voltage is applied. The high voltage causes an arc to form between the filament and anode, with the process repeating until the gas in the lamp is ionised enough to sustain the arc at lower voltages.

Once the arc is established, the molecular deuterium in the lamp is excited to a higher energy state and dissociates, emitting UV radiation. The dissociation of molecular deuterium into atoms results in continuous emission of UV light over a range of energy, from zero to the energy of excitation of the molecule. This is what gives deuterium lamps the ability to emit a broadband UV spectrum rather than discrete wavelengths as with atomic emission spectra.[7]

The tungsten incandescent lamp used emits light based on the principles behind conventional incandescent light bulbs. A tungsten filament in an inert atmosphere is heated electrically until white hot, generating a continuous spectrum over the visible and infrared ranges. Intensities of wavelengths at the shorter (blue) end of the spectrum are typically lower for tungsten lamps. As the intensity of light emitted is dependent on the temperature of the emitting filament, the current applied to the lamp must be very precisely controlled.

Detectors in UV spectrophotometers are typically either photomultiplier tubes (PMTs) or diodes. PMTs operate in a similar manner to the electron multiplier discussed in **section 2.4.** Incident photons strike a photoemissive cathode, which emits electrons in proportion to the intensity of the incident photons via the photoelectric effect. As discussed with the electron multiplier previously, the PMT is a vacuum tube containing a set of discrete dynodes, each held at a higher potential than the previous. The electrons generated by the photoemissive cathode strike the dynodes in the PMT causing secondary emission of yet more electrons. This process continues until the

electrons strike the final anode, which is the collector. The voltage generated at the collector is recorded, and used to generate the chromatogram for interrogation.

Alternatively, photodiodes may be used for detection. A photodiode is a semiconductor device used to convert light into current. In a standard diode, p-type and n-type semiconductors are joined, forming a p-n junction. In the p-type semiconductor, positive “holes” are used as charge carriers, while electrons are charge carriers in the n-type semiconductor. Without the external application of a potential (bias), the free charge carriers from both semiconductors diffuse into the other and recombine, with opposite charge carriers cancelling the other out. This causes a high-impedance “depletion layer” with no free charge carriers along the junction of the two materials.

When a positive voltage is applied to the p-type semiconductor and a negative voltage to the n-type semiconductor, this is known as “forward bias” operation. During forward bias, electrons are repelled by the negative potential of the n-type semiconductor towards holes repelled by the positive potential of the p-type semiconductor causing a reduction in the width of the depletion layer. This makes diffusion of the charge carriers from each semiconductor easier. If the energy supplied by the external voltage is greater than the energy of the potential barrier from the depletion layer, the opposition is overcome resulting in a flow of current.

If the potential applied to each side is reversed (reverse bias), the charge carriers in each semiconductor will migrate away from the junction, and the recombination process cannot occur. This results in an increase in the width of the depletion layer, presenting a high-impedance essentially non-conducting path, and no current flows.

In photodiodes, the diode operates in negative bias mode, with an increased depletion layer. When incident photons of with energy greater than the bandgap of the semiconductor material (the energy needed to promote an electron from the valence to the conduction band of a material) fall on the device, they are absorbed creating free electron and positive hole pairs. This is achieved through the photoelectric effect, with the number of electrons generated being proportional to the intensity of light falling on the surface. The newly formed charge carriers are accelerated towards the anode or

cathode (electrons and holes respectively), generating current. As with the PMT, the resulting voltage is recorded for data analysis.

2.6. Sol-gel Process

Since the mid-19th century, the sol-gel processing of ceramics and glasses has been of interest. Thomas Graham[8] noted in 1864 the condensation of silicic acid, $Si(OH)_4$, would yield a "colloidal glassy hyalite" in the presence of water, and that increased concentrations of silicic acid would cause faster "pectization" into the colloidal gel.

Hench and West[9] described in 1991 the 3 methods for the manufacture of sol-gel monoliths: 1) gelation of a solution of colloidal powders; 2) hydrolysis and polycondensation of alkoxide precursors followed by supercritical drying; 3) hydrolysis and polycondensation of alkoxide precursors followed by ambient drying. The method discussed here shall be method 3, as it was the one investigated in this study.

The fabrication of sol-gels involves a series of reactions: preparation of a *sol*, a solution of colloidal (dia. 1-100 nm) particles suspended in a liquid, gelation of the sol and removal of the solvent.[10] Typically beginning with hydrolysis of an alkoxysilane such as tetraethylorthosilane (TEOS), the reaction proceeds to either form the fully-hydrolysed tetrahydroxysilane or partially hydrolysed to yield silanols. The partially hydrolysed silanols link in condensation reactions with water and ethanol as by-products, and tetrafunctional $Si(OH)_4$ monomers allow complex branching of the polymer to occur. These reactions run in tandem, with the hydrolysis providing the necessary silanol groups for condensation, and the increasing degree of hydrolysis around the silicon centre allowing formation of the 3D network found in the gel.

Once a molecule has become macro-scale throughout the solution, the solution is described as a *gel*. The gel is the porous disordered network the colloidal particles form on aggregation or polymerisation. The solvent evaporates from the gel to give a solid xerogel. The physical properties of the bulk xerogel depend on the conditions of gelation, specifically pH, gel composition and drying mode.[10, 11] This process is illustrated below in **Figure 2.4**.

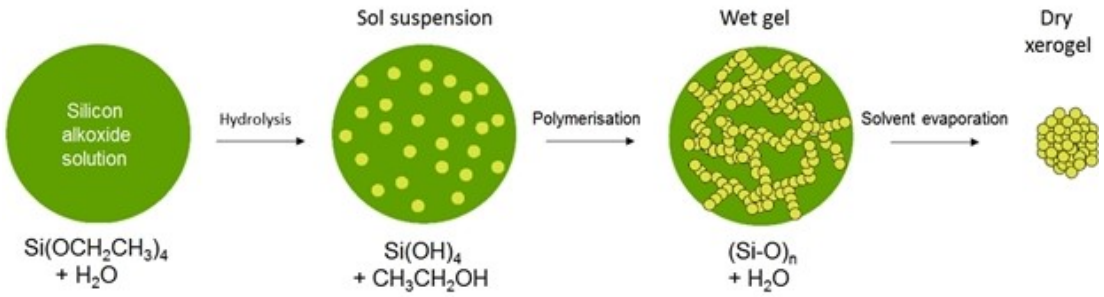

Figure 2.4: Diagram showing formation of glass xerogels using sol-gel process[11]

By adding different substrates to the silica matrix as it condenses, it is possible to alter the reactivity of the final bulk xerogel. Previous work in this area have included the incorporation of rhodamine 6G[12], Schiff's base[13], a number of acid/base indicators and metal chelating agents.[14, 15]

2.7. Agar gels

Agar is a hydrophilic gel isolated from algae and seaweed of the *Rhodophyceae* class, commercially from species of *Gelidium* and *Gracilariae*. The basic structural unit of agar is comprised of agarose, a neutral polymer and agaropectin, a sulfated polymer. Agaropectin forms a non-gelling fraction, with agarose forming the gelling fraction.

Agarose is a linear polymer with a molecular weight of approx. 120 000 Da. The repeating unit is comprised of alternating D-galactose and 3,6-anhydro-L-galactopyranose linked via α-(1→3) and β-(1→4) glycosidic bonds.[16] The agaropectin moiety is a heterogenous mixture of D- and L-galactose with modified sulfate and pyruvate side chains.

Linear agarose chains adopt double helical secondary structures, which aggregate into 3D bundle structures called suprahelices,[17, 18] with pores ranging from 50 nm to over 200 nm depending on the concentration of agar used. Water is held in the interstices of the framework, allowing diffusion of liquids through the pores of the gel.

Agar gels find extensive use as bacterial growth media, as well as stationary phases for column chromatography and electrophoresis. In the food industry, agar is commonly used as a thickening and gelling agent, especially in diets which animal-based gelling agents such as gelatine are not acceptable.

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3. Development of chromatographic methods for the determination of selected pesticides – part A: method validation

3.1. Introduction

3.1.1. Current methods for the determination of pesticides in museums

Heritage institutions present unique sampling environments in that the objects to be examined can be fragile, unique or rare, preventing the removal of samples for analysis, or the use of invasive methods such as those requiring destructive sampling. For example, detection methods for biocides may involve surface swabbing and as such sampling is not performed unless absolutely necessary to avoid damaging the object. Furthermore, it might be that the swabs taken do not collect sufficient mass of analyte to be detected. For inorganic biocides containing metals such as mercury and arsenic, X-ray fluorescence (XRF) spectroscopy has seen widespread use in heritage institutions in the identification of contaminants and pigment materials.[1-7] Detection of organic biocides is typically performed using swabbing or air sampling techniques.

As mentioned in **Chapter 1**, air sampling typically requires a chromatographic method to resolve the sample into its component analytes. Where gas chromatography (GC) is used for this purpose, an intermediate sample preparation step is required before injection of the sample into the instrument. Sample preparation typically involves desorbing the analytes from the sorbent using a solvent rinse. The solvent rinse is then injected into the instrument for analysis after appropriate clean-up and concentration steps have been completed.

Barro and co-workers[8] reported detection of 11 pyrethroids, a fungicide (2-phenylphenol), a carbamate (propoxur) and an insecticide synergist (piperonyl butoxide) were sampled in air using active sampling at a rate of 100 dm^3 min^{-1} over 25 mg Tenax. Analytes were solvent desorbed by emptying the sorbent into a vial and adding either 1 cm^3 hexane or ethyl acetate and sonicating for 10 min. The extractant solution was filtered using a 0.45 μm polyvinylidine fluoride membrane and analysed using gas chromatography – mass spectrometry (GC-MS) and GC-micro electron

capture detection (μECD). The limits of detection reported by the authors were 0.03-4.1 ng m^{-3} (μECD), 1.4-9.1 ng m^{-3} (MS), with recoveries of 81-114% for the analytes and a relative standard deviation (RSD) value of less than 10% for all analytes. While the authors reported a precise method, the use of very high sampling flow rates of 100 $dm^3\,min^{-1}$ is likely to have caused noise that would perhaps have been unwelcome in a heritage environment, and the high airflow through the tubes was shown to cause a decrease in recovery of the analytes of up to 50% over 10 minutes of pump operation leading to a high degree of uncertainty in any quantified results. The need to select an appropriate solvent for the extraction of the pesticides from the sorbent would have also required specialist knowledge that may not be available to heritage institutions. The applicability of the method to heritage environments is potentially low due to the analytes used. In this study, analytes of interest were predominantly pyrethroids which were typically employed after the use of chemical pesticides in museums was deemed less appropriate, and so-called "chemical free" integrated pest management strategies were adopted, meaning it is not likely these substances would be regularly detected in museum environments.[9]

Demel *et al.*[10] reported the trapping of 11 pesticides, 2 fungicides, 2 pyrethroids, a carbamate and a dinitroaniline on 100 mg Tenax using active sampling (2.1 $dm^3\,min^{-1}$ for 8 h). The trapped analytes were desorbed using 5 cm^3 MeOH followed by a 5 min incubation period with occasional shaking and subsequent 3 min sonication. After sedimentation had occurred, 1 cm^3 of the supernatant was filtered through a 0.45 μm glass fibre filter. An internal standard (phenacetin) was added, and the solution was adjusted to volume before analysis using high performance liquid chromatography-diode array detection (HPLC-DAD). The authors report detection limits of 1.0-9.1 μg m^{-3} with recovery values of 70-100% and RSD values of ≤4%. As with the work of Barro and co-workers,[8] the choice of biocides selected by the authors was not relevant to the heritage sector as the majority of the pesticides used were typically developed after the adoption of integrated pest management strategies in many heritage institutions. The authors also noted that a trivial but frequent source of error in results was due to leakage between the pump and sampling tube, suggesting that those unfamiliar with the use of such equipment may find it difficult setting up a sampling system that is sufficiently air-tight to achieve accurate results. As such, it is

believed the method would be of limited utility to this project, as one of the key objectives was to develop techniques that do not require any previous technical knowledge to implement.

Siebers *et al.*[11] analysed five insecticides and two fungicides in the air of greenhouses for 3 d and 4 d after the application of these substances to plants. The classes of substances analysed were three pyrethroids, and one phenylsulfonamide, dinitrocrotonate, organophosphate and carbamate each. Active sampling was performed using flow rates of 528 – 1261 cm^3 min^{-1} through Tenax sorbent tubes fitted with glass wool plugs for 60 min. Analytes were desorbed by emptying the Tenax from the sample tube along with the glass wool and added to a vial with 2 cm^3 acetone. Extraction was performed by shaking the vial for 5 min before extracting by shaking for 5 min in 2 cm^3 acetone. This extract was filtered with paper, rinsed with 2 cm^3 acetone and evaporated under N_2 before dissolution with *n*-hexane or acetone to the final volume. Analysis was performed using GC-ECD and gas chromatography – nitrogen/phosphorus detection (GC-NPD) with cool on-column injection. The authors reported limits of quantification (LOQs) of 0.1–0.2 μg m^{-3} and recoveries of 75-89%. However, the authors also note that the Tenax tubes collected particulate matter as well as vapour-phase fractions, which could have accounted for the high sensitivity of the method for detecting analytes with such low vapour pressures (0.002–1.3 mPa). Separate extraction of the glass wool and Tenax showed that the glass wool pre-filter behaved as a sorbent itself, and highlighted differing affinities of the analytes for Tenax and the glass wool. Parathion was mainly detected in the Tenax layer (>95%), whereas pirimicarb was found at an average of 65% in the glass wool and 35% in the Tenax layer, which indicated that mixed bed sorbents were effective at trapping a wide range of pesticides. The preparation of collected samples for analysis using this method involved the use of multiple steps including solvent extraction, filtration and preconcentration using a stream of gas. This results in a relatively complex and time consuming process, where care must be taken to ensure the analytes are quantitatively recovered at each stage of the sample preparation. It is possible that the lower recoveries stated by the author were a result of this multi-step process, which may not be suitable for the preparation of a large number of samples from a collection due to the time consuming nature of the extraction, filtration and preconcentration process.

In a review by Harper[12], the author stated that despite the high sensitivity of GC analyses, the dilution effect of solvent desorption (often in excess of 1000:1) or the collection of smaller sample volumes often meant that analytes were below the limit of detection or quantification for the analysis and required additional sample concentration. These additional steps were awkward, and prone to error. The use of thermal desorption mitigated the need for additional sample concentration as it is a non-diluting process.

Baroja *et al.*[13] reported a method for the analysis of fenitrothion and its metabolites in forestry air samples using Tenax sampling tubes. The sampled atmosphere was pumped through the tubes at a rate of 50 cm^3 min^{-1} before analysis by thermal desorption gas chromatography mass spectrometry (TD-GC-MS). Analytes were desorbed from the tubes at 300 °C onto a cold trap held at -100 °C for 5 min before heating the cold trap to 260 °C and transferring the analytes to the GC column. The authors reported detection limits of between 1.6 and 2.1 ng m^{-3}. The use of thermal desorption in place of the solvent extraction treatments discussed in other literature allowed fewer steps in the analysis and lessened the risk of analyte losses arising from multiple inefficient steps. This improved detection limits, reduced analysis time and potentially removed interfering peaks caused by the use of solvents. However, the study reported here only investigated measuring the presence of a single analyte and its degradation products in forest air, as opposed to a suite of biocides in air often already containing considerable concentrations of VOCs (as is often the case in heritage collection environments) so it is unclear if this method would be appropriate for such uses.

Clément *et al.*[14] developed a method for the determination of alachlor, atrazine, captan, formothion, lindane and phosalone in atmospheric samples using active sampling at a rate of 80 cm^3 min^{-1} onto sorbent tubes containing 125 mg of sorbents (Tenax, Carbopack Y, Carbopack B, Carbotrap, Carboxen, Chromosorb 106 and XAD-4). Collected samples were analysed by TD-GC-MS. Thermal degradation of the analytes was investigated by desorbing tubes in duplicate at temperatures of 250, 300 and 350 °C. The authors observed a decrease in peak area over the range of temperatures studied, but were unable to attribute this exclusively to high temperature as the

precision of the analysis was difficult to determine with only two replicates. The authors repeated this experiment analysing the samples in the order of high to low temperature and determined that a slight increase in sensitivity was shown for lower temperatures, presumably referring to higher recoveries of the analytes. This data was not presented in the publication, however. The authors reported that the use of thermal desorption was a good method for the determination of the majority of the pesticides analysed, however captan and phosalone could not be accurately determined. It was stated in the paper that thermal stability was more important than vapour pressure when determining the suitability of thermal desorption for an analyte, as trapped analytes must be stable enough to withstand aggressive desorption temperatures in order to be detected. Using the conditions detailed in the paper, Clément and co-workers determined that of the sorbents examined, only Tenax and Carbopack Y did not irreversibly adsorb compounds at the maximum desorption temperatures of the sorbents. Tenax was determined to be the most suitable sorbent, as Carbopack Y was only suitable for the measurement of lindane as other analytes adsorbed irreversibly to the sorbent. The authors conducted sampling for 72 h immediately after application of a range of pesticides, so it was likely that there would be a greater concentration of the aerosolised pesticide in the atmosphere, compared to detection of historical treatments where the only atmospheric source of the pesticide is typically volatilisation form the object itself. As the study was conducted over a short term following application of the pesticide, it is not clear if the method is suitable for longer term monitoring of pesticidal vapour. The authors noted that there was a night-day volatilisation cycle observed in the data, with warmer daytime temperatures causing an increase in the atmospheric concentration of the analytes. It was observed that atmospheric concentrations were equal to zero between 0000 and 0800 h but increased to a concentration of 53 ng m^{-3} between 1600 and 0000 h. As the study made use of short-term active sampling techniques which only take “snapshots” of the current condition of a sampling environment, there is a possibility that if sampling was to be conducted on a cooler day, lower vapour-phase concentrations of pesticide may be detected in the environment which may not be a true indication of the actual amount of pesticide present in the environment.

Briand *et al.*[15] reported the use of Tenax for the development of a multiresidue pesticide determination method in 2002. Atmospheric environments were actively sampled using calibrated pumps, followed by analysis using TD-GC-MS. Two sampling methods were developed. The first one connected a Tenax sampling tube to a pump with either a 1-2000 cm^3 min^{-1} flow rate or a lower flow rate of 50-350 cm^3 min^{-1}. The second sampler was a low-volume automatic air sampler which was modified to permit flow rates of 250-1500 cm^3 min^{-1}, with the previously described pumping systems. This collector permitted the sequential sampling of 24 tubes. Sampling duration was fixed between 1-2 h per tube for this study. The authors described that 100% recovery from Tenax tubes was achieved using a thermal desorption temperature of 350 °C. The authors claim that the method may be applied to atmospheric samples for the detection of their target pesticides, including lindane. However, the design of the atmospheric sampling was such that only one of the target analytes was applied to the test material so it was not possible to determine if the other analytes could be trapped. As such, it is not clear if this method can be applied within the heritage science community as it has not been demonstrated that the analytes can be trapped and analysed using the method described.

As discussed in **Chapter 1**, the use of Tenax sorbent for the trapping and analysis of volatile pesticides in museums has also been investigated by Schieweck and co-workers.[16, 17] In 2005, Schieweck *et al.*[16] used air sampling to determine organic pollutants in a German museum. Volatile organic compounds (VOCs) were sampled by pumping air through Tenax sorbent tubes for 40 min at a rate of 150 cm^3 min^{-1} before analysis by TD-GC-MS. Dichlorobenzene and camphor were measured at concentrations of 40 µg m^{-3} and 110 µg m^{-3}, and naphthalene was detected at concentrations between 2 and 9 µg m^{-3} across the sampling locations in the study. The authors were also able to detect the presence of lindane at a concentration of 0.16 µg m^{-3} through this method of air sampling. The authors were able to detect dichlorobenzene and lindane in air samples, but did not further investigate the detection of pesticides using the described methods in this paper, as the focus was on the detection of VOCs.

In 2007, Schieweck *et al.*[17] expanded this work by focussing specifically on biocidal contamination in museums. Tenax tubes were used in active sampling mode as previously, but also in passive mode to sample over 14 d. The authors were able to detect naphthalene, chlorinated naphthalenes and dichlorobenzene at concentrations ranging from >1 μg m^{-3} to 32 μg m^{-3}. These results are summarised in **Table 3.1**.

Table 3.1: Concentrations of biocides in air of museum cases[17]

Compound	**Lowest concentration detected (μg m^{-3})**	**Highest concentration detected (μg m^{-3})**
1,4-dichlorobenzene	4	24
Naphthalene	3	3
Monochloronaphthalene	2	32
Dichloronaphthalene	<1	30

The results presented in the table show that detected concentrations of dichlorobenzene were significantly increased in the cases sampled compared with the exhibition room. The increased concentration of the chlorinated naphthalenes compared to background samples was indicative that there was previous pesticidal treatment involving these substances on the objects contained within the cases. This study, however, focusses on a limited number of biocides, with each of the analytes being chlorinated aromatic substances. While the authors have been successful in detecting these substances, it is not clear if the method can be adapted for the trapping and analysis of a wider range of analytes with different functionalities.

Concentrations and types of VOCs found in indoor air vary considerably depending on the use of the indoor air space. For example, a workshop may contain higher concentrations of straight-chain hydrocarbons or solvent species from paints or lacquers etc. while a residential room with an open fire may contain higher than background concentrations of aromatic and polycyclic aromatic hydrocarbons from the combustion in the fireplace. Proximity to external sources also influences the indoor air pollutant (IAP) profile of a site. Nearby industrial sites, heavy road traffic

and surrounding land use all contribute to the VOCs found in a sample. When sampling indoor air in heritage environments, in addition to pesticides which may be present, it is also possible to trap other common indoor air pollutants. **Table 3.2** below lists some typical mono-aromatic compounds typically found in indoor air. Benzene, toluene, ethylbenzene and xylenes (often collectively referred to as BTEX) are common pollutants found in indoor air, especially in urban areas as they are often generated by vehicle exhaust.[18] While there appears to be a large difference in the concentrations found in these studies, the concentrations detected were approximately within one order of magnitude to each other, which at concentrations such as these, was an acceptable margin of error.

Table 3.2: Mean concentration of BTEX compounds (μg m^{-3}) found in indoor air sampling studies[19-22]

Substance	**Location**			
	Chicago	Helsinki	Hong Kong	Melbourne
Benzene	4.8	2.2	8.1	7.0
Toluene	15.3	20.4	52.8	14.0
Ethylbenzene	9.7	2.9	7.3	1.8
Xylene	11.2	2.5	5.1	8.9

A further study from 1987 detailed the VOCs found in 650 residences in seven US cities.[23] This study found that high concentrations of chemicals were emitted by cleaning products in particular. Perhaps indicative of the study being conducted in the late 1980s, the chemicals emitted by these sources were carbon tetrachloride, di- and tri-chloroethane and chloroform; all of these are now considered carcinogenic and are tightly controlled pollutants. This study indicated that for sampling in heritage environments, there are legacy chemical issues that must be considered when interpreting the data.

Although the use of Tenax and other sorbents as media for trapping VOCs by passive sampling is well established, the use of this method for the determination of pesticidal vapour in heritage environments has not been fully explored.

3.1.2. Target pesticides chosen for this research project.

The preservation of culturally significant objects has often involved the use of hazardous chemicals such as pesticides, fungicides or other biocides. A number of articles have been published highlighting the range of chemicals used to treat historical objects. Goldberg[24] reviewed records at the Smithsonian Institution (Washington, DC, US) and listed a wide range of chemical species used as pesticidal treatments. Objects may have been treated many times over their lifetime and chemicals that have been used at various dates include classes of compounds such as organochlorides, organophosphates, simple aromatic hydrocarbons and commercial biocidal formulations. Using tandem mass spectrometry (MS-MS) Purewal has also shown that mercury chloride ($HgCl_2$), arsenic trioxide (As_2O_3), and naphthalene could be detected on the surface of artefacts[25] while Sirois used X-ray fluorescence spectrometry to measure these inorganic species.[26] However, each of these studies required expensive X-ray fluorescence instruments, and only measures discrete points on the surface of collections items. In 2001, Hawks[27] discussed substances suspected of having been used for the pesticidal treatment of artefacts (see Table 3.3, where chemicals are listed under trivial and trade names). While not an exhaustive list, this table has been included to illustrate the breadth of substances previously used in conservation treatments. A comprehensive list of hazardous chemicals used in the heritage sector has been published by Unger *et al.*[28] in 2001.

Table 3.3: Organic and inorganic chemicals previously used in heritage collections[27]

Substance	**Trade/Other Name(s)**	**Chemical Family**
Carbaryl	Sevin	Carbamate
Bendiocarb	Ficam	Carbamate
Propoxur	Baygon	Carbamate

Substance	Trade/Other Name(s)	Chemical Family
Borax	-	Inorganic
Sulfuryl fluoride	Vikane	Inorganic
Boric acid	-	Inorganic
Alcohol	-	Organic
Edolan U	Eulan U33	Organic
Pyrethrins	-	Organic
Phenol	Carbolic acid	Organic
Hydrogen cyanide	-	Organic
Naphthalene	-	Organic
Carbon disulfide	-	Organic
Lauryl pentachlorophenate	-	Organic
Ethylene oxide	-	Organic
Formaldehyde	-	Organic
Heptachlor	Drinox, heptagram	Organochloride
Methoxychlor	Methoxy-DDT, methoxide	Organochloride
Aldrin	-	Organochloride
Endrin aldehyde	-	Organochloride
Endosulfan II	Phaser, thionex	Organochloride
o-Dichlorobenzene	-	Organochloride
Cyclohexane hexachlorides	HCH, lindane	Organochloride
Carbon tetrachloride	-	Organochloride
Dichlorodiphenyltrichloroethane	DDT, Anofex	Organochloride

Substance	Trade/Other Name(s)	Chemical Family
p-Dichlorobenzene	-	Organochloride
Chlordane	Belt, Chlor Kil	Organochloride
Pentachlorophenol	Acutox, Dowicide 7	Organochloride
Chloropicrin	PS gas	Organochloride
Dieldrin	Alvit, Octalox	Organochloride
1,2,4-Trichlorobenzene	-	Organochloride
Ethylene dibromide/dichloride	-	Organohalide
Methyl bromide	-	Organohalide
Dichlorvos	Vapona	Organophosphate
Hydrogen phosphide	Phosphine	Organophosphorus
Chlorpyrifos	Dursban	Thiophosphate
Diazinon	Knox-out, Dianon	Thiophosphate
Malathion	Carbophos	Thiophosphate

[a] Substances are grouped by chemical family and include trade names where available

As yet, and to the best of the author's knowledge, there is no general consensus as to which sampling approaches are most appropriate when sampling these analytes on heritage objects. While some publications have recommended trapping of analytes on polyurethane foams,[29, 30] or solvent extraction of collected dust,[16, 17, 31] the need to preconcentrate extracts adds complexity to these analytical procedures. This presents significant problems as the procedures may be carried out by those with no previous scientific experience. The range of analytes used is also vast and different geographical locations will focus on a different subset of pesticides traditionally used to treat the objects in their collections.

After discussion with project partners working in UK national institutions ten analytes were selected for study within this project. Substances were selected for study based

on their perceived occurrence within heritage collections based on the literature review contained in **Chapter 1**, and also due to concerns project partners from heritage institutions such as the British Museum and National Records of Scotland had about the hazards these substances present to those working with contaminated objects. As none of the previously published literature covered this specific combination of these ten pesticides that fulfilled the project criteria of being simple and relatively economical to implement, new methods of analysis were required. The target subset of pesticides chosen to reflect the range of chemicals commonly used in previous treatments included six organochlorides (hexachlorocyclohexane also known as HCH, dieldrin, endrin, aldrin, dichlorodiphenyltrichloroethane also known as DDT, and chloronaphthalene); one organophosphate (dichlorvos); two aromatic organic compounds (naphthalene and thymol) and one organic terpenoid compound (camphor). By developing a method to characterise any biocides present in the air, the working environment for museum staff and collection users can be made safer. While organophosphates are more acutely toxic than organochlorides, they are also less persistent in the environment and do not bioaccumulate to high levels in lipid tissues.[32] In a museum however, the environment is protected from many of the degradation pathways normally encountered by pesticides in industries such as agriculture such as hydrolysis and direct photolysis from sunlight. By definition, museums are designed to have static environments and as such, ultraviolet (UV) photolysis, hydrolysis and water run-off are not encountered in properly maintained collections.

The focus of the developed methods will be, where possible, non-invasive sampling of volatile analytes from the headspace around the treated object. This would be ideal for historical or heritage collections where direct sampling should be avoided if at all possible. Therefore, one key objective of the method development phase of the project was to isolate and concentrate volatiles emitted from an object using Tenax sampling tubes. The determination of less volatile pesticides in the museum environment such as mercury is discussed in **Chapter 5**. For the sampling of volatile analytes it was recognised that it may be necessary to enclose the treated object into a suitable container (plastic/glass storage box, or 'tent' around the object) during sampling. Sampling tubes could be used in either passive or active mode to collect the emitted volatiles.

3.2. Research aims and objectives

This study presented the development of two methods for the detection of biocides commonly found in heritage environments, before assessing the methods in case studies across UK heritage institutions. Target biocides were selected after consultation with conservators at project partner institutions. The work undertaken here has detailed the use of passive sampling using stainless steel tubes packed with Tenax. The use of these sampling tubes allowed them to be posted to collections, where they were easily deployed by collection staff in order to collect the samples for analysis. Key objectives of the study are discussed below.

Development of methods suitable for the detection of target analytes in museum environments

Methods for the determination of the 10 target analytes (aldrin, camphor, chloronaphthalene, DDT, dichlorvos, dieldrin, endrin, lindane, naphthalene and thymol) were developed based on the sampling needs for each substance. For example, volatile analytes such as camphor, naphthalene, chloronaphthalene, thymol, dichlorvos, aldrin, lindane and HCH were detected using TD-GC-MS from spiked Tenax tubes. More thermally labile analytes used an HPLC-UV method, which was able to detect naphthalene, thymol, chloronaphthalene, dichlorvos, aldrin, dieldrin, endrin and DDT. Additionally, a GC-FID method was developed allowing detection of all 10 target analytes. Sample preparation was investigated to determine the most suitable instrumental procedure for the detection of each analyte, with a preconcentration method being developed to allow detection of low masses of analyte recovered by solvent extraction. Chromatographic methods were appropriately validated to determine specificity of response and to investigate the correlation between peak area and concentration.

Creation of a sampling and analysis strategy for heritage institutions based on developed methods

A significant aim of this work was to provide museum staff from a non-scientific background and skill set with an effective tool for the detection and identification of pesticides in heritage environments. To this end, a flowchart has been designed which

incorporated sampling and analysis strategies for the target analytes, and a sampling protocol has been prepared detailing for heritage workers the procedure to follow for the collection of samples. The flowchart allows either an appropriate sampling strategy to be determined based on knowledge of chemical hazards present, or can inform conservation staff which analytes are likely to be detected based on the sampling needs of a particular object. The flowchart could potentially allow users to select an appropriate analytical strategy without any prior knowledge of analytical instrumentation or sampling techniques. It was proposed that this flowchart could enable heritage institutions to determine the presence of chemical hazards and legacy biocidal residues in their collections by empowering collection custodians to hire external laboratories to perform analyses tailored to the needs of their individual collections. The sampling protocol was prepared in order to fulfil the key objective that the developed techniques be simple for workers who may not be from a scientific background to follow. As such, the protocol is a step-by-step guide for the collection of samples for analysis. The protocol is found in **Appendix A**.

Assessment of developed methods using case studies in UK heritage institutions

Developed methods were assessed using case studies undertaken in several heritage institutions in the UK. The case studies demonstrated the utility of the methods in uncontrolled environments, aiming to investigate objects in an "as found" state. These case studies highlighted VOCs common across heritage environments as well as variations in type and concentration of VOCs present. The case studies also found a number of instances where camphor, naphthalene, chloronaphthalene, and lindane were detected in the vapour-phase, with the presence of these substances being attributed to previous pesticidal treatments. During the case studies, sampling was minimally invasive, non-contact and was performed *in situ*, allowing fragile objects to be sampled for analysis without fear of further damaging the object, fulfilling a key objective of this work. The analytes trapped on the sorbent were desorbed for analysis using either heat (for thermally stable analytes) or by solvent extraction (for less thermally stable analytes) and analysed by an appropriate chromatographic method.

3.3. Experimental

3.3.1. Part A: development of methods suitable for implementation in heritage environments

3.3.1.1. Calibration of GC instrument

Analytes trapped onto Tenax sampling tubes were recovered by thermal desorption using a Markes International Unity$_2$ thermal desorption unit connected to an Agilent 5890 GC-MS. Each sampling tube was heated for 10 min at 320 °C using He as the carrier gas at 1 cm^3 min^{-1} and desorbed analytes passed onto a cold trap held at -30 °C. In the second stage of the desorption process the cold trap was rapidly heated at approximately 99 °C s^{-1} to 300 °C permitting a sharp band of vapour to pass into the GC-MS instrument. Helium, at a flow rate of 1 cm min^{-1}, was used as the GC-MS carrier gas with a DB-5MS (30 m × 250 μm × 0.25 μm film thickness) capillary column. The GC column was heated using the following conditions: 65 °C for 5 min, 5 °C min^{-1} to 90 °C held for 5 min, 30 °C min^{-1} to 180 °C and held for 5 min before increasing at 20 °C min^{-1} to 220 °C and holding for 5 min to give a final analysis time of 30 min. Mass spectrometric detection was used in electron impact mode, scanning over the range of 30–450 amu, with an electron energy of 70 eV and a solvent delay of 2 min.

A standard solution was prepared by measuring approximately 20 mg each of aldrin (Pestanal analytical standard, Sigma-Aldrich, Gillingham), camphor (Purum ≥95.0%, Sigma-Aldrich, Gillingham), 1-chloronaphthalene (technical grade, Sigma-Aldrich, Gillingham), endrin (Pestanal analytical standard, Sigma-Aldrich, Gillingham), dichlorvos (Pestanal analytical standard, Sigma-Aldrich, Gillingham), dieldrin (Pestanal analytical standard, Sigma-Aldrich, Gillingham), DDT (Pestanal analytical standard, Sigma-Aldrich, Gillingham), HCH (1:1:1:1 α:β:γ:σ isomers, Pestanal analytical standard, Sigma-Aldrich, Gillingham), naphthalene (Scintillation grade, ≥99.0%, Sigma-Aldrich, Gillingham) and thymol (≥99.0%, Sigma-Aldrich, Gillingham) into a beaker before quantitative transfer into a 100 cm^3 volumetric flask and dilution to volume with 95:5 hexane (95% n-hexane, Fisher Scientific, Loughborough) and isopropyl alcohol (IPA, HPLC grade, Rathburn, Walkerburn).

This stock solution was then diluted 10-fold to yield a concentration of approx. 20 ng μL^{-1}. Calibration of the TD-GC-MS was performed by loading conditioned Tenax tubes with 2.5, 5.0, 7.5 or 10 µL of the diluted mixed standard solution with one tube being loaded with a solvent blank. This gave an analyte mass of 50, 100, 150 and 200 ng per tube, with a solvent blank. Loading was performed by injecting the necessary volume directly onto the Tenax in the tube using a Hamilton syringe. Linearity of the instrument was assessed over this range by determining the equation of straight line for the response and calculation of the R^2 value. Identification of analytes was performed by probability-based spectral matching with NIST reference spectra.

3.3.1.2. Calibration of HPLC instrument

A standard solution containing all 10 analytes of interest was prepared as above, to give a 200 ng uL^{-1} concentration using 95:5 hexane:IPA as a diluent. Instrument calibration was performed by injecting 25, 50, 100 or 250 µL of the standard solution onto individual Tenax tubes giving loaded analyte masses of 5, 10, 20, and 50 µg, respectively. Elution of calibration tubes using 10 cm^3 of 95:5 hexane:IPA gave calibration solutions of 0.5, 1, 2, or 5 ng μL^{-1}. A Tenax sampling tube which had not been loaded with the standard solution was eluted with 10 cm^3 of 95:5 hexane:IPA to provide a procedural blank.

Sampling tubes were back-flushed with 8 cm^3 of 95:5 hexane:IPA solution into a 10 cm^3 volumetric flask before being diluted to volume with the same solvent. Extracted solutions were transferred to a graduated centrifuge tube and reduced in volume, under a stream of N_2 at 30 °C, using a Techne FSD400D sample concentrator. The final solution volume was approx. 0.8 cm^3, which was diluted to 1 cm^3 using the hexane:IPA solution. Extracted solutions were analysed using a Thermo Separation Products HPLC equipped with a TSP UV1000 detector set to 225 nm. Separation was performed on a Jones C_{18} 4 µm, 4.6 x 250 mm column with an isocratic 90:10 acetonitrile:water mobile phase at 1.5 cm^3 min^{-1} and a 100 µL sample loop. Linearity of the instrument was assessed over this range by determining the equation of straight line for the response and calculation of the R^2 value.

Analyte recoveries were calculated by loading 250 µL or 25 µL of a 200 ng μL^{-1} mixed analyte standard solution onto Tenax tubes. Analytes were removed by solvent extraction by backflushing tubes with 8 cm^3 of 95:5 hexane:IPA as described. The solution eluted from the tube with the 25 µL loading was preconcentrated 10-fold to give both solutions a final concentration of 5 ng μL^{-1} giving two solutions of equal concentration. Both solutions were injected into the HPLC-UV system with a mixed standard solution prepared at 5 ng μL^{-1}. This procedure was repeated in triplicate with the resulting peak areas being compared to the calibration curve collected from analytes eluted from Tenax. The concentration values from the three replicates were averaged and expressed as a percentage of a theoretical 5 ng μL^{-1} concentration to give a recovery value.

3.3.1.3. Estimation of passive sampling rate onto Tenax sampling tubes for pesticides

The sampling rate was calculated based on the same Fickian diffusion principles discussed by Gibson *et al.*,[33] and in **Section 2.1.2**. The equation is shown below, where D is the diffusion coefficient of the analyte, A is the cross sectional area of the absorbing surface, and L is the length of the tube:

$$Rate = \frac{D \times A}{L} \quad \textbf{(1)}$$

The diffusion coefficient used was based on *t*-butyl toluene, a model substance with a molecular weight equal to the average molecular weight of camphor, naphthalene, thymol and chloronaphthalene as reported by Lugg[34] at 0.0571 $cm^2\ s^{-1}$. As the Tenax tube was open at both ends to give two air/sorbent interfaces, a sampling rate was calculated for each interface to give a total approximate sampling rate of 0.78 $dm^3\ d^{-1}$. Using the approximated sampling rate as an estimation of the passive sampling ability of the Tenax tube, a 28 d sampling period was selected to allow the collection of approximately 21.8 dm^3 of air. Using this model sampling rate, procedural limits of detection were calculated for 1 and 28 d sampling periods for the TD-GC-MS or HPLC-UV method of analyses (see **Table 3.4** and **Table 3.5**).

Table 3.4: Instrumental detection limit and lowest detectable concentration of analytes for fixed passive sampling periods for analysis by TD-GC-MS

Analyte	**Instrumental LOD[a] (ng)**	**Lowest detectable concentration – 24 h sampling (µg m^{-3})**	**Lowest detectable concentration – 28 d sampling (µg m^{-3})**
Camphor	7	10	0.34
Naphthalene	11	14	0.49
Thymol	8	11	0.39
Chloronaphthalene	12	16	0.55
Dichlorvos	3	4	0.13
Aldrin	53	36	1.28

Table 3.5: Instrumental and procedural detection limits for analysis by HPLC-UV

Analyte	**Instrumental LOD[b] (ng)**	**24 h sampling (µg m^{-3})**	**28 d sampling (µg m^{-3})**
Naphthalene	0.1	0.1	> 0.01
Thymol	35	45	1.62
Chloronaphthalene	22	28	0.98
Dichlorvos	22	28	1.00
Aldrin	77	99	3.53
Endrin/Dieldrin	39	50	1.79
DDT	36	46	1.65

[a] calculated as 3x standard deviation minus the intercept divided by the slope of the calibration curve, over 5 replicates

3.3.2. Part B: assessment of sampling methods using case studies in heritage environments

Several case study locations were identified after consultation with project partners. Locations were selected typically because it was suspected that there were biocidal residues present in the sampling location, but not confirmed. Sampling in **Section 3.5.1-3.5.5** and **Section 3.5.14** was conducted by museum staff by following the sampling protocol in **Appendix A**, and sampling in **Section 3.5.6-3.5.13** was conducted by the author. Methods of analysis used were as detailed in **Section 3.3.1**.

A further standard solution for TD-GC-MS analysis was prepared in addition to the biocide standard described previously in order to quantify some commonly identified VOCs. The standard was prepared by measuring approximately 20 mg each of toluene (≥99.9%, Sigma-Aldrich, Gillingham), ethylbenzene (puriss ≥99.0%, Sigma-Aldrich, Gillingham), furfural (ACS grade 99%, Sigma-Aldrich, Gillingham), acetophenone (puriss ≥99.0%, Sigma-Aldrich, Gillingham), *p*-dichlorobenzene (99%, Sigma-Aldrich, Gillingham), *o*-dichlorobenzene (98%, Sigma-Aldrich, Gillingham), cumene (98%, Sigma-Aldrich, Gillingham), and benzaldehyde (≥99.5%, Sigma-Aldrich, Gillingham) into a beaker before quantitative transfer into a 100 cm^3 volumetric flask and dilution to volume with 95:5 hexane (95% n-hexane, Fisher Scientific, Loughborough) and isopropyl alcohol (IPA, HPLC grade, Rathburn, Walkerburn). This stock solution was then diluted 10-fold to yield a concentration of approx. 20 ng μL^{-1}. Calibration of the TD-GC-MS was performed as previously by loading conditioned Tenax tubes with 2.5, 5.0, 7.5 or 10 µL of the diluted mixed standard solution with one tube being loaded with a solvent blank. This gave an analyte mass of 50, 100, 150 and 200 ng per tube, with a solvent blank. Loading was performed by injecting the necessary volume directly onto the Tenax in the tube using a Hamilton syringe. Linearity of the instrument was assessed over this range by determining the equation of straight line for the response and calculation of the R^2 value. Confirmation of analytes was performed by probability-based spectral matching with NIST reference spectra.

3.3.2.1. Swiss Cottage natural history case study

Swiss Cottage is an Alpine-style chalet constructed in the grounds of Osborne House on the Isle of Wight during 1853-4. The Swiss Cottage site contained a large avian taxidermy collection dating from the 19th century which was passively sampled over a period of 98 d. Several display cases were opened just enough to insert a Tenax tube before being resealed for the duration of the sampling. Sampling locations are shown in **Figure 3.1**.

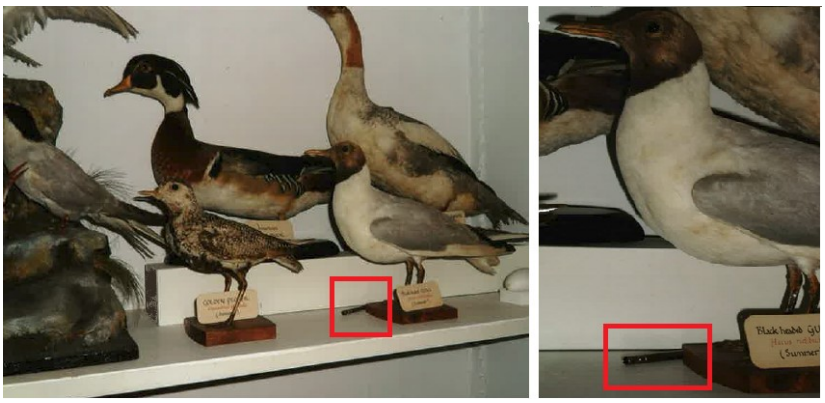


Figure 3.1: Image showing location of sampling tube within Swiss Cottage collection. Tenax tube highlighted for clarity.

3.3.2.2. British Museum rubber garments case study

A collection of rubber horse riding garments housed at the British Museum was sampled in order to determine the origin of a strong unpleasant odour emitting from the objects. The garments sampled were a rain cape (Am1988,08.672) and trousers (Am1988,08.670b) made from cotton calico which had been coated with rubber latex on the outer surface. Garments are shown in **Figure 3.2**.

Figure 3.2: British Museum rubber garments selected for study. From L-R: Trousers (Am1988,08.670b), and rain cape (Am1988,08.672)

The garments were placed in separate enclosures and sampled passively over a period of 25 d at a temperature of approximately 18-22 °C. Enclosures were created by heat sealing two polythene sheets around the object, with the diffusion tube already *in situ*. Although polyethylene is known to be gas permeable, this was not considered critical to the study as the objectives were qualitative only. Background measurements were taken from the Special Projects Room where the garment was stored for the duration of the sampling.

3.3.2.3. National Records of Scotland Station Road strongroom case study

The Station Road site to be monitored was a strongroom formerly used as a safe for storing pools coupons. At time of sampling, the room measuring approx. 200 m^3 contained a number of steel bracketed wooden shelves which held a number of maps as shown in **Figure 3.3**. Sampling was conducted in passive mode over a period of 33 d before analysis by TD-GC-MS.

Figure 3.3: Station Road strongroom sampling location showing papers on shelves

3.3.2.4. RAF museum case study

Sampling at the RAF museum presented an opportunity to determine if any of the 10 target analytes had been used across collections containing a broad range of material housed within a single institution. Samples were taken from a number of locations including four Portastor units housing collection material, two offices, two library/archival stores, a cleaning studio and a film store. The contents of each sampling location are summarised in **Table 3.6**. Locations were sampled for 28 d using Tenax tubes in passive mode. Images of the library storeroom and portastor 2 sampling locations are presented in **Figure 3.4**.

Table 3.6: Collection information at sampling locations in RAF Museum

Sampling location	Objects	Materials	Environmental conditions
Office	-	-	RH: 41% Temp: 26 ºC
Portastor 1 *(ammonia odour)*	Aircraft manufacturers' drawings, photographic material (from 1940s onwards)	Ink on paper, negatives, prints	RH: 40% Temp: 28 ºC
Portastor 2 *(vinegar odour)*	Aircraft manufacturers' drawings, photographic material (from 1920s onwards)	Ink on paper & waxed linen, ink on tracing paper, dyeline prints, blueprints, cellulose acetate film negatives, microfilm	RH: 43% Temp: 27 ºC
Portastor 3	Periodicals, technical manuals (from 1910s - 1990s)	Ink on paper	RH: 47% Temp: 26 ºC
Portastor 4	Technical manuals (from 1940s onwards)	Ink on paper	RH: 43% Temp: 26 ºC
Archive storeroom	Documents, photographic material, drawings, paintings (from 1900s – present)	Printed material, prints, microfilm	RH: 47% Temp: 26 ºC
Library storeroom	Books, periodicals, technical manuals (from 1900s – present)	Ink on paper	RH: 42% Temp: 25 ºC
DoRIS office	Various archival items	-	RH: 47% Temp: 27 ºC
Cleaning studio	Aircraft manufacturers' drawings (from 1920s onwards)	Ink on paper & on waxed linen, ink on tracing paper, dyeline prints	RH: 38% Temp: 27 ºC

Sampling location	Objects	Materials	Environmental conditions
Film Store	Audio-visual material (from 1900s – present)	CDs/DVDs, cassettes (video & audio), film (cellulose acetate)	RH: 41% Temp: 27 ºC

Figure 3.4: Images of library storeroom (L) and portastor 2 (R) with samplers in each location highlighted

3.3.2.5. National Records of Scotland Thomas Thomson House case study

The National Records of Scotland holds a large photographic and multimedia store in Thomas Thomson House. A total of 12 locations were selected for sampling as summarised in **Table 3.7**. Sampling was conducted over 28 d using tubes in passive mode.

Table 3.7: Collection information at sampling locations in Thomas Thomson House

Sampling location	Objects	Materials	Environmental conditions
GA 1 background	Photographic material store	Glass plate negatives, plastic film negatives, photographic albums, photographic prints and small number of cased photographs and framed photographic prints.	RH: 43% Temp: 17 ºC
GA 1 enclosure	4 flap paper enclosure with photographic material. Taken from GA1 drawer 6.4	Glass plate negatives	RH: 44 % Temp: 17 ºC
GA1 drawer 6.4	Cabinet drawer with photographic material	Glass plate negatives	RH: 44% Temp: 17 ºC
GA1 drawer 26.3	Cabinet drawer with photographic material	Plastic film negatives	RH: 45% Temp: 17 ºC
GA1 enclosure	4 flap paper enclosure with photographic material. Taken from GA1 drawer 26.3	Plastic film negative (Cellulose acetate negative)	RH: 44% Temp: 17 ºC
GA1 box	Box containing photographic material. Taken from GA1 drawer 13.3	Cased photograph – Ambrotype	RH: 43% Temp: 17 ºC
GA1 timecare box	Timecare box containing photographic material	B&W prints	RH: 45% Temp: 17 ºC
GA 1 print	4 flap paper enclosure with photographic material. Taken from above timecare box	B&W print	RH: 45% Temp: 17 ºC
GA1 album box	Box containing photographic material	Photographic album	RH: 45% Temp: 17 ºC

Sampling location	Objects	Materials	Environmental conditions
GA background	General store room	Mixed archival material.: Printed material and manuscripts 1 cabinet containing colour photographic prints in Timecare boxes	RH: 66% Temp: 16 ºC
GA cabinet 770	Cabinet containing photographic material	Timecare boxes with colour photographic material	RH: 51% Temp: 16 ºC
GA timecare box 770	Timecare box with colour photographic material	Colour prints	RH: 51% Temp: 16 ºC
GA print	4 flap paper enclosure with photographic material	Colour digital print	RH: 46% Temp: 16 ºC

3.3.2.6. University of Glasgow herbarium case study

In order to investigate the capacity of the sorbent to adsorb non-volatile substances in the presence of highly volatile substances, it was necessary to sample a treated object with a well understood VOC profile. The artefact chosen for study was a herbarium compiled in 1678 by a Scottish medical student named John Snodgrass while studying at the University of Leiden. The object is a bound volume of paper with pasteboard covers, with about 850 plant specimens from the Leiden botanical Garden and elsewhere in the Netherlands glued into the pages (**Figure 3.5**).

Figure 3.5: Photograph of University of Glasgow herbarium volume, removed from storage box

During the 1990s, a conservator who worked in the library conjectured that the book was treated with DDT, based on the presence of fine powdery residues being present on the pages. The box in which the book was stored (**Figure 3.6**) had been labelled "pesticide residues" rather than DDT specifically. Sampling comprised deploying four conditioned Tenax tubes to the Solander box containing the herbarium on day 0, and one tube removed after 7, 14, 21 and 28 d. At this point, the tubes were returned to Strathclyde and analysed by TD-GC-MS as described previously.

Figure 3.6: Storage box for University of Glasgow herbarium volume

3.3.2.7. British Museum Orsman Road scaredevil case study

The scaredevil, **Figure 3.7**, was a large wooden object, mounted to the front of a canoe used by some Oceanian cultures as a means of warding off evil. This carved wooden

figure would sit on the prow of the boat as a talisman. The scaredevil was stored in a wooden box and presented as an ideal case study due to the wooden storage crate being able to preconcentrate any VOCs present. This case study was selected as an extension of the work already completed in the University of Glasgow herbarium study as described in **Section 3.3.2.6**. The University of Glasgow study examined the capacity of the sorbent to trap marginally volatile substances such as DDT in the presence of more volatile cellulosic breakdown substances without exhausting the adsorption capacity of the sorbent, and was conducted in a Solander box that did not contribute any detectable VOCs to the emissive profile of the object. The scaredevil study was conducted within a softwood storage crate that readily produced VOCs associated with cellulosic materials. As such, the aim of this experiment was to determine if the more volatile analytes of interest, such as naphthalene and camphor, could be detected in the presence of high concentrations of cellulosic VOCs emitted from both the object and storage materials. As before, sampling was conducted using Tenax tubes collecting in passive mode over a period of 28 d at ambient conditions before analysis by TD-GC-MS.

Figure 3.7: Scaredevil from Nicobar Islands, Bay of Bengal, in storage crate

3.3.2.8. British Museum Orsman Road weapons room case study

The weapons room at Orsman Road was used to store weapons such as swords and spears which were part of ethnographic collections. A large amount of the material in the weapons rooms was of natural origin such as leather grips and scabbards for

weapons, as well as wooden staves and shafts as can be seen in **Figure 3.8**. Natural material such as this is susceptible to infestation, and was selected as likely to have been treated with pesticides, making the location ideal for inclusion as a case study. Sampling with Tenax tubes was conducted in passive mode for 28 d before analysis by TD-GC-MS.

Figure 3.8: Rack in weapons room containing a number of carved wooden objects

3.3.2.9. British Museum Orsman Road Arctic furs case study

The arctic furs studied were stored between layers of tissue paper in PVC bags kept in drawers (**Figure 3.9**). This experiment was performed to examine the emissive profiles of furs, a significant component of natural history collections. While natural history objects have previously been examined (see **Section 3.5.2**), this was the first example of furs being studied in this work. As mentioned in **Chapter 1**, crates of $HgCl_2$ and As_2O_3 were often employed for preservation of animal skins while travelling.[27] The VOC sampling methods would not be able to detect inorganic biocides such as these, but was instead used to determine the presence of organic biocidal treatments while collecting information on the volatiles emitted by skins and furs. Sampling was performed using Tenax tubes deployed in passive mode over a period of 28 d before analysis by TD-GC-MS.

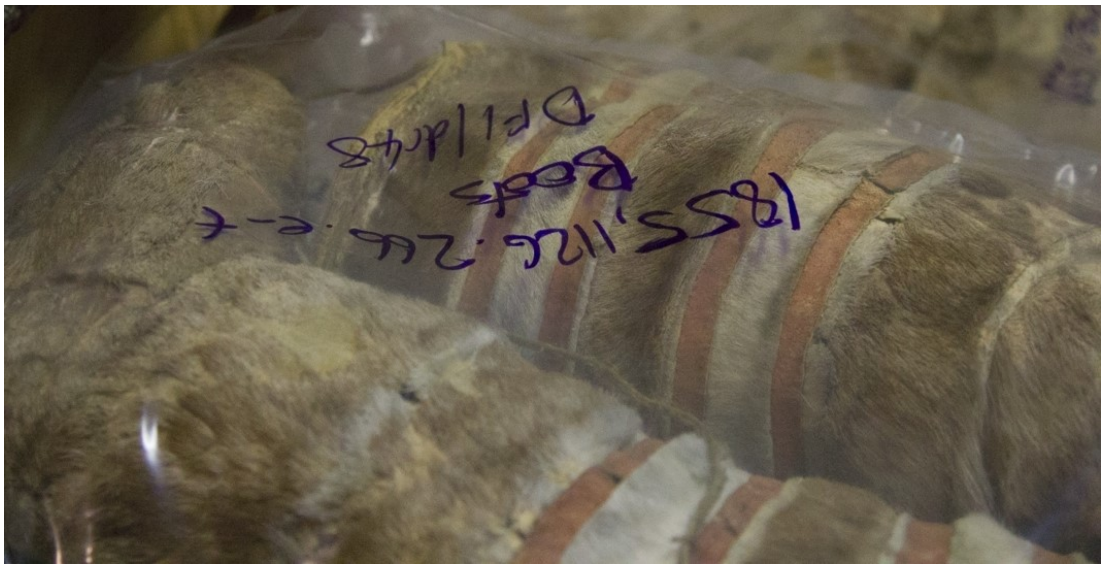


Figure 3.9: Arctic furs stored in bags at Orsman Road stores

3.3.2.10. British Museum Blythe Road large object store case study

The large object store (**Figure 3.10**) at Blythe Road was formerly a shipping container. The steel enclosure contained a number of wooden canoes, a detail of which can be seen in **Figure 3.11**, as well as various display cases, lighting stands and a small diesel powered generator. Sampling tubes were deployed next to two of the canoes (**Figure 3.12**) for 28 d before analysis by TD-GC-MS as described in **Section 3.3.1.1**.

Figure 3.10: Inside the large object store, Blythe road. Canoes are in racks, and a number of freestanding display cases are present also

Figure 3.11: Detail from canoe documenting vivid paintwork, suspected as a potential source of solvent VOCs if recently repainted

Figure 3.12: Canoe wrapped in plastic sheeting. Tenax tubes were placed on top of the sheeting for exposure

3.3.2.11. British Museum Blythe Road Mexican and Peruvian wool case study

Mexican wool from the textile conservation centre (TCC) was sampled *in situ* as conservation workers had complained that they had developed a strong smell similar to that of sour milk after pest control treatment using a fumigant gas. A number of samplers were deployed in passive mode for 28 d throughout the wool collection sampling the air inside wooden and fibre boxes containing the textiles. The locations sampled were TCC background air and boxes 28 and 30, each box containing a number of textile objects.

3.3.2.12. Bloomsbury Asia store case study

The Bloomsbury Asia store was a relatively small room measuring approximately 45 m^3. The room contained predominantly wooden artefacts from Eastern Asia. Objects were stored on steel shelving units lined with paper and foam padding (as seen in **Figure 3.13**, **Figure 3.14** and **Figure 3.15**). Sampling tubes were placed on open shelves in order to collect air from the room as seen in **Figure 3.16**. It was not possible to deploy a blank for this area, as the outer corridor to the room was open to outside air and would not produce a representative control. Sampling tubes were exposed in passive mode for 28 d before analysis by TD-GC-MS.

Figure 3.13: Objects stored in Asia room included a significant amount of wood, but also metals and gilding

Figure 3.14: Location of second set of Asia store samplers

Figure 3.15: Image illustrating the objects present in the Asia store

Figure 3.16: Exposure of Tenax tubes in Asia room on a shelf near an environmental monitor

3.3.2.13. British Museum crocodile mask case study

A crocodile-head dance mask (shown in **Figure 3.17**) from the Torres Strait Islands was selected for sampling after a collection worker experienced a sensitization reaction after exposure to the object. Debris from the object that had already become dislodged such as hair and fibres were extracted in 1 cm^3 95:5 hexane and IPA, and analysed by HPLC-UV.

Figure 3.17: Crocodile head dance mask used for sampling campaign © Trustees of the British Museum

3.4. Results and discussion, part A: development of methods suitable for implementation in heritage environments

Results and discussion for this chapter have been split into two sections, with part A describing development of the methods and part B detailing the case studies using the developed methods. Development of these methods was undertaken in order to investigate if 10 common heritage pesticides could be detected using non-contact, minimally invasive, *in situ* sampling devices.

3.4.1. Calibration of the TD-GC-MS for the determination of selected pesticides

The 10 key analytes were selected after consultation with project partners in heritage institutions. The analytes were selected based on their perceived prevalence of use in heritage collections. Analytes were analysed by directly injecting 2.5, 5.0, 7.5 or 10 μL of the diluted mixed standard solution plus a solvent blank onto conditioned Tenax tubes. This gave an analyte mass of 50, 100, 150 and 200 ng per tube, with a solvent blank, which were then analysed using the TD-GC-MS method described. Calibration data for the TD-GC-MS are shown in **Table 3.8**, with analytes listed in order of elution. High R^2 values were calculated for the calibration data for camphor, naphthalene, thymol, chloronaphthalene, dichlorvos and aldrin, indicating a high degree of correlation between peak area and concentration. However, the R^2 value for aldrin is

significantly lower than camphor, naphthalene, thymol, chloronaphthalene and dichlorvos, indicating the degree of correlation is lower for this analyte. Correlation coefficients for HCH and dieldrin were poor, indicating that there was not a strong relationship between peak area and concentration over the injections performed. It is likely that the high temperatures used in the thermal desorption of these analytes from the Tenax sorbent led to degradation of the analytes, giving poor correlation coefficients as a result of the poor recovery of these analytes.

Table 3.8: Linear regression data for analytes of interest

Entry	Analyte	Regression line	R^2 value
1	Camphor	y = 4946474x + 5	0.9936
2	Naphthalene	y = 7996573x + 2	0.9979
3	Thymol	y = 5392715x - 4	0.9979
4	Chloronaphthalene	y = 6692512x - 4	0.9958
5	Dichlorvos	y = 15422050x - 7	0.9937
6	Aldrin	y = 1062330x -24	0.8826
7	HCH	y = 736306x - 80	0.5360
8	Dieldrin	y = 413970x - 362	0.0642

The chromatogram from the injection of the 200 ng loading is shown in **Figure 3.18**, with the chromatographic region of interest from 12 min to 30 min expanded for clarity. The chromatogram confirmed that it was possible to desorb and detect 8 of the 10 target analytes from Tenax sampling tubes; the two exceptions being endrin and DDT. The low thermal stability of these substances is likely to have caused thermal breakdown during the desorption process, however the high desorption temperature was necessary to ensure full desorption and recovery of the analytes from the Tenax. Camphor, naphthalene, thymol, chloronaphthalene and dichlorvos were calculated to have highly correlating data, with R^2 values greater than 0.99 (refer to **Table 3.8**, entries 1-5). The R^2 value for aldrin was calculated to be 0.8826 (refer to **Table 3.8**, entry 6), which was lower than the value calculated for other analytes, although still indicated a high degree of correlation between the data. The low R^2 calculated for HCH

and dieldrin were due to the poor recovery values resulting from thermal degradation during desorption (refer to **Table 3.8**, entries 7 and 8).

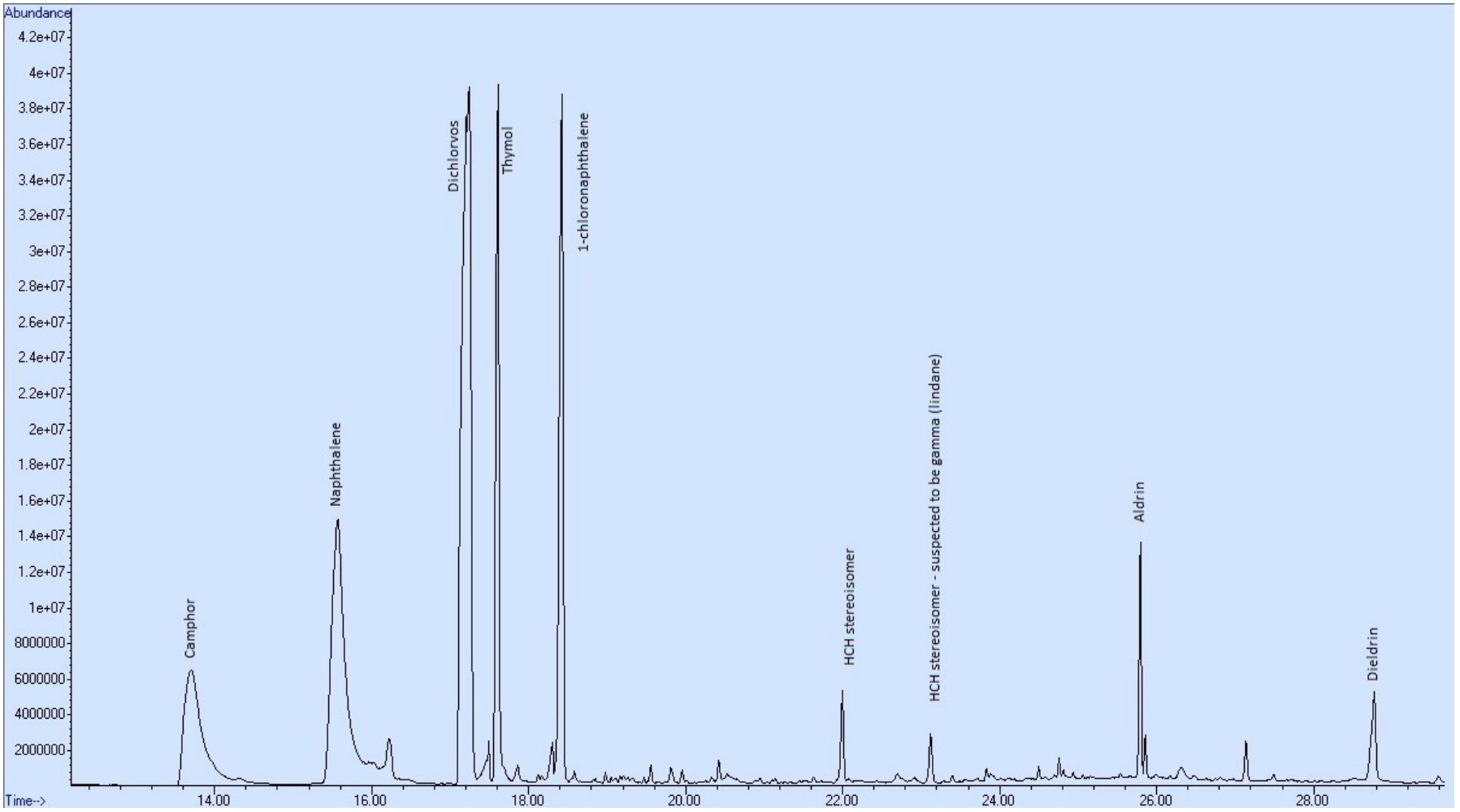


Figure 3.18: Region of interest (12-30 min) from TD-GC-MS chromatogram showing analyte resolution more clearly

3.4.2. Solvent extraction of pesticide loaded Tenax tubes and analysis by HPLC

Detection and quantification of the more thermally sensitive compounds (endrin and DDT) from Tenax tubes was examined using solvent extraction as an alternative to thermal desorption. Tenax tubes were loaded with known masses of pesticides and analysed by HPLC-UV using the operating conditions outlined in **Section 3.3.1.2** using a Jones C_{18} 4 μm, 4.6 x 250 mm column with an isocratic 90:10 acetonitrile:water mobile phase at 1.5 cm^3 min^{-1} and a 100 μL sample loop. Detector was a UV spectrophotometer set to 225 nm. The calibration data obtained (see **Table 3.9**) confirmed that this method of extraction could be used to quantitatively recover DDT, and also naphthalene, thymol, chloronaphthalene, dichlorvos and aldrin.

The chromatogram from the 7.5 ng μL^{-1} injection is shown in **Figure 3.19**. The figure shows that the majority of analyte peaks were baseline resolved, however isomers dieldrin and endrin co-eluted. An R^2 value of 0.9999 was calculated for the combined dieldrin and aldrin peak, indicating that there was still a highly correlating relationship between the area of this peak and concentration of the substances. R^2 values were calculated for all remaining peaks as being greater than 0.9700 which indicated a high degree of correlation between peak area and concentration. Camphor and HCH were not included as target analytes for this method as the molar absorptivity of camphor at 225 nm is very low, and HCH does not contain suitable UV chromophores.

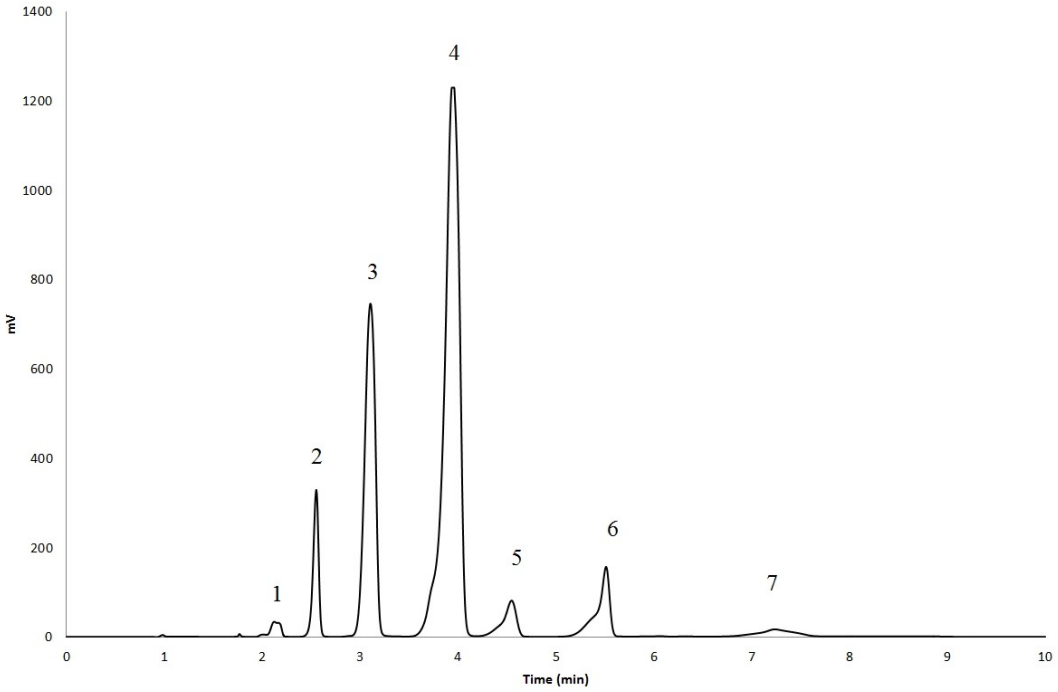


Figure 3.19: Chromatogram from 5 ng μL^{-1} calibration injection. 1 – dichlorvos, 2 – thymol, 3 – naphthalene, 4 – chloronaphthalene, 5 – endrin/dieldrin, 6 – DDT, 7 – aldrin

Table 3.9: Linear regression data for Tenax HPLC-UV method

Analyte	**Regression line**	**R^2 value**
Dichlorvos	y = 31141x - 0.4	0.9716
Thymol	y = 204833x - 0.02	0.9999
Naphthalene	y = 904422x - 0.3	0.9795
Chloronaphthalene	y = 1781937x - 0.1	0.9972
Dieldrin/Endrin	y = 144250x + 0.001	0.9999
DDT	y = 224620x + 0.004	0.9999
Aldrin	y = 62618x - 0.006	0.9999

3.4.3. Preconcentration of solvent extracted solutions

Desorption of trapped analytes using solvent washes was determined to be a more appropriate method for recovering thermally sensitive analytes than thermal desorption. The main drawback for solvent extraction was that due to the volume of solvent required for quantitative recovery, the analyte concentration was greatly reduced prior to analysis. In order to counter this effect, a method for preconcentrating solvent-extracted analytes was investigated.

Solutions containing analytes solvent extracted from Tenax tubes were successfully preconcentrated using the method described in **Section 3.3.1.2**. Tubes were prepared in triplicate by loading with known masses of analyte and preconcentrated before being analysed against an external standard for calibration and a sample eluted and analysed without the preconcentration step. Recoveries of the co-eluting endrin and dieldrin were calculated based on the mass of both analytes that had been applied to the tube. These results are shown in **Table 3.10**.

Recoveries for the solution eluted without the preconcentration were over the range of 98-102% mean recovery for individual analytes, with a mean recovery of 98% for all analytes. Values for relative standard deviation (RSD, calculated as the standard deviation divided by the mean, multiplied by 100) covered the range of 4-9%, with a mean of 6%. The low RSD values showed that the experiment had a high degree of repeatability and precision. Dichlorvos and aldrin were calculated to have the poorest RSD values at 9% and 7% respectively. As air sampling has inherently high uncertainty in quantifying analytes, concentrations of analytes measured in indoor air are typically considered equivalent within one order of magnitude.[16, 17, 35-37] Thus, RSD values in these recovery experiments of greater than 5% were considered acceptable.

Table 3.10: Recovery and RSD values determined from preconcentration experiment

	Recovered 5ng μl^{-1} standard (%)	**%RSD**	**Recovered 10x preconcentrated 0.5 ng μl^{-1} standard (%)**	**%RSD**
Naphthalene	98	4	96	6
Thymol	98	6	96	10
Chloronaphthalene	98	5	98	8
Dichlorvos	102	9	87	12
Aldrin	98	7	101	11
Dieldrin/Endrin	98	6	102	11
DDT	97	6	100	10

Analytes in solutions which underwent the preconcentration process were recovered over a range of 87-102% individually with a mean recovery of 97%. The lowest recovery was for dichlorvos at 87% and the highest was dieldrin/endrin at 102%. RSD values for the analyses were over the range of 6-12%, with an average of 10%. The higher RSD values of the preconcentrated samples were expected, as extra steps in sample preparation typically increases the imprecision of an analysis.

This experiment has shown that it was possible to preconcentrate the analytes to allow quantitative recovery of naphthalene, thymol, chloronaphthalene, dichlorvos, aldrin, dieldrin and endrin, and DDT with a mean recovery of 97%. The high recovery values for the most volatile analytes (naphthalene, 96%; thymol, 96%; and chloronaphthalene, 98%) confirmed that these analytes were not being lost in the preconcentration process allowing a single extraction and preconcentration process to be used for all analytes, regardless of volatility.

3.4.4. Analysis of solvent extraction solutions by gas chromatography-flame ionisation detection (GC-FID)

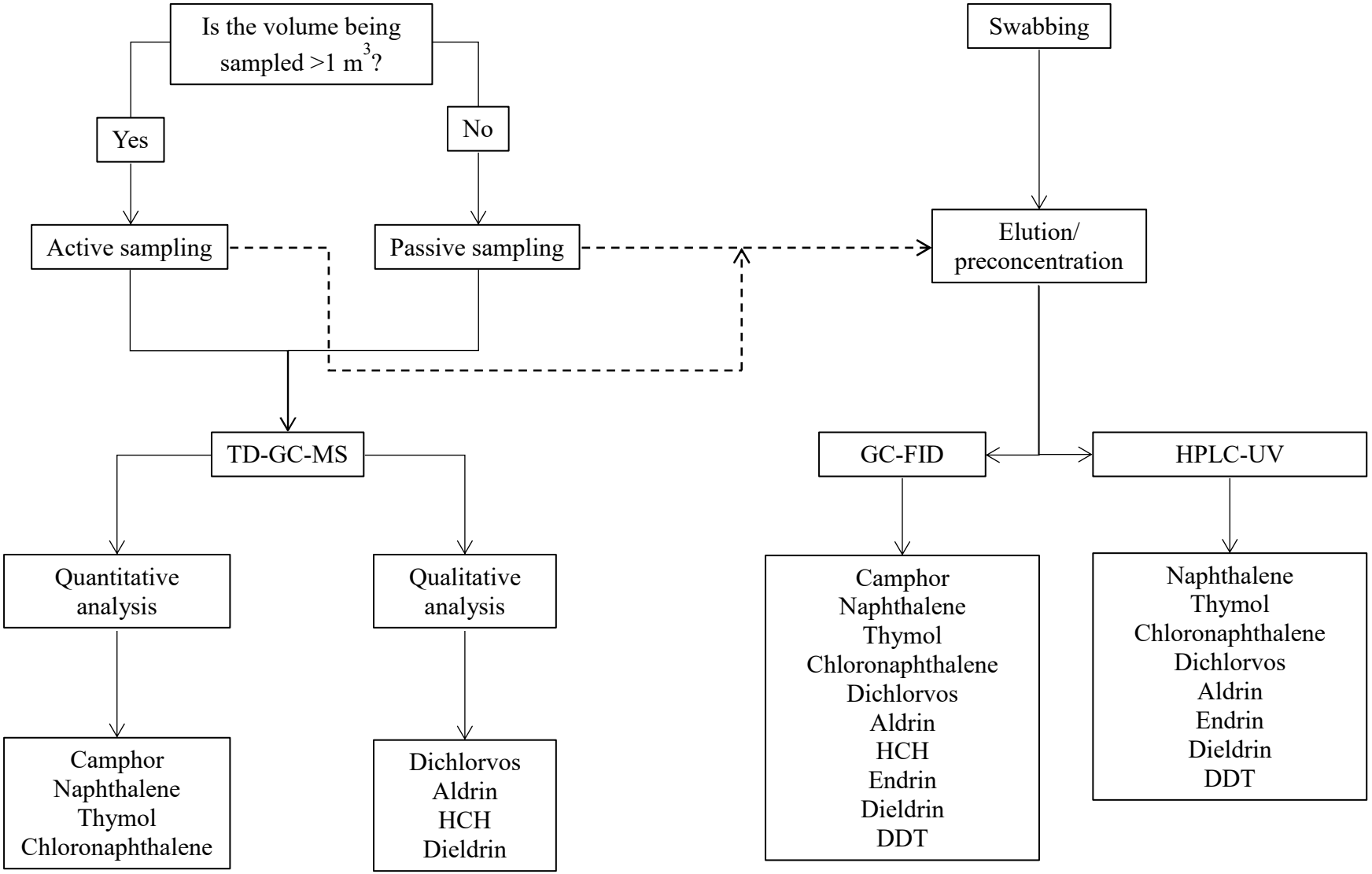
Analyte standard solutions prepared as in **Section 3.3.1.2** were solvent extracted from spiked Tenax tubes and analysed by GC-FID to assess the ability to determine the less thermally stable analytes. As the majority of the work in this method development study was performed using a mass spectrometer, the experiments undertaken using GC-FID were performed as qualitative indicators as to the suitability of the detector for these analytes only. Without using the high thermal desorption temperature, it was possible to detect previously detected analytes naphthalene, thymol, chloronaphthalene, dichlorvos and aldrin. Significantly, endrin and DDT were detected in the standard solution too, indicating that these analytes are detectable without the high temperature of the TD-GC-MS analysis.

3.4.5. Proposed sampling strategies for individual collections using developed methods

The instrumental methods presented above each have advantages and limitations which may not be immediately apparent to heritage institution staff who do not have a scientific background. As such, it was considered an important outcome of this research that museum personnel be able to use the methods described in this work appropriately, and according to the needs of their own collections. To this end, a flowchart is presented in **Figure 3.20**, which incorporates sampling and analysis strategies for the 10 target analytes in order to help collection staff identify appropriate sampling and analysis methods. For example, if collection staff suspected the presence of a particular analyte then the flowchart may be followed from bottom to top, allowing identification of a suitable instrumental method of detection and sampling strategy. Conversely, if collection staff wished to perform a broad screen of their collection but are restricted in terms of the sampling needs of a particular object, the flowchart may be followed from top to bottom which would highlight to staff which analytes were likely to be detected using a particular sampling method. This flowchart can potentially allow users from a non-scientific background to make informed choices about analytical strategies for collections without any prior knowledge of analytical instrumentation or sampling techniques. It was further proposed that this flowchart

may be able to empower collection custodians to hire external laboratories to perform analyses tailored to their individual collection needs by following the flowchart which could potentially significantly increase the utility of such external services to heritage institutions. A detailed sampling protocol for the collection of passive and active air samples and solvent swabs is presented in **Appendix A**.

Figure 3.20: Tenax sampling methods for pesticides in heritage environments



3.5. Results and discussion, part B: assessment of sampling methods using case studies in heritage environments

Case studies across UK heritage institutions were used to assess the methods developed in part A of this work. Following the flowchart presented in **Figure 3.20**, collections were typically sampled using Tenax tubes in passive mode for 28 d, before analysis of the tubes was performed using TD-GC-MS or HPLC-UV where appropriate. Concentration of the analyte in the vapour-phase was calculated by dividing the mass trapped by the sample volume and expressing in μg m^{-3}. Tenax sampling tubes were deployed in a number of locations in heritage institutions in the UK. The 10 key analytes were quantified using external standard solutions. The collection VOC data was not the focus of this investigation, but the data were interrogated to give better understanding of the commonalities and differences between the VOC profile of different sampling locations. As a result, there were too many VOC analytes detected to be able to quantify using individual reference standards. Instead, all but the most common of these substances have been quantified against a toluene external standard and expressed as toluene equivalents, following a precedent commonly used in indoor air publication.[37-43] The use of toluene equivalents should only be considered as an approximation of the VOC concentrations detected, and as such these results should not be interpreted as having the same degree of analytical rigour as the biocide results as the response factor for both the unknown analyte and toluene cannot be assumed to be the same. A list of analytes quantified from individual standards is shown in **Table 3.11**, while remaining analytes were calculated as toluene equivalents.

Table 3.11: Analytes quantified from individual standards

Analyte	Analyte type
Aldrin	Key biocide analyte
Camphor	Key biocide analyte
Chloronaphthalene	Key biocide analyte
DDT	Key biocide analyte

Analyte	Analyte type
Dichlorvos	Key biocide analyte
Dieldrin	Key biocide analyte
Endrin	Key biocide analyte
HCH	Key biocide analyte
Naphthalene	Key biocide analyte
Thymol	Key biocide analyte
Acetophenone	Common VOC
Benzaldehyde	Common VOC
Cumene	Common VOC
p-Dichlorobenzene	Common VOC/non-key biocide
o-Dichlorobenzene	Common VOC
Ethylbenzene	Common VOC
Furfural	Common VOC
Toluene	Common VOC

3.5.1. Risk assessment of results generated by sampling techniques

The purpose of this work was to allow heritage institution staff to adequately risk assess their collections. It may be difficult for staff not from a chemical sciences background to decide on an appropriate level of risk associated with their collections without guidance on the data generated through the use of the methods presented here. To aid this, a "traffic lights" system is suggested in order to add context to results generated by sampling activities. Traffic light colours of red, amber and green were assigned to each sampling location in order to show the associated risk of working within each sampling area, with the caveat that precautions should always be taken to ensure exposure to even low hazard chemicals should be as low as is reasonably practicable. The traffic light rating pertains

to biocidal analytes only, as VOC data were not considered. The criteria for assigning each colour are as follows:

Green – risk is presumed low. No biocidal substances were detected from samples of this environment. However, it is recommended to use nitrile gloves conforming to EU Directive 89/686/EEC and the standard EN 374 derived from it for handling objects from this location to further minimise risk of exposure.

Amber – risk is presumed medium. Biocidal residues have been detected in this sampling location, however they are below EU short term exposure limit thresholds, or such a threshold does not exist for the biocide detected. Detection of substances with H-phrases corresponding to sensitizing, mutagenic or carcinogenic properties as described in **Table 3.12** will also trigger an amber risk warning at minimum.

Table 3.12: Risk phrases used to determine medium "amber" risk objects

H-phrase	Hazard statement
H317	May cause an allergic skin reaction
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled
H340	May cause genetic defects
H341	Suspected of causing genetic defects
H350	May cause cancer
H351	Suspected of causing cancer

It is strongly recommended that nitrile gloves conforming to EU Directive 89/686/EEC and the standard EN 374 derived from it be used when handing objects form this area, and work on objects from this sampling location be performed in a well ventilated area.

Red – risk is presumed high. High concentrations of biocidal residues have been detected in this sampling location, that are approaching or exceeding EU short term exposure limits for this substance. Alternatively, a substance has been detected with indications of an LD_{50} value of less than 5 mg kg^{-1} suggesting extreme toxicity. It is strongly recommended

that access to this area be highly restricted, and that professional advice be sought for decontamination of affected objects. An individual substance specific COSHH assessment should be performed prior to working in this area.

The traffic light system has been used in case studies here in order to indicate the level of risk present. It should be stressed to the institution that these hazard assessments should be considered advisory only, and that each institution will be required to perform their own COSHH assessments. As such, the risk assessment procedure used here may be considered quite conservative as it was believed to be better overly cautious than risk unnecessary exposure to any chemical hazards detected.

3.5.2. Swiss Cottage natural history collection case study

The natural history collection in Swiss Cottage was selected as a case study location as the cases housing the collection were not opened often, allowing preconcentration of any vapour-phase analytes and VOCs present. It was anticipated that as the cases housed a natural history collection, the sampling tubes would collect a number of pesticides, most likely naphthalene and camphor. As the collection was housed in cases too small to allow active sampling without the introduction of a diluent flow, the cases were sampled in passive mode before analysing the tubes by TD-GC-MS.

Table 3.13 details the components in the sample with concentrations greater than 1.0 μg m^{-3} which were calibrated with individual standards. The background sample of air from the room containing the cases displays increased concentrations of chlorinated solvents trichloroethylene (TCE) and tetrachloroethylene (PCE). The concentrations were calculated as 1.46 and 2.63 μg m^{-3} for TCE and PCE respectively, which were well below the EU 8 hr time weighted average (TWA) exposure of 550 and 345 mg m^{-3}. The background sample also contained low concentrations of simple six-membered aromatics, toluene and acetophenone, at 1.34 and 1.19 μg m^{-3} as well as 1.73 μg m^{-3} furfural. Due to the presence of aged wood in the construction of the display cases, these VOCs were attributed to the hydrolysis of the lignin and cellulose in the wood.[30, 44, 45]

A number of pesticidal compounds were detected in the air sampled from the cases. Chloronaphthalene was detected in all sampling locations inside object cases and was

missing from the blank sample, indicating previous pesticidal treatment. Concentrations of chloronaphthalene ranged from 1.10 – 2.54 μg m^{-3} with a mean concentration of 1.58 μg m^{-3}. Naphthalene was detected in the background and case 33, which was sampled in duplicate. The mean concentration of naphthalene in case 33 was 1.48 μg m^{-3}. Camphor was detected between 1.07 and 2.68 μg m^{-3} over all cases with a mean concentration of 1.63 μg m^{-3}. The difference in concentration for the analytes sampled can be attributed to the precision of the passive sampling method which has been reported at between 12 and 17%.[46, 47] In addition to camphor, case 33 also contained the related compound borneol, calculated as a toluene equivalent. Borneol is the reduced form of camphor, which was detected at 1.25 and 1.33 μg m^{-3}. Of the pesticidal agents detected, only camphor has a UK workplace exposure limit.[48] The value of the workplace exposure limit was 13 mg m^{-3} over an 8 h period, which was significantly higher than the concentrations detected here, indicating that although pesticides have been detected, they are not at harmful levels.[48]

The remainder of the VOC profiles for these sampling environments was comprised of terpenoids 3-carene and cymene as well as wood-related breakdown products furfural and benzaldyhyde.[45] The aldehyde nonanal (1.18 μg m^{-3}) was also present in case 33 as was the corresponding carboxylic acid, nonanoic acid (1.32 μg m^{-3}). The presence of longer chain aldehydes were not considered out of the ordinary for indoor air samples such as these, as the substances are produced by a variety of emissive sources such as paints and adhesives.[30]

Table 3.13: Significant (>1.0 μg m^{-3}) peaks contributing to volatile profiles determined from Swiss Cottage sampling

Location	**Background sample**	**Case 41**	**Case 40**	**Case 33 (1)**	**Case 33 (2)**	**Case 31**
Compound	**Concentration μg m^{-3}**					
Trichloroethylene*	1.46	-	-	-	-	-
Toluene	1.34	-	-	-	-	-
Perchloroethylene*	2.63	-	-	-	-	-
Furfural	1.73	1.65	2.11	6.76	4.56	9.52
Acetophenone	1.19	-	-	-	-	-
Benzaldehyde	-	1.19	1.14	2.11	1.68	3.03
Camphor	-	1.09	1.07	1.60	1.71	2.68
α-Pinene*	-	1.10	-	-	-	1.20

Table 3.13: Significant (>1.0 µg m^{-3}) peaks contributing to volatile profiles determined from Swiss Cottage sampling

Location	Background sample	Case 41	Case 40	Case 33 (1)	Case 33 (2)	Case 31
Compound	**Concentration µg m^{-3}**					
Chloronaphthalene	-	1.31	1.10	1.17	1.78	2.54
Borneol*	-	-	-	1.25	1.33	-
Naphthalene	2.79	-	-	1.61	1.44	-
3-Carene*	-	-	-	1.15	-	-
Nonanal*	-	-	-	-	1.18	-
Nonanoic acid*	-	-	-	-	1.32	-
Cymene*	-	-	-	-	-	1.18
Phenylvinylacetylene*	-	-	-	-	-	2.31

*Calculated as toluene equivalents

The use of the Tenax tubes as passive sampling devices for determining the presence of vapour-phase pesticides has been shown with this case study. Both naphthalene and chloronaphthalene were detected in the locations sampled here, verifying the method selection flowchart presented in **Section 3.4.5**. All sampling locations in this case study were classes as amber risk level due to the presence of chlorinated solvents.

3.5.3. British Museum rubber garments case study

The British Museum rubber garments were selected for case study as the objects were reported by conservators as already having a strong unpleasant odour. It was suggested that these articles dating from the late 1980s, and may have been treated with pesticides. These objects provided a case study where high concentrations of VOCs attributed to the strong odour could potentially mask pesticide peaks on a chromatogram, providing a challenging sampling environment in which to assess the developed methods.

The results from the Tenax sampling tubes indicated that tetrachloroethylene, alkanes, methyl- and dimethyl-alkanes, cyclosiloxanes and benzoic acid were present at varying levels in both the background samples as well as the object enclosures. **Table 3.14** summarises the VOCs detected in each of the sampling locations, giving their concentration in µg m^{-3}. Mass spectrometry total ion chromatograms (TICs) obtained from the background samples and from the enclosures containing the rubber garments are shown in **Figure 3.21** and **Figure 3.22**. In the corridor areas, the most significant VOCs

were found to be straight chain and branched alkanes (isoalkanes) and tetrachloroethylene. Isoalkanes are typically generated by building materials such as synthetic resins, paints and residual solvents.[30] The source of the tetrachloroethylene could not be identified, but the concentration detected was significantly below the European Union time-weighted average workplace exposure limit (EU TWA WEL) of 138 mg m^{-3}.[49] Acetophenone, ethylbenzene and toluene were also detected. These substances are common VOCs emitted by cellulosic materials, and were likely to have been generated by natural ageing of materials in the sampling location.

All VOCs detected in the enclosures were present in the background samples and, with the exception of tetrachloroethylene, have been commonly reported in several museum environments.[16] The most significant difference noted between the enclosures and the background samples was in the concentration of cyclosiloxanes detected. The total concentration of the detected volatile cyclosiloxanes was 32.8 mg m^{-3} for the trousers and 37.3 mg m^{-3} for the rain cape. Background analysis of the corridors showed siloxane concentrations of 1.47 and 0.67 mg m^{-3} suggesting that as the garments were approximately 25 years old at the time of sampling, siloxanes may have been involved in the manufacture of the garments and could be attributable to a substance such as an aerosol-based waterproofing treatment, as these typically contain mixtures of cyclic siloxanes.[50]

Table 3.14: Summary of recurring contributing VOCs in each sampling location

Location	**Corridor background**	**Room background**	**Rain cape Am1988,08.672**	**Trousers Am1988,08.670b**
Compound	**Concentration µg m^{-3}**			
Acetophenone	1.59	8.16	-	-
Alkanes*	35.5	12.3	7.2	11.2
Benzoic acid*	13.3	33.2	17.2	24.6
Butanoic acid, butyl ester*	-	3.23	-	2.28
Ethylbenzene	2.03	0.90	-	-
Isoalkanes*	37.9	15.7	89.0	9.36
Nonanoic acid*	2.04	3.11	-	-

Pentafluoropropionic acid, octadecyl ester*	3.18	-	0.4	3.03
Siloxanes*	1.47	0.67	37.3	32.7
PCE*	36.3	18.8	-	16.8
Toluene	5.16	1.82	-	-

*Calculated as toluene equivalents

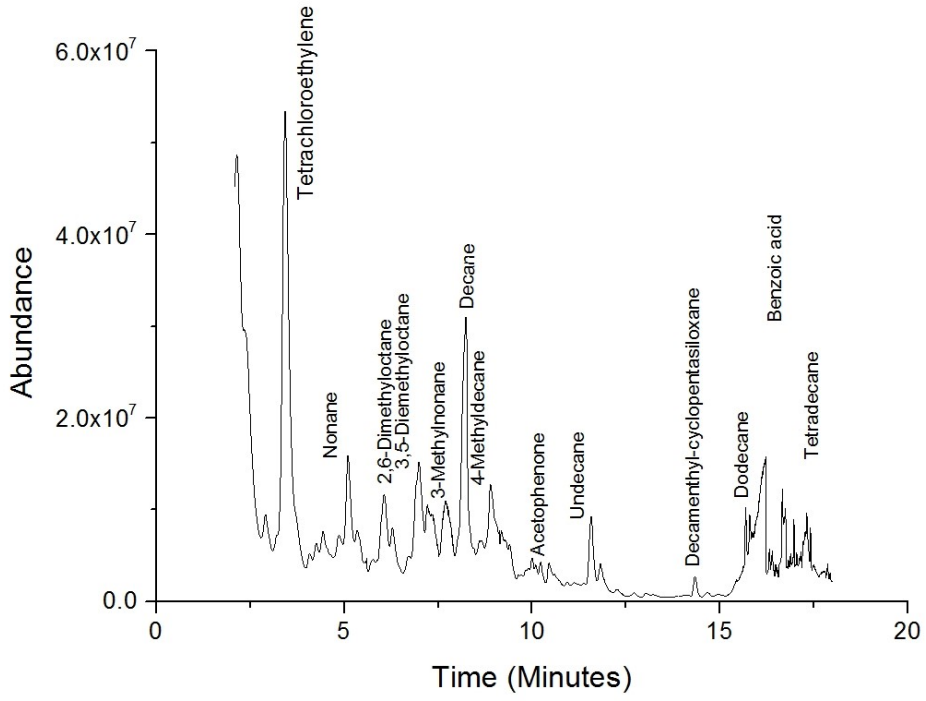


Figure 3.21: Typical TIC for the corridor and office backgrounds around British Museum rubber garment, shown here by the corridor location sample.

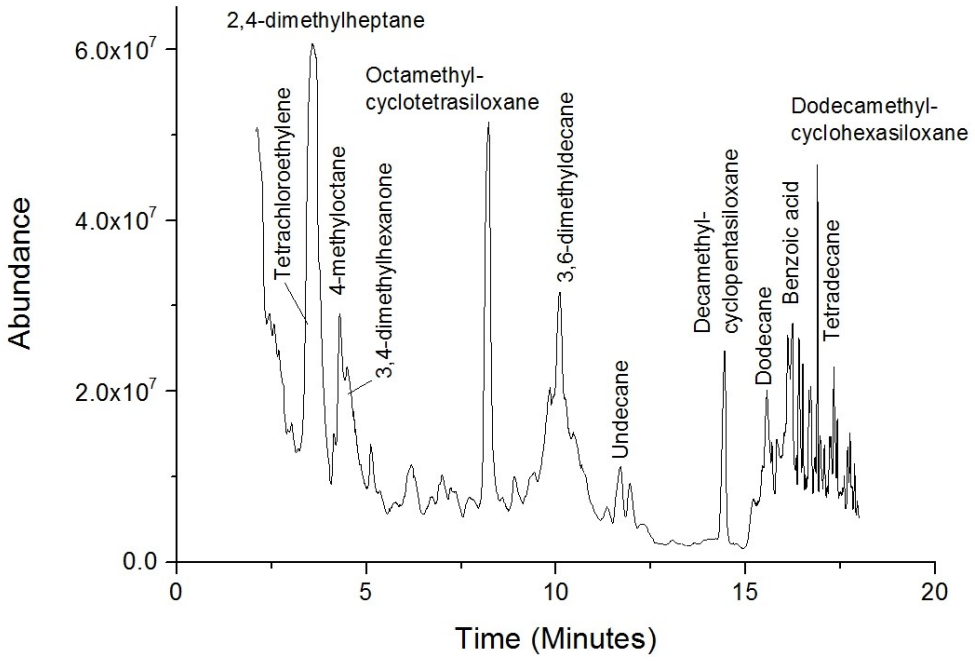


Figure 3.22: Typical TIC shown by Am1988,08.670b trousers and rain cape Am1988,08.672. Example above is from the rain cape.

None of the 10 target pesticides were detected in any of the samples associated with this case study. Further interrogation of the mass spectrometric data was performed by extracting *m/z* values associated with target analyte parent ions from the TIC.[51] This confirmed that none of the target analytes had been detected. It was also not possible to identify the source of the strong odour associated with the objects. This is likely to be attributable to the method used, which was not intended for use characterising low molecular weight organic acids or carbonyl sulfide compounds often responsible for such odours, but rather the identification of higher molecular weight pesticides.[52] The risk level associated with these case study samples was green as no biocides were detected.

3.5.4. National Records of Scotland Station Road strongroom case study

The National Records of Scotland strongroom was selected as a case study because the sampling location contained a number of paper items. Conservators of the National Records of Scotland reported that thymol had been used in other National Records of Scotland collections and it was possible that the substance was used here, although this

was not known for certain. The strongroom was sampled in duplicate and a third was used to collect a background sample from the area outside the strongroom. On visiting the strongroom to deploy sampling tubes, it was noticed that the strongroom contained a ventilation duct which prevented establishing a fully sealed sampling environment as expected. However, this allowed an opportunity to demonstrate the sampling method in an uncontrolled environment. The space outside the strongroom was a large warehouse storage area containing a number of smaller subdivisions previously used as office space. The room contained disused office furniture and stationery including paper files.

Peaks significantly contributing to the TIC are summarised in **Table 3.15** below. Examination of the TIC did not indicate that thymol had been previously used on the items in this strongroom. However, a low concentration of naphthalene was detected in the external blank (0.63 µg m^{-3}) and higher concentrations (1.55 and 2.12 µg m^{-3}) were detected inside the strongroom. The presence of naphthalene in the background at lower levels than the samples was taken to infer that naphthalene, perhaps as part of mothball formulations, had previously been used to control infestation of the archival material here. The remainder of the VOC profile was attributed largely to straight chain and branched alkanes, which are common in indoor air. The VOCs identified outside the strongroom contained increased concentrations of benzaldehyde and cymene (1.55 and 3.95 µg m^{-3}), attributed to the breakdown of cellulosic materials such as paper or wood products.

Table 3.15: Analytes corresponding to significant peaks in Station Road sampling site chromatograms

Location	**Background sample**	**Strong-room 1**	**Strong-room 2**
Compound	**Concentration µg m^{-3}**		
m-Menthane, (1S,3R)-(+)-*	-	14.25	-
Cyclohexane, butyl-*	-	13.12	-
Decane*	-	13.06	13.89
Decane*	-	11.26	12.97
Nonane, 3-methyl-*	-	10.36	5.78
Naphthalene, decahydro-*	-	10.15	10.37
Ethanone, 1-cyclohexyl-*	-	9.69	-
cis-1-Ethyl-3-methyl-cyclohexane*	-	9.25	-
9-Methylbicyclo[3.3.1]nonane*	-	7.17	8.22

Location	Background sample	Strong-room 1	Strong-room 2
Pentafluoropropionic acid, hexadecyl ester*	-	6.37	-
trans-1,2-Diethyl cyclopentane*	-	6.26	-
Decane, 3-methyl-*	-	6.02	6.45
Undecane*	-	5.65	6.36
1-Ethyl-4-methylcyclohexane*	-	5.44	5.13
3-Eicosene, (E)-*	-	5.39	-
Decane, 2,5,6-trimethyl-*	-	5.37	4.83
Octane, 2-methyl-*	-	4.98	-
Decane, 2-methyl-*	-	4.84	5.03
Cyclohexanone, 2-ethyl-2-propyl-*	-	3.92	4.40
Cyclohexane, 1-methyl-4-(1-methylethenyl)-, trans-*	-	3.70	-
Hexanal*	-	3.17	2.87
Benzoic acid*	4.89	5.76	4.42
Tridecane*	1.4	2.90	1.87
Cyclopentane, propyl-*	-	2.80	-
1-Nonadecene*	-	2.70	-
17-Pentatriacontene*	-	2.67	-
trans-Decalin, 2-methyl-*	-	2.64	3.08
Dodecane*	1.49	2.17	2.59
Cyclohexane, 1-methyl-2-pentyl-*	-	1.90	-
Spiro[4.5]decane*	-	1.77	-
2H-Inden-2-one, octahydro-3a-methyl-*	-	1.58	-
Naphthalene	0.63	1.55	2.12
Tetradecane*	0.72	1.44	1.41
2,6-Octadiene, 2,6-dimethyl-*	-	1.31	-
Toluene	-	1.30	1.46
Dodecanoic acid, 1-methylethyl ester*	-	1.30	-
2,5-Cyclohexadiene-1,4-dione, 2,5-diphenyl-*	-	1.09	-
2-Butenedioic acid, monododecyl ester	-	1.05	-
1,1-Dimethylethylamine, N-methoxycarbonyloxy*	1.18	-	-
Benzaldehyde	1.55	-	-
Cymene*	3.95	-	-
1-Hexanol, 2-ethyl-*	1.13	-	-
Nonanal*	1.62	-	-
Ethanol, 2-phenoxy-*	1.17	-	-
Ethanol, 2-(2-butoxyethoxy)-, acetate*	1.07	-	-

*non-target analytes quantified as toluene equivalents

This case study highlighted that the application of pesticidal treatments across institutions was not necessarily uniform, as no thymol was detected on this archival material but was reported as having been used in other collections housed by the same institution. The detection of naphthalene in the samples may be indicative of a previous interventive pest treatment and shows that the methods developed here can be used to collect concentrations of VOCs in excess of 10 μg m^{-3} in uncontrolled environments using 28 d sampling periods. All samples in this case study were assigned amber risk status due to the presence of naphthalene.

3.5.5. RAF Museum case study

The RAF museum was used as a case study in order to determine the presence of the target pesticides across several collection types within a single institution. Sampling was performed in a number of locations: a curatorial office; 4 Portastor modular storage units; an archive storeroom; a library storeroom; an office; a cleaning studio; and a storage room used for film. It was expected that sampling in each of the locations (which contained different materials), different emissive profiles would be collected and that pesticides used in these locations may be common to collection types.

Table 3.16 below shows the major peaks in the TIC from each of the sampling locations. Camphor, naphthalene and chloronaphthalene were the only target analytes identified in this case study. Camphor was detected in Portastor 2 at a concentration of 0.12 μg m^{-3}. The sampling location was being used to house a film collection, and also contained phenol at a concentration of 0.76 μg m^{-3}. The combination of phenol and camphor in a film store suggests that the presence of camphor in this instance can be attributed to its use as a plasticiser in cellulose acetate, as historically both camphor and phenol have been used for this purpose.[53, 54] The significance of this finding was that for these components to be off-gassing, it suggested degradation of the polymers comprising the film. As such, this indicated these methods could further be used to gain direct insight into the condition of the objects contained within collections. Similarly, this sampling location showed that care must be taken when attributing sources of VOCs, as camphor in this instance was much more likely to have been used as a plasticiser instead of a biocidal agent. Naphthalene was detected in two locations at the RAF museum: the archival store room

at a concentration of 0.09 µg m^{-3} and in the DoRIS office at a concentration 0.05 µg m^{-3}. It was possible that the presence of naphthalene was as a result of pesticidal treatments but the low concentrations present suggested that the application of naphthalene had not been performed recently. The film store contained a low concertation of chloronaphthalene at 0.03 µg m^{-3}. As this was the only location sampled in this institution containing chloronaphthalene, it was not considered likely that this was applied as a pesticide.

Other VOCs were also present in the samples. The curatorial office contained very low levels of VOCs, with the highest detected concentration being 0.04 µg m^{-3} for benzaldehyde. This is in contrast to the DoRIS office, which contained a greater number of VOCs (8 in total, versus 3 from the curatorial office), but also higher concentrations of these VOCs as the mean concentration in the DoRIS office was 0.56 µg m^{-3} compared to 0.03 µg m^{-3} in the curatorial office. As these locations were in use throughout the sampling, it is possible that the increased foot traffic in these areas contributed to a greater number of air exchanges and thus lower concentrations of VOCs.[55]

Excluding benzaldehyde, which will be discussed later, the siloxane species are the most abundant compounds present in the samples. In the chromatogram for the archive storeroom, 3 of the 10 major peaks were cyclic siloxanes. Cyclic siloxanes have been named in this thesis according to the convention of numbering according to the number of repeating $[(CH_3)_2SiO]$ subunits. For example, octamethylcyclotetrasiloxane is D4-siloxane, named for the 4 repeating subunits in the structure. The presence of the siloxanes can possibly be attributed to carpeting materials, sealants/caulks, or personal care products.[56] The use of personal care products may also be supported by the presence of low levels of limonene, a chemical often found in cleaning and fragrance products. Schieweck *et al.*[16] reported in 2005 siloxanes trapped in a similar manner ranging from 2-102 µg m^{-3}, and so the values here are in accordance with published literature. The presence of dimethyl and diethyl phthalate was detected in the samples. Phthalates are typically used as plasticisers in a number of materials and can be found in indoor air *via* off-gassing from said plastics. Examples of this can be found from electrical equipment

such as televisions or monitors and other plastics used for consumer electrical goods or where plastics may be encountered at temperatures higher than ambient conditions.[30]

Data collected from the archive storeroom refer to the presence of cellulosic and lignin breakdown products such as furfural (2.03 μg m^{-3}, calibrated against a furfural standard), toluene (1.08 μg m^{-3}), xylene (1.35 μg m^{-3}), pinene (2.39 μg m^{-3}), benzaldehyde (2.44 μg m^{-3}) and carene (2.54 μg m^{-3}). The concentrations detected here were in good agreement with previously sampled locations.[57] These compounds were also found in other locations containing largely paper based collections, such as the library storeroom, Portastors 1 and 2, and the DoRIS office, further reinforcing the correlation between cellulosic fibres and these analytes. The data regarding cellulosic and wood-based VOCs and their sampling locations have been summarised in **Table 3.17** below.

Table 3.16: Analytes corresponding to major peaks in chromatograms from RAF sampling campaign

Location	**Curatorial office**	**Portastor 1**	**Portastor 2**	**Portastor 3**	**Portastor 4**	**Archive storeroom**	**Library storeroom**	**DoRIS Office**	**Cleaning studio**	**Film store**
Compound	**Concentration µg m^{-3}**									
Decanal*	0.01	0.03	0.55	-	-	-	-	0.09	-	-
Butylated hydroxyanisole*	0.03	-	0.01	0.05	-	-	-	0.68	-	-
1,2-Dichloroethane*	-	0.14	-	-	-	-	-	-	-	-
Benzoic acid*	-	0.04	-	-	-	2.39	-	-	-	-
Benzaldehyde	0.04	-	-	0.04	-	2.44	2.44	0.09	-	-
Phenol*	-	-	0.76	-	-	-	-	-	0.22	-
Xylene*	-	-	0.28	-	-	-	-	0.13	-	-
Dimethyl phthalate*	-	-	0.04	0.04	0.03	0.34	-	-	0.01	-
Hexadecane*	-	-	-	0.02	-	0.28	-	-	-	-
Butyl butanoate*	-	-	-	0.04	0.02	1.75	-	-	-	0.05
Diethyl phthalate*	-	0.01	0.01	0.02	0.02	0.25	-	-	-	-
D3-siloxane*	-	-	-	-	-	1.77	0.13	-	-	-

Table 3.16: Analytes corresponding to major peaks in chromatograms from RAF sampling campaign

Location	**Curatorial office**	**Portastor 1**	**Portastor 2**	**Portastor 3**	**Portastor 4**	**Archive storeroom**	**Library storeroom**	**DoRIS Office**	**Cleaning studio**	**Film store**
Compound	**Concentration µg m^{-3}**									
1,3,5,7-Cyclooctatetraene*	-	-	-	-	-	2.09	-	-	-	-
D4-siloxane*	-	-	-	-	-	3.85	0.43	0.30	-	-
D5-siloxane*	-	-	-	-	-	5.34	-	0.54	-	-
Furfural	-	-	-	-	-	-	0.99	0.68	-	-
Limonene*	-	-	-	-	-	-	0.67	0.67	-	-
Nonanol*	-	-	-	-	-	-	-	-	0.02	-
Naphthalene	-	-	-	-	-	0.09	-	0.05	-	-
Chloronaphthalene	-	-	-	-	-	-	-	-	-	0.03
2-ethyl-1-hexanol*	-	-	-	-	-	-	-	-	0.11	-
Triacetin*	-	-	-	-	-	-	-	-	0.20	-
2-Methyl-2-ethyl-3-hydroxyhexyl ester Propanoic acid*	-	-	-	-	-	-	-	-	0.20	-

Table 3.17: Occurrence of cellulosic and wood-related breakdown products from RAF museum site

Location	**Curatorial office**	**Portastor 1**	**Portastor 2**	**Portastor 3**	**Portastor 4**	**Archive storeroom**	**Library storeroom**	**DoRIS Office**	**Cleaning studio**	**Film store**
Compound	Concentration μg m^{-3}									
Toluene*	-	-	0.55	-	-	1.08	0.25	0.09	-	-
Furfural	-	-	-	0.05	-	2.03	0.98	0.68	-	-
Xylene*	-	-	0.28	-	-	1.35	0.08	0.13	-	-
α-pinene*	-	0.03	-	-	-	2.39	0.20	0.19	-	-
Benzaldehyde	0.04	-	-	0.04	-	2.44	0.15	0.09	-	-
β-myrcene*	-	-	-	-	-	-	0.02	-	-	-
Carene*	-	-	-	-	-	2.54	0.12	0.12	-	-
Limonene*	-	-	-	-	-	2.21	0.67	0.67	-	-

*Calculated as toluene equivalents

The sampling performed in this location allowed comparison of different collection compositions. The use of uncontrolled environments allowed the sampling to be performed in as minimally invasive a manner as possible. Differences in the VOC profiles of each sampling location were observed based on the type of objects stored in each location, with paper-based collection items contributing larger amounts of cellulosic breakdown products and plasticisers camphor and phenol, which were detected in locations containing cellulose acetate films. It was possible to detect naphthalene, chloronaphthalene and camphor in sampling locations amongst other VOCs, but due to the widespread uses of these compounds it was not possible to identify emissive sources for naphthalene and chloronaphthalene. The archive store room, DoRIS office and film store were given amber risk status due to the presence of naphthalene and chloronaphthalene. All other sampling locations were considered to be low risk, and were given green status.

3.5.6. National Records of Scotland Thomas Thomson House case study

The sampling campaign performed at the National Records of Scotland was designed to look at primarily photographic and paper-based material. The sampling undertaken at the RAF museum detected the presence of phenol and camphor which were likely used as plasticisers for the cellulose acetate film as opposed to interventive pest control measures. Sampling of paper-based collections at RAF museum showed the presence of a number of VOCs commonly attributed to cellulosic breakdown products, which were expected in similar concentrations in samples collected from the Thomas Thomson House site. Identification of VOCs was performed by air sampling in passive mode over 28 d before analysis by TD-GC-MS.

Cellulosic breakdown products were seen in 4 of the 6 locations chosen for study at the National Records of Scotland, and these substances were approximately equivalent to the concentration seen at the other case study locations such as the RAF Museum (**Section 3.5.5**). As detected in other case study locations, peaks attributed to hydrocarbon chains such as nonane and decane were present. Major peaks for each sampling location have been summarised in **Table 3.18**.

Across the two rooms sampled, chlorinated solvents such as trichloroethylene (TCE) and tetrachloroethylene (PCE) are common. This sampling location also showed

evidence of the presence of siloxane species in common with the British Museum rubber samples (**Section 3.5.3**). The siloxanes detected in this campaign were of a typically lower level than the other VOC sampling sites, despite their presence being more widespread throughout the samples collected. In this instance, the concentration of the measured siloxane species was over the range of 0.1 – 3.12 μg m^{-3}, which still corresponded to a very low concentration in the indoor air environment.

Camphor, phenol and naphthalene were also detected during this case study. While in the RAF museum the presence of phenol and camphor was attributed to plasticiser used during the manufacture of the nitrocellulose photographic film in the store,[53, 54] the National Records of Scotland stores are predominantly print based so such objects were not present. The occurrence of cellulosic breakdown products is shown in **Table 3.19**. In common with other sites, the substances toluene, ethylbenzene, cumene, and furfural were detected at the National Records of Scotland.

Table 3.18: Analytes corresponding to major peaks in chromatograms from Thomas Thomson House sampling

Location	GA b/ground	GA cabinet 770	GA timecare box 770	GA Drawer 15.6	GA1 b/ground	GA1 Drawer 6.4	GA1 drawer 7.2	GA1 Drawer 26.3	GA1 Drawer 15.3	GA1 drawer 13.3	GA1 box cabinet 15.5	GA1 drawer 6.2	GA1 timecare box drawer 6.2	GA1 Album box drawer 15.5
Compound	**Concentration µg m^{-3}**													
Trichloro-ethylene*	1.66	-	-	-	1.07	-	0.62	-	-	-	-	-	1.49	-
Tetrachloro-ethylene*	9.86	-	-	4.91	-	0.46	0.33	1.48	3.12	0.45	-	3.39	9.63	1.26
Decane*	-	-	-	0.52	-	-	-	-	0.35	0.13	0.40	-	0.48	-
D3-siloxane*	-	4.34	-	-	-	-	-	-	-	-	-	-	-	0.32
D4-siloxane*	0.34	3.12	-	-	0.35	0.25	-	0.30	-	0.18	0.35	-	-	0.11
D5-siloxane*	0.12	0.45	-	-	0.10	0.09	0.10	0.10	0.13	-	0.12	0.15	0.14	-
Camphor	-	-	-	0.15	-	-	0.20	-	0.20	-	-	-	-	-
Naphthalene	0.12	-	-	0.13	-	-	-	-	-	-	0.11	0.13	0.13	0.09
1,3-Dichlorobenzene	-	-	-	0.72	-	-	-	-	-	-	-	-	1.03	-
1,4-Dichlorobenzene	-	-	-	-	-	-	0.46	-	-	-	-	-	-	-

*Calculated as toluene equivalents

Table 3.19: Occurrence of cellulosic and wood-related breakdown products from Thomas Thomson House site

Location	**GA background**	**GA cabinet 770**	**GA timecare box 770**	**GA Drawer 15.6**	**GA1 background**	**GA1 Drawer 6.4**	**GA1 drawer 7.2**	**GA1 Drawer 26.3**	**GA1 Drawer 15.3**	**GA1 drawer 13.3**	**GA1 box cabinet 15.5**	**GA1 drawer 6.2**	**GA1 Timecare box drawer 6.2**	**GA1 Album box drawer 15.5**
Compound	**Concentration µg m^{-3}**													
Toluene*	1.20	0.42	-	0.67	0.69	0.37	0.24	0.54	0.56	0.37	0.61	0.60	0.97	0.45
Furfural	1.09	-	-	-	-	0.29	-	0.86	1.01	0.29	1.36	1.00	2.49	0.25
Ethylbenzene	-	-	-	0.28	0.25	0.02	0.28	0.08	0.19	-	0.17	0.17	0.67	0.05
Cumene	-	-	-	0.30	0.12	-	-	0.03	0.23	-	0.10	0.20	0.45	0.05
Xylene*	0.34	-	-	0.82	0.44	0.16	-	0.38	0.29	0.15	0.18	0.16	-	0.14
α-Pinene*	-	-	-	-	-	0.08	-	-	-	-	-	-	-	-
Acetophenone	0.42	-	-	0.57	0.41	0.31	0.44	0.52	0.37	-	0.43	0.46	0.49	0.32
Benzaldehyde*	0.43	-	-	0.53	0.37	-	0.45	-	0.23	0.2	0.4	0.38	0.43	0.42
Phenol*	0.31	0.20	-	-	-	-	0.52	1.78	0.57	0.16	0.64	0.62	0.33	0.30
Limonene*	-	-	-	0.12	-	-	-	-	0.11	-	0.11	-	-	-

*Calculated as toluene equivalents

The sampling environment in this case study was a modern and purpose built for the conservation of archival materials. As such, environmental conditions within the sampling locations were more controlled than the RAF museum. The detection of camphor, naphthalene and dichlorobenzene showed that the sampling and analysis methodology developed here is suitable for use within archival and photographic collections, and can be used to sample objects *in situ* without the need to remove them from collection storage. The GA cabinet and GA timecare box locations were given green risk status as there were no detected substances with significant associated hazards. However, due to the presence of chlorinated solvents, the remaining locations were considered to be of medium risk, and designated amber risk status areas.

3.5.7. University of Glasgow herbarium case study

The case study at the University of Glasgow was performed after conservation staff reported that anecdotally an object in their library special collection was contaminated with DDT. During a visual examination of the herbarium, it was noted that the pages and pressed plant material contained within the book were frequently speckled with a white particulate material as noted by previous conservators. This speckling was confined to the inner pages of the book and was not visible on the book binding, cover or inside the Solander box containing the artefact. Due to the fragility of the object, it was not possible to collect this powder for analysis. Using the sampling and analysis methodology flowchart, passive sampling was selected due to the volume of the box, and was conducted over 28 d.

The main peaks in the chromatogram were attributable to cellulosic breakdown products as expected. The data are presented in Table 3.20 below. It was observed that the measured concentrations of these substances were lower than that of similar collections also measured (*cf.* RAF Museum, **Section 3.5.5**). As the herbarium volume was stored in a box with very little headspace, it was likely that as the analytes partition into the vapour-phase, the small headspace of the box would have quickly reached equilibrium and no further increase in concentration of these analytes would have taken place. Due to the small volume of air available, this equilibrium would have been established with very low masses of analytes, accounting for the low concentrations detected.

Table 3.20: Main peaks detected from sampling of University of Glasgow herbarium

Compound	**Concentration µg m^{-3}**
1-Butanol*	0.10
Toluene	0.08
Furfural	0.92
o-Xylene*	0.08
Benzaldehyde	0.14
Phenol*	0.09
D-Limonene*	0.08
Benzyl alcohol*	0.09

*Calculated as toluene equivalents

The type of analytes present was generally in good agreement with previously sampled locations containing paper based collections. As expected, detection of DDT was not possible using a vapour-phase approach and the object was too fragile to permit direct swabbing. **Figure 3.23** below shows an overlay of the chromatograms collected over the 28 d sampling period. This figure shows that with increasing time, the peak area of the analytes increased with no significant plateau effect. This implied that a 28 d sampling period was suitable for the trapping of VOCs over this period with no indication that the sorbent had become saturated with analytes. **Figure 3.24** shows this image with increased zoom.

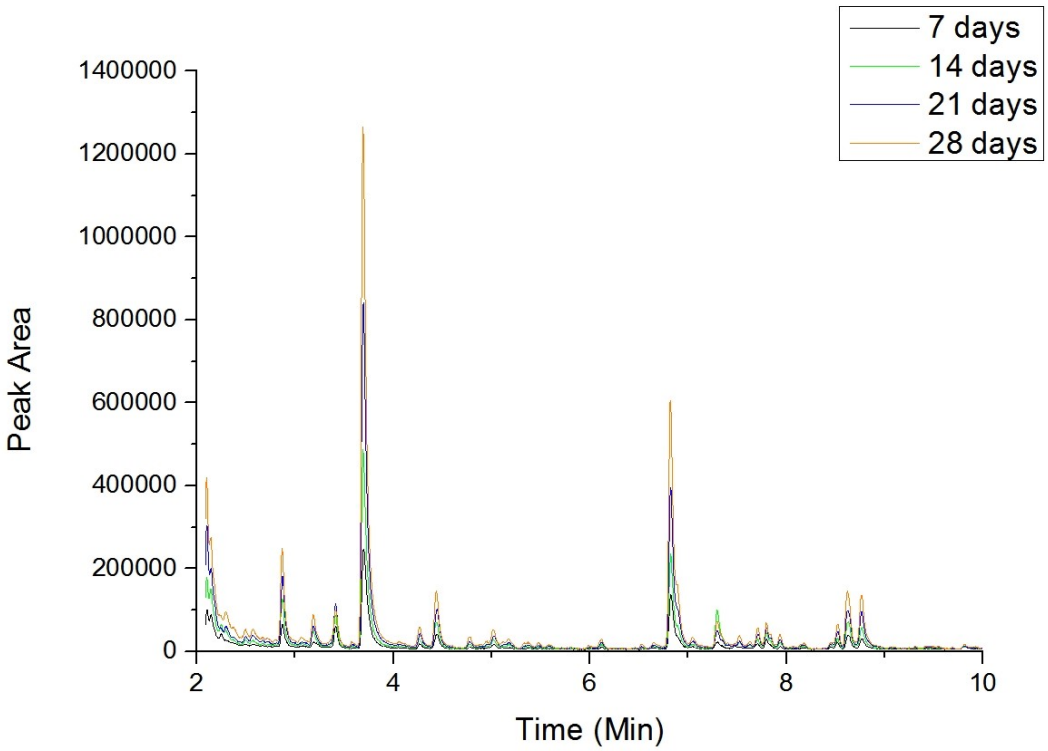


Figure 3.23: Overlay of chromatograms from University of Glasgow sampling

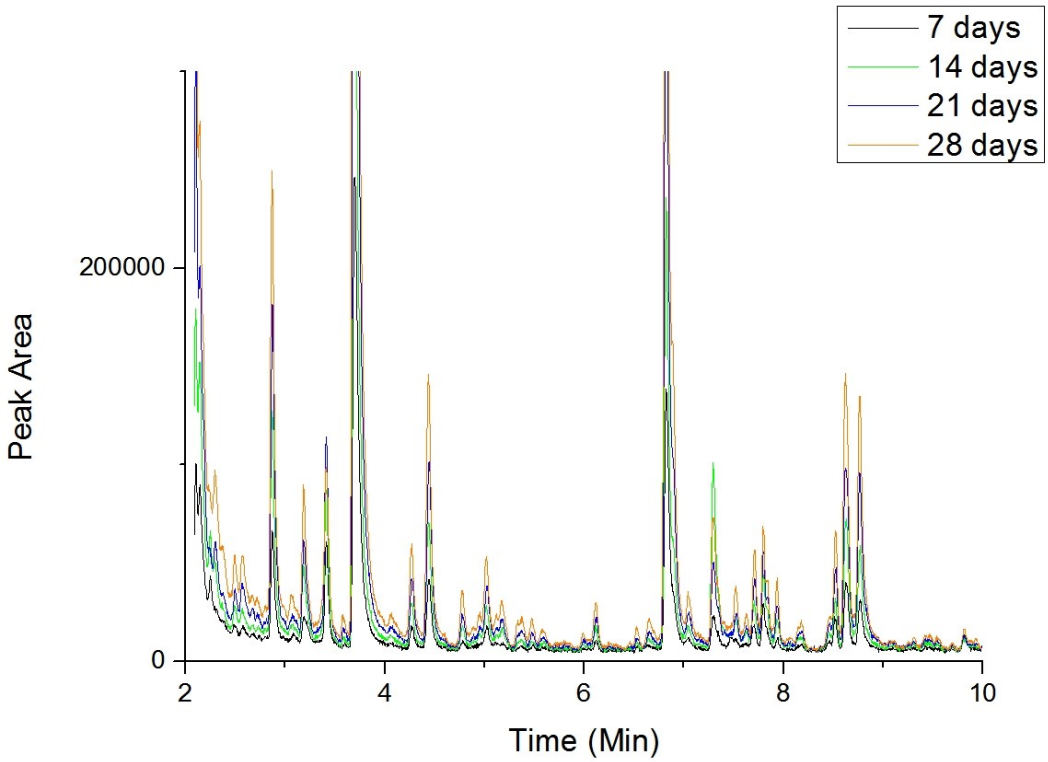


Figure 3.24: Zoomed image of the data collected from the University of Glasgow herbarium showing similarity of the TICs more clearly

Examining the change in peak area for these analytes as a function of time showed linear relationships. This implied that the air flow into the tube was constant and that the sorbent was not being saturated after 28 d sampling in close proximity to an emissive source. This provided justification for the length of sampling time in other case studies in this project. Used in this way, the methods described here allow the concentration and type of VOCs present in a sample to be used as a pseudo-quantitative indicator to infer the stability of an artefact, which can be explored in future work.

The samples collected from this object did not reveal any significant chemical hazard to be present in the air, and as such may be considered as belonging in the low risk category. However, due the suspected contamination of this object with DDT, a substance with very low vapour pressure, it is recommended that confirmatory swabbing be performed in order to determine the presence of DDT. Until then, the object may be considered to be of medium hazard status and should be handled with gloves.

3.5.8. British Museum Orsman Road scaredevil case study

In the British Museum scaredevil study presented here, the object was a carved wooden ornament for the prow of a boat and was stored in a chipboard box. This object was selected for study as conservators had reported that the object had previously been treated with lindane. As there was no electrical socket nearby to allow the use of a pump for active sampling, passive sampling was employed over a period of 28 d before analysis by TD-GC-MS.

Table 3.21 shows VOCs detected in the scaredevil crate. The pesticidal agent lindane was detected at a concentration of 0.29 μg m^{-3} confirming that previous conservation treatment using the substance had been carried out. The chromatogram for the scaredevil sample contained major peaks corresponding to cellulosic breakdown VOCs, substituted naphthalenes and polycyclic aromatic hydrocarbons (PAHs). The concentrations of these substances were in good agreement with other case study sites. Also present were substituted naphthalenes. The concentrations detected are summarised in **Table 3.22**. Concentrations for these substances are elevated for indoor air in an urban area, as determined by Kostiainen[58] as being an average of 0.08 μg m^{-3}.

Table 3.21: Occurrence of cellulosic and wood-related breakdown products from scaredevil crate at Orsman Road site

Compound	Concentration (µg m^{-3})
Furfural	13.7
p-Xylene*	0.89
Benzaldehyde	2.47
α-Pinene*	1.65
3-Carene*	1.60
Ethylbenzene	0.14
Phenol*	0.57
Acetophenone	0.37

*Calculated as toluene equivalents

Table 3.22: Concentrations of substituted naphthalenes detected in scaredevil crate

Naphthalene substitution pattern	Concentration (µg m^{-3})
1,4-Dimethyl-*	1.11
1,5-Dimethyl-*	0.76
1,6,7-Trimethyl-*	0.66
1,6-Dimethyl-*	1.21
1-Methyl-*	5.14
2,3,6-Trimethyl-*	0.88
2,3-Timethyl-*	0.57
2,7-Dimethyl-*	1.02
2-Methyl-*	1.77

*Calculated as toluene equivalents

The detection of lindane represented a significant finding for the work, as it was the first instance that one of the less volatile key pesticides was detected in a museum environment using the developed methods and procedure determined by the sampling flowchart. This result highlights the utility of this work for use in uncontrolled environments with minimally invasive sampling methods. Due to the presence of lindane in the sample collected from this area, these sampling locations have been designated as red high risk areas.

3.5.9. British Museum Orsman Road weapons room case study

As a continuation of the previous scaredevil case study, more wooden objects within the same institution were investigated in order to determine if lindane was applied to more wooden objects. This sampling location was situated on a different floor of the building and was used to store weapons which were part of ethnographic collections. The weapons room featured a mix of wood and metal objects, alongside leather and natural fibre material. Tenax tubes were placed on shelves within the weapons room to passively sample over a period of 28 d and analysed by TD-GC-MS.

The principle peaks in the chromatogram corresponded to naphthalene (45.9 μg m^{-3}) and furfural (45.8 μg m^{-3}) as summarised in **Table 3.23**. The concentration of naphthalene was significantly higher than had been detected at other case study sites and strongly suggested that naphthalene had been extensively applied to the collection. The high concentration of furfural was expected as the collection contained a large amount of wood. Other cellulosic breakdown products such as *p*- and *o*-xylene, α-pinene and 3-carene were also present. The compound 2-methylnaphthalene was detected at a concentration of 10.6 μg m^{-3} which was the highest concentration of a substituted naphthalene found in this sampling location. Other PAHs were also observed at low concentrations in the sample: acenaphthene and dibenzofuran were measured at 1.03 and 1.41 μg m^{-3} respectively, with benzo[b]thiophene recorded at 2.25 μg m^{-3}. Other compounds in the VOC profile of this air volume revealed concentrations of C6 and C7 carboxylic acids (5.44 and 0.51 μg m^{-3}) as well as longer chain alkanes such as decanal and nonanal (2.90 and 3.33 μg m^{-3}) common to many indoor air spaces.

Table 3.23: Cellulosic breakdown products from Orsman Road weapons room

Compound	Concentration (µg m^{-3})
Naphthalene	45.9
Furfural	45.8
2-Methylnaphthalene*	10.6
Hexanoic acid*	5.44
p-Xylene*	4.16
Nonanal*	3.33
o-Xylene*	2.92
Decanal*	2.90
Benzaldehyde	2.55
Benzo[b]thiophene*	2.25
α-Pinene*	1.52
Toluene	1.49
Dibenzofuran*	1.41
3-Carene*	1.10
Acenaphthene*	1.03
Ethylbenzene	0.74
Phenol*	0.73
Heptanoic acid*	0.51
Acetophenone	0.50

*Calculated as toluene equivalents

Lindane was not detected in the samples, contrary to expectations. However, the method of analysis used was still able to detect significant concentrations of naphthalene, which strongly suggest previous pesticidal treatment using the substance. Information regarding the concentration of other VOCs was also collected which showed the presence of a number of PAH compounds. It was not possible to identify a source of these compounds, but it is possible that these substances were present due to nearby traffic, as the combustion of fossil fuels is known to produce these compounds.[59] Due to the presence of naphthalene and PAH compounds, this sampling location was designated as an amber risk location.

3.5.10. British Museum Orsman Road Arctic furs case study

A number of Arctic furs were sampled in order to determine the presence of volatile pesticides on these objects. The furs were selected for study as many ethnographic collections contain fur materials and this study offered an opportunity to examine these types of objects more closely. The furs used in the case study were boots stored in PVC bags in a wooden storage case. The storage location also contained a number of wooden objects which were not directly sampled. Objects were sampled in passive mode for 28 d before analysis by TD-GC-MS.

Analysis of the sampling tube deployed to a bag of fur boots showed that peaks with the largest areas typically correspond to VOCs associated with cellulosic breakdown, in agreement with previous case studies stored in areas containing a large amount of wood (for example, see **Section 3.5.5**). The peak with the highest concentration in the sample was generated by furfural at 48.8 μg m^{-3}. Other notable cellulosic breakdown products are summarised in **Table 3.24**. Fewer identifiable cellulosic VOCs were detected here than in the Weapons Room, but were detected here in greater concentration. The boots were stored in a room containing a larger amount of wood used in the construction of object storage boxes, and also wooden objects themselves. As such, the presence of high concentrations of cellulosic VOCs was expected.

Table 3.24: VOCs of cellulosic origin found in Orsman Road fur boots sample

Compound	Concentration (μg m^{-3})
Furfural	48.8
o-Xylene*	4.12
m-Xylene*	2.20
Benzaldehyde	8.17
α-Pinene*	5.83
Limonene*	2.23
Toluene	2.09
3-Carene*	4.61
Ethylbenzene	1.76
Acetophenone	3.64

*Calculated as toluene equivalents

Other contributing peaks in the chromatogram are tabulated in **Table 3.25**. After furfural, the largest contributions to the chromatogram were from siloxane peaks. D3-siloxane and D4-siloxane are volatile siloxanes used in personal care products and were detected at 36.5 and 18.5 μg m^{-3} respectively. A number of PAH compounds were detected in the sample. Dibenzofuran was detected at a concentration of 4.38 μg m^{-3} and as with other case studies in this chapter, methylated naphthalenes were detected at this location also. Samples contained 1-methylnaphthalene and 2-methylnaphthalene at 8.44 and 3.93 μg m^{-3}, comprising two of the most abundant peaks in the chromatogram. As previously, it is suggested that the emissive source of these substances was the external traffic and that these analytes are present as a result of fossil fuel combustion. In addition to aromatic compounds, C14 and C15 alkanes were detected in concentrations of 3.32 and 5.71 μg m^{-3}. These substances are not uncommon in indoor air and have been measured at a number of sites during the project. It is likely that these VOCs are emitted from activities such as conservation treatments or cleaning.[35]

Three different substances with known insecticidal properties were detected in this sample. Naphthalene was quantified at 14.2 μg m^{-3}, along with *p*-dichlorobenzene at 6.63 μg m^{-3}. *p*-Dichlorobenzene has historically been used in conjunction with

naphthalene in mothball formulations and suggested that previously, similar formulations may have been applied to the collection here as the high concentration of naphthalene is likely as a result of pest control measures.[27] As with previous collections at this site, lindane was detected - here at a concentration of 0.96 μg m^{-3}. The addition of lindane as a pesticidal treatment was likely due to the number of wooden objects held in this sampling location. Although these objects were not sampled directly, it was possible that the volatility of lindane allowed the vapour to be sampled from elsewhere in the room, despite samplers not being placed near wooden objects.

Table 3.25: VOCs detected in Arctic furs case study

Compound	**Concentration (μg m^{-3})**
D3-siloxane*	36.5
D4-siloxane*	18.5
Naphthalene	14.2
1-Methylnaphthalene*	8.44
p-Dichlorobenzene	6.63
C15 alkane*	5.71
Dibenzofuran*	4.38
2-Methylnaphthalene*	3.93
C14 alkane*	3.32
Lindane	0.96

*Calculated as toluene equivalents

The analysis of the object here showed again that the Tenax sorbent was able to trap a wide range of VOCs, but also vapour-phase pesticidal analytes. The detection of *p*-dichlorobenzene further shows the utility of the method as this substance was not part of the set of 10 target analytes, but the concentrations detected here suggest it has been previously applied to the collection. As the Tenax sorbent is able to trap a wide range of analytes, this could allow users to sample with no knowledge of prior pesticidal treatments and be able to detect pesticides beyond the scope of the initial

target analytes. The detection of suspected carcinogens naphthalene and *p*-dichlorobenzene in this sampling location triggered an amber risk status.

3.5.11. British Museum Blythe Road large object store case study

The "large object store" was a shipping container housing a number of objects, including a number of carved canoes and a small diesel-powered generator. Sampling was conducted in passive mode over a period of 28 d before analysing the sorbent tubes by TD-GC-MS. This case study was selected as the large number of wooden objects meant there was a high likelihood of detecting lindane, and the shipping container was useful in providing a sealed environment for VOCs to concentrate for analysis. This was in contrast to some of the other case studies which were performed in open collection environments.

It was anticipated that the presence of the diesel-powered generator would be a likely source for PAHs and aromatic hydrocarbons; [60] this was found to be true as the most prominent peaks in the chromatogram belonged to PAH species. VOCs detected in this location are tabulated in **Table 3.26**. The peak with the largest area in the chromatogram corresponded to acenaphthene, with a concentration of 4.6 µg m^{-3}. Acenaphthene does not have a WEL, but is considered toxic to aquatic organisms. Other PAHs detected included 1-methylnaphthalene (5.3 µg m^{-3}), fluorene (2.2 µg m^{-3}) and anthracene (0.7 µg m^{-3}) and a number of di-methylated naphthalenes. Dibenzofuran was also detected in the sample at 1.7 µg m^{-3}. Dibenzofuran was reported to have been used as a pesticide as well as being present as a congener in many commercial formulations of other pesticides.[61] Naphthalene was measured at a concentration of 1.1 µg m^{-3}.

As expected from a sampling location containing a number of wooden objects, cellulosic breakdown products were present. Toluene (0.5 µg m^{-3}), *p*-xylene (0.4 µg m^{-3}), limonene (0.3 µg m^{-3}) and α-pinene (0.6 µg m^{-3}) were all detected. Two terpenoid compounds were detected: 3-carene (0.5 µg m^{-3}) and α-bergamotene (0.4 µg m^{-3}) which is expressed by sandalwood amongst other woods of that genus.

Table 3.26: VOCs detected in large object store

Compound	Concentration (µg m^{-3})
1-Methylnaphthalene*	5.34
Acenaphthene*	4.63
Fluorene*	2.22
Dibenzofuran*	1.72
Naphthalene	1.12
Anthracene*	0.73
α-Pinene*	0.64
3-Carene*	0.53
p-Xylene*	0.44
α-Bergamotene*	0.41
Limonene*	0.31
Toluene*	0.05

*Calculated as toluene equivalents

No lindane was detected in this location, however the detection of a number of PAH compounds would suggest that there is the potential to use this method for the analysis of PAH compounds in environmental analyses. The presence of PAH compounds designated this sampling location to be in the amber risk category, due to the suspected carcinogenicity of the PAH compounds.

3.5.12. British Museum Blythe Road Mexican and Peruvian wool case study

Mexican and Peruvian woollen items stored at the British Museum textile conservation centre (TCC) were sampled as part of a case study after conservation staff complained that there was a strong smell “like sour milk” coming from the objects. The odour had developed after disinfestation work had been performed using a fumigant gas. No information on the fumigant gas was available. It was not expected that it would be possible to detect the presence of commonly used fumigant gasses such as sufuryl fluoride (Vikane™, ProFume™), phosphine or methyl bromide, as due to their volatility they are unlikely to remain in the collection for long enough periods after

application to be sampled. However, the case study location was selected in order to assess the method in an environment already containing high concentrations of VOCs arising from the odour of the wool. As per the flowchart, all storage boxes in this section were sampled in passive mode over 28 d before analysis of the tubes by TD-GC-MS as the box volumes were less than 1 m^3.

3.5.12.1. TCC background air sample

A background sample of the TCC air was collected and subtracted from subsequent samples. Concentrations of VOCs detected are presented in **Table 3.27**. The background sample contained concentrations of naphthalene (67.2 µg m^{-3}) and substituted naphthalenes such as mono- and di- methylnaphthalene (presented as a summed total of 49.9 µg m^{-3}). The level of naphthalene detected in this location was higher than in previous case study locations and was likely to be indicative of previous pesticidal treatments involving the substance. While the concentration of naphthalene was higher than in previous sampling locations, no workplace exposure guidance was available for the substance and so it was not possible to determine if there was an impact on human health.[48] A number of PAHs were recorded: acenaphthene (15.9 µg m^{-3}), 2-benzothiophene (7.7 µg m^{-3}), fluorene (5.3 µg m^{-3}), anthracene (4.2 µg m^{-3}), naphtho[2,1-b]thiophene (2.3 µg m^{-3}) were each detected in the air sampling. It was not possible to identify an emissive source of these substances, as PAHs are typically generated by the combustion of carbonaceous material such as fuel oils.[62] In an indoor air environment such as this, it is likely that the source was infiltration of outdoor air contaminated with PAHs from heavy traffic.

As with previous case studies, volatile compounds associated with the breakdown of cellulose fibres were also present, as shown in **Table 3.27** below. Furfural (24.4 µg m^{-3}), *p*-xylene (19.5 µg m^{-3}), *m*-xylene (11.8 µg m^{-3}), benzaldehyde (9.8 µg m^{-3}) and α-pinene (6.1 µg m^{-3}) were all detected at elevated concentrations. The sources of these compounds can be attributed to the usage of a wooden box for the storage of the textiles sampled.

Table 3.27: Concentrations of VOCs detected in TCC air sample

Compound	Concentration (µg m^{-3})
Naphthalene	67.2
Total substituted naphthalenes*	49.9
Furfural	24.4
p-Xylene*	19.5
Acenaphthene*	15.9
m-Xylene*	11.8
Benzaldehyde	9.82
2-Benzothiophene*	7.73
α-Pinene*	6.11
Fluorene*	5.32
Anthracene*	4.21
Toluene	3.11
Naphtho[2,1-b]thiophene*	2.33

*Calculated as toluene equivalents

3.5.12.2. TCC – Americas, Peruvian textiles, wooden box 28 (TC/AM/PE/TXB 28)

The box containing the samples was a wooden box and contained a number of woollen items which had been previously treated with an unknown fumigant gas, producing a strong unpleasant odour. Sampling of the air inside the box detected a number of VOCs, presented in **Table 3.28**. Both naphthalene and *p*-dichlorobenzene were detected at concentrations of 57.0 and 3.3 µg m^{-3} respectively. It is likely that due to the high concentrations of naphthalene present in the samples that the dichlorobenzene was present as part of a mothball formulation.[24, 27] Another of the 10 target pesticides, lindane, was also detected at a concentration of 1.10 µg m^{-3}. The concentration of lindane detected is similar to concentrations detected in the Orsman Road furs (**Section 3.5.10**) where the concentration of lindane was measured at 0.96 µg m^{-3}. The presence of lindane was attributed to historical application, as it is a controlled substance under the Stockholm Convention on Persistent Organic Pollutants. It was not considered

probable that the application of lindane, naphthalene or *p*-dichlorobenzene were the cause of the textile odour.

o-Xylene (24.4 µg m^{-3}) and methyl-naphthalene (20.7 µg m^{-3}) were the greatest contributors to the VOC profile. Along with o-xylene, other cellulosic VOCs were detected due to the age of the wooden storage box used: α-pinene (10.7 µg m^{-3}), benzaldehyde (16.1 µg m^{-3}) and *m*-xylene (11.7 µg m^{-3}), 3-carene (3.5 µg m^{-3}) and limonene (3.6 µg m^{-3}). Cyclosiloxanes were also present at concentrations of 19.2 and 11.8 µg m^{-3} for D4-siloxane and D3-siloxane respectively. No PAHs were detected in the sample, which supports the conclusion that the source of these substances in the background sample was the result of infiltration of outdoor air. As the box was sealed from the background air, these compounds were not detected.

This case study has shown a significant concentration of lindane was present in the sample, as well as high concentrations of naphthalene and *p*-dichlorobenzene. This shows the ability of the method used to detect previous pesticidal treatment in locations where concentrations of other VOCs are similarly high.

Table 3.28: VOCs detected in box 28 of TCC, containing woollen items

Compound	Concentration (µg m^{-3})
Naphthalene	57.0
o-Xylene*	24.4
Methylnaphthalene*	20.7
D4-siloxane*	19.2
Benzaldehyde	16.1
D3-siloxane*	11.8
m-Xylene*	11.7
α-Pinene*	10.7
Limonene*	3.60
3-Carene*	3.50
p-Dichlorobenzene	3.30
Lindane	1.10

*Calculated as toluene equivalents

3.5.12.3. TCC – fibre box 30 (TC/AM/PE/TXB 30)

Box 30 contained similar items to the previous box 28; however, box 30 was constructed from fibre instead of wood. As a result, box 30 had a similar VOC profile to box 28. Both are stored in approximately the same location within the TCC.

Analytes detected in the sample are tabulated in **Table 3.29**. Of the project target pesticides, lindane was again present at a concentration of 0.61 µg m^{-3}, which was lower than the concentration detected in box 28. The greatest single contributing peak to the chromatogram corresponded to naphthalene, measured at a concentration of 46.2 µg m^{-3}. This was lower than the concentration found in box 28 and the surrounding store. The concentrations of both substances were suggestive that these compounds had been previously applied as pest control treatments.

Substituted naphthalenes comprised a large number of the larger peaks in the chromatogram, with the summed total of substituted naphthalenes measured at 70.4 µg m^{-3}. The concentrations of individual substituted naphthalenes are presented separately in **Table 3.30** below. A number of other PAHs were present in this sample,

including acenaphthene (6.6 µg m^{-3}), anthracene (0.8 µg m^{-3}) and cyclopenta[c]thiapyran (6.4 µg m^{-3}). The concentrations of these compounds was lower than the TCC air sample, suggesting that the storage box was again preventing ingress of these analytes from traffic exhaust contained in outdoor air.

A number of analytes contributing to the VOC profile of the sample may have been the products of cellulosic degradation of the box fibres. 3-Furaldehyde (12.5 µg m^{-3}), *p*-xylene (11.0 µg m^{-3}), 3-carene (3.70 µg m^{-3}) and α-pinene (3.73 µg m^{-3}) are all well documented products of the hydrolysis of cellulosic material. The concentrations detected here were of the same order of magnitude to similar VOCs detected in the sample from wooden box 28. Other similarities with box 28 include the presence of cyclosiloxanes measuring 6.1, 4.4, 1.8 and 3.4 µg m^{-3} for D3-, D4-, D5- and D6-siloxane respectively.

This case study demonstrated that it was possible to identify previous conservation treatment, having detected lindane, naphthalene and *p*-dichlorobenzene in both boxes 28 and 30 in the presence of a strong odour which suggested a high background VOC concentration. This was a potentially important finding in the case study as it illustrated that the selectivity of the method, and that information regarding the previous treatment of collections could still be inferred from a sample, even in the presence of competing analytes. It was not possible to identify the source of the odour by TD-GC-MS. While the cause of the reported "sour milk" smell was not identified, attribution of source was not the aim of this study. It is likely that the smell was caused by the fumigant gas used reacted with the proteins in the wool causing the smell, but the VOCs generated as a result did not have a high enough atomic mass to be detected by TD-GC-MS.

Detection of *p*-dichlorobenzene in both boxes containing objects allowed these boxes to be categorised as amber risk status, and the presence of naphthalene in the background sample of open workshop air also categorised this sampling location as having an amber risk level.

Table 3.29: VOC contributions to box 30 sample chromatogram

Compound	**Concentration (µg m^{-3})**
Naphthalene	46.2
3-Furaldehyde*	12.5
p-Xylene*	11.0
Benzaldehyde	7.03
Cyclopenta[c]thiapyran*	6.83
Acenaphthene*	6.62
D3-siloxane*	6.12
o-Xylene*	5.94
D4-siloxane*	4.40
3-Carene*	3.70
α-Pinene*	3.73
D6-siloxane*	3.42
Toluene	2.51
Ethylbenzene	2.12
D5-siloxane*	1.83
Anthracene*	0.82
Lindane	0.61

*Calculated as toluene equivalents

Table 3.30: Mono- and di-substituted naphthalenes found in TCC samples

Naphthalene substitution pattern	Concentration (μg m^{-3})
1,4,6-Trimethyl-*	1.70
1,6,7-Trimethyl-*	1.10
1,6-Dimethyl-*	6.60
1-Ethyl-*	5.40
1-Methyl-*	25.6
2,3,6-Trimethyl-*	5.60
2,3-Dimethyl-*	3.20
2,6-Dimethyl-*	6.40
2,7-Dimethyl-*	7.20
2-Ethenyl-*	5.50
2-Methyl-*	2.10
Total	**70.4**

*Calculated as toluene equivalents

3.5.13. Bloomsbury Asia store case study

The Bloomsbury Asia store contained a number of decorative wooden objects from East Asia, which were stored on metal shelves. Tenax tubes were placed on shelved and sampled in passive mode over a period of 28 d before analysis by TD-GC-MS.

Analytes detected in the sample are summarised in **Table 3.31**. Naphthalene was the only target analyte to be detected in this location, at a concentration of 51.9 μg m^{-3}, suggesting that the substance had been previously used in this collection as a pesticidal treatment. None of the other target analytes were detected in this location. By area, the largest peaks in the chromatogram corresponded to phenol at 62.4 μg m^{-3}; it was not possible to identify an emissive source of phenol in the sampling location. The presence of 2-ethyl hexanol 45.9 μg m^{-3} was determined in the sample also. This was likely to be a result of microbial action on plasticisers found in building materials.[63] As in previous case study locations, a number of cellulosic breakdown products were detected. The largest concentration of these substances was for furfural at 52.2 μg m^{-3}. α-Pinene (15.0 μg m^{-3}), limonene (8.35 μg m^{-3}) and 3-carene (6.24 μg m^{-3}) have also

been previously associated with emission from aging wood samples, explaining the presence of the substances here.[64, 65] As with previous samples, there were a number of substituted naphthalenes trapped. The total concentration of these species was 67.2 μg m^{-3}, and individual concentrations are presented in **Table 3.32**. The presence of these naphthalene derivatives and nearby busy roads suggests they are the product of fossil fuel combustion from passing traffic.[59] Other PAHs acenaphthene (16.4 μg m^{-3}), dibenzofuran (7.67 μg m^{-3}) and fluorene (2.92 μg m^{-3}) were also detected in the sample.

Table 3.31: VOCs trapped from the Asia store, Bloomsbury.

Substance	**Concentration (μg m^{-3})**
Phenol*	62.4
Furfural	52.2
Naphthalene	51.9
2-Ethyl hexanol*	45.9
Acenaphthene*	16.4
Heptanol*	16.3
α-Pinene*	15.0
Octanol*	12.6
Heptanol*	8.62
Limonene*	8.35
Dibenzofuran*	7.67
3-Carene*	6.24
Hexanal*	4.98
Toluene	3.29
m-Xylene*	3.12
Fluorene*	2.92
Quinoline*	2.59
Camphor	1.97
D5-siloxane*	1.56
Isoborneol*	0.47

*Calculated as toluene equivalents

Table 3.32: Substituted naphthalene compounds detected with splitless analysis

Naphthalene substitution pattern	Concentration (µg m^{-3})
1,4,6-Trimethyl-*	0.76
1,6,7-Trimethyl-*	1.15
1,6-Dimethyl-*	9.02
1,6-Dimethyl-4-(1-methylethyl)-*	0.72
1-Methyl-*	34.3
2,3,6-Trimethyl-*	0.99
2,3-Dimethyl-*	11.9
2,6-Dimethyl-*	8.33
Total	**67.2**

*Calculated as toluene equivalents

A second sampling tube was exposed for 28 d and analysed under the same TD-GC-MS conditions, except using an outlet split flow from the thermal desorber into the GC instrument. This analysis employed a 1:10 outlet split ratio on the thermal desorber in order to reduce the concentration of analytes passing on to the column in order to determine if there were any peaks corresponding to low-concentration analytes that may have been masked by higher concentration peaks in the previous sample.

Substances detected are presented in **Table 3.33**. Naphthalene and phenol corresponded to the highest area peaks of the chromatogram with concentrations of 66.9 and 59.9 µg m^{-3} being calculated. Phenol was not detected in the splitless analysis, however the concentration of naphthalene in both tubes was similar. Concentrations calculated from the split flow analysis of 2-ethyl hexanol (56.2 µg m^{-3}), dibenzofuran (10.4 µg m^{-3}) and octanol (13.4 µg m^{-3}) were also similar to those analysed in splitless mode. However, concentrations for furfural (7.7 µg m^{-3}) differed by a large degree. Substances detected on the split tube included cellulosic breakdown products furfural (7.37 µg m^{-3}), α-pinene (20.6 µg m^{-3}) benzaldehyde (5.18 µg m^{-3}) and 3-carene (13.7 µg m^{-3}).

Table 3.33: substituted naphthalenes detected in Asia store with 1:10 split ratio

Naphthalene substitution pattern	Concentration (μg m^{-3})
Phenol*	66.9
Naphthalene	59.9
2-Ethyl hexanol*	56.2
Acenaphthene*	28.4
2,7-Dimethyl*	20.6
α-Pinene*	20.6
Heptanol*	16.3
Butyl butanoate*	14.4
3-Carene*	13.7
Octanol*	13.4
Biphenyl*	12.7
Dibenzofuran*	10.4
1-Ethyl naphthalene*	8.18
Furfural	7.37
Nonanol*	6.55
Benzaldehyde	5.18
1,4-Dimethyl naphthalene*	4.60
2,3-Dimethyl naphthalene*	3.71

*Calculated as toluene equivalents

Fewer substances were detected using the split ratio on the TD-GC-MS compared to splitless mode; however, many of the analytes trapped were within an order of magnitude of concentrations detected from analysis of the splitless tube, and so were considered to be equivalent for the purposes of this experiment due to the large degree of imprecision associated with passive air sampling.[46, 47] The smaller number of compounds detected when analysing the tubes in split mode provided justification that for single-shot profiling analysis, tubes should be analysed in splitless mode so as to ensure detection of as low an analyte concentration as possible.

The detection of a number of PAH compounds as well as the potential mutagen phenol placed this sampling location in the amber risk category.

3.5.14. British Museum crocodile mask case study

The British Museum crocodile mask was used in a case study after a conservation worker reported an allergic reaction while working with the object. It was assumed that the sensitisation reaction was caused by the presence of a chemical treatment on the object, which could possibly have been related to previous pesticidal treatments. The fragility of the object had caused small flakes and fibres to dislodge from the body of the object which could be used for solvent extraction without swabbing the object itself.

Several microsamples from the British Museum mask were extracted using 3 cm^3 of 5% IPA in hexane. The extract solution was concentrated as per **Section 3.4.2** to 1 cm^3, and analysed by HPLC-UV. As UV detection is a non-specific technique, the resulting chromatograms contained a number of analyte peaks that did not correspond to analytes in the reference standard solution and could not be identified. Comparison of these peaks' retention times against peaks in the chromatogram of the standard solution showed that the majority of substances did not have similar enough retention times to be considered as positive identification the key project analytes.

Samples taken from a shell and from the barb of a feather gave chromatograms containing a peak with a retention time of 2.06 min, matching the retention time of dichlorvos. Confidence in the dichlorvos peak integration was established in **Section 3.4.3** where repeatability and linearity were established, demonstrating that despite the proximity of the analyte peak to the solvent front of the chromatogram, repeatable integration of the dichlorvos peak was possible. The concentration of dichlorvos measured was 149.7 and 401.4 ng μL^{-1} for the shell and feather respectively, indicating a high concentration of the analyte was present. However, positive identification can only be tentatively made using the HPLC data alone and further identification of the analyte with a secondary analytical technique such as the organophosphate detection kit developed in **Chapter 5** should be performed. Due to the suspected presence of dichlorvos and the sensitization reaction experienced by a previous worker, this object should be considered as amber risk status. Due to constraints placed upon the object,

as it was being prepared for exhibition, it was not possible to sample the object further in order to get confirmatory testing. This indicated that while identification of analytes is possible through analysis by HPLC-UV, a second confirmatory method is needed in order to validate the results due to the complexity of the sample matrix.

3.6. Conclusions

This study shows the development of chromatographic methods for the successful determination of pesticide residues in heritage environments. The analytical methods developed in this chapter have been demonstrated as robust, minimally invasive techniques through laboratory testing. A sampling and analysis flowchart was presented which could potentially allow collection custodians to identify the most appropriate sampling and analysis techniques for their collections. The flowchart allows the determination of pesticidal residues using the developed methods to be tailored to the needs of their individual collections, based on any suspected pesticidal treatment or handling requirements for objects. Finally, the methods developed here were assessed in case studies using real objects in heritage collections.

Part A - method development

Consultation with heritage science personnel identified 10 target analytes considered to be of importance to heritage institutions (aldrin, camphor, chloronaphthalene, dichlorodiphenyltrichloroethane (DDT), dichlorvos, dieldrin, endrin, hexachlorocyclohexane (lindane), naphthalene and thymol). Of these 10 analytes, the TD-GC-MS method developed here was able to successfully detect volatile analytes camphor, chloronaphthalene, dichlorvos, naphthalene and thymol with high coefficient of determination, as the R^2 values were greater than 0.99 for these analytes. Aldrin was detected with a lower R^2 value of 0.88, however the linear regression model was still able to explain a large percentage of response variable variation and was suitable for use. Dieldrin was tentatively identified through comparison of the experimentally determined mass spectra to those in the NIST library. DDT was not detected by TD-GC-MS due to thermal breakdown during the desorption process. The main advantages of the TD-GC-MS method were that the need for a complex sample preparation step was eliminated, offering the potential to perform high throughput

analyses using an automated thermal desorption unit. Desorption of analytes onto a cryofocussing cold trap allowed recovery of analytes without risking the loss of analytes typically associated with preconcentration steps. This method of analysis would be most suited to the sampling of environments where the swabbing of the museum objects cannot be performed, and that the analytes suspected of being present are sufficiently volatile to allow trapping.

Using the developed HPLC method, aldrin, chloronaphthalene, dichlorvos, DDT, dieldrin, endrin, naphthalene and thymol were all successfully and reproducibly detected with R^2 values in excess of 0.97. Dieldrin and endrin co-eluted, however the peak area for both substances was reproducible, and an R^2 value greater than 0.99 was calculated from the regression model. HCH and camphor were not detected by HPLC-UV detection, as camphor was only weakly absorbing and HCH has no active chromophore at 225 nm. The use of HPLC-UV allowed detection of thermally sensitive analytes not seen in the analysis by TD-GC-MS. The linear model used to calculate concentration from peak area for the HPLC analysis of aldrin, dieldrin, DDT and endrin had a greater R^2 values than in the corresponding GC analysis. Importantly, DDT could be repeatably detected using the HPLC-UV method, offering a method of detection for one of the most commonly used pesticides of the 20th century.

Recovery of the target analytes after solvent extraction was in the range of 87-102% with RSD between 4-12%. This was comparable to similar published studies by Barro *et al.*[8] but is presented here without the need for time consuming disassembly of sampling tubes for removal of the sorbent or ultrasound-assisted extraction. The use of this technique is recommended for the detection of thermally sensitive analytes which would not be detectable using the thermal desorption analysis. The HPLC-UV analysis can also be used if swabs can be collected from the object to be sampled, allowing for the detection of less volatile analytes.

Sample processing for analysis by GC-FID was performed using solvent elution from sample tubes; without the high thermal desorption temperatures it was possible to detect endrin and DDT, as well as the other eight pesticides of interest. As the majority of the work in this thesis was performed using MS as the detection method, the GC-

FID analyses were not studied in as great detail and were used as proof of concept that the analytes would be detected by GC-FID as well as GC-MS and by HPLC-UV.

A flowchart enabling selection of appropriate sampling and analysis methods for individual collections has been developed, alongside a sampling protocol (**Appendix A**) giving precise instructions on how to perform the sampling was prepared. The flowchart Is designed to aid conservation workers to determine which methods are able to detect which substances, while the sampling protocol enables them to collect the samples without prior training. This allows analytical chemist to coordinate the sampling without having to visit the site, and still have confidence that samples have been collected properly.

Part B - case studies

The utility of the developed methods was assessed by performing 13 case studies in UK heritage institutions. A number of the key pesticides selected for study were detected in the vapour-phase at case study sites. Camphor, chloronaphthalene, naphthalene, and lindane were all detected in low concentration at several sites. The concentrations of these substances detected was typically less than 3 $\mu g\ m^{-3}$, with the exception of naphthalene which was detected at a maximum concentration of 60 $\mu g\ m^{-3}$. Naphthalene, chloronaphthalene and lindane do not have controls on workplace exposure limits. The detection of lindane in only 5 of the 13 case study locations showed that the substance was only found in a minority of locations and in each instance was not detected in high concentration. Less volatile pesticides aldrin, dieldrin, endrin and DDT were not detected in any of the sampling locations. Across all case studies, VOCs common to indoor air such as C6-C9 aldehydes and organic acids were detected as well as products associated with cellulosic breakdown. These analytes were quantified as toluene equivalents (with the exception of furfural, cumene, ethylbenzene, benzaldehyde, *o*- and *p*-dichlorobenzene, and acetophenone), and as such should not be considered as having the same analytical rigour as the biocide data, as it was not possible to complete an investigation into the response factors of all VOC substances detected, compared to that of toluene. Nevertheless, the concentrations of these VOC substances were found to be in good agreement with similar reported studies.[16, 17, 36]

Although the vapour-phase sampling of the analytes was limited in that it was not able to detect all analytes by air sampling alone, this work is of potential benefit to the heritage science community in that it describes a selection process that many be used by untrained personnel to determine the most appropriate choice of method for a particular collection. The methods reported here may be used to determine the presence of commonly used pesticides using minimally invasive *in situ* sampling in order to aid conservators in the risk assessment of their collections. To aid this, a "traffic light" system has been developed based on risk associated with handling contaminated objects.

3.7. References

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4. Development of an optical organophosphate sensor

4.1. Introduction

4.1.1. Development, toxicology and usage of organophosphate compounds

In health, agriculture and legislation, the term "organophosphate" is used to describe a substance which inhibits esterase enzymes, specifically acetylcholinesterase (International Biochemistry and Molecular Biology enzyme code EC 3.1.1.7) in synapses and erythrocyte membranes, and butyrylcholinesterase (International Biochemistry and Molecular Biology enzyme code EC 3.1.1.8) in plasma.[1] It is in this sense that the term "organophosphate" has been used throughout this chapter. Organophosphates are a class of compounds used widely in pest control applications. The lower tendency of organophosphates to bioaccumulate, as well as their shorter half-lives and higher acute toxicity allowed them to largely replace organochloride pesticides, particularly in environmental applications.[2, 3]

Organophosphates act on the parasympathetic nervous system to inhibit the action of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Although acute BuChE inhibition does not seem to cause clinical features, acetylcholinesterase inhibition results in accumulation of acetylcholine in neuromuscular junctions and synapses of the autonomic and central nervous systems, causing overstimulation of acetylcholine receptors. As organophosphates phosphorylate the enzymes necessary for the degradation of the acetylcholine in these receptors (and thus the relaxation of the neuron) the muscle or organ is unable to relax the contractions, leading to paralysis and death.[4] Clinical features of organophosphate poisoning include: salivation, lachrymation, urination, sweating, diarrhoea and vomiting, miosis and muscle spasm.[1, 3]

The first organophosphate with the ability to inhibit the action of AChE was tetraethylpyrophosphate, synthesised in 1854.[5, 6] Originally developed as a substitute for the natural pesticide nicotine, its utility was limited as it was rapidly inactivated by hydrolysis.[6] Development of organophosphate pesticides continued and in 1944 IG

Farben chemist Gerhardt Schrader first described parathion, which became one of the most popular and well-known organophosphate insecticides.[7] Throughout the 1950s, the pattern in insecticide usage was dominated by organochlorines (led by dichlorodiphenyltrichloroethane, (DDT)) through to the mid-1970s where organophosphate moieties became the leading functionality in pesticidal agents.[8] However, by 2014 usage had declined with organophosphates accounting for only 1% of pesticide usage in British arable farming, with 93% of pesticides used in that same period being pyrethroids.[9]

A number of high profile cases of organophosphate poisonings have also been documented. In 1930, thousands of Americans were poisoned by ingesting Jamaica Ginger Extract contaminated with triorthocrescyl phosphate (TOCP).[10] The extract, also known as Jake, contained a high concentration of ethanol and was a popular means of circumventing Prohibition laws at the time. The TOCP was added by bootleggers as a means of cheating regulatory requirements that the extract contain a minimum requirement of ginger solids per cm^3 of extract, as TOCP could be substituted for the extra ginger (which made the Jake unpalatable) without affecting the drinkability. The ingestion of the TOCP caused axonal neuropathy and an upper motor neuron syndrome causing victims to lose the use of their hands and feet – a condition that became known as Jake leg.[11]

The high toxicity of organophosphates has led to a number of these compounds to be developed for use as chemical weapons. In 1995, the religious sect Aum Shinrikyo released the organophosphate nerve agent sarin on several lines of the Tokyo subway system in a coordinated attack, killing 12 and injuring approximately 5500.[12, 13] More recently, in 2013, rockets containing liquid sarin were fired upon Damascus during the Syrian civil war.[14]

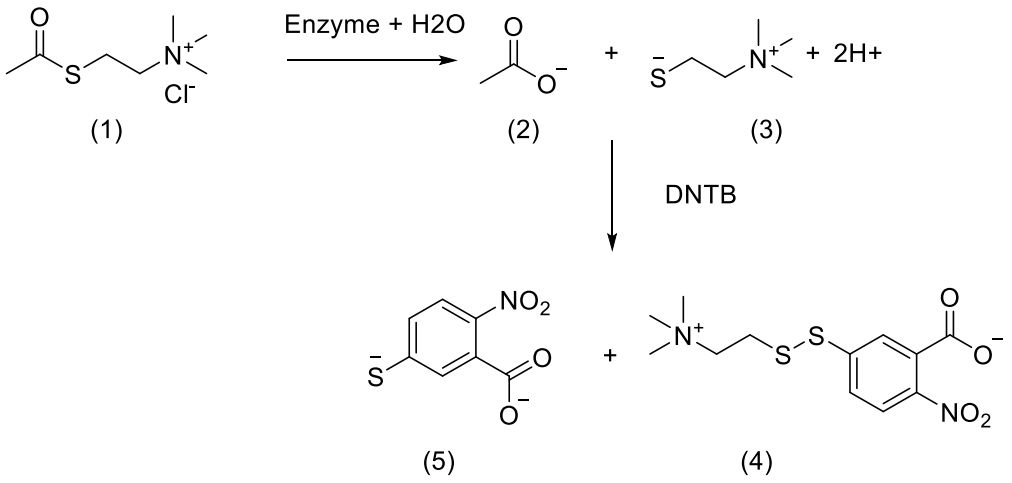
4.1.2. Use of organophosphate compounds in heritage environment

Dichlorvos was commonly used in heritage institutions as organochloride pesticides became increasingly regulated, and in the case of DDT, banned.[15-17] The use of dichlorvos-impregnated strips of polyvinyl chloride, marketed as Vapona™ No-Pest strips allowed fumigation of enclosed spaces with the volatile pesticide.[18-20] Goldberg[15] noted that this practice was becoming unpopular from the mid-1980s due

to concerns about the safety of the substance. Hawks[21] further noted that the organophosphate substances chlorpyrifos, malathion and parathion were also used in heritage collections. However, information regarding the extent of use in collections is typically limited, as the use of insecticides in heritage collections is often not documented.

4.1.3. Current methods of detection of organophosphate compounds

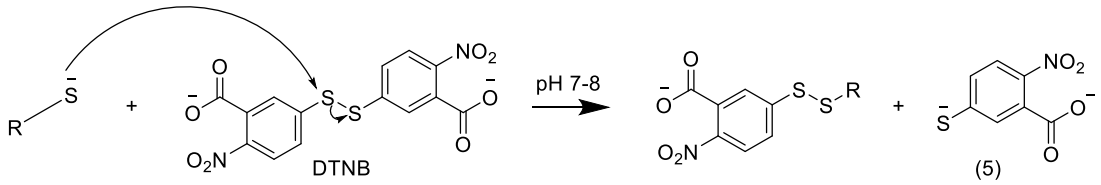
Organophosphates are often detected by measuring their inhibitory effect on reactions catalysed by AChE. Ellman's assay[22] is widely regarded as the standard assay for the determination of AChE activity. Initially developed as a method for the determination of thiols, Ellman's assay can be used to determine the activity of AChE through the catalytic hydrolysis of acetylthiocholine as a substrate. In this reaction a sulfur containing substrate, acetylthiocholine (**1**), is hydrolysed by AChE to form acetate (**2**) and thiocholine (**3**). The thiocholine reacts with dithiobisnitrobenzoate (DNTB) in neutral to mildly alkaline conditions to yield a thiocholine disulfide compound (**4**), and a yellow 5-thio-2-nitrobenzoate dianion (**5**). A proposed reaction is presented in **Scheme 4.1**. The concentration of the dianion compound can be determined by measuring the intensity of the yellow colour using a spectrophotometer at 412 nm.



Scheme 4.1: Hydrolysis of acetylthiocholine chloride followed by reaction with DNTB to form a dianion species (5) with λ_{max} = 412 nm

The proposed reaction of thiols with DTNB is clarified in **Scheme 4.2**, showing the nucleophilic attack of the DTNB by the thiol to form a new disulfide with the DTNB

and the yellow, doubly charged 2-nitro-5-thiobenzoate dianion (**5**) under the basic reaction conditions.



Scheme 4.2: Reaction of a thiol with DNTB to form yellow 2-nitro-5-thiobenzoate dianion (5) in neutral – mildly basic media

The colour change of this reaction from colourless to yellow is difficult to observe in artificial light, especially in low concentrations. In 2010, Pohanka *et al.*[23] used the ability of organophosphates to inhibit the action of AChE by developing a pH sensing colorimetric method. This method used a pH indicator to determine the increasing concentration of acetic acid resulting from the AChE catalysed hydrolysis of acetylcholine to choline and acetic acid.

4.1.4. Colorimetric detection of organophosphates

In 2011, Pohanka *et al.*[24] reported the use of AChE and indoxyl acetate as an alternative method to Ellman's assay for determination of AChE activity. Using a spectrophotometer, the authors measured the absorbance at 670 nm 30 min after mixing AChE and the indoxyl acetate substrate, in comparison with an Ellman's assay benchmark. The indoxyl acetate method was developed for use with biological samples taken from cases of suspected organophosphate poisoning. The reaction had the benefit of overcoming the limitations of Ellman's assay as the development of a blue colour was easier to observe than yellow. The author's method also allows the concentration of organophosphates to be determined in the presence of antidotes to organophosphate poisoning such as oximes which are known to cleave the DNTB and give false positive reactions while using Ellman's assay.[25] Other methods for colorimetric detection of organophosphates include the cleavage of indophenyl acetate, which is hydrolysed directly to give blue-purple coloured indophenol, the concentration of which can be measured as a function of absorbance at 625 nm using a spectrophotometer.[26]

Pavlov *et al.*[27] developed a method for the determination of organophosphates using thiol-inhibited growth of gold (Au) nanoparticles (NPs). Solutions of 0.1 M Tris buffer (pH 8.0) containing variable concentrations of thiocholine were inoculated with 16 μL of 0.13 U cm^{-3}[3] AChE solution in Tris buffer. The solutions were incubated at 35 °C for 15 min before mixing with 3.2 cm^3 1.25 mM $HAuCl_4$ solution. A glass plate was functionalised with an aminopropylsiloxane film and Au NPs before immersion in the enzyme solution for 5 min. The slides were washed with water and their absorbance was measured using a spectrophotometer. In the presence of an AChE inhibitor, plasmon absorbance bands of the NPs decreased, leading to a decrease in the intensity of a blue colour (λ_{max} = 570 nm). Due to the complexity of the protocol as well as the expense and technical challenge of synthesising Au NPs, this method is not suitable for use in heritage environments.

Climent *et al.*[28] also explored silica NPs functionalised with both thiols and aliphatic alcohols. The thiol subunit was selected as it was active towards a squaraine chromogenic probe, which reacts with the thiol and induces a loss of aromaticity in the probe causing a "bleaching" of the blue colour. An alcohol moiety was selected as alcohols are known to undergo acylation reactions with organophosphate substrates. The principle of the method was that the phosphorylation of the alcohol inhibits the reaction between the thiol and probe. The chromogenic response was measured using a spectrophotometer at 643 nm, with the presence of organophosphates inhibiting the reaction between the thiols and squaraine probe. The study also reported that a NP functionalised polyethylene terephthalate stick was exposed to air containing 5 ppm diisopropylfluorophosphate for 15 min. After immersion of the stick in a solution containing the squaraine probe, the solution remained blue, which indicated that phosphorylation of the alcohol had taken place as the thiol had not reacted with the probe. Exposure of the NP stick to air free of organophosphate vapour caused the blue solution to lose colour upon addition of the exposed stick, indicating a successful qualitative test for the absence of organophosphate vapour. As previously, the use of

[3] NB Units are used in place of mass for enzymes: 1 U corresponds to 1 mmol substrate catalysed min^{-1} at 25 °C.

NPs adds a technical complexity to this method which would limit its utility in heritage environments.

Species with azo functionality have also been used in optical sensors to measure organophosphates, as the resulting product has a strong colour and can be measured spectrophotometrically, as discussed by Costero.[29] This short communication reported an azo probe adsorbed onto silica gel underwent an intramolecular cyclization process causing a colour change from yellow to colourless on interaction with organophosphates in both vapour-phase and solution-phase.

White, Legako and Harmon[30] developed an assay using monosulfate tetraphenyl porphyrin (TPPS), a reversible competitive inhibitor of AChE which exhibits unique spectral characteristics on complexation with the enzyme. The addition of an organophosphate inhibitor to the porphyrin-enzyme complex led to displacement of the porphyrin form the active site of the enzyme. This displacement was observed as a decrease in absorbance at 446 nm, the λ_{max} value of the porphyrin-enzyme complex.

These publications show that while there is a small range of colorimetric methods for the determination of organophosphates using AChE-based assays and other chromogenic reactions, the preparation of these tests often requires synthesis of nanomaterials or complex protocols for immobilising reagents. As a further consideration, the methods reported above required the use of a spectrophotometer to determine the result which makes these methods more expensive and less useful for heritage institutions.

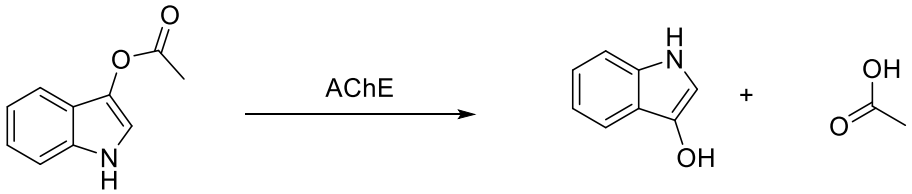
4.2. Research aims and objectives

Since organophosphates have been used in commercial pesticide formulations for both museum and agricultural uses, the rapid determination of these substances has become increasingly important. While organophosphates can be detected by chromatographic methods, on-site colorimetric detection could offer a more cost-effective option for field work, and using the methods presented here, does not require specialist equipment or training to use. The development of an organophosphate sensor is therefore of interest to the heritage and environmental science communities and as

such, finding safe and non-toxic methods of detection has become a global priority in recent years.

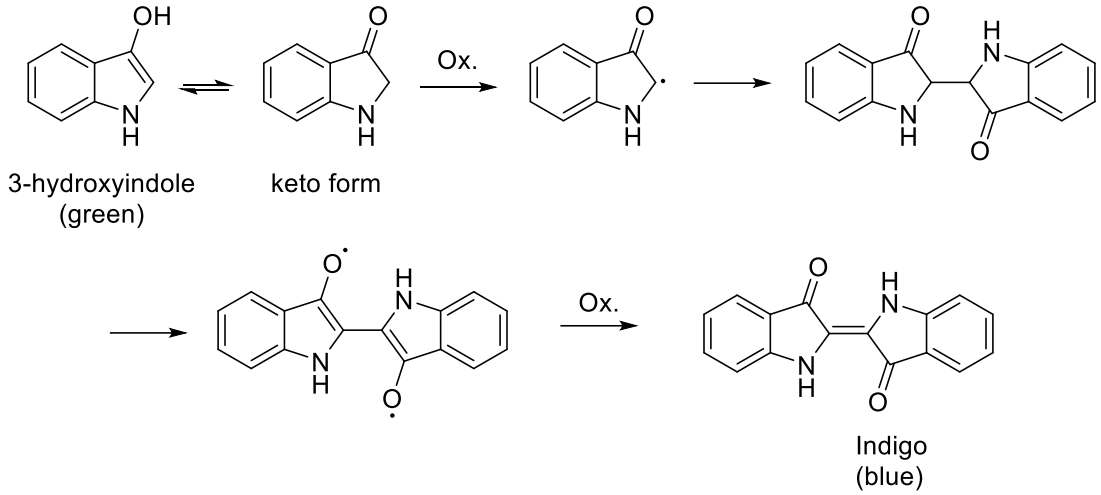
In this work, AChE has been immobilised in an agar gel matrix, and used to develop a swabbing kit using indoxyl acetate as a substrate for the enzyme. The development of an easily observed blue colour (compared to the yellow of Ellman's assay) allows for these swabbing kits to be used as disposable alternatives to currently available assays, allowing faster, easier, in-the-field detection. The utility of this sensor has been demonstrated for use with both contact swabbing and vapour-phase sample collection. The demonstration of the sensor in the vapour-phase is an important advantage over existing techniques for use in heritage collections as sampling fragile objects often prohibits direct contact.

The principle of the detection method used in the kit was based on the formation of indigo, a strongly coloured blue dye, and was intended to be a screening test offering on-site, near real-time detection of organophosphate pesticides by eye, without the need for instrumental analysis. The chromogenic reaction taking place is the AChE catalysed hydrolysis of indoxyl acetate to form a green coloured intermediate, 3-hydroxyindole, and acetic acid as shown in **Scheme 4.3**.[31]



Scheme 4.3: AChE catalysed hydrolysis of indoxyl acetate to 3-hydroxyindole[31]

The 3-hydroxyindole rapidly interconverts between the keto and enol tautomers. The subsequent dimerisation and oxidation of 3-hydroxyindole to form indigo dye causes a colour change from green to blue as the indigo product forms. A proposed reaction scheme, **Scheme 4.4**, shows the formation of indigo proceeding from the keto form of the 3-hydroxyindole.



Scheme 4.4: Oxidation and dimerisation of 3-hydroxyindole to form indigo

As with the development of the mercury sensor in **Chapter 5**, the key objective in this work was to develop an economical way to determine the presence of a chemical hazard by designing an easy to use and easy to interpret sensor. This allows collection custodians to make informed choices regarding the exhibition and handling of objects in their collections. Further key development objectives are outlined here:

Examination of solution chemistry

Initial experiments were performed to examine conditions required for the reaction of indoxyl acetate with AChE to form the indigo dye. AChE was immobilised in an agar matrix, as the success of this material in the formation of a mercury sensor was demonstrated in **Chapter 5**. These gels containing immobilised enzyme were exposed to solutions of indoxyl acetate in order to determine their effectiveness at catalysing the indoxyl acetate into the indigo precursors. The reaction was then inhibited by adding increasing volumes of dichlorvos to reaction mixtures in order to determine a detection limit.

Assessment of spiked materials

Agar hydrogels loaded with AChE were formed in a centrifuge tube containing a swab loaded with indoxyl acetate substrate. The swab was wetted and used to take samples from filter papers and soft toys spiked with dichlorvos, which were used as analogues for treated collection items.

Vapour-phase determination of dichlorvos

A vapour-phase sensor kit was prepared and included an enzyme gel in a centrifuge tube containing an indoxyl acetate swab, which had previously been shown to be able to measure dichlorvos in the vapour-phase.

4.3. Materials and methods

4.3.1. Assessment of activity using Ellmans's assay

Approximately 216 mg of acetylthiocholine chloride was dissolved in 10 cm^3 of deionised (DI) water for use in the Ellman's assay. Approximately 40 mg of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, ≥98% BioReagent, Sigma-Aldrich, Gillingham) was dissolved in 10 cm^3 of 0.1 M phosphate buffered saline (PBS, Sigma-Aldrich, Gillingham) which was prepared by dissolving 5 PBS tablets in 100 cm^3 of DI water. An enzyme solution was prepared by dissolving approximately 500 U of AChE (type VI-S, from *Electrophorus electricus,* Sigma-Aldrich, Gillingham) in 100 cm^3 of 0.01 M PBS solution giving a 5 U cm^{-3} solution, to which 2 mg of bovine serum albumin (BSA, lyophilized powder, >96%, Sigma-Aldrich, Gillingham) was added. The enzyme solution was stored at approximately 4 °C in a separate refrigerator to the dichlorvos standard. The solution was stored for no longer than 2 w after which time it was re-prepared.

In a cuvette, 3 cm^3 of 0.1 M PBS, 20 µL acetylthiocholine chloride (≥99% (TLC), Sigma-Aldrich, Gillingham) solution, 100 µL of DTNB solution and 50 µL of 5 U cm^{-3} AChE were mixed well by inversion before the mixed solution was measured using a spectrophotometer. Control experiments were conducted by repeating the analysis without acetylcholine chloride. The solutions were measured at 412 nm every 5 min over a 2 h period and the analysis was performed in triplicate.

4.3.2. Solution phase proof of concept and optimisation of reaction between AChE and indoxyl acetate

4.3.2.1. Initial proof of concept reaction and observation of colour development

The following reactions were undertaken in order to verify the chemistry taking place in the hydrolysis of indoxyl acetate by AChE. Indoxyl acetate (>95% (TLC), Sigma-Aldrich, Gillingham) solution was prepared by dissolving approximately 500 mg of indoxyl acetate in 10 cm^3 of absolute ethanol (HPLC grade, Fisher Scientific, Loughborough), and the solution was sonicated for approximately 5 min to yield a light pink solution. The solution was stored at approximately 4 °C for 24 h. PBS solution was prepared at a concentration of 0.01 M by adding 1 PBS tablet to 200 cm^3 of deionised (DI) water.

Solution phase chemistry of the organophosphate sensor was investigated by adding 1 cm^3 of 0.01 M PBS to the wells of a polypropylene cuvette holder. A 50 µL volume of indoxyl acetate solution and 50 µL of 5 U cm^{-3} AChE were added to the well and the potential colour change observed over 10 min.

4.3.2.2. Effect of buffer ionic strength on colour development

50 µL aliquots of indoxyl acetate solution and 50 µL of 5 U cm^{-3} AChE were added to a well containing 1 cm^3 of 0.01 M or 0.1 M PBS. Colour change was observed over 10 min.

4.3.2.3. Effect of pH on colour development

The effect of solution pH was investigated by altering the pH of the buffer solution used. The 0.01 M PBS solution was adjusted to pH values of 8, 9 or 10 by the dropwise addition of 0.1 M KOH (certified ACS, Fisher Scientific, Loughborough). Solution pH was measured using a Jenway 3505 pH probe. The volumes of adjusted buffers and blank Milli-Q water, measuring 1 cm^3, were added to cuvette wells as shown in **Table 4.1** below.

Table 4.1: Preparations used to investigate effect of pH on enzyme activity in indoxyl acetate reaction optimisation

Well no.	Reaction medium	pH
1	Milli-Q water	6
2	0.1 M PBS	7
3	0.01 M PBS	8
4	0.01 M PBS	9
5	0.01 M PBS	10

Solutions containing 100 µL of 5 U cm^{-3} AChE and 50 µL of indoxyl acetate were then added to each well. Control wells were also prepared as above but without addition of the enzyme. Five replicates were performed for each experiment.

4.3.2.4. Effect of enzyme concentration on colour development

Enzyme concentration was varied by adding 250, 500 or 750 µL of 5 U cm^{-3} AChE to wells containing 1 cm^3 of 0.01 M PBS and 50 µL indoxyl acetate solution. A fourth control experiment was prepared as above but without the addition of AChE. The colour change of each solution was observed over 20 min.

4.3.2.5. Effect of dichlorvos on colour development

Inhibition of the hydrolysis of indoxyl acetate was investigated using dichlorvos (Pestanal analytical standard, Sigma-Aldrich, Gillingham) as a model inhibitor. A 200 ng μL^{-1} dichlorvos solution was prepared by mixing 20 µg of dichlorvos with approximately 80 cm^3 of DI water in a 100 cm^3 volumetric flask and diluting to volume. Dichlorvos solutions used throughout this chapter were prepared immediately before use.

Volumes of 1 cm^3 of 0.01 M PBS, 50 µL of indoxyl acetate and 200 µL of 5 U cm^{-3} AChE were added to act as a negative control; the same solutions and volumes were

then used in a separate well with the addition of 200 μL of 200 ng μL^{-1} dichlorvos as a positive control.

To investigate the effect of increasing volumes of dichlorvos on enzyme activity, 1 cm^3 of 0.01 M PBS, 50 μL of indoxyl acetate and 200 μL of 5 U cm^{-3} AChE were each added to increasing volumes (0, 50, 100, 200, 300 or 400 μL) of 200 ng μL^{-1} dichlorvos solutions. The colour change was observed after 20 min. Once the approximate amount of dichlorvos required to inhibit the formation of indigo was determined, the experiment was repeated. Here, 1 cm^3 aliquots of 0.01 M PBS, 50 μL of indoxyl acetate and 200 μL of 5 U cm^{-3} AChE were added to 0, 25, 50, 100, 150 or 200 μL of 200 ng μL^{-1} dichlorvos solution; with colour development observed over 20 min.

4.3.3. Immobilisation of enzyme onto agar support

AChE was immobilised into gels by preparing 5% w/v concentrations of agar in 0.01 M PBS by heating 2.5 g agar powder (laboratory grade, Fisher Scientific, Loughborough) and 50 cm^3 of 0.01 M PBS in a steam sterilisation unit until a thin, clear liquid was formed. Replicate 1 cm^3 aliquots of this liquid were removed and pipetted into cuvette trays for mixing with AChE solution before being allowed to set. Loading of enzyme into the gels was performed by pipetting 200 μL of 5 U cm^{-3} AChE solution into individual cuvette wells, to which 1 cm^3 of the agar solution was added once the agar solution had cooled to approximately 35-40 °C. Each gel contained approximately 1 U of enzyme. A second series of agar gels each containing 10 U AChE were prepared by adding 200 μL of a 50 U cm^{-3} AChE solution to 1 cm^3 5% w/v agar.

A further series of gels was prepared using 1% w/v agar in order to investigate the effect of decreased agar concentration in the hydrogel matrix. PBS buffered 1% (w/v) agar gel was prepared by adding approximately 500 mg of agar powder to 50 cm^3 of 0.01 M PBS and heated as described above. The 1% agar gels were each loaded with 1 U of AChE by pipetting 200 μL of a 5 U cm^{-3} solution of AChE into a cuvette well containing cooled agar solution and the resulting gels were mixed well with a pipette tip. A series of 1 and 5% gels were also prepared with no added enzyme to act as controls.

Dried gels were prepared by drying 5% gels loaded with 1 U AChE, and allowing the gel to dry under ambient conditions over approximately 48 h. Enzyme activity was not checked in agar and relies on a constant systematic error to prevent false results.

4.3.4. Reaction of prepared agar gels with indoxyl acetate substrate

Recognising that the colour change reaction progressed more rapidly in PBS than water, all agar gels were subsequently prepared using 0.01 M PBS solution.

4.3.4.1. Effects of rinsing prepared AChE gels

Duplicate solutions of approximately 2 cm^3 of 0.01 M PBS were each mixed with 100 µL indoxyl acetate solution and added to a beaker. A 1 cm^3 5% agar gel with a 1 U loading of enzyme prepared as described in **Section 4.3.3**, was added to one beaker, whilst a second identical gel was first rinsed in DI water before being added to a second beaker. Potential colour change was observed over 1 h.

4.3.4.2. Comparison of colour development in freshly prepared and dried AChE gels

An experiment was performed to compare the colorimetric response of dried gels to freshly prepared gels, each prepared from 1% agar with a 1 U loading of AChE. Gels were added to 2 cm^3 of 0.01 M PBS containing 100 µL of indoxyl acetate solution. Potential colour change was observed over 1 h.

4.3.4.3. Effect of enzyme concentration in AChE gels

A comparative study of 1% w/v agar gels containing 1 U of enzyme and 10 U was conducted by placing each gel in 2 cm^3 of 0.01 M PBS containing 100 µL indoxyl acetate solution. Potential colour change was observed over 10 min.

4.3.4.4. Determination of detection limits for developed AChE gels

Inhibition of the enzyme gel was investigated by adding 200 µL of 200 ng μL^{-1} dichlorvos solution to 2 cm^3 of 0.01 M PBS and 100 µL indoxyl acetate solution. A 1% agar gel loaded with 400 µL 5 U cm^{-3} AChE. The experiment was repeated (n = 5) in the presence of a negative (no dichlorvos present) control.

Increasing volumes of dichlorvos solution were used in further replicate experiments to establish an approximate detection limit for the gels. Volumes of 50, 100, 150 or 200 μL of a 200 ng μL^{-1} aqueous solution of dichlorvos were added to 2 cm^3 of 0.01 M PBS buffer containing 100 μL indoxyl acetate solution. A 1% w/v agar gel with a 1 U loading of AChE was added to each reaction vessel and the colour changes were observed. A control reaction was also performed using 2 cm^3 of 0.01 M PBS and 100 μL of indoxyl acetate without the addition of dichlorvos.

4.3.5. Assessment of developed sampling kits

After initial experiments were prepared to understand the solution-phase and gel based chemistry of the reaction, the method was adapted to prepare a near real-time, *in situ* colorimetric method that could be used for the detection of organophosphates by swabbing objects suspected of contamination.

Gel kits were prepared by pipetting 1 cm^3 of 1% agar gel solution into the bottom of a centrifuge tube and once the gel cooled to approximately 30-40 °C, 400 μL of 5 U cm^{-3} AChE solution was added and the resulting mixture was stirred manually using a Pasteur pipette tip, before being allowed to solidify. The enzyme activity in the prepared gel was approximately 2 U. Cotton swabs were then dipped into an indoxyl acetate solution and allowed to dry completely before being added to the enzyme tubes, inserting so that the bud loaded with substrate was not in contact with the gel (**Figure 4.1**). These sampling kits were capped and stored at approximately 4 °C until used.

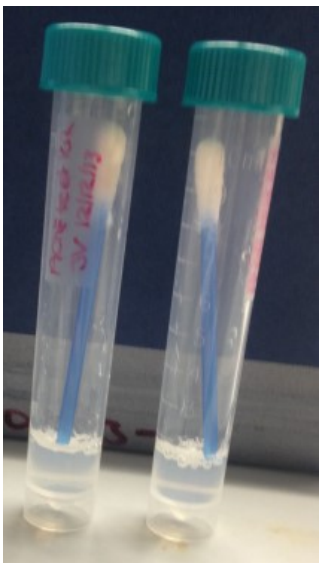


Figure 4.1: Prepared organophosphate test kits with a set AChE gel at the bottom of the tube and indoxyl acetate loaded buds at the top of the tube.

4.3.5.1. Sampling kit detection limit

To determine the lowest concentration of dichlorvos required to inhibit the reaction of the sampling kit, indoxyl acetate buds were wetted with 100 µL of DI water and had 5 µL, 20 µL, or 100 µL of 200 ng μL^{-1} dichlorvos solution pipetted directly onto the cotton bud, before being introduced to the enzyme gel in the centrifuge tube. A control experiment was performed by pipetting water onto the bud in place of dichlorvos.

4.3.5.2. Response of sampling kit after storage

To assess the response of the sampling kit to dichlorvos after storage, batches containing five freshly prepared sampling kits each were stored under the conditions described in **Table 4.2**. After approximately 6 w, sampling kits were allowed to equilibrate to room temperature before 100 µL of 0.01 M PBS buffer was pipetted onto the tip of the bud, which was returned to the tube inverted so that it was in direct contact with the gel.

Table 4.2: Storage conditions of sampling kit batches

Batch number	**Light/dark**	**Approximate temperature (°C)**
1	Light	20
2	Dark	20
3	Dark	5
4	Dark	5
5	Dark	-18

4.3.6. Simulated field study using prepared kits

The utility of the sampling kit for a museum application was assessed by preparing fur analogues spiked with dichlorvos as an inexpensive means of replicating contaminated objects in a natural history collection. Approximately 100 µL of 200 ng μL^{-1} aqueous dichlorvos solution was pipetted onto a polyester fur cloth from a soft toy (approx. 2 cm x 2 cm) and the cloth was left to dry for approximately 1 h. Cotton swabs from the sampling kit were wetted with 100 µL distilled water before being used to collect 10 horizontal and 10 vertical strokes from the cloth before being re-inserted in to the centrifuge tube so that the bud was in contact with the enzyme gel. The kit was stored at room temperature and the colour change was observed after 2 h. The experiment was repeated with a second section of cloth.

A second material chosen for investigation was filter paper, as an analogue for paper-based collections. A number of applications of 100 µL of 200 ng μL^{-1} aqueous solution of dichlorvos were made to a series of 6 filter papers (110 mm Whatman no. 1) as detailed in **Table 4.3** to give increasing masses of dichlorvos available for swabbing.

Cotton swabs from the kits were wetted with distilled water and used to take 10 vertical and 10 horizontal strokes from each of the filter papers, before being applied to the gels to develop the colour. Potential colour change was observed after 24 h at ambient temperature.

Finally, 75 µL applications of a 1000 ng μL^{-1} solution of dichlorvos were applied to six filter papers. Swabs from three kits were wetted with 100 µL distilled water, the first swab was used to collect 10 horizontal and 10 vertical strokes from a single filter paper before being placed in contact with the gel inside a centrifuge tube. The second swab was used in the same way to collect organophosphate residue from two filter papers, and the third swab used to collect the residue from three filter papers. After collection of the samples, swabs were returned to the centrifuge tubes so that they were in contact with the enzyme gel and potential colour changes were observed over 1 h.

Table 4.3: Spiking of filter papers with dichlorvos solution to investigate effect of dichlorvos on colour development in developed kits

Filter paper	No of applications	Total mass dichlorvos applied (µg)
A	0	0
1	1	0.2
2	4	0.8
3	6	1.2
B	0	0
4	1	75
5	2	150
6	3	225

4.3.7. Vapour-phase analysis of dichlorvos using developed testing kit

As a key aim of this work was to develop a method to determine the presence of organophosphates in the vapour-phase, a proof of concept experiment was performed to support this aim. Approximately 100 µL of a 2000 ng μL^{-1} solution of dichlorvos in distilled water was placed in the cap of an inverted centrifuge tube and tightened into the body of the tube. The bottom of the tube contained a 1 cm^3 of 1 U AChE gel. The tube was allowed to equilibrate for 5 d and kept inverted, separating the dichlorvos and enzyme gel throughout.

After exposure, the cap was discarded and a swab containing the indoxyl acetate was wetted with 100 μL PBS and placed in contact with the gel. The procedure was replicated using 100 μL distilled water in place of the dichlorvos as a control. Both kits were left to develop for 1 h after introduction of indoxyl acetate swab.

4.4. Results and discussion

4.4.1. Assessment of activity using Ellman's assay

Ellman's assay was performed in order to provide a benchmark value for the activity of the AChE used in the indoxyl acetate assay. Absorption was measured at 413 nm and plotted as a function of concentration until a maximum of approximately 1 absorbance unit (AU) was reached. A linear trendline was fitted to the data with an R^2 value of 0.9905 indicating highly correlating data with a slope of 0.1870 AU min^{-1}. Using the Beer Lambert law to calculate the concentration of the absorbing species, a molar absorptivity of 13 970 mol^{-1} cm^{-1} was used as published by Riddles *et al.*[32] correcting an error in the original Ellman paper.[22] Dividing the slope by the molar absorptivity (multiplied by a pathlength of 1 cm) gave a concentration rate of 1.34×10^{-5} mol min^{-1}. As 1 μmol of substrate catalysed per minute is equal to one enzyme unit (U), the activity here were measured at 13.4 U. The specific activity of the enzyme (activity divided by the volume of enzyme used) was calculated at 0.268 U μL^{-1}.

The assay was repeated 5 times, the results of which are tabulated in **Table 4.4**. The low relative standard deviation of these data indicated a high degree of repeatability and precision.

Table 4.4: Precision of Ellman's assay over 5 replicates

Replicate	**Slope**
1	0.1911
2	0.1863
3	0.1824
4	0.1923
5	0.1946
Mean	0.1893
% Relative standard deviation	2.6

4.4.2. Solution phase proof of concept and optimisation

The intensity of all colour change reactions in this chapter were observed without instrumentation as a key objective of this work was to be able to develop a method of determining organophosphates without the need for analytical instrumentation. This would enable the use of these methods by untrained collection staff working on-site in heritage environments.

4.4.2.1. Solution phase proof of concept and optimisation of reaction between AChE and indoxyl acetate

Initial reactions between the AChE and indoxyl acetate in PBS allowed the blue colour to be observed after 10 min. This developed to an apparent maximum over the course of 12 h. Mixing of the enzyme with the indoxyl acetate and PBS solutions in the wells with a Pasteur pipette did not have an observable effect on the rate of colour development compared to an unmixed control after 35 min, so it was assumed that the solution in the wells was uniform, as the turbulence caused by the injection of solutions into the well was enough to cause sufficient mixing.

4.4.2.2. Effect of buffer ionic strength on colour development

A comparison of MilliQ water (measured at pH 5.6), 0.01 M PBS (pH 6.9) and 0.1 Mol PBS (pH 7.3) was performed to determine the effect of ionic strength on the ability of the enzyme to catalyse the indoxyl acetate substrate. Approximately 1 cm^3 each of MilliQ water, 0.01 M PBS or 0.1 M PBS were mixed with 50 µL indoxyl acetate solution and 50 µL of 5 U cm^3 AChE solution. Colour change was observed over 10 min. After this time, the darkest blue colour was observed in the 0.01 M PBS buffer, indicating the reaction was faster in this medium and that it was the most suitable solution to perform future experiments in.

4.4.2.3. Effect of pH on colour development

As the investigation into the ionic strength of the buffer solutions used buffers at slightly different pH values, an experiment was performed to examine the effect of pH on the rate of colour development. The effect of further modifying the buffer pH values within a range of pH 6-10 is summarised in **Table 4.5**. Colour change of the solutions was observed after 10 and 20 min and documented qualitatively. Addition of indoxyl acetate to pH 10 wells caused immediate colouration of the solution. This was observed in the control well also, indicating that the highly basic conditions were causing the reaction to proceed without the need for AChE. The presence of the hydroxide in the reaction medium at sufficiently high pH allowed nucleophilic attack of the carbonyl present on the indoxyl acetate, yielding the 3-hydroxyindole intermediate without the use of the enzyme.[33]

A less intense blue colour was observed in pH 9 solutions, supporting the above explanation regarding strongly basic solutions. No colour change was observed in either of the pH 6 solutions over the course of the experiment. Blue colouration was observed in pH 7 and 8 solutions containing enzyme, while the control solutions remained colourless, as expected. This supported conclusions regarding the suitability of PBS as a reaction medium drawn from earlier experiments as well as showing that pH ranges of approximately 7-8 were the most suitable for the progression of the reaction by enzyme catalysis. The use of neutral to slightly basic media allowed the reaction to produce a colour change without the need for strongly basic reagents. This

lower hazard approach is likely more suited to museum staff without a chemical sciences background who may not wish to handle strongly basic reagents.

Table 4.5: Effect of buffer pH on observed colour from AChE and indoxyl acetate

Buffer pH	Reaction solution		Control solution	
	10 min	20 min	10 min	20 min
6	No change	No change	No change	No change
7	Pale green	Light blue/green	No change	No change
8	Green	Blue/green	No change	No change
9	Blue/green	Dark blue	Light blue/green	Blue/green
10	Dark blue	Intense blue	Dark blue	Intense blue

The top well in **Figure 4.2** shows the solution at pH 8 with the enzyme added, exhibiting the dark blue colour of the uninhibited reaction. The bottom control solution (no enzyme) did not react. The image was taken after 20 min.

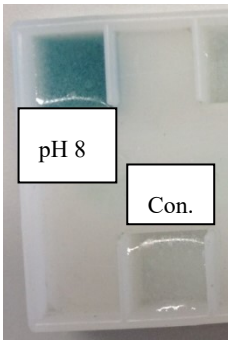


Figure 4.2: Image of solution phase reaction between indoxyl acetate and AChE after 20 mins at pH 8 (top) against a control not containing enzyme (bottom)

4.4.2.4. Effect of enzyme concentration on colour development

Having established a suitable pH range for the AChE inhibition assays, the effect of enzyme concentration was studied in order to optimise the assay conditions further.

For these experiments, it was found that the effect of increasing the concentration of enzyme present in the sample did not significantly alter the overall speed of colour development. Solutions containing larger concentrations of enzyme underwent faster conversion of indoxyl acetate to the 3-hydroxyindole intermediate, however the dimerisation of the 3-hydroxyindole occurred at approximately the same rate for each well and thus no difference in the intensity of the developed blue colour was observed.

The experiment was repeated using the same volumes of a more concentrated enzyme solution (50 U cm^{-3} compared to 5 U cm^{-3}) in order to lessen colour development time. In the solutions containing higher concentrations of enzyme, evidence of the faster production of the 3-hydroxyindole intermediate was provided by the development of a strong lime green colour. The green colour slowly turned to indigo over a 1 h period, showing that this secondary reaction was the rate determining step. As the development of the indigo from the 3-hydroxyindole intermediate took a longer time than the initial 5 U cm^{-3} experiment, there was no advantage gained by using more concentrated solutions of AChE. The use of more concentrated solutions of AChE is made more impractical by being significantly more expensive to make due to the amount of AChE used.

It was possible to increase the rate of colour development by performing the experiment in a sealed centrifuge tube and shaking by hand to introduce more oxygen into the liquid. Initially the unshaken control reacted at the same rate as the tube being shaken. Tubes were observed after 4 min to both be exhibiting similar green colours. At 12 min, the tube being shaken was noticeably darker than the control. This experiment acted as further evidence that the secondary oxidation/dimerisation reaction is the rate determining step. It was not possible to use the intensity of the green colour from the initial hydrolysis step of the reaction as the end point for the indicator due to the presence of the dimerisation reaction, as the secondary process would make it difficult to compare samples.

4.4.2.5. Effect of dichlorvos on colour development

Finally, the inhibition of the reaction by organophosphates was investigated using both negative controls (containing PBS, AChE and indoxyl acetate) and positive controls (containing PBS, AChE, indoxyl acetate and 40 μg dichlorvos). The reaction was

performed by mixing 1 cm^3 0.01 M PBS, 50 µL indoxyl acetate and 200 µL 5 U cm^{-3} AChE with increasing volumes of dichlorvos solution which was used as a model organophosphate substance. The intensity of the blue colour was observed after 20 min. **Table 4.6** summarises the results of these experiments.

As expected, increasing masses of dichlorvos inhibited the development of the indigo colour by blocking the hydrolysis of the indoxyl acetate. The most intense blue colour was observed in the negative control containing no dichlorvos, with lighter blue observed in the solution with 50 µL added dichlorvos solution, (a mass of 10 µg dichlorvos), with lighter still blue was observed in the solution containing 100 µL dichlorvos solution (20 µg mass of dichlorvos), while solutions containing higher concentrations did not appear to have formed any indigo as the solution remained colourless.

Table 4.6: Mass of dichlorvos required to inhibit action of 1 U AChE

Volume 200 ng μL^{-1} dichlorvos added (µL)	**Mass dichlorvos (µg)**	**Colour observed at 20 min**
0	0	Dark blue
50	10	Blue
100	20	Light blue
200	40	Colourless
300	60	Colourless
400	80	Colourless

Using the information from the previous experiment, a range of lower concentrations around 100 µL dichlorvos solution (20 µg dichlorvos) were used in repeat experiments to examine the lower concentrations of dichlorvos required to inhibit the reaction, see **Table 4.7**.

As with the higher concentration experiments, the control solution was most darkly coloured, with a clear trend of reduced colouration with increasing concentrations of dichlorvos. Solutions containing 100, or150 µL of 200 ng μL^{-1} dichlorvos solution was very pale blue in colour compared to the control solution indicating the reaction had been inhibited by dichlorvos. The solution containing 200 µL of dichlorvos did not colour, indicating that 40 µg of dichlorvos was enough to inhibit 1 U AChE with these experimental conditions.

As the reaction is catalytic, the enzyme was not consumed during the reaction, meaning that the success of the reaction must be determined at a specific time point as opposed to waiting for a maximum colour development. If left long enough, even a very low concentration of AChE would catalyse the unreacted indoxyl acetate in the sample, leading to a false negative result. For this reason, it was recommended to select a time point of 20 min for the recording of results.

Table 4.7: Lower masses of dichlorvos required to inhibit 1 U AChE

Volume 200 ng μL^{-1} dichlorvos added (µL)	**Mass dichlorvos (µg)**	**Colour observed at 20 min**
0	0	Dark blue
25	5	Blue
50	10	Light blue
100	20	Very light blue
150	30	Very light blue
200	40	Colourless

4.4.3. Immobilisation of enzyme onto agar

After the solution-phase chemistry had been investigated, work was performed to immobilise the enzyme into a gel matrix in order to allow easier handling of the material by untrained personnel. Initial experiments with agar were performed during

the development of a colorimetric sensor for mercury (see **Chapter 5**) and the same 5% w/v concentration of agar was initially used here. The 5 % agar gel was selected as it provided gels physically robust enough to withstand handling and transfer between containers using tweezers, but was still porous allowing permeation of the reagent solution. A 10% w/v solution was also assessed, but found to be too difficult to work with as it was very viscous and set much faster than other solutions, giving little working time for pipetting solutions. Therefore, a 1 cm^3 gel containing 200 µL of a 5 U cm^{-3} solution of AChE in a 1% w/v agar solution was compared to the same reagents loaded into 5% w/v agar solution.

On exposure to 100 µL indoxyl acetate solution in 2 cm^3 0.01 M PBS, colour change in the 1% gel was observed after approximately 15 min while the 5% gel did not show colouration until approximately 22 min. This comparison showed that a 1% w/v gel allowed faster reaction times with the substrate, but also offered a longer working time with the liquid gel prior to the gel solidifying. This increase in working time was likely due to the lower number of crosslinks present in the gel structure as a result of less agar per unit volume and was of benefit when loading a large number of gels with enzyme solution. The combination of a longer working time with the agar solution and faster colour development times led to the use of the 1% gels in future experiments.

4.4.4. Reaction of prepared AChE gels with indoxyl acetate substrate

Suitable pH conditions, enzyme concentration and proof of concept were established with solution-phase chemistry, as well as a method for immobilising AChE in agar. Enzyme activity was not checked in agar as it was in buffer solution, therefore the method relies on a constant systematic error to eliminate false positive results. The following experiments were conducted in order to assess the suitability of the enzyme gels.

4.4.4.1. Effects of rinsing prepared AChE gels

Distribution of the enzyme throughout the prepared gels was investigated by preparing two identical 1 cm^3 enzyme gels containing 1 U AChE and rinsing the surface of one with DI water. Gels were each exposed to 2 cm^3 0.01 M PBS mixed with 100 µL

indoxyl acetate solution and the resulting colour change was observed over the course of 1 h.

Similar changes from colourless to blue were observed from each gel, indicating that rinsing the gels did not remove a significant portion of the enzyme from the surface. This suggested that the enzyme was distributed through the gel matrix as opposed to bound on the surface of the gel.

4.4.4.2. Comparison of colour development in freshly prepared and dry AChE gels

Freshly prepared "wet" gels were compared to desiccated gels that had been dried at ambient conditions for 2 d. Both gels were prepared from 1% w/v agar solutions and had a 1 U AChE loading. After exposure to 2 cm^3 0.01 M PBS mixed with 100 μL indoxyl acetate solution, colour development was observed over 1 h. As expected, the dried gels experienced considerable shrinkage during the drying process, yielding small semi-transparent films. The reaction speed of these dried films was shown to be significantly slower than the wet gels, making them less appropriate for use as organophosphate sensors. It was assumed that the pore size of the dried films was smaller than that of the fresh wet gels, causing slower ingress of the reactant solution into the interior of the gel for reaction with the bound enzyme. Investigation with dried gels was not continued further.

4.4.4.3. Effect of enzyme concentration in agar gels

The effect of enzyme concentration within the wet gels was assessed by preparing 1 cm^3 gels containing 1 or 10 U of AChE and the rate of colour development was examined after the addition of 2 cm^3 of 0.01 M PBS mixed with 100 μL of indoxyl acetate solution. The 10 U gel reacted similarly to the solution phase experiment, producing a green colour after approximately 4 min. After 10 min, no further colour change was evident. The production of the green colour from the 3-hydroxyindole was anticipated, it was expected that this colour would fade to blue as the intermediate 3-hydroxyindole was oxidised to indigo. As this secondary oxidation did not take place, it was suggested that as the increased action of the enzyme hydrolysed a larger number of indoxyl acetate molecules, leading to increased acetic acid concentration

within the gel. This increased acid concentration would cause the pH to drop below 6, inhibiting the secondary reaction. This hypothesis is in agreement with solution phase results obtained in **Section 4.4.2**. In contrast, the 1 U gel, as expected, coloured blue after approximately 10-15 min. Due to the small volume of the test reaction, it was not possible to check the pH of the solution with a pH probe, and the highly coloured solution made estimation with pH paper impossible.

4.4.4.4. Determination of detection limits for developed AChE gels

With an appropriate concentration of enzyme selected for use with the agar gels and wet gels selected over dry gels, inhibition of the action of the enzyme gels by dichlorvos was investigated by adding 200 μL of 200 ng μL^{-1} dichlorvos solution to 2 cm^3 of 0.01 M PBS and 100 μL indoxyl acetate solution. A 1% agar gel loaded with 200 μL of 5 U cm^{-3} AChE solution was added to catalyse the reaction. Gels added to the control (no dichlorvos present) solutions began to show colouration after approximately 15 min, in a similar timescale to the liquid reactions. This colouration after 15 min was shown to be repeatable over the five replicates used. Solutions containing dichlorvos caused slower colouration of the gels over this period, with the blue colour of the gels being notably less intense. It was noted, however, that at this concentration of dichlorvos (approximately 0.09 ng μL^{-1}) the reaction was only retarded, not inhibited.

Determination of the minimum amount of dichlorvos required to inhibit the reaction was performed by adding enzyme gels to solutions of substrate and buffer containing increasing volumes of dichlorvos. Volumes of 50, 100, 150 or 200 μL of a 200 ng μL^{-1} aqueous solution of dichlorvos were added to 2 cm^3 0.01 Mol PBS buffer containing 100 μL each of indoxyl acetate solution. Control experiments were performed without the addition of dichlorvos. After 15 min, gels in solutions containing 50 or 100 μL of the dichlorvos solution showed colouration similar to that of the control gel, indicating only partial inhibition of the enzyme. Gels exposed to 150 and 200 μL dichlorvos did not display colouration, indicating that the reaction was completely inhibited at this level of dichlorvos contamination. This shows that the developed gels have a limit of detection in solution of approximately 13 μg dichlorvos per cubic centimetre.

4.4.5. Assessment of developed sampling kits

Gel kits were prepared in order to have the enzyme gel and indoxyl acetate solution in a form that could be easily transported and used by unskilled personnel. The kits were formed by pipetting 1 cm^3 of a 2 U cm^{-3} gel solution into the bottom of a centrifuge tube and allowing the gel to solidify, holding the gel in place at the end of the tube. A cotton bud dipped in indoxyl acetate solution was inserted shaft-first to the tube to stand inverted with the bud and gel not in contact with one another. It is recommended that a reagent blank, showing a negative response is used with these sampling kits in order to minimise reading errors.

4.4.5.1. Sampling kit detection limit

The swabs from four gel kits were removed from the tube and wetted with 100 µL DI water before being spiked with either 5, 10 or 100 µL of dichlorvos solution by directly pipetting the solution onto the swab. The swab was placed back in the tube with the bud in direct contact with the enzyme gel. A control kit was prepared without the application of dichlorvos. Colour changes in the gel were observed after 6, 14, 23 and 40 min, and are presented in **Table 4.8**.

At the 6 min time point, a light blue colour was observed in the gel with the 5 µL dichlorvos loading that was approximately equal to the blue colour in the control kit. A very light blue colour was observed in the gel which had 20 µL of dichlorvos added, and no colouration observed at all in the gel containing 100 µL dichlorvos. At the 14 min time point, the control gel was now a darker blue, which differentiated it from the lighter blue of the gel containing 5 µL dichlorvos. The gel containing 20 µL dichlorvos was a very light blue, and no colouration was observed in the gel loaded with 100 µL dichlorvos solution. After 23 min, colour had further developed in all kits, which were now darker than the previous time point. The control was now an intense dark blue, and rank ordering was noted in the 5 – 100 µL gels, with each being a sequentially paler blue with increasing loading of dichlorvos solution. At this time point, the gel with the highest loading of dichlorvos solution was a very light blue colour, indicating that the enzyme had not been completely inhibited. At this time the control, 5 or 20 µL loaded gels were indistinguishable, with a blue colour developed

in each, which had diffused deeply into the gel. The 100 µL loaded gel displayed a very light blue colour which had not penetrated deeply into the gel.

Table 4.8: Colour development in kits from direct dichlorvos application on swabs

Volume of dichlorvos applied (µL)	**Time of observation (min)**			
	6	**14**	**23**	**40**
0	Light blue	Blue	Dark blue	Dark blue
5	Light blue	Light blue	Blue	Dark blue
20	Very light blue	Very light blue	Light blue	Dark blue
100	None	None	Very light blue	Very light blue

The data collected here highlighted the necessity to examine the colour of the loaded gels at a specific time, as the catalytic nature of the enzyme means that even small amounts of AChE that have not been inhibited by dichlorvos will still continue to develop observable colour changes in the gels. It is recommended that 20 min be used as this cut off point as it allows enough time for colour to develop, without allowing development of colouration in heavily contaminated samples where enzyme inhibition is not 100%. Based on a 20 min measurement time, the threshold for observable inhibition of the 2 U AChE loading of the gel in these test kits was 20 µL of a 200 ng μL^{-1} dichlorvos solution, which is the equivalent of 4 µg dichlorvos. However, for complete inhibition of the enzyme to be observed at the 20 min reading point, 100 µL of the 200 ng μL^{-1} solution of dichlorvos (20 µg) was required in this test.

4.4.5.2. Response of sampling kit after storage

In order to provide an assessment of the performance of the sampling kits over time, a number of test kits were stored under different conditions for 6 w prior to use. When removed from cold storage, kits were allowed to warm to room temperature before swabs from the kits were wetted with PBS. Wetted swabs were returned to the tubes

and placed in contact with the gel to develop the colour over a period of 40 min. The results are shown in **Table 4.9**.

Examination of the intensity of colour development indicated that that there was no apparent difference in the use of the gel after storage at 20, 5, 8 or -18 °C in either light or dark. It was anticipated that due to the presence of agar gel, a known microbiological growth media, that gels stored at temperatures greater than -18 °C would be contaminated with the growth of microorganisms after the 6 w storage period. However, the use of a high temperature steam bath to prepare the agar meant that gels were free of visible microbial growth after 6 w. As there was no observable difference in the colour development times of kits from each of the storage conditions tested, kits may be stored at ambient temperature without the need to be placed somewhere dark. This overcomes a potentially important practical point, in that enzyme solutions typically require refrigerated storage prior to use, which may not be available to users.

Table 4.9: Response of sampling kits after 6 w in storage under different conditions

Storage conditions and approximate temp	**Time after addition of swab (min)**			
	10	20	30	40
Room temp, light (20 °C)	Light blue	Blue	Dark blue	Dark blue
Room temp, dark (20 °C)	Light blue	Blue	Dark blue	Dark blue
Refrigerator (5 °C)	Light blue	Blue	Dark blue	Dark blue
Refrigerator (8 °C)	Light blue	Blue	Dark blue	Dark blue
Freezer (-18 °C)	Light blue	Blue	Dark blue	Dark blue

4.4.6. Simulated field study using prepared kits

The results shown in **Section 4.4.3** indicated that an agar gel support was satisfactory for immobilising the AChE enzyme, and that the indoxyl acetate colour change

reaction was suitable for the qualitative determination of dichlorvos. Several experiments were performed using filter paper and fur from soft toys which had been spiked with dichlorvos to mimic materials found in archives and natural history collections as a means to illustrate the application and ease of use by non-scientists working in heritage environments.

The fur analogues were spiked with either a 100 μL of a 200 ng μL^{-1} or 2000 ng μL^{-1} solution of dichlorvos. Using the indoxyl acetate swab from the kit, 10 vertical and 10 horizontal strokes were made on the material after the swab was wetted with DI water. The swab used to rub the material spiked with 200 ng μL^{-1} of dichlorvos turned blue confirming that the mass of dichlorvos spiked onto the substrate was not enough inhibit the action of the enzyme. In contrast, the swab used to rub the surface of the substrate spiked with 2000 ng μL^{-1} of dichlorvos solution did not change colour after 20 mins, indicating full inhibition of the enzyme was achieved by the mass of dichlorvos collected.

Table 4.10 lists the results observed over the full range of the swabbing experiment conducted on filter paper. Numbered filter papers were loaded with a number of applications of dichlorvos solution. Dichlorvos solution was applied to discrete points on the filter papers, forming individual points of contamination on each filter paper. The dichlorvos solution was applied to one point on filter paper 1, four points on filter paper 2 and six points on filter paper 3, applying 100 μL of a 200 ng μL^{-1} solution each time.

A second set of papers (4-6) were prepared through multiple 75 μL applications of a more concentrated 1000 ng μL^{-1} solution.

Table 4.10: Preparation and results of dichlorvos filter paper swabbing experiments

Filter paper number	No of applications	Volume dichlorvos applied (µL)	Concentration of dichlorvos solution applied (ng μL^{-1})	Total mass dichlorvos applied (µg)	Result for presence of dichlorvos
A	0	0	0	0	Negative
1	1	100	200	0.2	Negative
2	4	100	200	0.8	Negative
3	6	100	200	1.2	Negative
B	0	0	0	0	Negative
4	1	75	1000	75	Slight positive
5	2	75	1000	150	Positive
6	3	75	1000	225	Positive

Results from filter papers 1, 2, and 3 showed that the concentration of dichlorvos recovered from the surface of the paper was not sufficient to inhibit the reaction as colour was observed in all test kits (in **Table 4.10** experiments are labelled A, and 1-3).

Using higher masses of dichlorvos (filter papers B, and 4-6) the results were more promising as no colour was observed in the test kits that were used to swab the surface of filter papers loaded with 150 or 225 µg of dichlorvos. This showed that the swab can be used to pre-concentrate samples prior to performing the assay. This will allow end users of the kit to collect samples over larger areas, should they suspect very low level contamination. Slight blue colouration was observed on the kit used to test filter paper 4. The intensity of the blue colour from filter paper 4 was clearly less than that of the control kits, indicating that the reaction has been significantly, if not completely inhibited. Should end users to the kit wish to collect sufficient quantities of dichlorvos to inhibit the reaction, small areas of the object surface could be swabbed to inhibit the reaction giving a positive result. However, if the objects were contaminated with lower concentrations of dichlorvos, a large area of the object would need to be swabbed to collect a sufficient mass of dichlorvos to inhibit the reaction. It was recommended that users of these kits in the field perform the test with both positive and negative controls

in place so that partial inhibition of the enzyme may be compared to both positive and negative results for the presence of organophosphates.

As the principle of the sensor presented here is the inhibition of AChE by organophosphate compounds, it should be acknowledged that there are substances present in heritage institutions that may act as interferents. Some heavy metals are reported to have an inhibitory effect on AChE due to binding to the anionic active site on the enzyme.[34-39] Irreversible inactivation of 15 nM AChE from *Torpedo californica* was reported by Frasco *et al.*[39] after exposure to solutions of 1-10 μM $HgCl_2$. However, the same study reports that inhibition of an equal concentration of AChE from *Electrophorus electricus* (as used in this study) required $HgCl_2$ in the range of 1-10 mM, a 1000-fold higher concentration of mercury. The authors noted that the inhibition of the *E. electricus* AChE was reversible. The authors suggested that a free cysteine at position 231 on the *T. californica* AChE was able to react with mercury in the bulk solution causing permanent inactivation. As *E. electricus* does not contain a free cysteine, the inhibition reaction progressed *via* a different mechanism using other sites on the enzyme, resulting in reversible inhibition. The 1 mM $HgCl_2$ solution was the lowest concentration found to inhibit AChE from *E. electricus*. This is equivalent to 271.5 mg dm^{-3}, which would indicate that significant mercury contamination of an object being sampled would need to have occurred in order to have an effect on the determination of organophosphates using the AChE method. Due to heritage institutions often containing a wide range of mercury concentrations it is suggested that when swabbing case study objects for organophosphate contamination, swabs also be used with the mercury sensor developed in **Chapter 5**.[40-44] As the lowest concentration of mercury required to inhibit the enzyme is almost three times greater than the highest concentration used in the development of the mercury sensor in **Chapter 5** it is expected that concentrations of mercury high enough to act as interferents with the organophosphate sensor will be readily detected.

Other heavy metals historically used in pesticide formulations such as arsenic may also inhibit AChE function. Patlolla and Tchounwou[38] reported a positive correlation of increasing doses of arsenic trioxide with decreasing AChE activity in Sprague-Dawley rats. However, the authors based their conclusions on the dose administered to the rats,

not as serum concentration of arsenic. As such it is difficult to determine what concentration arsenic begins to have an inhibitory effect on the action of AChE in this paper. Bocquené *et al.*[45] reported IC_{50} values (the concentration of an inhibitor where the activity of the enzyme is reduced by half) of arsenic trioxide in the presence of dichlorvos as being in the range of 4.7-10.7 mg dm^{-3}. The authors also reported that the combined presence of arsenic trioxide and dichlorvos had a synergistic effect, enhancing the inhibitory effect of the organophosphate. This was found to be in agreement with the results of Forget *et al.*[46] who reported similar findings, where arsenic and dichlorvos reduced AChE activity after 96 h of exposure by at least 65% when mixture levels were only a quarter of the concentration required to kill 50% of the *Tigriopus brevicornis* population used in the study.

While the additive effect of arsenic and organophosphate compounds on the inhibition of AChE would still allow the organophosphate sensor to indicate the presence of a hazard, the device may not be able to distinguish these two substances. Therefore, it highlights a limitation of the device in that it should only be used as a presumptive, rather than confirmatory test for the presence of organophosphates. However, chromatographic analysis according to the protocol described in **Chapter 3** may be used to further indicate the presence of organophosphate agents. False or exaggerated positive responses of the organophosphate sensor may also be ruled out by testing for the presence of heavy metals. While Odegaard, Zimmt and Smith[47] suggest a test for the presence of arsenic through the production of arsine gas and its subsequent reaction with mercuric bromide, it is recommended that institutions use x-ray fluorescence spectrometry in order to quantify the presence of arsenic if available, due to the lack of any "wet" chemical techniques involved.

There are also several species of microbial fungi which may have an effect on the action of AChE. The dominant fungal species found in indoor air typically belong to the *Aspergillus*, *Penicillium* and *Cladosporium* genera.[48-51] Of these species, it has been reported that *Aspergillus terreus*,[52, 53] *Penicillium citrinum*[53-55] and *Cladosporium cucumerinum*[51] which produce natural products with inhibitory effects on the action of AChE. In their comprehensive review, Houghton, Ren and Howes[55] listed several AChE-inhibiting compounds made by microbial fungi as having IC_{50} values ranging

from 0.06 μM (terreulactone C) to 25.8 μM (arisugacin B) as well as noting that a number of arisugacin compounds (E-H) did not produce inhibitory effects. With respect to inhibitory substances produced by microbial action, it was not considered likely that this would induce false positive reactions in the sensor. This was believed to be a fair assumption as any inhibition of the enzyme requires both a microbial species capable of synthesising an AChE-inhibiting compound to be present, and the ability of that species to produce a great enough quantity of the inhibitor to significantly reduce the binding ability of the enzyme so that the sample was indistinguishable from a positive control. Typically, water activity values (the ratio of the vapour pressure of water in a sample to the vapour pressure of pure water under the same experimental conditions) in excess of 0.7 are required to support development of fungal microbes.[56] Water activity values in this range suggest damp and humid conditions which are unlikely to be encountered in a properly maintained heritage institutions, as the same conditions are also likely to be damaging to objects.[50, 57-59] However, although the risk of enzyme inhibition by microbial natural products is considered low, it is recommended as a precautionary measure to further reduce this risk that swabs from objects for analysis are not taken from areas of the object visibly contaminated with fungal colonies.

4.4.7. Vapour-phase analysis of dichlorvos using developed testing kit

Approximately 100 μL of a 2000 ng μL^{-1} solution of dichlorvos was places in the cap of a centrifuge tube, with the bottom of the centrifuge tube containing 1 cm^3 of a 1 U AChE gel. The cap containing the dichlorvos was screwed back on to the body of the centrifuge tube, which was held inverted for 5 d to allow the dichlorvos vapour to equilibrate throughout the headspace of the tube, while not allowing the liquid dichlorvos to come into direct contact with the enzyme gel. After the equilibration time, the body of the centrifuge tube containing the gel was removed from the dichlorvos source in the cap and a swab dipped in the ethanolic indoxyl acetate solution before being placed in direct contact with the gel. The colour change was observed after 1 h against a control using water in place of dichlorvos as seen in **Figure 4.3**.

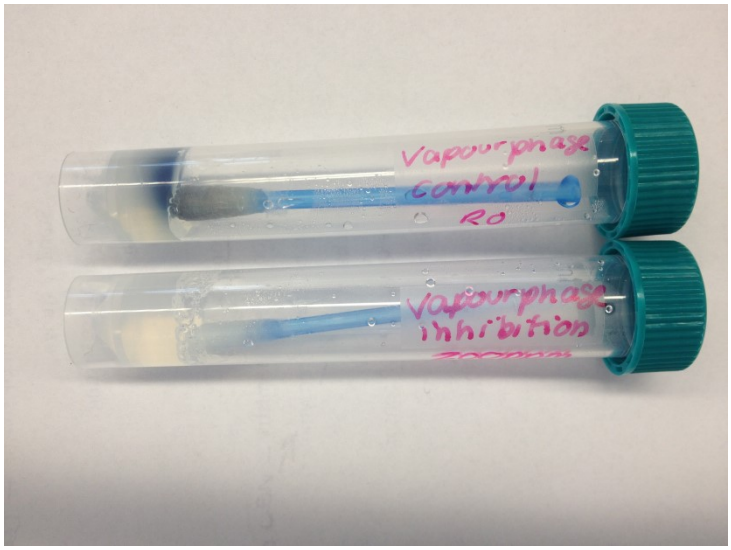


Figure 4.3: Control (negative, top) and dichlorvos sample (positive, bottom) from vapour-phase sampling trial

The picture in **Figure 4.3** indicates that the control tube had developed the indigo colour in the interior of the gel, not just the surface. In comparison, no indigo colouration has been observed in the gel exposed to dichlorvos. This shows the wet gel was sufficiently porous to allow diffusion of vapour-phase analytes throughout the gel material, showing the potential of the kit as a tool for atmospheric sampling. This is an important result as it highlights the utility of the gel as a potential vapour-phase sensor for dichlorvos, allowing minimally invasive, *in situ* detection of organophosphates in the atmosphere without the need for instrumentation or specialist training.

A current limitation of this work is that there is no calibration scale available to enable approximate quantitation of the results. As such, it is possible that the utility of the method developed here could be expanded by developing such a scale to enable quantification of the colour change of the gels. For example, a number of colorimetric apps have been developed making use of the widespread distribution of smartphones wit camera functionality for the detection of key analytes in particular assays.[60-64] With this in mind, it may be possible to develop a method to quantify the colour development of the sensor here. One such method by Oncescu *et al.*[65] reported the use of a custom external accessory on a smartphone to perform light reflectance measurements from an end-point enzymatic reaction to determine cholesterol in blood

samples. The accessory used test strips which separated plasma from the whole blood sample and directed an aliquot of this to an analyte-specific reaction pad. Here, cholesterol oxidase enzyme converted total cholesterol and high density lipoprotein cholesterol to cholest-4-en-3-one and hydrogen peroxide. The peroxide then reacts with di-substituted aniline to form quinone-imine dyes, producing a colour change. The smartphone camera is used with the accessory to capture reproducible images with a 100 by 100 pixel area, using the smartphone flash fitted with a siloxane diffuser to ensure uniform illumination. A dedicated app is then able to analyse the luminosity, hue and saturation data from the images to quantify the cholesterol level, displaying the value on the screen. The challenge with the approach reported by the Oncescu *et al.*[65] is that it would require development of the software itself, as well as external accessories in order to reproducibly perform the quantification. This is due to the difficulty in capturing standardised images using a typical smartphone, thanks to the camera's inbuilt colour- and light-balancing functions. These functions typically altering ratios of red, blue and green in the image to give the appearance that the image has been captured in bright ambient light, so lighting conditions must be carefully controlled. As such, it may be difficult to perform accurate quantitative measurements with such devices without developing handset-specific external housing and specific software to manage the settings of the phone's camera in order to achieve this degree of control. If these challenges were suitably addressed in the development of such a device, it may present a means to quantify the response of the organophosphate sensor developed here, addressing one of the limitations of this preliminary work.

4.5. Conclusions

This study successfully demonstrated the use of indoxyl acetate and AChE immobilised in agar as a suitable means of investigating the presence of dichlorvos in solution and in the vapour-phase. It is suggested that the results present promising new test kits that could be used to provide simple, on-site test kits for the presence or absence of dichlorvos in solution of the vapour phase. Moreover, the test kits may also be used to swab the surface of a material and detect dichlorvos if present at reasonable concentration. Although the results presented here were at the proof of concept stage, the promising results indicate that the utility of this method should be probed further.

Moreover, this work has simplified current colorimetric methods of analysis for organophosphates by using a reaction that produced a clearly visible colour change in ambient conditions that was easily be detected without the need for instrumentation. As the developed kit relies on the action of AChE upon an indoxyl acetate substrate, it is potentially able to determine the presence of other AChE-inhibiting substances like carbamate pesticides such as carbofuran.

As with the agar gels developed for use as mercury sensors in **Chapter 5**, the AChE agar gels developed here exhibited the properties of the robust and easy-to-handle agar, while retaining the fast colour development times observed in solution. AChE gels developed here were shown to retain reactivity towards the indoxyl acetate substrate after a storage period of 6 w, potentially allowing the enzyme gel to be prepared at another location and transported to the sampling location for use.

A key result of this work is that it demonstrated the potential of the developed test kit for the detection of dichlorvos vapour without the need for contact sampling. The benefit of such a kit to the heritage community would be that objects considered too fragile for contact sampling can potentially be tested for the presence of organophosphate pesticides without touching the object.

However, consideration must be made for the presence of other AChE inhibiting substances, particularly heavy metals which may be present as part of historical biocide formulations applied to collections. In these instances, it is recommended to use either the presumptive testing sensor for mercury developed in **Chapter 5** or employ a technique such as XRF spectroscopy in order to determine the presence of these substances.

Overall, this work has successfully demonstrated a means of the determination of vapour-phase dichlorvos through the preliminary development of a sampling kit that can be used with traditional swab-based sampling too. The advantages of the prepared sampling kit are that does not require any technical knowledge to be able to operate, and can give a fast, easily understood result in near real-time without the need for instrumentation. It is expected that the ease of use, low cost and lack of instrumentation

could potentially allow the developed sampling kits to be successfully used in heritage environments for the sampling of organophosphate pesticides.

4.6. References

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5. Development of optical sensors for the selective determination of mercury

5.1. General toxicity of mercury compounds

Mercury is among the most hazardous of the potentially toxic elements (PTEs). It exists in different chemical and physical forms with elemental mercury (Hg^0), inorganic (Hg^{2+}), methylmercury (MeHg) being considered the most important in terms of toxicity and impact on the environment.[1] Mercury undergoes a biogeochemical cycle, interchanging between the terrestrial, aquatic and atmospheric environments[2] (see **Figure 5.1**.).[3]

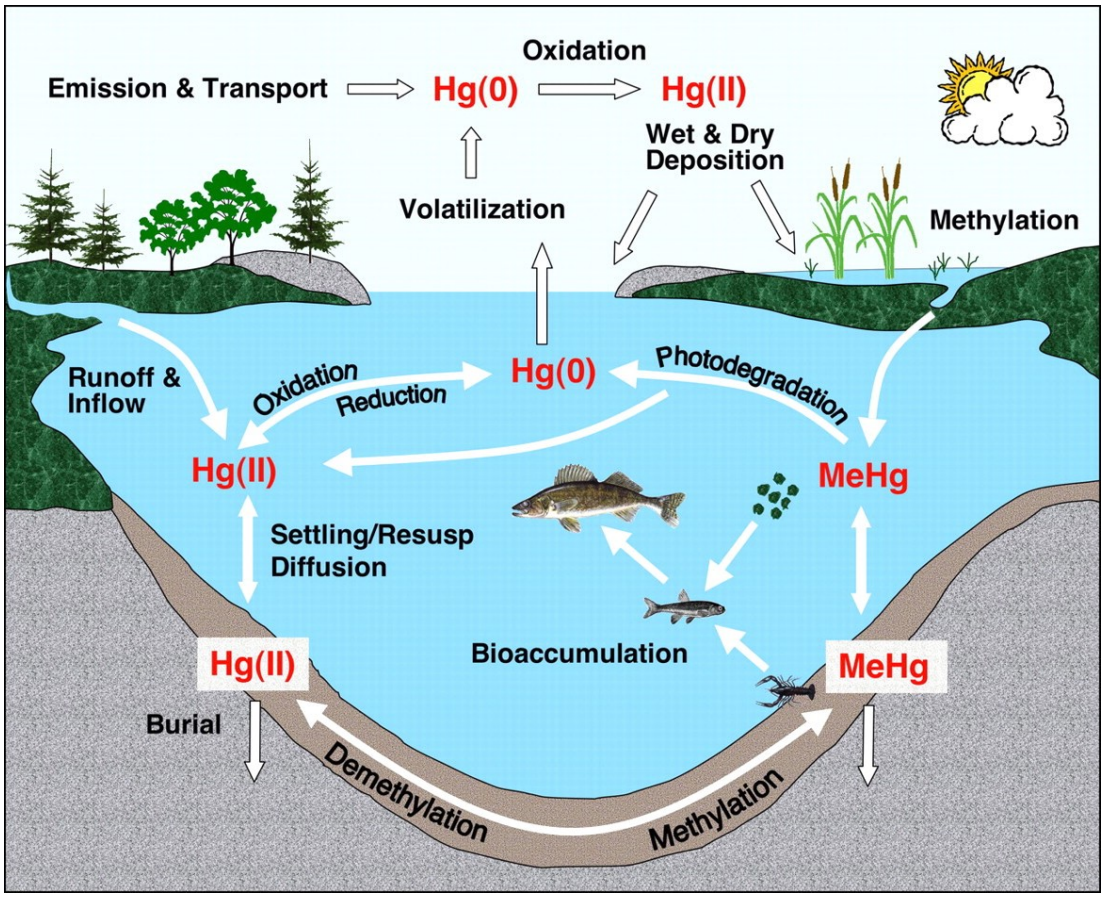

Figure 5.1: The mercury cycle in a lake and its watershed.[3] Hg(0) and Hg(II) refer to elemental and Hg^{2+} mercury, and resusp indicates resuspension.

As mercury is a liquid at standard temperature and pressure with a vapour pressure of 171.3 Pa at 20 °C, elemental mercury is significantly volatile.[4] This volatility allows

degassing from rock, soils and surface water to the atmosphere. Once in the atmosphere, photo-oxidation converts the elemental mercury to Hg^{2+} which can combine with water vapour and return to the surface with rainfall, depositing the Hg^{2+} in soils and water.[2, 3] Once in soils and water, the Hg^{2+} can be converted by anaerobic bacteria to the significantly more toxic MeHg which is bioaccumulated in the food chain.[3] The toxicity of mercury depends on its form; MeHg is considerably more toxic than elemental and inorganic mercury due to its ability to bind with sulfur-containing amino acids cysteine and methionine.[5, 6] This binding allows transport across the blood-brain and placental barriers where it affects the central nervous system and foetal development. A significant mercury poisoning case was reported, and subsequently extensively studied at Minimata Bay, where consumption of contaminated fish led to toxic levels of mercury in humans.[7] Alkyl-mercury antifungal seed treatments also led to widespread poisoning in Iraq in 1972 after accidental human consumption of the contaminated grain.[8] While Hg^{2+} is less toxic than MeHg, it is still acutely toxic by oral administration and is listed as a category 2 reprotoxin. [9]

5.2. Detection of mercury compounds in heritage environments

The use of Hg^{2+} as a biocidal treatment in heritage collections is well documented. Mercury compounds, particularly mercuric chloride ($HgCl_2$), have been previously used to protect artefacts from attack by various insects and their larvae.[10-15] Briggs *et al.*[16] described a method in which 60 g of $HgCl_2$, and 60 g of phenol were dissolved in 2 dm^3 methylated spirits before being painted onto dried herbarium specimens using a fine brush. Briggs and co-workers further noted that "it is not [the Cambridge Herbarium's] custom to indicate… that specimens have been treated with mercuric chloride".[16] The continued persistence of mercury residues such as these may present a problem to those handling treated collections; however it is difficult to determine which objects have been previously treated and if so, the concentrations of mercuric chloride that still exist on the surface of the object.[11] Therefore, monitoring of mercury compounds is becoming increasingly important to heritage environments.

Current methods for the detection of mercury in heritage environments typically include taking measurements with handheld X-ray fluorescence (XRF) detectors. However, these XRF instruments are expensive, require specialist training for

handling the radioactive source and only measure discrete points where the beam focusses. Dussubieux *et al.*[17] described the capability of XRF as "semi-quantitative at best" and noted that the technique did not take matrix or surface variation into account.

Purewal[18] reported that the National Museum of Wales had employed an external contractor, Anachem Analytical Laboratories, to measure the concentration of mercury vapour present in the air of the botany division. A calibrated pump was used to draw air through a 0.8 μm mixed-cellulose ester filter at a rate of 2 dm^3 min^{-1} for 3 h. After this time, the filter was removed and digested in nitric acid before analysis by cold vapour atomic absorption spectrometry (CV-AAS). All concentrations of mercury detected were below 0.1 μg m^{-3}, which is under the UK Health and Safety Executive guideline of TWA 20 μg m^{-3}. The objects in the collection were examined by exposing samples to ultraviolet light at 366 nm to observe fluorescent areas. Visibly fluorescing areas were cut from the object and analysed by atomic absorption spectrometry (AAS) and proton-induced X-ray emission (PIXE) after nitric acid (HNO_3) digestion. After the analysis of over 500 samples, it was clear that low mercury concentrations (μg g^{-1}) were readily detected, although the expense of analysis by AAS and PIXE as well as the destructive nature of the sampling process would likely make this a less practical approach for heritage institutions.

Oyarzun *et al.*[19] described the use of a portable AAS instrument to measure mercury in the air of MAF Herbarium in Madrid, where real-time concentrations of 404 – 7977 μg m^{-3} were measured. The concentrations reported in desiccated plant material by Oyarzun *et al.*[19] were 1.2 – 11 967 μg g^{-1}. The study reported that not all objects were treated and that higher concentrations of mercury were typically found on older specimens, as opposed to objects that had been acquired over the last 40 years. Plant material was reported to be the source of the mercury present in the air. This approach is not likely to be adopted on a large scale in heritage institutions due to the requirement of specialised instrumentation and technical skill in the interpretation of the data.

Kataeva, Panichev and van Wyk[20] monitored mercury concentrations in the air of two South African herbaria by bubbling air through a dilute HNO_3 impinger for 1.5 h at a rate of 1.1 dm^3 min^{-1}. Further samples of plant material were collected and digested in

concentrated HNO_3 and hydrogen peroxide at 180 °C at a pressure of 300 psi for 10 min. The impinger solution, and sample extracts were analysed by electrothermal AAS. High mercury concentrations in the range of 114 – 432 μg g^{-1} were measured on treated plant material and up to 1 μg m^{-3} of mercury in air was reported in both South African herbaria, suggesting that mercury contamination in heritage environments is both widespread and severe. As with the technique used in the MAF Herbarium, the method used here requires specialised instrumentation to analyse samples, as well as staff comfortable with the use of concentrated acids and oxidising agents at high temperatures. It is therefore not presumed likely that heritage institutions would adopt this technique.

Direct-reading sampling tubes are also commercially available for the determination of mercury.[21] These pseudo-quantitative devices are glass tubes packed with a colorimetric reagent through which a volume of air is pumped. The length of colour change that develops throughout the reagent bed is used as an indicator of the approximate concentration of the pollutant being measured. The reagent, copper iodide, turns grey-white to yellow/beige in the presence of mercury. It was reported that these tubes have an operational range of 50 – 2000 μg m^{-3} elemental mercury.[21] However, the manufacturers also warn that mercury cannot be detected in the presence of halogens, so for heritage environments where the principle source of mercury is $HgCl_2$, these devices are unsuitable.

Copper iodide colorimetry was also used for the determination of mercury in fish, by screen-printing a slurry of copper iodide onto a filter paper.[22] The filter paper was exposed to the headspace of a vessel containing a solution for the digestion of samples. This solution contained 1:1 solution of HNO_3 and sulfuric acid (H_2SO_4) in the presence of 0.1% vanadium oxide (V_2O_5). Sample oxidation was completed by the addition of 5% potassium permanganate ($KMnO_4$) solution. Mercury vapour was generated by a 50% antinomy chloride ($SnCl_2$) solution in 50% hydrochloric acid (HCl). The authors reported exposing the filter paper to the generated mercury vapour for 5 – 40 min to produce the characteristic white/grey to yellow/beige colour change in the copper iodide. The same author also reported the use of a coated filter paper for the detection of mercury in soil, sediment and gold mining residues.[23] While this work demonstrates

that it was possible to detect vapour-phase mercury, it also required destructive testing of the sample and a complicated digestion reaction using concentrated acids. It is therefore not considered suitable for use in heritage insitutions.

The use of 4,4'-bis-(dimethylamino)-thiobenzophenone, also known as Michler's thioketone, has been reported as a colorimetric reagent for the determination of mercury. In acetate buffered media, Michler's thioketone reacts to form a red complex with an absorbance maximum at 565 nm.[24] However this method is unlikely to be suitable for use in heritage collections as interference from noble metals (Au, Ag, Pt) is likely to be present on objects containing these metals held in heritage collections.[25]

Xylenol orange has been reported as a complexing agent for Hg^{2+}, forming ternary complexes in basic solutions.[26-28] This reaction produces a colour change in the presence of the mercury cation with the absorbance maximum changing from 582 to 590 nm (giving an orange to yellow colour transition).[26] The preparation of a basic solution (such as hexamine) to facilitate this reaction adds complexity that would make the method unsuitable for heritage environments.

Dithizone is one of the most widely used colorimetric reagents for the detection of mercury.[29] Irving and co-workers[30] reported preparation of mercury dithizonate as a scarlet solid which reacts with mercury in a 2:1 ligand:metal stoichiometry, with nitrogen and sulfur donating electrons to bind to the cation.[31] An orange-yellow complex is produced with an absorbance maximum of 490 nm.[32] The same authors also remarked that the complex exhibits photochromism in bright light, with the mercury complex solution turning an intense royal blue before reverting to orange over time when stored in the dark. Wichmann[33] reported favourable complexation of mercury and dithizone in acidic media, whilst the chemistry at higher pH values was poorly understood.

In 2000, Odegaard, Carroll and Zimmt[34] published a collection of tests for pesticide-treated museum objects, including one describing the use of diphenylcarbazone (see **Figure 5.2**) for mercury. Odegaard advised that the diphenylcarbazone (DPC) be prepared as a 1% ethanolic solution and applied to swabs collected from the contaminated object. The approach was not widely adopted perhaps because it required

collection workers to prepare and use solutions that they were unfamiliar with, and that handling of chemical solutions was typically enough to dissuade cautious institutions of their use.

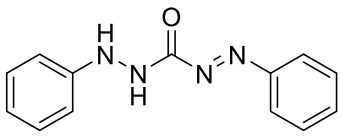

Figure 5.2: Chemical structure of diphenylcarbazone

The use of DPC and the reduced form, diphenylcarbazide, for the determination of mercury outside the field of heritage science has been well documented[35-38] having first been discovered by Cazenueve.[39] The theory of DPC's colour chemistry is not well known and published papers have only discussed it in general terms.[40] However, it was reported that DPC complexed with Hg^{2+} ions in a similar 2:1 ratio.[41-45] This stoichiometry was determined by Balt and van Dalen[44, 45] by reacting HgO and DPC in 80/20% v/v water and ethanol, however no structure was published alongside these data. A 2:1 structure was published in 2015 by Mergola *et al.*[46] as part of an ion imprinted polymer used for the sequestration of Hg^{2+} from drinking water supplies, the Hg-DPC complex is shown in **Figure 5.3**

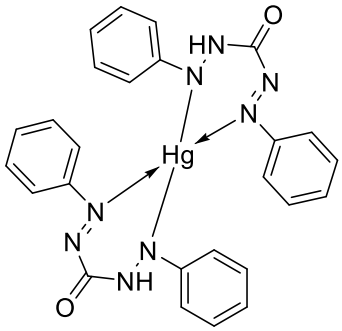

Figure 5.3: Mercury-diphenylcarbazone complex structure[46]

This agrees with the 2:1 diphenylcarbazone structure seen in the mercury complex of the sulfur analogue dithizone, shown in **Figure 5.4**, tentatively suggesting the cation:metal ratio of the DPC-Hg complex structure may be comparable due to the molecular structural similarities of DPC and dithizone.[47] The dithizone structure was determined by X-ray diffraction from crystals formed by the reaction of dithizone and

mercury chloride in sulfuric acid before being washed with acid and recrystallized from pyridine.

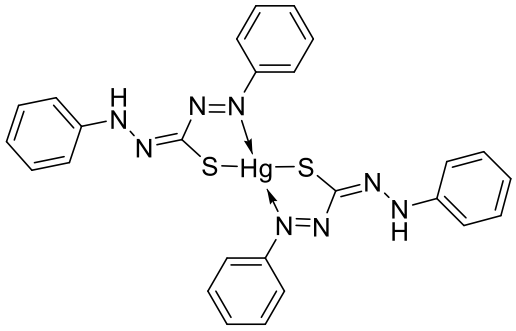

Figure 5.4: Structure of mercury(II) bis(dithizonate)[47]

Contrary to the results of Balt and van Dalen,[44, 45] and Mergola *et al.*,[46] Blaton *et al.*[48] described a complex with a DPC:cation ratio of 1:2, as shown in **Figure 5.5**, with each mercury forming an almost linear bond with the nitrogen and chlorine atom, sandwiched between the places of two benzene rings. The structure presented features two tetravalent nitrogen atoms, assumed to be coordinating to the metal atoms *via* the lone pair with the positive charges balanced by free Cl^-. Goodgame *et al.*[49] reinterpreted these data (without reanalysing the substance) and concluded the mercury interacts with only one benzene ring.

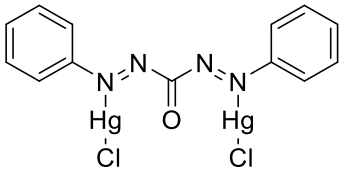

Figure 5.5: Blaton *et al.* structure showing 2:1 cation:DPC stoichiometry

However, it is difficult to determine the applicability of this structure to the current work as the authors did not state how the complex was synthesised, only that it was recrystallized from methanol and water. As such, and with no similar metal halide complexes discovered in the Cambridge Crystallographic Database, this author has concluded the structure in **Figure 5.3** to be the more likely molecular structure in accordance with published literature described above.

5.2.1. Use of immobilised supports for colorimetric reagents

By incorporating a colorimetric reagent into a matrix such as a silica xerogel or polymeric hydrogel, on-site chemical detection can still be achieved without having to prepare reagent solutions in the field. If the reagents used provide a colour change on interaction with the target analyte, users can interpret the results by eye in near-real time without the need for instrumental analysis. Khan *et al.*[50] described the preparation of DPC-doped sol-gel silica monoliths to investigate solid phase extraction of Hg^{2+} from aqueous media. The monoliths were prepared via a base-catalysed process, using ammonium fluoride as the catalyst, with an ethanolic solution of DPC being added to the reaction slurry to incorporate the ligand into the silica matrix. After formation, gels were dried at 48 °C causing shrinkage and shattering. After 7 d, the pieces were ground and sieved to obtain particles of approximately the same size. The doped silica powder was soaked in water to extract any unbound DPC before oven drying. After drying, the powder was added to aqueous solutions of radiolabelled mercury nitrate and equilibrated for 5 min before centrifuging and analysing the supernatant for residual ^{203}Hg by gamma scintillation. The paper reported the ability of the functionalised silica to remove 0.028 mmol g^{-1} Hg^{2+} from the solution compared to undoped sol-gel silica, as well as being able to recover the silica and regenerate it by eluting the Hg^{2+} with 0.05 mol nitric acid. While the authors noted that the DPC changed colour from red to violet, it was not the focus of the paper and the potential for developing the silica here as a colorimetric test for mercury was not discussed.

In addition to using the sol-gel process to synthesise inorganic xerogels, a large number of synthetic and biological polymers are suitable for the formation of organic hydrogels. Hydrogels are defined as gels in which the fluid in the interstices of the gel (the swelling agent) is water.[51] Hydrogels have seen increased use as colorimetric supports for the detection of metal ions.[52-57] Lin *et al.*[56] reported the use of a DNAzyme – a DNA molecule able to perform a catalytic reaction – as a crosslinker in an acrylamide hydrogel used as a catalytic platform for the sensing of copper(II) ions (Cu^{2+}). The crosslinker consisted of a poly-DNAzyme and poly-substrate forming a "cage" filled with gold nanoparticles. In the presence of Cu^{2+} the substrate underwent site-specific catalytic self-cleavage, releasing the nanoparticles from the cage and subsequently produced a strong absorption in the visible spectrum at 520 nm.

Joseph *et al.*[55] immobilised a mercury-binding thymine-rich DNA on the polymer backbone. The introduction of Hg^{2+} caused site-selective bridging between thymine bases resulting in a hairpin structure. This allowed the fluorescent dye (SYBR green I) to bind to the hairpin, emitting green fluorescence. In the absence of Hg^{2+}, an intense yellow fluorescence was observed, attributed to the dye binding to the unfolded DNA. Similarly, Helwa *et al.*[54] used thymine-rich Hg^{2+} binding DNAs immobilised in a biopolymer to detect the metal ions in the presence of SYBR green I. This methodology used a blue light transilluminator and the fluorescence was recorded using a digital camera. Fluorescence was also observed under a fluorescence microscope, but the authors reported that it took up to 1 h to obtain a stable optical signal due to long diffusion distances. Dave *et al.*[53] utilised a polyacrylamide backbone, thymine-rich DNA and SYBR green I dye to detect Hg^{2+} ions by photographing the fluorescence before quantitatively recording the intensity of the light emitted using a gel documentation system with an excitation wavelength of 365 nm.

Despite the previously reported success of these methods, they are unlikely to be applied in heritage environments for a number of reasons. First, production of the polyacrylamide gels present a safety concern as they require synthesis from acrylamide solution, which is acutely toxic by inhalation and oral exposure, a skin sensitiser, an irritant, reprotoxic, carcinogenic and displays germ cell mutagenicity.[58] Similarly the production of binding DNAs require specialist training in order to successfully synthesise the target aptamers. The use of fluorescence lamps to view the results of the experiment, while not necessarily a problem for many institutions, will add further complexity to the procedure.

5.2.2. Research aims and objectives

The work in this chapter presents the development of a novel Hg^{2+} sensor for use in heritage environments. Hg^{2+} was determined to be the species of mercury most prevalent in historical collections after a survey of literature naming $HgCl_2$ as a commonly used heritage biocide.[10, 11, 16, 19, 20, 59-61]

DPC was selected as the colorimetric reagent because of its unambiguous colour change in the presence of Hg^{2+} and because it is a well-established reagent for the

detection of heavy metals. As the use of DPC in solution form may present a problem for workers in institutions without laboratory facilities, immobilisation of the reagent was investigated in order to develop an optical sensor.

Two support matrices (silica xerogel and agar hydrogel) were assessed. The choice of matrix was determined by their ease of manufacture, ease of handling and low toxicity of both product and precursor materials. The sensor was based on the colour change of DPC in the presence of Hg^{2+} and was intended to be a screening test offering on-site, near-real time detection by eye without the need for instrumental analysis. The key objective was to develop an economical, easy to use sensor that could be used as an 'early warning device' to detect mercury contamination. This would allow custodians of collections to prioritise object treatment and ensure the safe exhibition and handling of objects in their collections. Moreover, the proposed sensors benefit from the use of non-toxic matrices compared to the linear polyacrylamide hydrogels used elsewhere in literature. The key developmental objectives are outlined below:

Examination of solution chemistry

After initial tests examined the colorimetric reaction between DPC and Hg^{2+}, the reagent was immobilised into both matrices. The 'sensors' were then exposed to Hg^{2+} solutions of known concentration to ensure a colour change was observed. The xerogels were then used in a case study to sample a Native American headdress previously treated with $HgCl_2$. Here, the xerogels were used to determine the viability of passive sampling using the silica matrix sensor.

Assessment of spiked materials

Agar hydrogels loaded with DPC were used to examine two materials spiked with $HgCl_2$ to simulate Hg^{2+} contamination on furs and aged parchment. Natural history collections typically house furs which contain large concentrations of biocidal contamination.[13, 62] In this project modern polyester soft toys were selected as an inexpensive fur analogue. Parchment dating from 1860 was supplied by the National Records of Scotland (previously the National Archives of Scotland) was also used as a test material to simulate contamination in herbaria as reported by Purewal.[12, 18, 60]

Selectivity of the chosen sensor

As DPC will change colour in the presence of more than one metal ion, the study was expanded to investigate the interaction of DPC with hexavalent chromium ions. While chromium was not identified as a priority pollutant in heritage environments, the ability of the sensor to selectively detect more than one target increased the utility of the work by addressing needs in the environmental sector. Instrumental colorimetric determination of the gels was not investigated due to the desire to develop a method that did not require instrumentation to read.

5.3. Materials and methods

5.3.1. Investigation of solution phase DPC colorimetry

5.3.1.1. Initial investigation of solution phase chemistry

Spectrophotometry was performed using a Model 440 UV-visible spectrophotometer (SI Photonics Inc., Tucson) over a wavelength range of 390 – 700 nm. 2 cm^3 of 50 mg dm^{-3} solutions of either $HgCl_2$ (ACS reagent, ≥99.5%, Sigma-Aldrich, Gillingham) or potassium dichromate ($K_2Cr_2O_7$, ACS reagent, ≥99.0%, Sigma-Aldrich, Gillingham) were added to a 1 cm^2 quartz cuvette, with 50 µL of 1% w/v DPC (mixture with diphenylcarbazide ~60%, Sigma-Aldrich, Gillingham) in ethanol (absolute, HPLC grade, Fisher Scientific, Loughborough) added. Blanks were collected from $HgCl_2$ and $K_2Cr_2O_7$ solutions prior to adding DPC. Measurements of pH values were recorded using a calomel electrode with platinum reference electrode using a Jenway 3505 pH meter.

5.3.1.2. Selective determination of Hg^{2+} in mixed metal solutions

Solutions of 4 cm^3 volume were prepared using 2 cm^3 each of a 100 mg dm^{-3} solution of $HgCl_2$ and $K_2Cr_2O_7$. Increasing volumes of 2.73 g dm^{-3} HCl were added to acidify the solutions, and the pH values were measured. To act as controls, four 2cm^3 solutions of distilled water, with the same volumes of HCl added, were used as sampling blanks. A summary of the eight solutions prepared are given in **Table 5.1**. One DPC gel was added to each solution and the colour change was visually observed after 5 min.

Table 5.1: Sample preparation for determination of Hg^{2+} in mixed metal solutions

Tube	Vol. $HgCl_2/K_2Cr_2O_7$ used (cm^3)	Vol. distilled water used (cm^3)	Vol. 2.73 g dm^{-3} HCl added (µL)	pH value
1	2/2	-	0	4.64
2	2/2	-	25	3.30
3	2/2	-	75	2.82
4	2/2	-	150	2.50
5	-	4	0	5.32
6	-	4	25	3.31
7	-	4	75	2.82
8	-	4	150	2.45

5.3.2. Sol-gel derived xerogels as immobilisation media

5.3.2.1. Synthesis of sol-gel derived xerogels

Gibson synthesis[63]

A 11.6 cm^3 aliquot of tetraethyl orthosilicate (TEOS, ≥99.0% (GC) Sigma-Aldrich, Gillingham) was added to 10.3 cm^3 of EtOH and stirred for 30 min. Approximately 11.5 cm^3 of distilled water, acidified to pH 1 with hydrochloric acid (HCl, 10 N certified, Fisher Scientific Loughborough), 1 cm^3 of glycerol (≥99%, Sigma-Aldrich, Gillingham), 1 cm^3 of a 1% w/v solution of DPC in EtOH and 14.3 cm^3 of EtOH was added to the TEOS mixture and stirred for 1 h. From the mixed solution, 1 cm^3 aliquots were removed and pipetted into a polypropylene cuvette holder and left to cure under ambient conditions. Concentration of DPC in the mixed solution was approximately 200 µg dm^{-3} before curing. After curing, glassy orange shards were found in the cuvette tray wells after approximately 48 h. Final DPC concentration was approx. 280 µg per 1 cm^3 gel.

Ferrer synthesis[64]

A reduced acid, co-solvent free sol-gel synthesis was also attempted, based on the work of Ferrer *at al.*[64] To begin, 56 mL TEOS was mixed with 19 mL distilled water and 1.25 mL 0.62 M HCl. The mixture was stirred at high speed using a magnetic stirrer (Fisher Scientific, Loughborough) for 30 minutes. Aliquots of 1 cm^3 were pipetted into a polypropylene cuvette tray and allowed to cure under ambient conditions. However, this new route was unable to give reproducible formation of a xerogel. The cured xerogels were less physically robust, crumbled easily when handled and were not used further in this project.

5.3.2.2. Preliminary evaluation of sol-gel derived xerogels

Investigation into response to Hg^{2+} ions

The contents of a cuvette well containing shards of cured DPC-doped xerogel prepared using the method used by Gibson *et al.*[63] were added to an excess of 100 mg dm^{-3} aqueous $HgCl_2$ solution, with the colour change observed over 10 min.

Native American head-dress field study

A case study was conducted in collaboration with the University of Arizona and Arizona State Museum. DPC xerogels were exposed to a Native American head-dress (**Figure 5.6**) known to have been previously treated with $HgCl_2$. The head-dress was made from linen and was decorated with feathers. The object was placed into a glove box (volume approximately 1 x 0.5 x 0.5 m), which was sealed for 3 d to allow the atmosphere in the headspace to equilibrate with any volatiles emitted from the head-dress. After this time, two xerogels were placed inside the glove box and left for a period of six months (**Figure 5.7** and **Figure 5.8**). To assess the potential colour change of the sensor in contamination-free environment, xerogel fragments were placed in the laboratory and left to sample the indoor air for six months also. After sampling the xerogels were removed and examined visually.

Figure 5.6: Contaminated Native American head dress, used for sampling of xerogels.

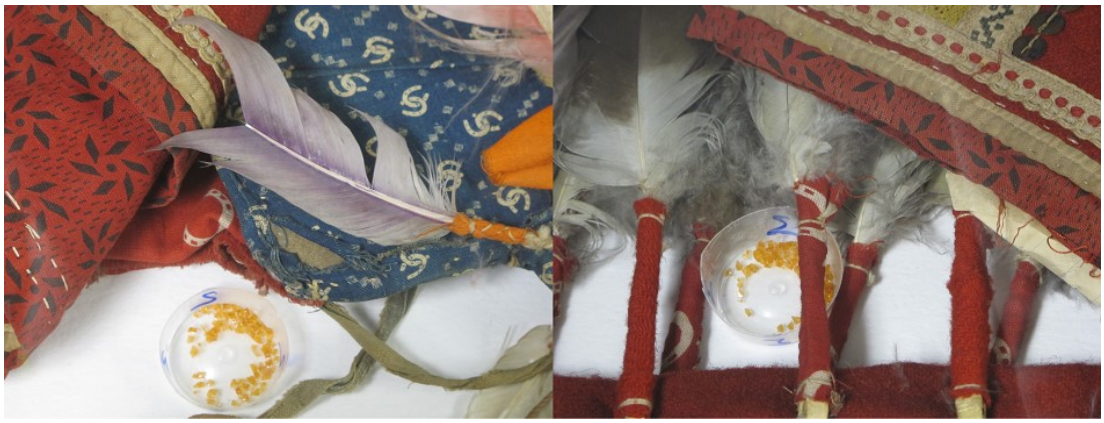

Figure 5.7: Cups of DPC xerogel placed with Native American head-dress for sampling of $HgCl_2$ vapour

Figure 5.8: Sampling set up of head-dress and xerogels inside sealed sampling box (bottom right and centre left) for detection of $HgCl_2$

5.3.3. Use of agar as an immobilisation medium

5.3.3.1. Synthesis of agar hydrogels

Approximately 6 g of agar agar (laboratory grade, Fisher Scientific, Loughborough) powder was dissolved in 120 cm^3 of distilled water using an electric steriliser unit (Boots Plc., Nottingham) to give a concentration of 5% w/v agar agar (hereafter reagent is referred to as agar). Once the resulting solution was a clear, thin liquid it was allowed to cool slightly before the addition of 10 cm^3 of 1% DPC in EtOH (as prepared above) and mixed well. Replicate 1 cm^3 aliquots of the loaded agar solution were removed and pipetted into cuvette trays and allowed to set. The final DPC concentration was approximately 800 µg per 1 cm^3 gel.

5.3.4. Preliminary evaluation of DPC hydrogels

5.3.4.1. Response of DPC agar hydrogels to Hg^{2+} and Cr^{6+}

DPC agar hydrogels were immersed in 4 cm^3 of aqueous $HgCl_2$ solution at concentrations of 1, 10 or 100 mg dm^{-3}, and also distilled water which was used as a sampling blank. The colour change was observed by eye and photographed over a

10 min period after which time the gels were removed to a Petri dish for further examination and dried using paper towels.

The colour change reaction with Cr^{6+} was evaluated by diluting 7.5 cm^3 of 100 mg dm^{-3} $K_2Cr_2O_7$ with 2.5 cm^3 of 2.73 g dm^{-3} HCl to give a 75 mg dm^{-3} solution containing Cr^{6+}. This solution was further diluted (1:10 or 1:100 with distilled water) to give solutions of 7.5 or 0.75 mg dm^{-3} $K_2Cr_2O_7$. The DPC agar hydrogels were again added to distilled water to act as a sampling blank. A single DPC agar hydrogel was added to 4 cm^3 of each solution and the colour change was observed by eye and photographed over a 10 min period. As before the DPC agar hydrogels were removed dried and visually examined on clean Petri dishes.

5.3.4.2. Vapour-phase investigation with DPC agar hydrogels

To assess the potential of the DPC agar hydrogels to change colour in the presence of volatile mercuric species, a 1 cm^3 volume of the gel solution was pipetted into the bottom of a centrifuge tube and left to set. The tube was then inverted to situate the 'sensor' at the top end of the tube while 20 mg of $HgCl_2$ was placed in the cap which was used to seal the tube. This placed the source of mercury at the opposite end of the tube to the sensor, which remained sealed under ambient conditions for six weeks.

5.3.5. Simulated field study using agar films

Parchment dating from 1860 and a polyester fur from a soft toy were prepared for analysis by applying 100, 50, 10 or 5 μL of 100 mg dm^{-3} $HgCl_2$ solution to pieces of test material weighing approximately 0.2 g. DPC agar films were formed by preparing the DPC agar solution as described in **Section 5.3.3** and pouring approximately 10 cm^3 into a Petri dish and allowing to set. The set gel was allowed to dry under ambient conditions for approximately 48 h, forming a thin, plastic-like film. The $HgCl_2$ solutions were doped onto the parchment and soft toy using a micropipette. After drying for approximately 4 h, swabs of these test materials were collected using cotton buds wetted with distilled water by taking 10 horizontal 10 vertical strokes across a 2 cm^2 area where the $HgCl_2$ solution had been applied. The swabs were then drawn over the agar DPC film (10 horizontal and 10 vertical strokes) to develop the colour change and determine if the sensor could be used in surface swabbing of objects.

5.4. Results and discussion

5.4.1. Investigation of solution phase DPC colorimetry

5.4.1.1. Initial investigation of solution phase chemistry

The viability of DPC as a colorimetric reagent was demonstrated by adding 50 μL DPC to 1 cm^3 of 100 mg dm^{-3} $HgCl_2$ (pH 4.6), which caused formation of a vivid purple complex with a λ_{max} measured at 532 nm. The colour of the complex remained when stored at ambient conditions for eight weeks, after which time the solution was discarded. As DPC is known to interact with several metals, producing chromogenic reactions,[41] the reagent was added to solutions containing potentially interfering Cr cations.

A solution of 50 μL DPC added to 1 cm^3 of a 100 mg dm^{-3} solution of $K_2Cr_2O_7$ did not change colour with solution pH values between 4 and 7. When the chromate solution pH was decreased to 2.8 a pink colour was observed with a λ_{max} of 540 nm. As the diphenylcarbazone was used as received, it contained approximately 60% diphenylcarbazide resulting in the Cr^{6+} oxidising diphenylcarbazide to diphenylcarbazone.[40, 41] The Cr^{6+} was reduced to Cr^{3+} and subsequently complexed with the diphenylcarbazone giving the colour change observed. It was observed that increased acidity favoured the redox-complexation reaction of the chromium, selectively forming the pink colour as opposed to the complexation reaction favoured by the mercury at higher pH values. It should be noted that for these reasons, attempts to selectively complex Cr^{3+} will also result in complexation of Cr^{6+} unless total Cr is reduced to Cr^{3+}. Selectivity towards Cr or Hg cations of DPC-based sensors can therefore be influenced by controlling the pH of the sample media. Houghton[65] has also stated that chromate may be eliminated by reduction with hydrogen peroxide.

While pH can be used to alter the selectivity of DPC to chromium and mercury, it should be noted that DPC is sensitive to other metals as well.[66-69] Elements such as vanadium, molybdenum and manganese may be discounted in heritage collections as their presence is not anticipated due to their principally being used as alloying agents.[70-72] Lead is also known to react with DPC under alkaline conditions, and is more likely than vanadium, molybdenum and manganese to be found in heritage

environments. Molybdate ions may also be masked *via* the addition of oxalic acid.[41, 65] The intensity of absorption corresponding to the lead-DPC complex has been measured over a range of pH values, with a maximum measured in the pH range of 9.5-10.4.[66] Interferences from lead in the determination of Hg^{2+} can therefore be considered unlikely as the determination of Hg^{2+} is performed under mildly acidic conditions. Feigl[41] also reports that the reaction of DPC is specific towards mercury in 0.2 M HNO_3.

5.4.1.2. Selective determination of Hg^{2+} in mixed metal solutions

The use of DPC reagent for the detection of Cr^{6+} suggested that the developed procedure may extend into applications in environmental chemistry. The ability of the agar sensor to selectively determine Hg^{2+} or Cr^{6+} in aqueous solution was an important factor in the usability of the sensor. As shown in **Table 5.2** and the preliminary solution phase chemistry in **Section 5.4.1,** controlling the pH of the reaction solution allowed the DPC hydrogel to selectively complex with either Hg^{2+} or Cr^{6+} ions. More specifically, when solution pH modified to within a range of 3.3 – 4.6, the sensor selective interacted with Hg^{2+} ions in the presence of Cr^{6+} and formed the purple DPC-Hg^{2+} complex. At lower pH 2.8 – 2.5, the sensor selectively reacted with Cr^{6+} forming a different product which was pink coloured. Therefore, it is proposed that DPC agar hydrogel could be used as a sensor which could be tuned to give specific colour changes to Hg^{2+} or Cr^{6+} in contaminated water.

Table 5.2 Mixed metal study results, showing pH dependent selectivity between Hg^{2+} and Cr^{6+}

Tube	Vol. $HgCl_2/K_2Cr_2O_7$ used (cm^3)	Vol. distilled water used (cm^3)	Vol. 2.73 g dm^{-3} HCl added (µL)	pH value	Colour observed
1	2/2	-	0	4.6	Purple
2	2/2	–	25	3.3	Purple
3	2/2	–	75	2.8	Pink
4	2/2	–	150	2.5	Pink
5	–	4	0	5.3	–
6	–	4	25	3.3	–
7	–	4	75	2.8	–
8	–	4	150	2.4	–

5.4.2. Preliminary evaluation of sol-gel derived xerogels

5.4.2.1. Description of DPC xerogels

As the curing process of the xerogel material synthesised *via* the method described by Gibson *et al.*[63] was performed under ambient conditions, the uncontrolled temperature and pressure led to fast evaporation of solvent from the pores of the material. This resulted in formation of xerogels with high internal stresses, rendering them fragile and only occasionally produced intact glass cuboids.[73] As a result, the majority of xerogels shattered into small shards of varying sizes (see **Figure 5.9**). Each time 20 cuvette wells were prepared only 1 – 2 xerogels per batch formed intact cuboids measuring approximately 5 mm x 5 mm x 1 mm.

Figure 5.9: Shards of acid catalysed DPC xerogel as synthesised

When the DPC xerogel fragments were added to $HgCl_2$ solutions, the small fragments experienced further stress causing them to shatter further (see **Figure 5.10**), making handling of the xerogels difficult. In addition, in a small number of batches it was observed that the DPC reagent was not distributed uniformly through the xerogel. These glass shards were not dark orange as expected but were colourless to slightly yellow with DPC blooming observed on the surface of the glass. As EtOH was produced as a by-product during condensation of the sol, it is proposed that the DPC reagent would selectively partition into this solvent without dispersing throughout the gel; as the EtOH evaporated from the surface of the gel, this caused a capillary effect throughout the pores of the material leading the migration of the DPC to the surface of the gel where it was crystallised forming efflorescence and non-uniform dispersal of the colorimetric reagent throughout the gel matrix.

5.4.2.2. Evaluation of DPC xerogel response to Hg^{2+} ions

Aqueous solutions containing Hg^{2+}, K_2CrO_4, or $K_2Cr_2O_7$ were added to xerogel fragments and agar hydrogels to determine if the immobilised DPC would change colour in a similar manner to that observed for the solution phase work.

Approximately 1 cm^3 of a 100 mg dm^{-3} solution containing $HgCl_2$ was added to a well containing a shattered DPC xerogel. After 5 min there was no observable colour change. The pH of the solution was initially estimated using pH paper to be 7, but 5 min after the solution was added the pH decreased to approximately 2. The low solution pH was due to extraction of HCl from the silica matrix. It was clear that

residual acid from the synthesis migrated through the pores of the DPC xerogel into the aqueous solution. The experiment was repeated using pH 9.0 buffer in place of water. Leaching of acid from the xerogel decreased the pH of the solution to approximately 7 – 8, under which conditions DPC-Hg^{2+} complexation was evidenced by the development of a purple colour in the solution and some of the gel fragments after 10 min (the results of this experiment can be seen in **Figure 5.10**). While it is proposed that a washing step in the preparation of these xerogels could remove any residual acid from the material, to do so thoroughly would likely require milling or grinding of the xerogels to a powder, the preparation and handing of which would offer no direct benefit to heritage users over commercially available DPC crystals.

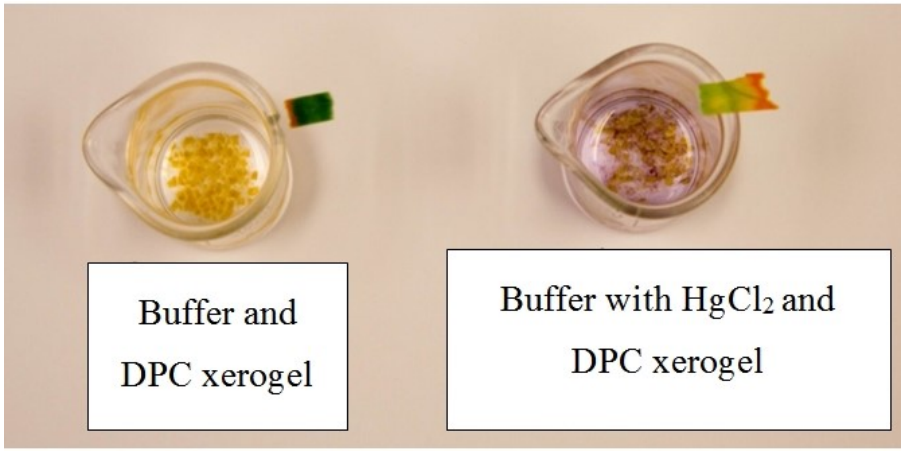

Figure 5.10: Further shattering of DPC xerogels in the presence of liquid, photographed approximately 10 min after addition. DPC has been released from the xerogel matrix into the $HgCl_2$ solution upon shattering.

The xerogels presented here were considered unsuitable for further development as metal sensors in heritage environments. The difficulty in preparing the material, in addition to the finished material shattering easily and requiring careful handling of the shards meant it did not offer any appreciable benefit over using DPC crystals as received from a supplier. The limitations of the xerogels as described here strongly suggests this material is not suitable for use as a support for colorimetric reagents, and barring the Native America headdress case study below, was not investigated further.

Native American headdress case study

Two xerogels were placed in a sealed glove box with a Native American object known to be contaminated with Hg^{2+} residues. After six months, DPC xerogel fragments were removed from the box containing the head-dress (see **Section 5.3.2.2**) and examined closely for any colour change. No change from orange to purple was visible in any part of the xerogels used in the experiment and close inspection revealed the DPC xerogels exposed to the headspace of the mercury contaminated object were indistinguishable from the control gels. As the headdress had previously been determined to be contaminated with Hg^{2+} by XRF analysis, and the equilibration and sampling time was sufficient to allow any mercury present to volatilise into the headspace, it was concluded that the DPC xerogel fragments did not react with Hg^{2+} in the vapour phase. It was proposed that coordination of Hg^{2+} with DPC was inhibited by the presence of residual acid in the xerogel. In this case the acid would have protonated the coordinating nitrogen atoms of the DPC, thus preventing chelation with Hg^{2+} and the development of the violet complex seen in other experimentation. This conclusion is in good agreement with published literature regarding the pH range of the DPC-Hg complexation reaction.[50]

5.4.3. Use of agar as an immobilisation medium

The sol-gel process generated materials that were difficult to synthesise reproducibly, difficult to handle, and required the use of buffer solutions in order for the colour change reaction with mercury to proceed. By changing from a silicate polymer to a biological one (agar), many of these challenges were overcome. Agar gels cured rapidly compared to the silicate xerogels prepared in **Section 5.3.2**. The hydrogels solidified from hot agar solution within 15 min. DPC hydrogels were immediately useable as there was no solvent evaporation stage necessary and they were produced without the need of a slipping agent or access to specialist equipment such as a magnetic stirrer or sonicator. The DPC hydrogels produced were orange to reddish-orange 1 cm^3 cubes and did not fragment during the curing process, making them very easy to handle once cured. Water in the interstices of the gel wetted the surface of the mould, allowing easy removal of the agar blocks from the moulding wells once cast.

5.4.4. Preliminary evaluation of DPC hydrogel response to selected metal ions

Agar hydrogels were formed in a number of shapes, highlighting the potential of the material to be applied in a number of ways. **Figure 5.11** demonstrates the freshly cast DPC hydrogel, a DPC hydrogel that had been dried and also DPC agar gel coating the inside of a Palmes diffusion sampler. To the tube, a 1 cm^3 solution containing 100 mg dm^{-3} of $HgCl_2$ was added, resulting in development of the purple complex.

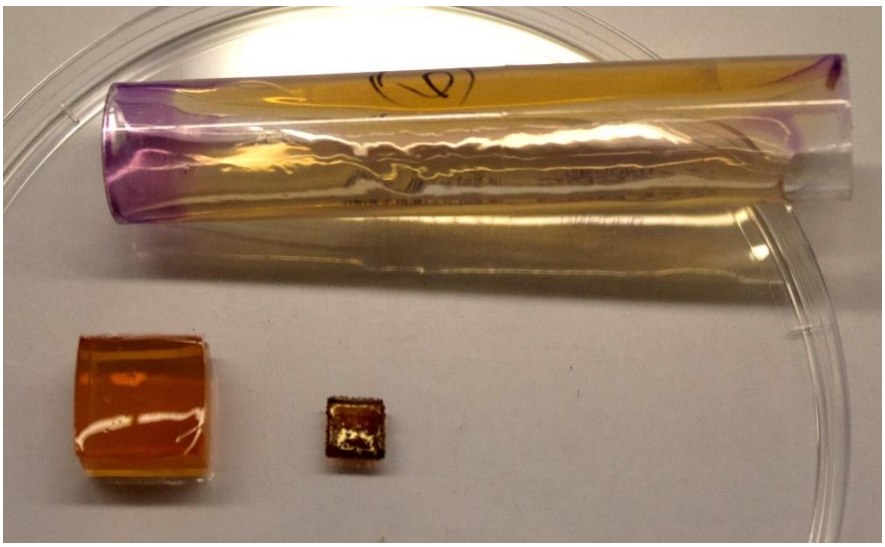

Figure 5.11: DPC-doped hydrogels cast on the inner surface of a Palmes tube, as a fresh cube, and as a dried cube

Longer residency time of the solution within the tube was needed to fully develop the complex throughout the entire length of the tube, thus the purple colour was only visible at the bottom where droplets of $HgCl_2$ were retained. However, as the colour change was observed after approximately 10 s, this figure nevertheless shows that the material has potential application outside the heritage sector as an environmental sensor which can be used to detect Hg^{2+} in fluidic channels.

5.4.4.1. Response of DPC agar hydrogels to Hg^{2+} and Cr^{6+}

Aqueous solutions containing Hg^{2+}, K_2CrO_4, or $K_2Cr_2O_7$ were added to agar hydrogels to determine if the immobilised DPC would change colour in a similar manner to that observed for the solution phase work. The test used chromium as both dichromate and

chromate forms, both of which have historical use as pigments and may be present on heritage samples contaminated with mercury.

Freshly cast agar gels were immersed in a 3 cm^3 solution containing 100 mg dm^{-3} of $HgCl_2$. Colouration of the outer edges of the hydrogel was observed after approximately 4 s, with full colour change taking place throughout the interior of the DPC hydrogel after 8 min. This was evidence that the Hg^{2+} ions could easily diffuse through the pores of the hydrogel.

The colour changes observed after the DPC hydrogel was immersed in different concentrations of $HgCl_2$ are shown in **Figure 5.12**. The colour change was visible at Hg^{2+} concentrations of 10 mg dm^{-3}, but at this concentration of Hg^{2+} ions, most of the colour change was observed in the solution and not in the interior of the DPC hydrogel. This indicated that the hydrogel released DPC into the solution where it reacted with Hg^{2+} ions forming the purple complex. As shown in **Figure 5.12**, solutions with Hg^{2+} concentration of 1 mg dm^{-3} did not produce an observable colour change.

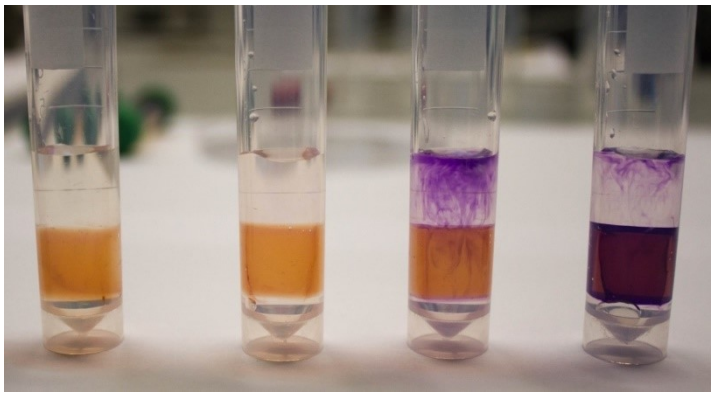

Figure 5.12: DPC hydrogels after being exposed to (L-R) 0, 1, 10 and 100 mg dm^{-3} solutions of $HgCl_2$ for 5 min.

A clear illustration of the different colour formed can be seen in **Figure 5.13**. This allowed the sensor to differentiate between Hg^{2+} or Cr^{6+} after having been exposed to mixed 100 mg dm^{-3} solutions of mercury and dichromate under conditions with varying pH. The intense purple of the Hg complex is noticeably different from the pink of the chromium based colour.

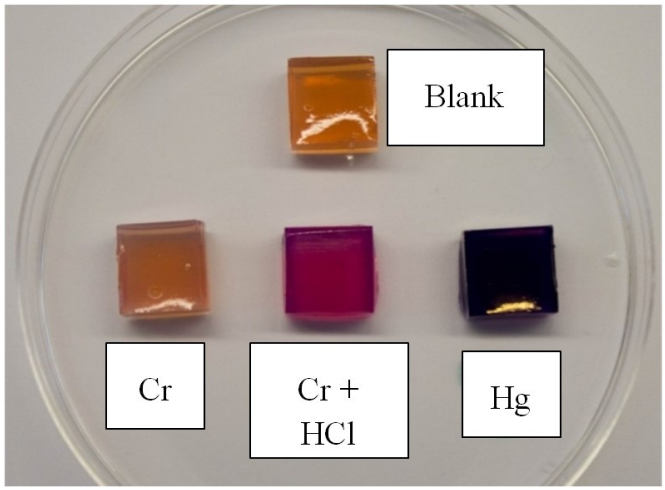

Figure 5.13: Agar DPC gels exposed to 100 mg dm^{-3} $HgCl_2$ and $K_2Cr_2O_7$ solutions for 10 min, at optimised pH levels

At concentrations of 7.5 mg dm^{-3}, the pink chromium complex was easily distinguishable from the purple mercury complex. As the agar gels were still reliant on the ingress of media through the pores of the agar material, the long contact time with the outside of the sensor allowed deep coloration to occur for even modest concentrations, as seen in **Figure 5.14**.

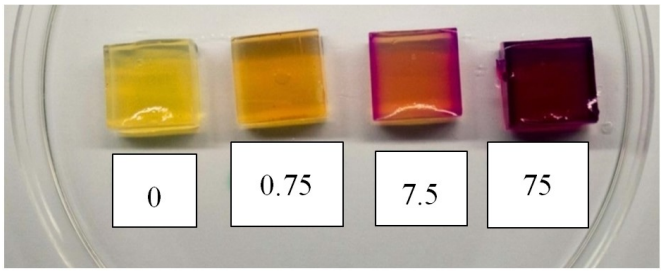

Figure 5.14: Approximate detection limits for chromium using agar sensor. Measurements taken after 10 min exposure to 0.75, 7.5 and 75 mg dm^{-3} $K_2Cr_2O_7$, acidified with HCl.

Hydrogels were also shown to be suitable for Hg^{2+} measurement after drying at ambient temperature and pressure. Fresh agar hydrogels were approximately 95% water by mass at time of gelation, but reduced in size to approximately 5 x 5 x 2 mm as the water evaporated over the course of 2 – 3 d (see **Figure 5.15**). The dried DPC agar hydrogel, a darker orange colour in comparison to the freshly prepared gel, was still shown to change colour when immersed in a solution containing 100 mg dm^{-3} of

$HgCl_2$ (see **Figure 5.16**). The dried DPC agar hydrogel immersed in the 10 mg dm^{-3} solution of $HgCl_2$ changed colour around the outer edges of the material.

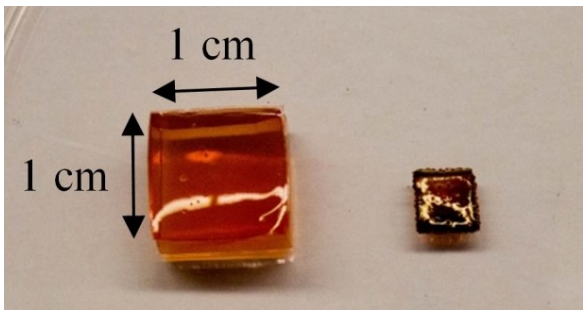

Figure 5.15: Size comparison of freshly prepared agar DPC gel (L) and DPC gel dried for 4 d at room temperature (R).

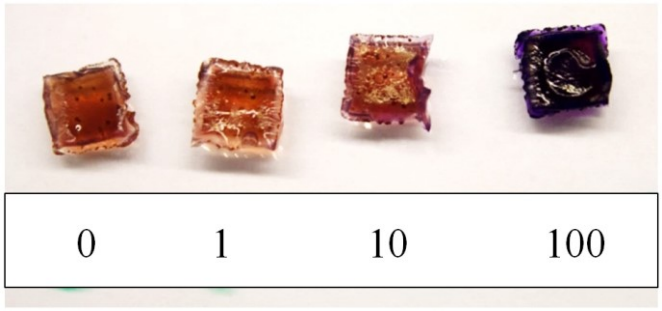

Figure 5.16: Agar gels dried for approximately 8 weeks and exposed to (L-R) 0, 1, 10 and 100 mg dm^{-3} solutions of $HgCl_2$ for 10 min.

Colouration of dried gels was slower than their freshly prepared counterparts, typically occurring over several minutes, as opposed to the wet gels which reacted in seconds. However, the intensity of the colour change of the dried DPC hydrogel was more pronounced than the freshly prepared "wet" counterpart when added to the $HgCl_2$ solution at 10 mg dm^{-3}. It is suggested this was due to the dry gel having the same mass of DPC in a smaller volume, allowing detection of a lower concentration of Hg^{2+}. The dried gels were stored and periodically added to the 100 mg dm^{-3} solution of $HgCl_2$. Colour development was observed for the 8 week testing period. The results highlighted the utility of the DPC agar hydrogel as a sensor. Specifically, here the DPC solution could be prepared and loaded into xerogels well in advance of their exposure to Hg^{2+}. This provides a significant advantage to the use of DPC solution where it has

been stated that it needs to be prepared immediately before use in order to prevent oxidation of the compound.[36] This is a major result in terms of developing a DPC-based sensor that retains the ability to form an easily observed, highly coloured complex with Hg^{2+} without the need to freshly prepare solutions immediately prior to analysis.

The formation of the mercury complex within the dried gels in solution is shown in **Figure 5.17**, with these gels having been exposed to 0, 1, 10, or 100 mg dm^{-3} $HgCl_2$ for 10 min. Very slight colouration of the gel in the 1 mg dm^{-3} solution was observed after 10 min. Development of the purple complex was easily determined when the dried DPC agar hydrogel was added to the 10 or 100 mg dm^{-3} solution as the colour intensity was stronger.

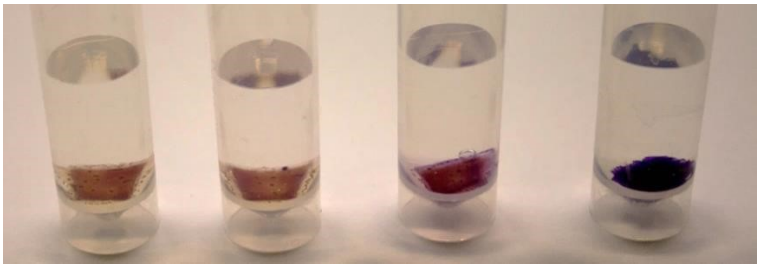

Figure 5.17: Dried DPC hydrogels after being exposed to (L-R) 0, 1, 10 and 100 mg dm^{-3} solutions of $HgCl_2$ for 5 min.

A control set of freshly cast gels were stored for 8 weeks in a sealed container to prevent dehydration of the gel. These gels did not retain reactivity and substantial loss of colour intensity from the gel was observed, indicating extensive oxidation of the DPC had taken place. This is in agreement with literature previously published advising that the DPC solution was not stable and must be freshly prepared before use.[36, 37, 41, 74]

The work presented here was intended to act as proof of concept, and as such the results suggest the sensor may be of utility to the heritage sector. As the use of liquids is ideally avoided in heritage environments, the study here was expanded by using case studies based on heritage items.

5.4.4.2. Vapour-phase investigation with DPC agar hydrogels

Vapour-phase exposure of a wet gel placed above crystals of $HgCl_2$ in a sealed container (see **Section 5.3.4.2**) did not give any positive results; no purple colour was produced. It was suspected that as the sealed environment prevented evaporation of the water from the agar gel, stopping the sensor from drying out, the excess liquid in the DPC agar hydrogel may have led to oxidation of DPC to the colourless diphenylcarbadiazone which is known to be inactive towards metals.[36, 37, 41, 74]

As the dried sensors were resistant to the oxidative process that deactivates the DPC-Hg^{2+} reaction, the experiment was repeated using dried sensors. The dried sensors were exposed to the headspace of solid $HgCl_2$ in the same manner as the wet sensors. No colour change reaction was apparent after 14 d. It is therefore not recommended to use agar sensors for the vapour-phase determination of volatile mercury. As sampling using vapour-phase techniques was not possible using the sensors developed here, a modified strategy was developed that would allow the use swabbing on the surface of contaminated heritage objects.

As stated in **Section 5.4.1**, DPC is sensitive to a number of metals, but the response is specific for mercury in 0.2 M HNO_3.[41, 65] Development of this sensor did not use pH modifiers as part of the composition of the DPC hydrogels, as it could be potentially damaging to heritage objects to collect swabs using acidic or basic solutions. However, it may be possible for future work to incorporate 0.2 M HNO_3 into the hydrogel itself, thereby allowing the swabs to be collected using water as is preferred, but still achieving selectivity within the sensor for mercury.

5.4.5. Simulated field study using agar films

Gel films cast in Petri dishes were used to provide DPC supports for swabbing tests. Test materials included a parchment deed dated from 1860 and the outer material of a 100% polyester soft toy which was used as a fur analogue. Both materials were spiked with a solution of $HgCl_2$, as outlined in **Section 5.3.5**, giving concentrations below 50 μg g^{-1} in contrast to the high values detected on objects reported in literature.[19, 20] After spiking, the materials were swabbed using a cotton bud wetted with distilled water to extract any Hg^{2+} from the surface. Using the same cotton bud, this swabbing

procedure was repeated across the surface of a dried DPC agar hydrogel to develop the coloured mercury complex.

The results observed are shown in **Table 5.3**. A positive result (i.e., confirmation of the presence of Hg^{2+} ions) was indicated by the visual observation of a purple colour on the cotton bud after swabbing both the test material and the gel film. A negative result was indicated when no purple colour was developed on the swab after exposure to both the test material and gel film. As shown in **Table 5.3**, the cotton bud that had been used to swab the parchment and then the DPC agar hydrogel did not change colour. However, application of DPC solution directly to the parchment using a Pasteur pipette resulted in a positive colour change for mercury. This indicated that the mercury was still present on the parchment and had not been removed by the swab. The result further demonstrated that the parchment did not inhibit the reaction. In contrast, after swabbing the spiked fur analogue, a strong purple colour was observed on the cotton bud where mercury had been transferred from the fur as well as DPC from the gel film.

Table 5.3: Results of swabbing for mercury from parchment and fur analogue

Item	**Vol. 100 mg dm^{-3} $HgCl_2$ spiked (µL)**	**Mass $HgCl_2$ spiked (µg)**	**Concentration of $HgCl_2$ on material (µg g^{-1})**	**Positive or negative result for mercury**
Parchment 1	0	0	0	Negative
Parchment 2	5	0.5	2.5	Negative
Parchment 3	10	1	5	Negative
Parchment 4	50	5	25	Negative
Parchment 5	100	10	50	Negative
Fur 1	0	0	0	Negative
Fur 2	5	0.5	2.5	Positive
Fur 3	10	1	5	Positive
Fur 4	50	5	25	Positive
Fur 5	100	10	50	Positive

This experiment demonstrated that the DPC hydrogel could be used in a swabbing procedure to positively identify Hg^{2+} ions on the surface of fur. The method had a fast response time and as fur was a good material candidate it is suggested that this method may have potential use in natural history collections. By using aqueous swabs and the sensors developed here to perform the test, the presumptive determination of mercury in heritage environments was no more invasive than cleaning practices currently in widespread use. However, without the need for instrumentation to perform the screening test the method could be used by users with no specialist training and at low cost. The same sensor also allows flexibility in the method of determination used, as samples can be used in aqueous media to offer a semi-quantitative result in conjunction with a calibration curve, showing intensity of colour development over a set time. This was an encouraging result for the use of the sensor in heritage applications.

5.5. Conclusions

This preliminary study has successfully shown that agar is a suitable support matrix for DPC when investigating mercury contamination in heritage environments. While the use of DPC in the detection of mercury is not in itself novel, the immobilisation within an agar hydrogel to form a colorimetric sensor is new. The use of agar had significant benefits over the alternative xerogels synthesised here.

DPC-doped hydrogel supports are presented here as a sensitive and selective method for the visual, rapid, on-site and economical determination of Hg^{2+} ions in solution. The gels exhibited hybrid properties of the robust and stable agar, while still retaining fast colour development times and vivid colouring of DPC in solution. Initial experiments into the ease of use of the agar allowed casting gels in a number of different shapes allowing functional flexibility for a variety of uses. This approach also allowed casting of unconventional shapes such as coating the inner surface of a tube, potentially allowing a flow sensor to be developed. The shapes focussed on in this work were typically thin films and cuboids.

Dried DPC hydrogels had significant advantages over more established techniques for the determination of mercury in heritage environments. The use of dried films would allow reagents to be prepared elsewhere and posted to institutions in a ready-to-use

form. The dried gel films also conferred stability to the DPC, preventing oxidation to diphenylcarbadiazone observed in ethanolic solutions of DPC. As a result, the gel films can be prepared and stored at ambient conditions until required for use. This is an advantage over published methods where DPC is used as a solution and has to be prepared fresh immediately before use.

The purple colour was observed at concentrations as low as 10 mg dm^{-3} $HgCl_2$. The sensor developed here was also shown to be sensitive towards Cr^{6+}, with 7.5 mg dm^{-3} solutions of both Cr^{3+} and Cr^{6+} showing colorimetric responses to the DPC reagent in the gel. As this reaction favours more acidic pH levels than the mercury complexation, DPC can be selectively reacted with chromium in the presence of mercury by altering the pH of the reaction media. It is anticipated that this will be of benefit to the environmental sector more than heritage institutions.

The sensors were used by immersion in a solution containing the analytes of interest and as "blotting pads" for swabs. By using swabs, the samples were preconcentrated on the bud, allowing for sensitive detection of Hg^{2+} on surfaces. This was shown through the use of simulated objects contaminated with $HgCl_2$ which were swabbed with water and offered a qualitative determination for the presence of mercury. While swabbing was not quantitative, this method allowed simple and fast presumptive testing for the presence of Hg^{2+} in heritage collections without the need for instrumentation or training.

While the technique was limited in that it was not non-contact as initially hoped, the technique still represents a step forward in terms of making the reagents a less undesirable prospect within collections. The swabbing method shown in this work was no more invasive than swabbing techniques currently used in cleaning of objects, which use only water as a solvent. It is anticipated that this technique could be widely adopted in heritage collections for fast and cheap detection of inorganic mercury.

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6. Conclusions and future work

6.1. Development of chromatographic methods for the determination of selected pesticides in heritage environments

6.1.1. Conclusions

At the outset of this work, discussions with project partners revealed a lack of minimally invasive methods suitable for the determination of substances historically used in museum pest control strategies. Further consultation with project partners identified aldrin, camphor, chloronaphthalene, dichlorodiphenyltrichloroethane (DDT), dichlorvos, dieldrin, endrin, hexachlorocyclohexane (lindane), naphthalene and thymol as priority pollutants within heritage collections, which were subsequently used as target analytes. With this goal, two methods for determination of the 10 target analytes were successfully developed.

A significant driver for this work was to develop methods that would allow sampling of key pesticidal residues without the use of potentially damaging contact sampling techniques. The methods developed in this thesis therefore used stainless steel tubes packed with Tenax TA sorbent to passively sample indoor air in order to trap analytes for analysis. Volatile analytes such as camphor, naphthalene, chloronaphthalene, thymol, dichlorvos, aldrin, and lindane were successfully detected on spiked sorbent tubes using thermal desorption – gas chromatography – mass spectrometry (TD-GC-MS). The advantage of using TD-GC-MS analysis was thus that complex sample preparation could be eliminated; more thermally labile analytes used an HPLC-UV method, which was able to detect naphthalene, thymol, chloronaphthalene, dichlorvos, aldrin, dieldrin, endrin and DDT.

Further to the need to leave heritage samples intact, the methods may be required to be used by non-scientists, therefore simplicity of execution was another key requirement. To achieve this goal, a sampling and analysis flowchart was incorporated into this work in order to provide museum staff from a non-scientific background and skill set with an effective tool for determining an appropriate sampling and analysis strategy tailored to their individual collections. The flowchart potentially allows users

to select appropriate analytical strategies without prior knowledge of the instrumental or sampling techniques available. It was proposed that this flowchart would inform collection custodians when hiring external laboratories and allow selection of sampling and analysis strategies appropriate for their collections. In addition to the flowchart, a sampling protocol detailing precise instructions regarding the collection of samples and how to send them to laboratories for analysis was also included to aid conservation staff in collecting useful samples.

The utility of the developed methods was assessed by sampling collections from UK heritage institutions as part of a series of 13 case studies. Of the target pesticides, concentrations of camphor, chloronaphthalene, lindane and naphthalene were detected at low concentration at a number of sites. Lindane was detected in 5 of the 13 case studies, while less volatile analytes such as DDT, dieldrin and endrin were not detected. In order to aid heritage institution staff by adding context to the results generated, a "traffic" light system was adopted based on the hazards detected. The traffic light colours of red, amber and green were assigned to each sampling location in order to show the associated risk of working within each sampling area, with the caveat that precautions should always be taken to ensure exposure to even low hazard chemicals should be as low as is reasonably practicable. Green samples presented minimal risk; amber samples or medium risk contained a substance known or suspected to be to sensitizing, mutagenic or carcinogenic; red, or high risk samples, contained substances approaching or exceeding EU short term exposure limits. An additional criterion was that a substance had been detected with indications of an LD_{50} value of less than 5 mg kg^{-1} suggesting extreme toxicity.

This work has shown the ability of the developed methods to detect a number of legacy pesticides with historical use in heritage collections. The sampling was performed *in situ* and was minimally invasive, furthermore, the flowchart has potentially armed conservation staff with a toolbox for effective determination of the extent of pesticidal contamination in their collections, or other substances historically used in museum pest control strategies.

6.1.2. Suggestions of future work for the development of methods suitable for the determination of chemical hazards in heritage environments

In this work, two methods were successfully developed allowing detection of a number of pesticides in heritage environments using minimally invasive, *in situ* sampling techniques before assessing the utility of the developed methods in case studies in heritage collections. The methods assessed in this study were developed using 10 target pesticides, identified after consultation with project partners in heritage institutions. These pesticides were not intended to be a complete list of substances applied in heritage environments; given the range of pesticides currently and previously marketed, the work could be expanded by increasing the number of pesticides investigated. As more than one of the case study sites contained dichlorobenzene, another known pesticide, it is possible that a wider range of analytes was present than was initially studied.

In order to address the limitations of the thermal desorption method with respect to thermal degradation of some analytes, a solvent desorption and preconcentration method was described here. A recommendation is that in order to retain the capability to collect structural data by using mass spectrometric detection, liquid samples from the preconcentration method may be analysed using liquid chromatography with tandem mass spectrometry (LC-MS/MS). When looking for particular mass/charge ratios of specific pesticides, the chemical background of a sample can be significantly reduced when using LC-MS/MS systems with selected reaction monitoring, allowing very low limits of detection. The "soft" ionisation techniques used in electrospray LC-MS/MS often produce molecular ions which considerably reduces the number of compounds that can be matched to peaks of that particular mass/charge ratio. This has the potential to make identification of pesticides in complex matrices such as indoor air much simpler.

As the Tenax sorbent used to sample indoor air matrices trapped a wide range of volatile organic compounds (VOCs), chromatographic resolution of the sample could potentially be improved by using multidimensional gas chromatography (GCxGC). This is an established technique allowing increased peak capacity (by separating more peaks than a single column), potentially faster separations and the removal of

interfering compounds. This analysis could offer easier identification of target analytes by better resolving the sample mixture, allowing more comprehensive analysis and quantitation of pesticidal analytes.

An important factor in the development of the methods presented in this work was that the sampling be as non-invasive as possible, leading to the use of *in situ* sampling using Tenax tubes. This method relied on placing of sorbent tubes for a fixed period of time before recollection of the tubes and analysis off site. A potential alternative option to sampling with sorbent tubes and off site analysis is the use of instrumentation such as portable MS instruments to allow samples to be collected and analysed in real-time. Portable MS technologies have become increasingly attractive to those looking for easily transportable analytical instruments. This project briefly considered applying techniques such as ion mobility spectroscopy (IMS) to detect analytes of interest. Unfortunately, indoor air proved too difficult to interpret as a matrix with the limited resolution power available to IMS even with the aid of a specialist, so was not investigated further. However, if front-end separation of analytes was achieved, it is possible that this would allow more selective analysis using these instruments, with the added benefit of short sampling times and portability.

6.2. Development of an optical organophosphate sensor

6.2.1. Conclusions

The work undertaken on the colorimetric determination of organophosphates is presented as an extension of the work undertaken by Odegaard, Zimmt and Smith.[1] Here, aqueous and pH-neutral agar gels were investigated as to their suitability for immobilising the enzyme acetylcholinesterase (AChE) for use as part of an enzyme-based organophosphate assay. The key aim of this work was to develop a fast, sensitive and easy to use method for the visual determination of the presence of organophosphates by untrained personnel. The development of a sampling kit suitable for the determination of organophosphate pesticides in heritage environments was successfully completed and the results obtained conclusively demonstrate the potential for this sampling kit to be a useful and effective method for the determination of organophosphate pesticides by non-scientific personnel.

Proof of concept was established with solution-phase experiments for the reaction between AChE and indoxyl acetate to yield the product 3-hydroxyindole. The product 3-hydroxyindole then oxidises to give the blue coloured indigo. The initial ester hydrolysis of indoxyl acetate was inhibited in the presence of a model organophosphate substance (dichlorvos) due to deactivation of the enzyme. In this instance, the blue colour of the indigo did not develop showing that positive test results for organophosphates would remain colourless as opposed to blue coloured negative results.

The AChE enzyme was successfully immobilised into an agar hydrogel matrix, allowing easier handling of the reagents. The response of the immobilised enzyme to the indoxyl acetate substrate in terms of speed and intensity of colour development was not observed to be significantly different from the experiments conducted with solution-phase experiments. The immobilisation of the enzyme allowed a sampling kit to be developed, using AChE gel and an indoxyl acetate swab to be contained with the same centrifuge tube without the reagents coming into contact with one another until needed.

The utility of the sampling kit was demonstrated by simulating field studies on museum objects. Fur, paper and parchment were spiked with known levels of dichlorvos which were successfully assessed using the developed sensor showing inhibition of colour development after swabbing an area spiked with as little as 75 μg dichlorvos. In an important result, the sampling kit was also shown to be a suitable method for the determination of dichlorvos in the vapour-phase. After exposure to vapour-phase dichlorvos over five days, inhibition of the colour development reaction was observed in the sampling kit. This potentially allows use of the sampling kits without contact sampling, which could be of benefit to heritage institutions where objects are too fragile to sample using contact methods.

Overall, this work demonstrates that it is possible to develop a swab-based sampling method to test for the presence of organophosphate pesticides in heritage environments, in a similar manner to the testing developed for mercury pesticides. The samples can be used in both passive vapour-phase sampling mode and also using direct contact swabs. The results of the sampling are fast and offer near real-time results that

can be easily determined by untrained personnel by comparing the intensity of an easily observed blue colour with a control. This approach is benefitted by being low-cost, portable and does not require the end user to handle substances other than water, making this a safe and effective technique for the detection of organophosphate residues in heritage collections.

6.2.2. Suggestions for future work on development of an optical organophosphate sensor

In this work, the use of acetylcholinesterase (AChE) immobilised into agar gel was successfully shown to react with indoxyl acetate to cause development of a blue colour through the production of indigo. Further work is required in order to quantify the colour change occurring in the sensor. It is recommended that spectrophotometric readings be taken of the colour change response to increasing concentrations of organophosphate. This could be used to determine the concentration of organophosphate present in the sample, allowing a quantitative device to be developed. It may also be possible to develop a smartphone application to fulfil this measurement role without the cost of purchasing a spectrophotometer thanks to the near ubiquity of smartphone devices. [2-5] Further understanding is also required in order to determine the activity of the enzyme in the agar phase in order to rule out any possible interference.

In the presence of dichlorvos, an organophosphate pesticide, this colour change reaction was inhibited. As the method was developed using a single model organophosphate substance, dichlorvos, further research is needed to determine the sensitivity of the method towards a wider range of commonly used organophosphate pesticides such as malathion, parathion and chlorpyrifos among others. As the principle behind the development of the colour is based on the inhibition of AChE, it is also likely that the study could be expanded to include pesticides with similar inhibitory effects on AChE, such as carbamate pesticides. These substances have toxicity similar to organophosphate pesticides and have historically seen application in heritage institutions under the trade names Sevin, Ficam and Baygon amongst others.[6, 7] It is suggested that in order to build upon the work undertaken here, that field

studies be performed in order to assess contamination of real objects in heritage institutions.

In order to expand the utility of the developed method, it is possible that applications outside heritage science could be explored. Chemical warfare agents such as sarin and VX also use inhibition of AChE as their mode of action. The recent use of sarin in the Tokyo subway terrorist attack and Syrian civil war has underscored the need to develop fast, accurate and simple methods of detection for this and other chemical warfare substances. Chromogenic assays such as the one developed here could allow rapid determination of these substances without the need for complex synthesis of fluorescent or nanoparticle probes as discussed in literature.[8, 9] Significantly, this technique has the potential to allow much cheaper and simpler analysis than many other techniques. Furthermore, without the need for extra reagents other than water for sampling, the centrifuge tubes are highly portable allowing easy use in the field away from a laboratory environment.

6.3. Development of optical sensors for the selected determination of mercury

6.3.1. Conclusions

The chromatographic methods successfully developed in the first section of this project were focussed on organic analytes and highlighted a need for the development of counterpart analytical techniques for the determination of inorganic pesticides. To this end, a simple, sensitive, selective and inexpensive method for the determination of mercury chloride was developed, allowing the presence of mercury(II) ions to be determined by observing near a real-time colour change reaction.

To achieve this goal, a colorimetric reagent for the determination of mercury (diphenylcarbazone, DPC) was investigated as a means of developing a sensitive and selective method for the visual, rapid, on-site and economical determination of mercury(II) ions. Solution-phase proof of concept reactions were performed in order to establish ideal reaction conditions. Once these conditions were determined, the DPC reagent was immobilised in both a silica matrix formed *via* an acid catalysed sol-gel process and an agar hydrogel matrix in order to assess the suitability of these materials

as a mercury sensor. A comparative study was conducted on the two matrices before the agar matrix was chosen for further experimentation.

Agar hydrogels were found to be reactive over a period of several weeks once dried, offering a significant advantage over more established techniques as the dried gel prevented the oxidation of the DPC to the inactive diphenylcarbadiazone. The use of dried gels potentially allows the DPC gels to be prepared elsewhere and dispatched to institutions in a ready-to-use form, which is an important advantage over published methods where DPC must be prepared immediately before use. The DPC gels were shown to give easily detectable responses through an orange to purple colour change which was observable by eye without the need for any analytical instrumentation, with colour changes observed at concentrations as low as 10 mg dm^{-3} mercury chloride at pH values of approximately 3.3 – 4.6. In more acidic media (pH less than 3), the DPC gels were shown to produce a colour change reaction with chromium ions, generating a pink colour as opposed to the purple seen with mercury. By altering the pH of the reaction media, the DPC gels can selectively react with either chromium or mercury. The selective determination of chromium potentially allows the DPC gel to be used in areas where chromium determination is important, such as environmental chemistry. As the focus of this work was mercury, the chromium chemistry was not investigated further.

Following this proof of concept study, DPC gels were cast into Petri dishes and allowed to dry, forming thin films. These films were successfully demonstrated as "blotting pads" allowing sensitive detection of mercury(II) on surfaces. This was further demonstrated by preparing simulated museum objects (parchment and fur) contaminated with mercury chloride, which were swabbed with water before swabbing the film allowing fast, simple, low-cost and instrument-free qualitative detection of the presence of inorganic mercury on these simulated objects.

In summary, this work demonstrates the use of a colorimetric reagent for the determination of mercury chloride, a known inorganic pesticide used in heritage museums, by using an inexpensive, fast and easy-to-use method that does not require specialist training or equipment to interpret. Additionally, swabbing of the contaminated objects does not require the use of any solvent other than water, so it is

possible that this method may be used as part of swabbing techniques currently used for the cleaning of objects. The use of dried gels conferred resistance to oxidation on the reagent, extending the storage life significantly beyond published studies which recommend preparation of the reagent immediately prior to use.

6.3.2. Suggestions for future work for optical sensors for the selective determination of mercury

Diphenylcarbazone (DPC) was immobilised into an agar gel was successfully demonstrated to change colour in the presence of mercury chloride, a pesticidal agent historically used for the preservation of ethnographic materials. Studies have noted that the presence of chloride ions interfere with the chelation of the DPC and mercury(II).[10, 11] It is predicted that despite the sensor having the ability to detect concentrations of approximately 10 mg dm^{-3} in solution for $HgCl_2$, this limit would be lower when testing for non-halogenated inorganic mercury species such as nitrate or oxides.

While the vapour-phase detection of mercury(II) was not successful in this study, it is possible that a deliquescent material may moisten the dried gels enough that a reaction takes place. By using a deliquescent substance such as calcium chloride as a wetting agent, the moisture absorbed by the calcium chloride may also act as a sink for the soluble mercury chloride, allowing preconcentration and subsequent detection of the mercury(II).

The focus of this work was the determination of the presence of mercury. As the DPC reagent complexes with mercury(II), it also serves as a method of sequestration of the cations from aqueous media. The use of the agar gels presented here could offer an inexpensive method for the scavenging of metal ions from water supplies. Investigation into this possibility should determine the concentration of mercury in a solution before and after the addition of a DPC gel in order to quantify the amount of mercury the gels are able to chelate over time.

In this work, the DPC reagent was shown to selectively produce a colour change with both chromium and mercury cations. As the reaction with mercury is specific in 0.2 M HNO_3, it may be possible to incorporate the acid into the DPC hydrogel, thereby

achieving a specific analysis for mercury but still enabling the use of water-based swabs for the collection of the sample. This would be preferred over the use of acid-based swabs.

It is well established that DPC will also react with other metals such as vanadium, lead and molybdenum.[12-14] It is perhaps possible that a colorimetric analysis could be developed in order to analyse these targets using the DPC-doped agar developed here, by modifying the pH that the sample is analysed at. It is expected that such a sensor could be of use to environmental chemists performing field analysis as the increased shelf life conferred on the DPC by the agar matrix would mean that the DPC reagent would not need to be prepared immediately before use.

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Appendix A: Sampling protocol used for the collection of museum object samples by non-technical staff

Preparation for sampling

- Prior to sampling, select appropriate sampling locations for the sampling tube deployment. Of these locations, one will be a sampling blank, tube A (i.e. in a corridor or office), whereas tubes B and C will be placed in the sampling site of interest. The tubes are approximately 9 cm in length by 0.5 cm in diameter.
- If the area being sampled is greater than 1 m^3 then it may be possible to use active (with a pump) sampling, provided there is a source of electricity. If the area being sampled is less than 1 m^3 it is recommended to use passive (diffusion) sampling.
- Sampling locations (if cupboards, drawers etc.) should remain closed prior to sampling. Opening the environment will allow gases to diffuse out and concentrations will be significantly reduced.
- No sampling preparation is required for collecting swab samples.

On receiving the sampling tubes

- Each tube will be labelled with an identifying code etched into the tube body. Select two tubes that are to be placed in the chosen location and enter the information into the **Sampling Information Form**. Please do not attach any adhesive labels, or write directly onto the body of the tubes.

Passive sampling

- To initiate sampling the brass nuts are removed from the end of the sampling tube.
- Position each sampling tube in the sampling location and remove the brass nuts from either end of the tube. Sampling tubes may be supported using a piece of blu-tac (or an alternative tie) if there is no suitable level surface on

which to lay the tubes. Take care not the cover the open end of the tube as this might restrict the sampling.

- Note that if the sampling location is inside a closed space (e.g. cabinet or drawer) the tubes should be uncapped prior to opening the cabinet door to minimise the time the space is "open" to air. Close all doors as quickly as possible, and keep closed for the duration of the sampling where possible. If it is necessary to access this space during the sampling period a comment should be inserted into the **Sampling Information Form**.
- Immediately after the tubes are deployed, the exposure start time and date are recorded, to the nearest minute, on the **Sampling Information Form**.
- If possible a photograph should be taken of the sampling tubes when in position.
- If known, the environmental conditions of the location should be recorded (e.g. average temperature, relative humidity etc.) on the **Sampling Information Form**.
- Sampling tubes should be left undisturbed for the duration of the sampling period - approximately 28 days. The end date will be agreed at the deployment stage.

Active sampling

- To initiate sampling the brass nuts are removed from both ends of the sampling tube and the sampling end of the tube is attached to the pump tubing. The sampling end will be marked with an arrow showing the direction of flow.
- Connect the pump to an electrical socket.
- Position each sampling tube appropriately. Sampling tubes may be supported using a piece of blu-tac (or an alternative tie) if there is no suitable level surface on which to lay the tubes. Take care not the cover the open end of the tube or cause kinks in the pump tubing as this might restrict the sampling.
- Turn on the pump. The pump will already be calibrated to a flow rate of 100 mL min^{-1}.

- Immediately after the pump is started, the exposure start time and date are recorded, to the nearest minute, on the **Sampling Information Form**.
- If possible a photograph should be taken of the sampling tubes when in position.
- If known, the environmental conditions of the location should be recorded (e.g. average temperature, relative humidity etc.) on the **Sampling Information Form**.
- Sampling tubes should be left undisturbed for the duration of the sampling period - approximately 8 hours. The end time will be agreed at the deployment stage.

Swabbing

- Swabs may be collected with a cotton bud wetted with hexane and isopropyl alcohol in a 95:5 ratio.
- Measure an area of approximately 2 cm by 2 cm, and draw 10 horizontal strokes over this area before taking 10 vertical strokes over the same area.
- Using scissors, snip the bud used for swabbing into a vial.
- Number the vial, and detail the object swabbed on the **Sample Information Form**.

Post sampling

- Please complete the **Object Information Form** and either include with any tubes and vials to be returned, or send an electronic copy to iain.rushworth@strath.ac.uk.
- After exposure the sampling tubes are removed from their location and securely re-capped with the brass nuts. Immediately after removal, record the date and time (to the nearest minute) on the **Sampling Information Form**.
- Attempts should be made to return the sampling tubes on the same day to the University of Strathclyde (see full postage details below). Use of the Royal Mail overnight delivery service is appropriate (please note that parcels need to be at the Post Office before 4pm). Please note, DO NOT POST on a Friday night as the tubes will remain in the postal system over

the weekend. If there is a delay to posting, please place the tubes in a refrigerator until it is convenient to do so.

- Please mark all parcels **URGENT ATTN. Mr Iain Rushworth**
- On receipt of the sampling tubes they will be analysed using previously published methods. Analyses and reports should normally be completed within one month of receipt of tubes at the University.

Return To: Mr Iain Rushworth

Dept of Pure and Applied Chemistry

University of Strathclyde

295 Cathedral Street, Thomas Graham Building

Glasgow, G1 1XL

Sampling information form

Sampling location: ***Institute Name, Address.***

EXAMPLE LOCATION : **e.g. Positioned on a drain pipe directly outside the rear entrance to the building.**							
TUBE No.	**Label ID**	**Start Date**	**Start Time**	**End Date**	**End Time**	**Environmental Conditions**	**ADDITIONAL COMMENTS**
1	**BL01250108**	**25/01/08**	**10.47**	**22/02/08**	**14.23**	*23°C, 33% RH*	None
2	**BL01250182**	**25/01/08**	**10.48**	**22/02/08**	**14.23**	*23°C, 33% RH*	Noticed tube missing on 10/02 ??
LOCATION A:							
1							
2							

LOCATION B:							
TUBE No.	**Label ID**	**Start Date**	**Start Time**	**End Date**	**End Time**	**Environmental Conditions**	**ADDITIONAL COMMENTS**
1							
2							

Object information form

Institution name	
Contact name: **address/telephone/email:**	
Object name	
Object Identification Number	
Object picture included?	☐ **Yes**
Please be as detailed as possible when filling out the following: **What is/are the object(s) made of:**	

How is the object stored (i.e. climate controlled cabinet, dedicated store, next to other samples of different type etc.)?
How long has the object been in its current location?
Is anything known about previous chemical treatment of the object or its current location, such as pesticides, fumigants, antifungal sprays, mothballs etc.?