

Analysis of Pesticides in the Environment

1 The investigation

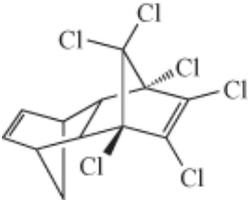
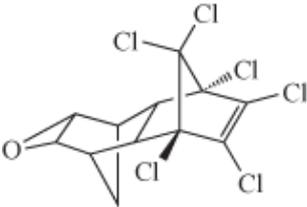
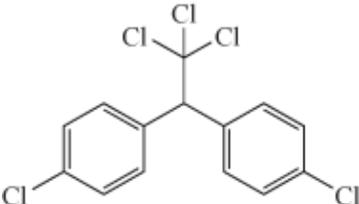
This investigation considers the presence in water of organic compounds. These are carbon-containing compounds, generally man-made in origin, and include crude oil and its derivatives, detergents, and pharmaceuticals and their metabolites. But perhaps the class of organic compounds that cause the most concern when present as contaminants in water, owing to their toxicity, is pesticides.

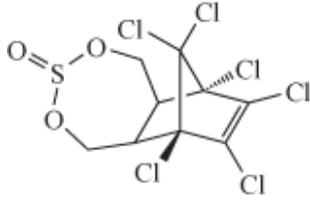
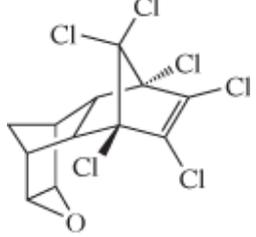
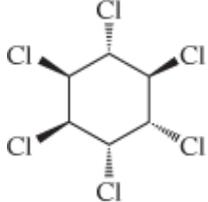
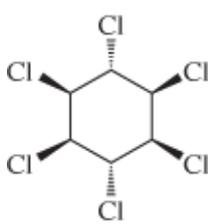
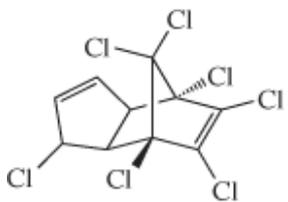
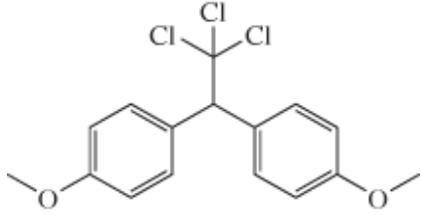
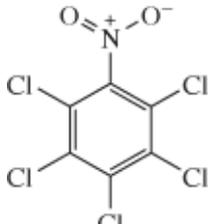
The term 'pesticide' encompasses a wide range of organic compounds used to control weeds (herbicide), fungi and moulds (fungicide), insects (insecticide) and pests (pesticide). Traces of these compounds may enter rivers and surface water as run-off from agricultural land (where they are used to protect crops) or via non-agricultural use (for example, the use of herbicides to control weeds along motorway verges). They may also percolate through soil into ground water.

The EU directive on drinking water has set a standard of $1 \mu\text{g l}^{-1}$ for individual pesticides and $5 \mu\text{g l}^{-1}$ for the total pesticides present. There are stricter standards for the organochlorine pesticides (OCPs), such as dichloro-diphenyl-trichloro-ethane (DDT), which are now banned in many countries. This class of pesticides is very persistent, remaining active in the environment for a long time after initial contamination. Such chemicals may still be observed in samples many years after they have been banned. A number are, however, still in use in lower- and middle-income countries.

In this investigation you will be using the technique of gas chromatography together with mass spectrometry (a combination also known as 'GC-MS') to evaluate the environmental contamination of a tidal bay in China by this class of persistent organochlorine pesticide. Examples of the pesticides that you will be analysing are given in Table 1.

Table 1 Examples of the class of OCPs

Pesticide	Molecular formula	Structure	Information ^a
Aldrin	$\text{C}_{12}\text{H}_8\text{Cl}_6$		pesticide (banned but restricted use as a local insecticide)
Dieldrin	$\text{C}_{12}\text{H}_8\text{Cl}_6\text{O}$		insecticide (banned but restricted use for agricultural purposes)
Dichlorodiphenyltrichloroethane (DDT)	$\text{C}_{14}\text{H}_9\text{Cl}_5$		pesticide (banned but restricted use for malaria control)

Endosulfan	$C_9H_6Cl_6O_3$ S		insecticide (banned only recently, still in use in China and India)
Endrin	$C_{12}H_8Cl_6O$		insecticide and rodenticide (banned)
Hexachlorocyclohexane (α HCH)	$C_6H_6Cl_6$		pesticide (banned)
Lindane (γ -HCH)	$C_6H_6Cl_6$		insecticide (banned but restricted pharmaceutical use for treatment of lice and scabies)
Heptachlor	$C_{10}H_5Cl_7$		insecticide (banned but restricted use as a termiticide)
Methoxychlor	$C_{16}H_{15}Cl_3O_2$		insecticide (banned in US and EU)
Pentachloronitrobenzene (PCNB)	$C_6Cl_5NO_2$		fungicide

^a Banned status according to the United Nations Treaty (known as the Stockholm Convention on Persistent Organic Pollutants), which has been signed by the majority of countries. One notable exception is the USA, which has its own list of banned substances covering many of the same pesticides.

In this investigation you will first develop a hypothesis and sampling plan, choosing which sites to sample in the bay. You will then use GC–MS qualitatively to identify any pesticides present in your samples and quantitatively to determine the concentrations of the pesticides you have found.

By the end of this investigation you should be able to:

- devise a sampling plan
- understand how chromatography is used to separate mixtures of compounds
- compare mass spectra of individual pesticides with a ‘pesticide’ library to identify particular compounds present in a mixture
- appreciate the significance of percentage recovery in extraction processes
- use response factors and percentage recovery to determine the concentrations of individual pesticides present in a sample.

2 Analytical method

In this investigation you will be using GC–MS to identify pesticides present in a sample of water (qualitative analysis) and their concentrations (quantitative analysis).

The analysis involves the following steps:

- the organics are first extracted into an organic solvent using solvent extraction (the samples in water cannot be analysed using GC–MS)
- any residual water is removed using a drying agent, such as anhydrous sodium sulfate
- the organic solution is concentrated using an evaporation process
- the mixture of organics in the organic solvent is separated using gas chromatography.

Figure 1 shows an example of a chromatogram for a range of different pesticides. In this example, the conditions in the GC have been optimised to allow separation of the pesticides, each shown as an individual peak with different retention time.

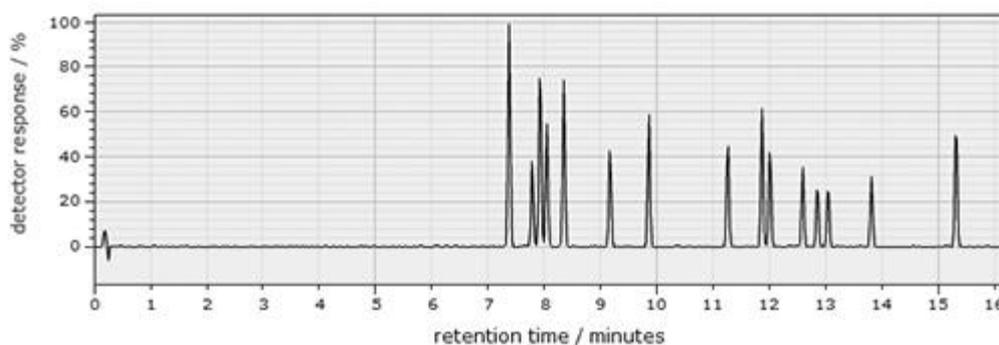


Figure 1 Example chromatogram

- How could retention time be used to identify the pesticides in a mixture?
- Standards containing the different pesticides could be used to determine the retention times of particular pesticides, which can then be compared to the retention time of the peaks in the sample.

Although the retention time of a particular compound can be matched against that of a standard, the differences in retention time between different compounds can be small. Chromatography combined with mass spectrometry (GC–MS) is often used to conclusively identify a substance.

Mass spectrometry can be used to provide a ‘fingerprint’ of the different pesticides or compounds. The mass spectrum of the pesticide lindane shown in Figure 2.

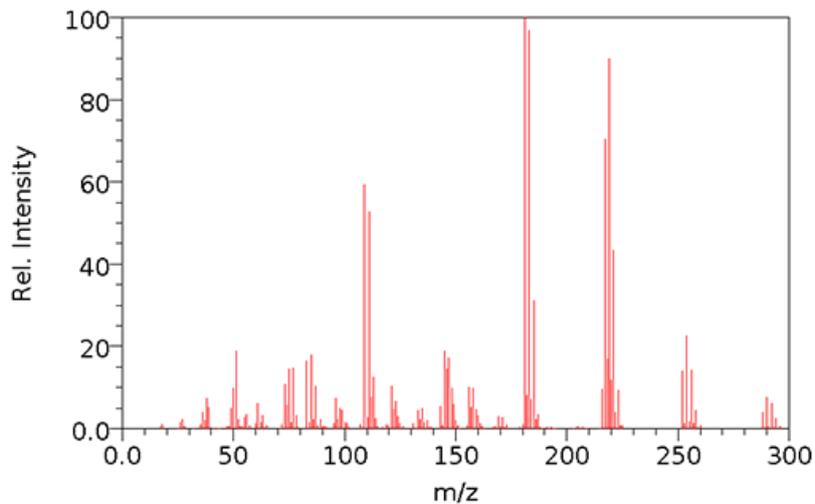


Figure 2 Mass spectrum of lindane

Although a small peak due to the molecular or parent ion is present, the mass spectrum itself is complex, reflecting the different possible fragment ions that are produced. Rather than analysing the peaks in the mass spectrum of each pesticide and building up the structure in that way, the spectrum can be compared with a 'library' of mass spectra of a range of different pesticides/related molecules. This is what you will be doing in this investigation to identify any pesticides present in your samples.

3 The task

The key objective of the investigation is to determine the presence/level of organochlorine pesticides (OCPs) in the Chinese coastal bay shown in Figure 3, together with a reference grid. The bay covers an area of approximately 380 km² and is fed by a number of rivers running from mainly agricultural land, where the use of OCPs has, in the past, been widespread.

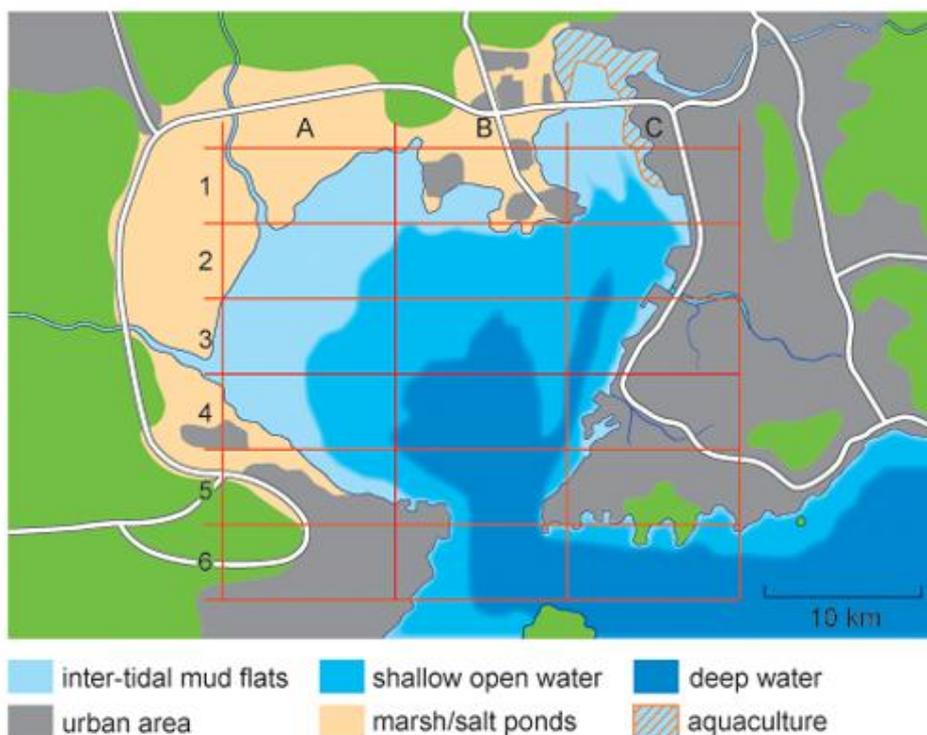


Figure 3 Map of the bay and surrounding area. The bay is of variable depth; the area highlighted as shallow open water does not exceed 6 m in depth at low tide.

The area surrounding the bay is densely populated; the eastern shore is a largely built up, urban and industrial area with a major port. The bay is tidal with a small opening in the south into the Yellow Sea. The northern shore consists of intertidal flats, which are submerged at high tide but exposed at low tide, salt ponds and a number of ponds used for the cultivation (aquaculture) of shellfish. The bay, like many coastal regions in China, suffers from algal blooms due to high nutrient levels.

In order to determine the levels of OCPs in the bay you should use the map and information given in Table 2 about the particular sampling location for each grid area to decide which sites that you wish to sample. It is recommended that you develop a hypothesis and sampling plan for this investigation. This should be based on the assumption that this is an initial study and you are only able to sample a limited number of sites within the bay.

You should complete both before you start to collect experimental data.

Table 2 Information about the sampling location in each grid area

Grid reference	Description of location
C1	intertidal flats, close to an area developed for aquaculture
A2	intertidal flats
B2	shallow open water
C2	shallow open water, not far from an estuary of a major river
A3	intertidal flats
B3	shallow open water
C3	deep sea water
A4	shallow open water
B4	deep sea water
C4	shallow open water, in an estuary at the mouth of a major river; is also close to a major port
B5	deep sea water in the inlet into the bay
B6	deep sea water in the inlet into the bay
C6	shallow open water

Note that no samples were taken in the following sections of the grid: A1, B1, A5, C5 or A6.

An analytical sampling plan would typically consider the following questions.

- Where is the best site for the sampling?
- How many samples should be taken?
- How often samples should be taken?
- How much of each sample is needed for analysis?
- How will each sample be collected?

As you will not actually physically take the samples yourselves, you do not need to consider the last points in any great detail and just concentrate on the first two. Details of the others are given at the end of this section in Box 1, which is about the sampling protocol used in the analysis.

Box 1 Sampling protocol

In this investigation assume that samples (1500 ml) are taken at three different depths below the surface of the bay and stored at 4 °C in amber-coloured bottles to maintain the integrity of the sample. The sample you will be analysing is a composite of the three samples taken at each sampling site. As this is a preliminary study, we will not consider how often samples will be taken and will collect only one set of samples at each site.

3.1 GC–MS qualitative identification of the pesticides in the standard

Activity 1 Qualitative analysis of standard sample

Allow approximately 40 minutes

In this activity you will run the GC–MS of a standard sample containing a mixture of pesticides and their metabolites, and use a pesticide library to identify the organic compound that elutes at each retention time.

1. Access the interactive pesticide determination experiment and run the GC–MS spectrum of the standard mixture. (Instructions for doing this are available via the ‘Reference’ tab within the software.)
2. Note the retention time of each peak in the table in your notebook.
3. Click on the number above a peak in order to see the mass spectrum of this pesticide. A library of spectra from known pesticides will also open. Use this pesticide library to identify which pesticide/metabolite is present at that particular retention time and make a note of this in your notebook. Repeat this process until you have assigned all the peaks in the standard.

Record the retention times and peak areas in your notebook. You should then answer the question below.

Question 1

A peak in the chromatogram has a retention time of 8.05 min. Using the information you derived from the standard and the pesticide library, identify the pesticide that elutes at this time.

Answer

Pentachloronitrobenzene, PCNB, has a retention time of 8.05 min.

3.2 GC–MS qualitative identification of the pesticides in your water samples

Activity 2 Qualitative analysis of samples from the bay

Allow approximately 30 minutes

1. Select the ‘Sampling’ tab within the software, and choose one of the sampling sites that you selected in your sampling plan to analyse using GC–MS.
2. Move to the ‘Analysis’ tab in the software and run the GC–MS spectrum of your first sample. The retention time of each peak is provided in the table in the software, together with the peak areas. You should make a note of these data in your notebook. To run replicate analyses, press ‘reset’ then ‘run’. You should take the mean of the peak areas for the different peaks identified.
3. Use the data that you derived from the standard and the pesticide library in Activity 1 to identify any pesticides present and record your findings in your notebook. (You may view the mass spectrum of each pesticide by clicking on the number above each peak to confirm this, should you wish to.)
4. Repeat this process for the other samples sites that you have selected.

Now that you have identified the pesticides present at each of your sample locations your next action will be to use the peak areas observed to complete a quantitative analysis of the samples. You will do this in Section 3.3.

3.3 Data interpretation: quantitative analysis of pesticides

Activity 3 Quantitative analysis of samples from the bay

Allow approximately 30 minutes

Use the following expression to calculate the concentration of each pesticide (see Box 2 for details):

$$\text{concentration of pesticide in initial sample} = \frac{\text{peak area of sample} \times \text{response factor}}{15 \times \text{percentage recovery}} \quad (1)$$

Table 3 lists the response factors for the range of pesticides analysed, as well as their percentage recovery. The initial volume of the water sample was 1500 ml, which was concentrated during the preparation step to 1 ml.

Table 3 Response factor and percentage recovery for some OCPs

Pesticide/metabolite	Response factor/ $\mu\text{g l}^{-1}$	Recovery/%
Aldrin	0.0078	75.3
<i>p,p'</i> -DDD ^c	0.0379	93.1
<i>p,p'</i> -DDE ^c	0.0088	90.0
<i>o,p'</i> -DDT ^d	0.0158	79.1
<i>p,p'</i> -DDT	0.0175	88.2
Dieldrin	0.0123	94.4
Endosulfan	0.0109	89.9
Endrin	0.0152	88.9
α -HCH ^e	0.0064	102.1 ^f
β -HCH ^e	0.0159	101.6 ^f
γ -HCH (lindane)	0.0078	110.7 ^f
δ -HCH ^e	0.0087	102.9 ^f
Heptachlor	0.0097	72.1
Methoxychlor	0.0098	97.0
PCNB	0.0089	79.5

^aThe response factor for each pesticide is the ratio of pesticide concentration to peak area, and can be determined by the slope of a calibration graph for that pesticide that shows peak response of calibration standards (*y*-axis) versus concentration of calibration standards (*x*-axis). In practice, the instrument is calibrated with a mixture of the pesticides at a range of different concentrations and the resulting calibration curves are used to determine a response factor for each pesticide.

^bThe percentage recovery ('recovery %') is the percentage of the pesticide compound that can be recovered during the solvent extraction phase. It is determined using a standard where a known mass has been introduced into a sample *before* the extraction and then measured *after* the extraction process to see how much of that mass is recovered. Generally, the extraction process is controlled so that a percentage recovery of approximately 90% is achieved.

$$\text{percentage recovery} = \frac{\text{mass of standard after recovery}}{\text{mass of standard added}} \times 100\%$$

^c metabolite resulting from the breakdown of *p,p'*-DDT in the environment. The *p*, *p'* and *o* is notation given to different isomers.

^d minor isomer present in commercial DDT

^e present in commercial HCH (also with γ -HCH)

^f recoveries of greater than 100% can result from uncertainties in determination of the analyte concentration

You may like to try the following example before you attempt your own calculations.

Question 2

The peak area of the pesticide aldrin is 600 using GC. What is the concentration of aldrin in the sample? (You can assume that the peak area of the pesticide is within the linear range of the calibration curve.)

Answer

$$\begin{aligned}\text{concentration of aldrin} &= \frac{600 \times 0.0078 \mu\text{g l}^{-1}}{15 \times 75.3} \\ \text{in initial sample} &= 4.1 \text{ ng l}^{-1}\end{aligned}$$

Once you have calculated the average concentration for each pesticide at each of the sampling sites that you selected you should also calculate the total pesticide concentration at each site.

Do your results support your hypothesis? You should consider whether your samples are truly representative and how you might improve your sampling plan

Box 2 Initial concentration – derivation of equation 1

The percentage recovery ('recovery %') can be used to calculate the recovery correction factor (RCF), where

$$\text{RCF} = \frac{100}{\text{recovery \%}}$$

From the GC, the amount of analyte,

$$A = \text{peak area of sample} \times \text{response factor} \times \frac{\text{final extract volume}}{\text{initial sample volume}}$$

In this investigation,

$$\frac{\text{final extract volume}}{\text{initial sample volume}} = \frac{1 \text{ ml}}{1500 \text{ ml}}$$

and

$$A = \frac{\text{peak area of sample} \times \text{response factor}}{1500}$$

Concentration of analyte in initial sample is

$$\begin{aligned}A \times \text{RCF} &= \frac{\text{peak area of sample} \times \text{response factor}}{1500} \times \frac{100}{\text{recovery \%}} \\ &= \frac{\text{peak area of sample} \times \text{response factor}}{15 \times \text{recovery \%}}\end{aligned}$$