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INTRODUCTION

Pesticides used in agriculture, public health and agricultural pest control programmes can enter the environment in a number of ways depending upon the method and proficiency of application, as a result of accidents or through the unauthorized dumping of unwanted pesticide products or their containers.

Pesticide residues are the deposits of pesticide active ingredient (a.i.), its metabolites or breakdown products present in some component of the environment after its application, spillage or dumping. Residue analysis provides a measure of the nature and level of any chemical contamination within the environment and of its persistence. It is often difficult to correlate pesticide residues in the environment with effects on fauna and/or ecological processes. They can, however, show whether an animal or site has been exposed to chemicals and identify the potential for future problems. Selected sampling programmes can be used to:

- investigate residual levels of pesticide in the environment, their movement and their relative rates of degradation
- identify contaminated areas and/or sources of contamination
- examine the uptake of pesticide by food chain components
- determine whether pesticides were a cause of mortality.

All pesticides are subject to degradation and/or metabolism once released into the environment. The rates of degradation and dissipation vary greatly from pesticide to pesticide and situation to situation. The object of residue analysis is to indicate the residues present at the time of sampling and every precaution must be taken to ensure that the sample arriving at the laboratory has not been allowed to deteriorate in such a way that the results are meaningless. Some losses of and/or changes in the chemicals are inevitable and these will vary depending upon the conditions and the nature of the pesticides present. When sampling for residue analysis, the aim is to minimize these losses and thus maximize the correlation between the result obtained from the sample taken and the residue level actually present at the sample site.

The difficulties of sampling biotic and abiotic materials for pesticide residues in tropical countries are exacerbated in areas remote from suitable storage facilities or from the analytical laboratories themselves. Any delay in preserving the sample or extracting the pesticide residues means that there is an increased risk of degradation of any residues present, with a corresponding increase in the uncertainty regarding the analytical results and their interpretation. If analysis of shorter lived compounds (such as organophosphates or carbamates) is required, then the risk of loss is great. However, with some pesticides (particularly the more persistent chlorinated pesticides and some herbicides), the risks of loss are less. The rate of loss for all types of compounds is greater under tropical rather than temperate conditions.

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PROPERTIES OF PESTICIDES

Knowledge of the properties and characteristics of pesticides is vital in developing a sampling plan for residue analysis. Although it is difficult (and risky!) to generalize, the following briefly outlines the relevant environmental characteristics of the various pesticide classes.

After each of the following sections, a brief summary of reported data (from *The Pesticide Manual* and *EXTOXNET* files – see suggested ‘Further Reading’ on page 147) on water solubility, stability of residues in soil and on mammalian metabolism/excretion of residues is given for examples of each of the classes. This will give some idea of the general characteristics of the class and of the potential variation in environmental persistence. It is difficult to make any general statements on interpretation of this data as the individual compounds are so markedly different. However, increased water solubility indicates the *potential* for greater movement/leaching from the soil (although the type of soil in the treatment area is important in such considerations, e.g. clay soils are more retentive than sandy soils). Soils with a high organic matter content are also more retentive to certain residues. The half-life data (i.e. the times taken for half of the active ingredient to have been lost through degradation or dissipation) is a useful indicator of likely persistence and will help shape, particularly with regard to time scales, any proposed sampling programme. The significance of known metabolites/breakdown products should also be taken into account.

Organochlorines

Mobility of organochlorines in soil is generally limited, although it is greater in sandy soil. They tend to be bound in clay soils with limited leaching. Residues of the parent compound or metabolites can be found in soil, sediment, vegetable samples and in vertebrates/invertebrates for extended periods. Their solubility in water is low, although residues can be detected in water where there is extreme contamination and, particularly, on suspended matter in water.

Examples of water solubility, persistence in soil and mammalian excretion are given below.

- **lindane (gamma isomer of benzene hexachloride)**
Water solubility: 7.3 mg l⁻¹ (25 °C), 12 mg l⁻¹ (35 °C). Half-life of 15 months (temperate) when incorporated into the soil; much shorter if sprayed on the soil surface. Shows a low soil affinity and may be mobile in certain soil types. Fairly readily metabolized by animals to pentachlorocyclohexane, 1,2,4-trichlorobenzene and isomeric trichlorophenols and excreted as glucuronic acid derivatives. Other isomers of benzene hexachloride can be more persistent.
- **dieldrin**
Water solubility: 0.19 mg l⁻¹ (25 °C). Persistent in soil under temperate conditions; at average application rates (3.1–5.6 kg ha⁻¹), it is estimated that roughly 95% will disappear in 12.8 years on average. In bright sunlight, photo-dieldrin can be formed, which is a more toxic product. Some accumulation of dieldrin occurs in animal tissue, particularly fat; dieldrin is very slowly metabolized to water-soluble products which are excreted from the body.
- **DDT (p-p' isomer)**
Practically insoluble in water. Reported half-lives are 28 days (river water) and 56 days (lake water). Residues are lost by volatilization, photodegradation, adsorption on particulate matter and sedimentation. In soil, DDT is chemically and microbially degraded. In temperate climates, a half-life of 2–15 years is reported; under tropical conditions, the half-life is 5–12 months. In the tropics, initial dissipation is rapid, through volatilization. Metabolized (very slowly) to a range of saturated and unsaturated products by progressive dechlorination. Residues accumulate in fatty tissues and are excreted in milk.

- **heptachlor**

Water solubility is low: 0.056 mg l⁻¹ (25–29 °C). Heptachlor is rapidly hydrolyzed in water with the product then converted to the epoxide. Loss from water by volatilization, photodegradation and sedimentation. Persistent in soil with a reported half-life of 250 days; substantial variation reported depending on soil type. In soil, it undergoes hydrolysis and then microbial epoxidation. Half-life in soil (temperate climate) is 9–10 months at agricultural rates of application. In animals, heptachlor metabolizes to the epoxide which can be found in most body organs but it particularly accumulates in body fat.

- **endosulfan**

Water solubility: 0.32–0.33 mg l⁻¹ (22 °C). Stable to sunlight. In neutral river water, residues will disappear in approximately 4 weeks; persistence extended under acidic conditions and substantially so (5 months) under basic conditions. Half-life in soil is 30–70 days and the main metabolite is endosulfan sulphate which is degraded more slowly and is thus an important metabolite. The soil half-life for total endosulfan (both isomers plus sulphate metabolite) is 5–8 months. Endosulfan sulphate again is the primary metabolite on plants; plant half-life is 3–7 days (varies with species). Rapidly metabolized and excreted by mammals.

Note: With the organochlorine pesticides there is substantial variation in the published data for soil half-lives with some authors quoting periods of years instead of months. These materials can be extremely persistent under certain conditions, particularly in temperate climates from where much of the available data is obtained. Under tropical conditions, however, persistence can be substantially reduced. The data presented above although from reputable published sources should be regarded as merely a guide.

Organophosphates

Organophosphates have a fairly limited environmental persistence and residues in living specimens generally are not detected, or only as metabolites in specific cases.

Water solubility is variable but higher than with the organochlorines; residues generally break down quite quickly in water (hydrolysis) and are not generally detected except where the contamination is quite recent. Soil residues are similarly short-lived. Residues are probably only of interest for 5–15 days after spraying unless in shaded areas or where the concentrations applied are high.

Examples of water solubility, persistence in soil and mammalian excretion are given below.

- **fenitrothion**

Water solubility: 21 mg l⁻¹ at 20 °C. Half-life in soil is 12–28 days, less in submerged conditions (4–20 days). Rapid mammalian metabolism and excretion. The most important metabolites are dimethylfenitrooxon and 3-methyl-4-nitrophenol. Plant metabolism to similar products (and their decomposition products) with a half-life of the parent compound of about 4 days.

- **fenthion**

Water solubility: 4.2 mg l⁻¹ at 20 °C. Rapid degradation in soil and water (half-life is approximately 1 day). Elimination of residues in mammals by excretion of hydrolysis products. Major metabolites are fenthion sulfoxide and sulfone and their oxygen analogues. Further degradation of these metabolites to the corresponding phenols can occur. Similar degradation pattern occurs on plants.

Carbamates

Residues of parent compounds are generally not environmentally persistent; metabolites are rapidly excreted by vertebrates. Water solubility is moderate; greater for the metabolites. Most carbamates are relatively stable in water of neutral pH. Stability and mobility in soil varies between compounds. Environmental residues are

probably only of interest for 10–20 days after spraying, although in certain soils and in water, extended monitoring may be required.

Examples of water solubility, persistence in soil and mammalian excretion are given below.

- **aldicarb**
Water solubility: 4.93 g l⁻¹ at 20 °C. Residues are oxidized in soil but residues are persistent and effective for approximately 10 weeks. Aldicarb is toxic to mammals but sub-lethal doses are metabolized rapidly with over 90% excreted in 3–4 days. Major metabolites are the sulfoxide and sulfone. In plants the metabolism pattern is similar but the sulfoxide has a systemic action and is 10–20 times more active as a cholinesterase inhibitor than the parent compound.
- **carbaryl**
Water solubility: 120 mg l⁻¹ at 20 °C. In soil under aerobic conditions, 1 ppm carbaryl is degraded with a half-life of 7–14 days in a sandy loam and 14–28 days in a clay loam. In mammals, carbaryl does not accumulate and is rapidly metabolized to non-toxic substances, particularly 1-naphthol, and excreted.
- **propoxur**
Water solubility: 1.9 g l⁻¹ at 20 °C. Mobility in soil is high although degradation is rapid in different soils. In mammals, metabolism, principally to 2-hydroxyphenyl-N-methylcarbamate and 2-isopropoxyphenol, and excretion in the urine, is rapid.

Pyrethroids

Pyrethroid insecticides are generally non-persistent in the environment, being rapidly degraded in the presence of strong sunlight. Residues are probably only of interest for 5–7 days after spraying, unless in shaded areas and where the concentrations applied are particularly high. Proper and accurate detection of residues requires a specialist laboratory.

Examples of water solubility, persistence in soil and mammalian excretion data are given below.

- **cypermethrin**
Water solubility: 0.004 mg l⁻¹ at 20 °C. In river water, rapid degradation is reported (a half-life of approximately 5 days). In soil, it is fairly persistent; degrades by hydrolysis (approximately 16 weeks). Mammalian metabolism/excretion is similar to that for deltamethrin (see below).
- **permethrin**
Water solubility: 0.2 mg l⁻¹ at 20 °C. Rapidly degraded in soil and water. In mammals, elimination is by hydrolysis, hydroxylation and elimination as glucoside conjugate. In the rat, an orally administered dose is completely eliminated within 12 days. The metabolism of the trans isomer is more rapid than that of the cis isomer.
- **deltamethrin**
Water solubility: <0.2 µg l⁻¹ at 25 °C. In soil, it is microbially degraded in 1–2 weeks. Residues strongly bound in the soil with little risk of leaching. In rats, it is virtually eliminated from the body within 8 days with extensive metabolism occurring.

Insect growth regulators

Benzoyl urea IGRs generally act by inhibition of chitin synthesis and moulting, thus interfering with the formation of the insect cuticle. They are increasingly used for the control of leaf-eating insects (mandibulate herbivores) in forestry, ornamentals and fruit. Their low water solubility and adsorption by soil reduces their environmental

impact and in general use, residues are only likely to be detected in soil. There may be some, limited non-target effects in treated areas.

There are also IGRs which act as juvenile hormone mimics, disrupting or preventing maturation of immature invertebrates.

- **diflubenzuron – benzoyl urea IGR**

Water solubility is low: 0.08 mg l⁻¹ at 20 °C (pH 5.5). Diflubenzuron is strongly bound to the soil/humic acid complex and is virtually immobile. Stable to sunlight. Non-systemic and not metabolized in plants. In mammals, excretion of ingested diflubenzuron is quite rapid, partly as the parent compound but also as hydrolyated metabolites.

- **teflubenzuron – benzoyl urea IGR**

Water solubility is low: 0.019 mg l⁻¹ at 23 °C. Half-life in soil varies from 2 to 12 weeks depending upon soil type and conditions with microbial degradation to 3,5-dichloro-2,4-difluorophenylurea. Almost no uptake or metabolism by plants. In mammals (rats), teflubenzuron and metabolites are rapidly excreted in the faeces and urine.

- **triflumuron – benzoyl urea IGR**

Water solubility is low: 0.025 mg l⁻¹ at 20 °C. Half-life data in soil are not available but the half-life is reported to be fairly rapid; no accumulation of residues has been detected where application to soil is repeated over a period of 3 years. After application at normal rates, no residues could be detected after a few months. In mammals (rats), metabolized residues are excreted quite rapidly.

- **methoprene – terpenoid IGR (juvenile hormone mimic)**

Water solubility is low: 1.4 mg l⁻¹ at 20 °C. Rapidly degraded in soil with a half-life of ±10 days. In plants, it is degraded through ester hydrolysis. In mammals, it is metabolized with cholesterol as one of the secondary metabolites.

- **fenoxycarb – bridged diphenyl carbamate IGR (juvenile hormone analogue)**

Water solubility: 6 mg l⁻¹ at 20 °C. Shows low mobility in soil and relatively rapid breakdown in soil and water. Does not bioaccumulate. Rapidly metabolized in plants.

Herbicides

Although of relatively low acute toxicity to most animals, herbicides can indirectly affect a variety of species through the removal of vegetative cover. Environmental persistence of the herbicides varies; some are readily absorbed by and degraded in soil (e.g. paraquat) whilst others are more persistent and, with relatively high water solubilities, considered to be quite mobile (e.g. triazine materials). Residues transferring (leaching) to waterways is a recognized problem. Residues in wildlife are generally transient with rapid metabolism and excretion.

The significance of residues depends upon the applied material e.g. with 2,4-D, residues decline quite quickly with a half-life of <7 days in soil; with the triazine herbicides or with products such as linuron/diuron, persistence is considerably greater and residues can be present for months. The persistence of sulphonyl urea herbicides varies although at the extremely low rates they are applied under normal use, the residues present are particularly low and the analysis can be difficult.

Examples of water solubility, persistence in soil and mammalian excretion are given below.

- **2-4-D**

Water solubility: 46 mg l⁻¹ at 25 °C; 311 mg l⁻¹ at 25 °C (pH 1.0). Rapidly degraded in soils (by microbial activity; half-life <7 days). Rapidly eliminated from mammals (as parent compound), often within 24 h. Maximum organ concentration is reached in approximately 12 h.

- **atrazine**
Water solubility: 33 mg l⁻¹ at 20 °C. In water, it has an extended half-life (e.g. 100→200 days in groundwater). In soil, the half-life is 35–50 days; longer under dry, cold conditions. The major metabolites are desethylatrazine and hydroxyatrazine. In mammals, rapid and complete metabolism of ingested residues is primarily by oxidative dealkylation of the amino groups.
- **linuron**
Water solubility: 81 mg l⁻¹ at 25 °C. In soil, degraded microbially with a half-life of 2–5 months.
- **chlorsulfuron**
Water solubility: 27.9 g l⁻¹ at 25 °C (pH 7.0). Hydrolyzed in soil in 4–6 weeks; hydrolysis is more rapid in moist conditions, at raised temperatures and in the presence of a high moisture content. Further breakdown is microbial.

Fungicides

Some fungicides can have adverse environmental effects but, although they are used extensively in the field for cereal production, their use patterns suggest limited scope for environmental contamination except as the result of disposal (e.g. from large-scale dip treatment operations) or accidental contamination (spillage, etc.).

Water solubility and stability are variable; some fungicide residues can be detected in water for periods of days through to months.

Examples of water solubility, persistence in soil and mammalian excretion (where available) are given below.

- **carbendazim**
Water solubility: 8 mg l⁻¹ at pH 7; 29 mg l⁻¹ at pH 4.0 (24 °C); water half-life of 2–25 months under aerobic and anaerobic conditions. In soil, it is microbially degraded with a half-life of 3–12 months. It is rapidly metabolized and excreted by mammals.
- **chlorothalonil**
Water solubility: 0.9 mg l⁻¹ at 25 °C. Soil residues are degraded fairly rapidly, 5–36 days under aerobic/anaerobic conditions, much quicker (hours to days) under flooded conditions. Soil residues are not considered to be mobile. Residues are largely unabsorbed by mammals.
- **metalaxyl**
Water solubility: 8.4 g l⁻¹ (22 °C), residual activity in soil is approximately 70–90 days.

Soil fumigants

Materials such as methyl bromide (use now heavily restricted under the Montreal Protocol) and 1,3-dichloropropene are examples of materials used as soil fumigants. Under controlled use, soil fumigants do not pose a substantial environmental problem unless they are allowed to contaminate watercourses (methyl bromide is highly soluble in water, 13.4 g l⁻¹ at 25 °C, 1,3-dichloropropene is less soluble, 2 g l⁻¹ at 20 °C). The materials are volatile and dissipate to atmosphere on aeration of the soil.

STUDY DESIGN

The construction of a comprehensive residue sampling programme is a huge subject and beyond the scope of this text. It is not possible to define a sampling regime for all circumstances and the local conditions will need to be taken into account in each case. However, the following section summarizes the key points to bear in mind when taking and preserving environmental samples.

Residue analysis might be considered as part of an environmental assessment for:

- planned pesticide application
- accidental localized spillage
- major site contamination
- long-term pesticide application or exposure
- unexplained wildlife mortality.

The nature of the sampling exercise and the collection of the samples themselves requires careful thought and planning. Samples taken in the wrong way or without due care can be misleading, resulting in incomplete or wrongly directed conclusions.

General information on the properties and relative persistence of the different pesticide groups (see pp.126–130) and their methods of application will help the investigator to determine the samples which need to be taken, e.g. whether the pesticides used and the area and method of treatment will potentially affect biotic or abiotic factors (or both) and help in the development of an appropriate residue sampling programme.

The exact nature of the study and the material of interest (soil, vegetation, insects, animal tissues, etc.) defines the way in which the samples are taken and preserved prior to analysis. Chapters 7–13 of this handbook consider specific faunal components of the ecosystem or ecosystem processes and include notes on the collection and preservation of samples for pesticide residue analysis, together with some guidance on the potential problems in the interpretation of residue data. There are a few general principles that are applicable to all types of sample and these are discussed below.

Appraisal studies can be either single sampling missions or more structured, e.g. an immediate assessment followed by further, periodic sampling (surveillance) visits. In general, the latter will provide the most useful results to help interpret monitoring studies on fauna, however, it will also significantly increase the costs. The type of study will often reflect the nature of the pesticides used and whether these are likely to be persistent or relatively short-lived. Treatment histories for the area, where available, will be helpful in identifying survey sites and in data assessment.

Background residues

Before proceeding with studies involving the application of pesticides, it is worthwhile establishing baseline data by screening for background pesticide levels. Where pesticide use has not been recent, the only residues likely to be detected will be of the more persistent organochlorine materials, benzoyl urea insect growth regulators (IGRs), phenyl pyrazoles and their metabolites or certain herbicides. These residues may be found in the soil, groundwater or the sediment of waterways. Residues may also be found in vertebrates/invertebrates associated with the treated area, through direct contact or food chain effects.

Planned pesticide application

Intense spray operations for the control of major pests such as locusts, African armyworm, tsetse fly or quelea, can result in broad off-target spray deposition and may warrant a detailed assessment (see chapter 4).

Sampling for the purposes of residue analysis will, in most cases, only involve surviving non-target species and samples of vegetation, soil and, perhaps, surface water or sediment. The analysis of vertebrates or invertebrates killed by direct spray application is generally not meaningful. Residue analysis will only determine the residue present in the body at the time of analysis and interpretation of the significance of that residue is not straightforward.

Where the details of the spray operations are precisely known, and particularly when samples can be obtained immediately before and immediately after application, then sampling for residue analysis can be used to estimate:

- rates of pesticide adsorption or degradation by vegetation and by fauna
- the rate of adsorption by, and movement through, soil
- the rate of loss from soil
- the rate of transfer to groundwater.

Where the pesticide is a relatively non-persistent material (such as an organophosphate, carbamate or pyrethroid), residues are likely to decrease quite quickly, depending upon climatic conditions, and sampling should commence immediately after spraying and then at short intervals thereafter. The half-life of any pesticide will be significantly reduced where it is hot and humid, where they are exposed to direct, bright sunlight or to high microbial populations. The persistence of even some of the more stable pesticides (such as the organochlorines) will be less under tropical climates than in temperate conditions. Sampling programmes must take account of these factors.

Methods of application

The method and precision of application (and the purpose of the pest control operation) usually determines the quantity of pesticide applied and its overall distribution (see chapter 4 on pesticide application). Poor application can result in over-spraying of an area (i.e. an excessive dose), excessive spray drift, or poor targeting with scope for greater non-target contamination.

The types of field treatment used are briefly reviewed in the following paragraphs.

Spray operations

Spray operations refer to the distribution of a pesticide solution or suspension through a nozzle system producing a fine spray of droplets of a controlled or variable size. The size of the droplet, which depends upon the nature of the equipment used and the target pest, generally controls the rate at which the droplet settles; larger droplets settle more quickly than small droplets. Smaller droplets are more likely to drift from the target area, particularly when applied in windy conditions or where there are thermal currents. Application systems can be *high volume*, using a pesticide concentrate that has been diluted with water, or *ultra-low-volume* (ULV) where the concentrated pesticide is dispersed as a fine mist, without any dilution. The former generally result in larger droplets, the latter in smaller droplets. In between these extremes lie a range of modified techniques producing different droplet spectra (see chapter 4).

The purpose of the operation will determine the way in which the pesticide is applied. For example, spray operations where the objective is to drench the target (e.g. certain quelea control techniques) will use coarser droplet sizes and heavy dose rates. Where the target is a smaller flying target, a finer droplet size may be more appropriate although this approach can be used for quelea where a ULV formulation is sometimes used. The capacity for small droplets to drift can be used deliberately to deposit the spray downwind on to the target, but can also result from poor application practice. The height from which the pesticide is applied can also be a factor (i.e. ground or aerial spraying). In all these situations, the extent of pesticide drift determines the frequency of sampling and the extent of the sample area.

Where pesticides have been applied by ULV, and where the droplets are much smaller, residues are likely to be more widespread and to adhere to vegetation (trees, shrubs or grass) with a much smaller proportion reaching the soil. Vegetative cover (unless absent) will thus generally form the primary focus of the sampling programme.

Where pesticides have been applied in aqueous solution at high volume, whatever the target, a larger proportion of the spray solution will reach the ground. Soil and covering vegetation should show, at least initially, the highest residues and should form the primary focus of the sampling programme. The secondary focus for samples will be those species living in the soil/vegetative cover and higher species which may accumulate residues through food chain effects.

Dust treatments

Field dust treatments (e.g. those used in locust control operations) involve the dispersion of a dilute dust (a fine powder formulation generally containing 0.5–2% a.i.) with a vehicle-mounted power sprayer. Application by hand may also be conducted where facilities are limited. Such treatments can involve high levels of application with a clearly visible dust deposit; smaller quantities can drift away from the target site and non-target effects are possible. A range of pesticides can be used, some of which are persistent, e.g. hexachlorocyclohexane (HCH, also known as benzene hexachloride, BHC). Residues of these materials can be detected for extended periods and are most likely to be detected by sampling soil, vegetation and vertebrates/invertebrates coming into contact with treated soil or vegetation.

Dip treatments

Dip treatments are generally used in veterinary medicine or for post-harvest protection of fruits. With dip systems a solution, or suspension, of pesticide is prepared into which the animal or fruit is immersed. The scale of the operation depends upon the quantity and size of the material to be treated. Depending upon where the operation is performed, there can be localized contamination, from splashing or run-off. More significant is localized contamination that can arise at the site of disposal of the pesticide used, particularly where this is effected by tipping on to open ground, draining into a stream or into a soil pit. Subsequent leaching or disturbance of the site can spread the contamination further. Where the site is adjacent to a watercourse, or where treated animals may enter the water, broader, downstream contamination can occur. There is also a slight possibility of contamination of dung from dip use (see pour-ons). Sampling for residues should thus concentrate on water (although contamination is generally transient), sediment, aquatic vegetation, fish and molluscs collected downstream of the contamination site. Sampling of dung fauna may also be informative.

Granule application

Granular pesticide formulations can fulfil two distinct functions. The first is where the active ingredient is particularly toxic and where there is a risk to the operator associated with its use as a dust or as a dilute spray. In such a case, the product is formulated as a heavier granule substantially reducing the risk from movement of the dust and small particles or droplets.

The second covers those active ingredients formulated as a granule with a slow-release mechanism, such that the release of the active ingredient from the granule can be controlled to give an extended active control period.

Granules are generally used as a treatment against soil pests such as nematodes, slugs, cut worms or termites and are distributed around the base of plants or susceptible structures. Sometimes the granule or capsule is deposited on the ground or, more often, it is incorporated into the soil to protect it from disturbance. This also provides protection for non-target species. Environmental contamination is thus localized, but there is a deliberate intention for the soil in the vicinity of the granules/capsules to carry a residue of the active ingredient. The persistence of the residue depends upon the active ingredient and the characteristics of the granule. Some localized contamination of surface water may occur where granules are spread near to irrigation ditches, small streams, ponds etc. This can be through direct broadcast, or from run-off after heavy rain. Underground water will only become contaminated in extreme cases or where the water table is particularly high. Some localized effects on non-target soil-dwelling species and on higher species through food chain effects, may be observed.

Also, birds are particularly susceptible to eating granules and can suffer acute or chronic effects as may occur following improper use or poor incorporation into soil.

Baits

Baits are generally used against infestations of specific pests and are only infrequently used in open areas. The commonest form of bait is either compound blocks or treated cereal feeds used for the control of rats and mice. On rare occasions they may be used in plantations but generally they are used to control infestations in domestic premises, factories or warehouses. As such, their release is controlled and because of the nature of the pesticides used in the baits, there is only limited scope for environmental contamination.

However, insecticidal baits (generally insecticide-treated bran) are sometimes used for the control of locust and grasshopper species and for certain ants and termite pests. These baits have the potential for uptake by non-target species and so are generally used in areas where such risks are minimal (e.g. desert areas). The potential for environmental contamination is limited, although in areas where the bait is laid in strips or broadcast there will be localized contamination. Residues will mainly be detected in soil where the bait has become incorporated with time and in soil-dwelling species; residues in vegetation will be unlikely.

Fogging

The application of pesticides by fogging is now rarely practised in the field and is a technique generally restricted to warehouse use where a very fine mist of pesticide in oil is generated by blowing an oil/pesticide mixture across a hot exhaust nozzle. The technique is more akin to a fumigation and although used occasionally to treat dense forest canopies, plantation crops or orchards, there is significant drift of the fine pesticidal mist and its range of application is limited. If fogging has been used, then significant residues are more likely to be found on vegetation than in the soil. Water contamination will only occur if the fogging has been performed close to an open water body.

Pour-ons

Pour-ons are insecticides (generally synthetic pyrethroid compounds) used for veterinary purposes that are applied along the backs of cattle for the control of biting/sucking flies. They are being increasingly used in areas of Africa where the tsetse fly is prevalent. Contamination of the ground from direct run-off of the insecticide is likely to be minimal, although this could become significant should recently treated cattle be exposed to heavy rain. Similarly, waterways could be contaminated should treated cattle enter rivers or streams to drink. Residues of these materials have been detected in the faeces of cattle at low levels, although these residues may be significant to species such as the dung beetle which feeds on cow dung (Vale and Grant, 2002). The most appropriate samples for analysis will be cow (or other stock animal) dung (both fresh and aged material) and beetles found in and adjacent to the treatment area. The analysis of soil samples is not likely to be productive.

Accidental localized spillage

Spillages generally affect a relatively small area, although the concentration of the spilled product is generally far higher than that of a diluted spray, and thus consequences may be of prime importance to local communities and wildlife.

In selecting appropriate samples for analysis the following factors should be considered.

- Is the contaminated area contained in any way by natural or constructed barriers?
- Is the contaminated area fenced off or can fencing be erected?

- What pesticides were involved in the spillage, what were the quantities involved and how where they formulated?
- If the spillage is on open, natural ground, what is the soil structure like (sandy/clay)?
- How far is the spillage from open watercourses or known underground streams or springs?
- If the area is not fenced, do grazing animals have access to it?
- Which indicator species exist naturally in the area?

The answers to these questions will help to define the types of samples to take.

When examining the focal point of the contamination, protective clothing should be worn (see chapter 3 on safety/precautions). With major incidents, preliminary residue analysis of soil/ground samples should be undertaken as a matter of urgency to define the extent of the main contamination and the nature and concentration of the residues. This will define the safe working areas and help in defining the assessment plan.

Major site contamination

Where the release of pesticide into the environment is major, the chances of broader contamination through soil migration and leaching are significantly greater, particularly where the product is formulated as a water-miscible material and where the formulating agents assist the spread of the material. The implications of a major site contamination (e.g. from an industrial manufacturing or formulating plant or from a large pesticide store that has been destroyed in some way) will generally be long lasting, with a greater reservoir of material available for dispersion. This poses considerable problems for effective site decontamination. The environmental implications can be immense and the scale of any assessment exercise proportionately high.

The considerations outlined above for localized spillages are equally applicable to major incidents. The problem of personal contamination can be significantly greater and the requirement to wear protective clothing, at least until the preliminary analytical findings have been considered, is particularly important.

Unexplained wildlife mortality

The cause of wildlife mortality may be revealed from post-mortem analysis of tissues for residues. Samples should be collected and transferred as quickly as possible to the analytical laboratory. Where delays in transportation are likely, the use of formalin (see below) can be helpful.

Acetylcholinesterase measurements in warm-blooded animals are useful indicators of exposure to organophosphorus and carbamate pesticides and these measurements can be carried out in the field using blood samples taken from the animal. Portable kits are commercially available from veterinary suppliers. Samples of brain tissue require deep-freezing and specialist handling and interpretation.

Samples taken from the habitat of the dead specimens will also be of some use, although the point of ingestion or absorption may have been some time/distance from the point of death, depending on the pesticide and animal in question.

THE PREVENTION OF SAMPLE CONTAMINATION

The physico-chemical properties of individual pesticides affect their behaviour in the environment and their fate. Sampling for residues must take this into account.

Personal protection

There may be some risk of personal contamination when entering a heavily treated or contaminated area. A good precautionary measure is to wear protective clothing and masks if the spraying has been within 24 h of entry. Even after that period, gloves should be worn when collecting samples and bare skin should ideally be covered; do not enter a contaminated area with bare feet. Also remember that gloves and clothing can become contaminated which can then contaminate the samples being collected; wear clean, disposable gloves for each sample. Clothing used during sampling in a contaminated area should be washed as soon as possible using hot water containing detergent (see chapter 3). If going from a pesticide-contaminated to an area contaminated with a different pesticide, an unsprayed or uncontaminated area, protective clothing should be changed.

Sample selection

Each of the component parts of the process of collecting samples for residue analysis, i.e. the nature of the samples and their selection criteria, their location, quantity and preservation, is critical and the analysis will be meaningless if the sample is not representative or if it has been compromised in any way, e.g. if it becomes contaminated during or after sampling or it is allowed to deteriorate through exposure to light, high temperatures, etc.

The nature of the sampling will be directed by the objectives of the exercise. A proper plan for the area and the material to be sampled must be properly established and clearly defined beforehand. Wherever possible, an appropriate, statistically based, sampling scheme should be adopted (see chapter 2). The sampling points **must** be established and marked in such a way that they can be re-visited should further samples be needed to confirm or extend earlier findings.

Sample containers and the prevention of contamination

All sample containers must be clean (internally and externally). New containers are preferred; if containers are to be re-used they should be thoroughly washed with high purity solvent (hexane or acetone) between use. Glass, teflon or aluminium extrusion containers are preferred. Solid samples may be wrapped in aluminium foil and placed in polyethylene or polypropylene bags/containers. **Poly-vinyl chloride (PVC) materials are not to be used as these can be a source of sample contamination.** Filter or blotting paper may be needed to wrap vegetation samples. Sample containers or packing materials used in sample collection or transportation must not come into contact with pesticides of any description and must be stored away from any source of pesticides. Similarly any other materials used during sampling (shovels, trowels, augers, nets, etc.) must be clean and not exposed to any pesticides. Disposable gloves worn during sampling or sub-sampling should be used only once and not re-used.

Tools used during sampling (soil corers, shovels or knives) should be cleaned after use. Washing with water (or water plus detergent) followed by rinsing with acetone is the most effective. Failing this, the tool should be washed with acetone, using a clean acetone-soaked cloth or similar (wear solvent-resistant gloves when handling acetone).

Individuals collecting samples should themselves be clean and have not been involved in spraying operations before sampling unless they have washed and completely changed their clothes. Clothes worn during sampling should not have been worn for any process of pesticide application or previous visits to pesticide-contaminated areas, even if it was some time (e.g. days) before sampling.

All sample containers must be adequately and effectively labelled. Two types of labelling should be used, internally (with a pencil on paper) and externally with all relevant details with permanent marker pen. Samples can be individually and uniquely coded with details of the codes appearing on a separate sheet, a copy of which should accompany the samples at all times.

Sample preservation and pesticide degradation

Pesticide residues in the samples collected can degrade through biological and chemical processes and at a rate dependent on the nature of the pesticides present. Chlorinated pesticides (such as aldrin, lindane or DDT) will deteriorate relatively slowly, but organophosphorus or carbamate materials (such as fenitrothion or carbaryl) degrade at a much faster rate. In hot, damp conditions, degradation will be much faster, even for the chlorinated materials, thus it is important that the samples are transported without delay to the analytical laboratory. Where this is not possible, the samples must be treated in such a way as to minimize the risk (and rate) of deterioration.

Field samples should generally be placed in a cool-box held at 4–8 °C after collection. The rate of pesticide degradation is reduced at lower temperatures. They should be transferred to a refrigerator immediately on return to base. With most samples for residue analysis, it is recommended that the sample should be deep-frozen unless the sample can be analysed (or extracted) within 24–36 h. Tissue samples, or samples of high moisture content (bird or animal tissue, fish, vegetation, etc.) should not be frozen unless:

- storage before shipment to the laboratory is going to be 2–3 days or longer. This period can vary considerably depending upon the nature of the sample and the chemical nature of the residues.
- it can be guaranteed that the sample, once frozen, can be transported to the analytical laboratory without being allowed to thaw.

Where samples are not frozen, alternative arrangements should be made as described later in the individual method sheets. These alternative procedures are not 100% effective in countering degradation processes and some pesticide loss will still occur. The procedures will, however, reduce the rate of deterioration during transfer to the laboratory or to suitable storage facilities.

Where the identity of the pesticide(s) in the analytical sample is known, field recovery ('spiked', see below) samples can be prepared and then subjected to the same delays, conditions of storage and transportation as the actual field samples. Analysis of these 'spiked' samples, in parallel with the samples themselves, will provide an indicator of the rate and extent to which pesticide degradation has occurred in the samples. **Spiked' samples should, therefore, be prepared wherever possible.**

Field recovery samples are prepared by adding known quantities of the pesticide to untreated material of a similar nature (from another source if all the local material is thought to be contaminated) or to further samples of the contaminated field material (i.e. increasing the residue burden). Pesticide(s), generally in organic solvent or as the formulated material, can be prepared by the collaborating analytical laboratory for field application using a simple pipette or hypodermic syringe. Detailed advice/instructions should be obtained from the laboratory together with storage and safety instructions for the pesticide(s) in question.

Transportation to the analytical laboratory

It is important that the samples be delivered to the laboratory conducting the analysis at the earliest opportunity. It is equally important that the laboratory should be advised exactly when to expect the samples so that they are adequately prepared. Such notification (particularly where the laboratory is remote and the samples are being transported by air and/or a courier rather than directly by hand), can prevent unnecessary delay and potentially a further loss in the sample residues. This is particularly important where international travel is involved. Adequate information provided to the recipient laboratory can often expedite customs clearance and sample delivery.

SAMPLING TECHNIQUES

Soil sampling

This will generally begin with the examination of a soil profile. Residues in initial samples are normally confined to the top 5 cm (mainly in the top 1 cm, but this can vary with soil structure). With time after application, downward movement of the pesticide may be observed, particularly where there has been heavy rainfall and the soil is of a fine, sandy texture (see chapter 5). With organophosphorus pesticides, it is likely that the relatively short persistence will not allow time for any significant soil dispersion of residues.

The nature of the soil sample will reflect either the need to monitor for vertical pesticide movement or to determine what pesticides are present and at what concentration. In the former case a depth profile will have to be collected. In the latter case, a large grab sample (to perhaps one spade depth) and appropriately mixed, is generally adequate. To take a depth profile sample, a soil auger or other tool capable of taking a soil core is normally required. The core is cut at selected depths and these sub-samples separately packaged for analysis. In the absence of a suitable tool, and as long as the soil is reasonably firm and does not crumble, a depth profile can be obtained using a clean spade. To do this dig a hole in the soil to the depth of the spade, with one vertical edge and with a clear area in front of the spade to facilitate its removal with soil on the blade. Once this hole has been prepared, the spade is inserted vertically into the soil at a distance of 5–7 cm behind the vertical edge of the hole and a slice of soil removed. This soil slice can then be cut to give the desired soil profile.

Soil samples should always be carefully screened to remove stones, leaves and other vegetable material.

The importance of cleaning any tools used in sampling was stressed earlier in the text; use water containing detergent as the primary wash and follow this with an acetone rinse. Allow the tool to dry before re-use. Where detergent/water is not readily available, wipe or brush the tool and then thoroughly clean using acetone. **Limitations** There can be rapid loss of pesticide from the top few centimetres of soil under extremely hot conditions and shallow sampling can miss significant residues.

Processing Samples require an analytical laboratory with significant resources to allow the extraction of residues and then processing to remove interfering co-extractives prior to analysis by gas liquid chromatography (GLC) and high performance liquid chromatography (HPLC).

Resulting data Identity of the pesticide(s) present and concentration. Soil profile data can help to determine the persistence of the pesticide(s) present and the rate of leaching (which is dependent on soil type and organic matter content).

Number of samples Depends upon size of the sampling area and the statistical sampling pattern. A minimum of five cores should be bulked for a composite sample and a minimum of two replicate samples taken from the bulk for analysis.

Sampling period Immediately after treatment or after contamination has been detected and then at intervals of 2–3 months (chlorinated pesticides) or 5–14 days (other classes of pesticide). Rainy and dry season samples should be collected wherever possible for comparison.

Equipment Sampling scoop (trowel or spade) or soil auger (corer), glass or aluminium sample containers, cleaning materials (for sampling tools), labels and cold-box. A soil cover can be made from metal (steel) tubing.

Staff required 1 or 2 depending upon sample numbers.

Water sampling

Water, particularly from any over-sprayed watercourses, will only tend to show pesticide residues for a short time after application. There are some exceptions, but generally even where the solubility is reasonably high or where rates of degradation are low, the pesticide often absorbs on to sediment or other organic matter and is removed from aqueous solution. With some pesticide formulations the residue may form a surface film rather than being dispersed.

Water samples often contain suspended matter. In most cases, the suspended matter can contain a significantly higher pesticide residue than the water itself and its inclusion should be carefully considered. For many purposes, the water and suspended matter are often considered together although for others, there will be merit in separating them, by filtration, for separate analysis. Suspended matter can also pose difficulties for the analyst and separation may be a practical necessity. Where the components are analysed separately, the values can then either be considered in isolation or together.

The process of water collection requires thorough consideration, and the starting point is the question “why is the sample being taken?”. The answer to this question will help to identify the correct sampling point. Additional considerations are:

- Is the sample to be taken from close to the shore or further out in the river/lake? If the latter, a boat may be needed.
- At what depth is the sample to be taken – at surface level, sub-surface or mid-water? (There can be differences depending on temperature, whether there are surface water films from pollutants or decaying vegetation or whether sediment is present at particular depths.) This will affect the sampling apparatus used and details of the methodology.
- Are streams entering a river or lake to be sampled and the results compared with samples from elsewhere in the river or lake, e.g. above the point of entrance of the stream?

Water analysis (Barcelo, 1991) is difficult in that if the laboratory extraction of the sample is significantly delayed, any residues present can degrade or be absorbed on to the walls of the sample container. There is thus a need to keep the sample chilled and to transport it to the laboratory as soon as possible. Alternative methods exist whereby the sample can be extracted in the field, using solid phase extraction (SPE) technology (International Sorbent Technology, 1995; Font *et al.*, 1993; Albanbis and Hela, 1993; Hendriks, 1993; Land, 1994) assuming there is access to some basic equipment (for further information refer to manufacturers’ specifications or specific procedures published in the scientific press). Samples extracted in this way are more stable than in solution, although to ensure reliable analysis, they should again be transported to the laboratory as soon as possible after extraction. The volume of water required for analysis varies with the analytical sensitivity of individual pesticides and the method of extraction. Volumes used are commonly in the range of 0.5–2 litres.

The data may indicate a contamination. Significance can only be determined by follow-up monitoring to see whether residues remain or have spread, e.g. further down a river or across more wells/boreholes accessing the same aquifer at the same depth.

Containers used to carry/store water samples for residue analysis should be washed with clean water, followed by an acetone rinse and then allowed to dry before re-use.

Limitations Water residues can be transient in nature depending upon water flow, rains, etc. Different analytical techniques are required if the samples are to be analysed for a range of pesticides representing different chemical groups/characteristics. The levels of analytical sensitivity are also significantly different.

Processing Analysis by GLC and HPLC after extraction of the residues from the water into an appropriate organic solvent and concentration of the resultant extract.

Resulting data The identity of the pesticide and or metabolites and approximate concentration.

Number of samples Each identified collection point should be sampled in duplicate (minimum).

Sampling period Immediately after treatment or when contamination is suspected. Routinely during the wet and dry seasons (twice for each season).

Equipment A sampling device for surface water sampling can be made from a locally available 0.75–1 litre with a screw-top glass bottle (thoroughly cleaned with soap and water and rinsed with acetone). The metal cage to contain it should also be possible to construct (or adapt) from locally available materials (see illustration on the

method sheet). A device for sampling from a defined depth can also be made from a similar glass bottle, a rubber or cork bung to fit the bottle neck, a wooden, bamboo or metal pole and thick wire or thin metal (see method sheet for design). Clean glass containers with teflon caps for the water samples, labels, cold-box, map and/or global positioning system (GPS).

Staff required 1 (2 preferable).

Sediment sampling

Sediment samples can be difficult to collect but are important in a residue sampling exercise. *Bottom sediment* is generally found in stagnant water or, in rivers and streams, away from fast-flowing currents and must be sampled using an appropriate device dependent upon the depth of the water. Commercial 'grab-sampling' devices are available but can be relatively expensive and are not always convenient for transportation; a scoop or other container tied to a pole, or similar, can be effective in relatively shallow water (see method sheet).

Suspended solids in flowing water can be collected by filtering the water. Relatively large volumes of water need to be filtered to get a meaningful sediment sample and this can be both tedious and time consuming. The use of portable vacuum pumps where available, and Buchner flask filtration systems, can considerably speed up this process.

Limitations It is difficult to access samples away from the river/lake bank without a boat; restrictions on sampling in deep water.

Processing Samples require an analytical laboratory with significant resources to allow the extraction of residues from tissue and then processing to remove interfering tissue co-extractives before analysis by GLC and HPLC.

Resulting data The identity of the pesticides or metabolites and an indication of the approximate concentration for such residues.

Number of samples At least two replicate samples from each identified collection point.

Sampling period Immediately after pesticide use or when contamination is first detected. Frequency of sampling is dependent upon pesticide type; 2–3 months for chlorinated materials, 1–2 weeks for other pesticide classes.

Equipment A suitable sampling device can be made from a small locally available metal pot or dish (e.g. empty baked bean can cut to a height of 6 cm) attached to pole 2–3 m long. Glass or aluminium sample containers, waterproof boots or waders, labels and cleaning materials (for sampling device).

Staff required 1 or 2 depending upon sample numbers.

Vegetation sampling

Vegetation will often show the highest deposits from spraying, dusting or other operations (apart from in desert situations) and is a good indicator of likely rates of ingestion of residues by grazing animals or vertebrates/invertebrates that live in/on such vegetation. Care should be taken in handling sprayed vegetation samples as initial residues will be surface deposits and easily dislodged by hand. Depending on the nature of the pesticide, there may be little long-term adsorption of residues by the leaf and residues may stay as surface residues (but not so easily dislodged) until they degrade by exposure to sunlight/rainfall; some residues will be washed off and fall to the soil immediately below.

Vegetative samples can pose particular problems. If they are kept in sealed polyethylene bags or glass jars, they quickly lose moisture which condenses as free water, altering the nature of the sample and, where the sample cannot be refrigerated, lead to the rapid development of moulds which can promote microbial degradation of pesticides and, in extreme cases, pose a health hazard to the handler. These conditions should be avoided wherever possible.

Depending upon the nature of the vegetation (size, shape, etc.), one useful method is to enclose the sample in clean filter paper or blotting paper and to put the wrapped sample inside a clean, paper envelope. The addition of a small sachet of silica gel to the envelope, which is then sealed, helps to reduce the moisture content of the

system. Where filter or blotting paper is not available, paper towels or tissue can be used. However, samples of these should be provided to the analytical laboratory to check for possible co-extractives which could interfere with the analysis. Wherever possible, analytical checks on the suitability of the material should be completed before sampling commences. Again, rapid transportation to the analytical laboratory is recommended.

Limitations Substantial variation may be detected in surface residues on vegetation depending on the nature of the application method.

Processing Samples require an analytical laboratory with significant resources to allow the extraction of residues from tissue and then processing to remove interfering tissue co-extractives before analysis by GLC and HPLC.

Resulting data The data will identify the pesticide involved and its concentration at the time of collection.

Number of samples Dependent upon the size of the treated area, nature of the treatment and nature and density of the vegetative cover. As a rule, it is better to collect too many samples than too few; it is easier to throw some samples away once preliminary results have been obtained than to regret that vital additional information is missing. Fewer than 25 samples will rarely be adequate.

Sampling period Samples should be collected before, immediately after treatment and then again at 7-day intervals.

Equipment Scissors, blotting paper or filter paper, paper envelopes, labels, silica gel, disposable gloves and a cold-box.

Staff required 1 or 2 depending upon sample numbers.

Tissue sampling

In the field and where immediate access to chilled storage (3–5 °C) is not practicable, whole body or muscle tissue, organs and viscera from fish, birds, amphibians, reptiles or small mammals (see individual chapters for capture methods) can best be preserved in dilute (8–9%) formalin solution. Deep-freezing should be avoided unless the sample is guaranteed to stay frozen until it reaches the analytical laboratory; freezing/thawing/re-freezing can promote enzymatic and bacteriological breakdown of residues and invalidate the analytical results. The use of formalin may affect some organophosphorus pesticides and, where possible, this should be clarified in advance of sampling. Body lipids are not, generally, soluble in formalin; where this appears to be a problem, separate analysis of the specimen and of the formalin (residue and lipid content) can be undertaken, although this is rare.

The formalin solution should be prepared by diluting a commercial solution (generally at a concentration of 40–45%) in a ratio of *one part formalin solution to four parts distilled (or de-ionized) water*. Wherever possible, this should be carried out in a fume cupboard (see chapter 3 on safety). Where this is impossible, it should be done outside or in a well-ventilated area. Plastic or rubber gloves and safety glasses or goggles should be worn during this process. A face mask would also be helpful and although conventional masks give little protection against solvent vapours, there is some temporary relief.

The diluted solution should preferably be stored in a *clean* glass container (although aluminium or other metal containers can also be used). Ensure that the container screw-cap is lined with teflon or aluminium foil. Where possible, a sample of the formalin solution should be analysed by an analytical laboratory before it is used in the field or, if necessary, after, to ensure that there are no interfering contaminants which could affect the analysis. Also, in cases where the identity of the pesticide(s) in the field samples is known, or the analysis is targeted against specific pesticides, then an experienced pesticide chemist should be consulted to check whether formalin is known to affect those compounds.

Limitations With chlorinated residues it cannot always be determined whether the residues detected are from recent or past exposure.

Processing Samples require an analytical laboratory with significant resources to allow the extraction of residues from the tissue and then processing to remove interfering tissue co-extractives before analysis by GLC and HPLC.

Resulting data The identification and quantification of pesticide detected in live or dead collected samples. Data

provides information on the rate of uptake by different species, tolerance to pesticide, and comparison with published environmental/toxicological data.

Number of samples Depends upon the extent of the sampling area, number of relevant species to be sampled and whether whole samples or dissected samples are to be considered (e.g. analysis of specific body organs). Generally, fewer than five samples of a given species will not give representative results.

Sampling period Sampling should commence immediately after pesticide application or when contamination is first detected and then at intervals to be decided once the identity of the contaminant is known. For example, if the contaminant is a chlorinated pesticide then sampling at intervals of 2–3 months may be appropriate. For other classes of pesticide, the sampling interval will be significantly reduced (days or weeks).

Equipment Sample containers (glass), formalin solution, disposable gloves, forceps, cold-box and labels.

Staff required 1 or 2 depending upon sample numbers.

Vertebrate/invertebrate sampling

Fauna from treated areas should be collected as described in chapters 8–13. Although the collection of samples at periodic intervals after application will give some indication of residue accumulation or the rate of loss/metabolism of any ingested pesticide, this is not always the case with field samples and the data will need careful assessment. Specimens collected from recently sprayed areas may be contaminated on their outer surfaces through contact with treated surroundings and they should be washed/brushed (to remove adhering soil or other material).

Invertebrates such as worms can deteriorate rapidly if not kept in a suitable medium. Unless metabolism of possible ingested pesticides is a problem (see guide to pesticides earlier in this chapter), the specimens may best be preserved alive until immediately before transfer to the analytical laboratory. Storage at reduced temperature (refrigerator, 5 °C) is also required. The specimens can, alternatively, be kept in formalin as described above for tissue samples.

Insects are best preserved dry and intact in ventilated jars or bottles. In cases where extreme delay before analysis is likely, or where the samples are considered likely to deteriorate, formalin preservation can be used.

Limitations With chlorinated residues it cannot always be determined whether the residues detected are from recent or past exposure.

Processing Samples require an analytical laboratory with significant resources to allow the extraction of residues from the tissue and then processing to remove interfering tissue co-extractives before analysis by GLC and HPLC.

Resulting data The identification and quantification of pesticide detected in live or dead collected samples. Provides information on the rate of uptake by different species, tolerance to pesticide, and comparison with published environmental/toxicological data.

Number of samples Depends upon the extent of the sampling area, number of relevant species to be sampled and whether whole samples or dissected samples are to be considered (e.g. analysis of specific body organs). Fewer than five samples per species will rarely be adequate.

Sampling period Sampling should commence immediately after pesticide application or when contamination is first detected and then at intervals to be decided once the identity of the contaminant is known. For example, if the contaminant is a chlorinated pesticide then sampling at intervals of 2–3 months may be appropriate. For other classes of pesticide, the sampling interval will be significantly reduced (days or weeks).

Equipment Sample containers (glass with aluminium-lined lids), formalin solution, disposable gloves, forceps, cold-box and labels.

Staff required 1 or 2 depending upon sample numbers.

DATA COLLECTION AND RECORDING

Whenever carrying out field sampling, it is extremely important to record carefully all the information about sample sites and number at the time of sampling. It is essential to take an additional data sheet along to the field with you.

The format for the data sheet depends on what type of sampling is being conducted. The minimum requirement is for all necessary data to define the sample, and where and when it was collected. However, the researcher may wish to collect supplementary information on, for example, weather conditions at the time or any unusual observations in the sampling area (Figure 6.1). This type of information could be useful in interpreting the results obtained from the sampling/analysis and can only be properly defined at the time of sampling. Never try to remember what the conditions were or any other factors after several weeks or months, as this can be misleading.

Thus there is no perfect model for the data sheet; you develop it for your specific purpose. You do not want an over complicated sheet but you do want to collect all of the necessary information. An example of a completed basic data sheet with a few initial entries is given below. Think what extra information you may need.

A blank data sheet is provided along with the method sheets, to be photocopied and taken out to the field.

Date	Sample type	Site reference	GPS coordinates	Sample code	Weather conditions	Other comments or observations
20.6.02	Soil	1. Farm of Mr Ngata; Area 1	N12.23.41 W87.01.91	SS1	Dry, 28 °C	Fallow field, no recent disturbance
20.6.02	Soil	Farm of Mr Ngata; Area 2	N12.23.42 W87.01.88	SS2	Dry, 28 °C	As above
20.6.02	Soil	Farm of Mr Ngata; Area 3	N12.23.42 W87.01.84	SS3	Dry, 28 °C	As above
20.6.02	Soil	2. Farm of Mr Mwangi; Area 1	N12.22.81 W87.00.34	SS4	Rain, 26 °C	Cultivated soil
20.6.02	Soil	Farm of Mr Mwangi; Area 2	N12.22.81 W87.00.88	SS5	Rain, 26 °C	Cultivated soil
20.6.02	Fish	3. Stream adjacent to site 2	N12.22.81 W87.00.22	F1	Rain, 26 °C	Shallow (30–100 cm), slow moving, little vegetation
20.6.02	Fish	As above	N12.22.81 W87.00.22	F2	Rain, 26 °C	As above
20.6.02	Fish	As above	N12.22.81 W87.00.22	F3	Rain, 26 °C	As above

Figure 6.1: Example sample data sheet: pesticide residue sampling

DATA PRESENTATION AND INTERPRETATION

Data interpretation is critically important. The care taken in sample selection, preservation, transportation and analysis can be wasted if the analytical findings are not properly understood or if they are misinterpreted. It is essential that the worker fully understands both the way in which the results are expressed and their significance. In cases of doubt, the analytical laboratory should be asked to explain the findings; most laboratories will be pleased to help.

It is worthwhile, however, to consider briefly the basic ways in which the analytical data may be presented; this will, in part, depend on what has been requested from the laboratory.

Data are generally expressed as milligrams of pesticide per kilogram of analytical substrate (mg kg^{-1}) for solid materials or milligrams of pesticide per litre (mg l^{-1}) for residues in a liquid medium. These terms both equate to one part per million (ppm), an expression commonly used in the past but now less so, in favour of the above 'comparative units' which give a real, quantitative value. Residues can be expressed as decimal fractions of these comparative units, e.g. a residue of 0.001 mg kg^{-1} , or it could also, correctly, be expressed as $1 \mu\text{g kg}^{-1}$. With increasingly sensitive analytical methods being used, residues at this level (and below in some cases) are increasingly being quoted. For reference, commonly used units include:

- one part per million in a solid material can be written as either 1 mg kg^{-1} , $1 \mu\text{g g}^{-1}$ or 1 ng mg^{-1} ; all these terms are equivalent to each other
- one part per million in an aqueous medium can be written as either 1 mg l^{-1} , $1 \mu\text{g ml}^{-1}$ or $1 \text{ ng } \mu\text{l}^{-1}$
- aqueous pesticide residues, however, are generally low and are often expressed in terms of $\mu\text{g l}^{-1}$ which is one part in one thousand million or ng l^{-1} or part per billion.

These expressions can be confusing and it is essential that the recipient of the data is comfortable with the terms and the units and can manipulate the data without error.

The relationship within the terms is one of quantity (mass) of pesticide compared to the quantity (mass) or volume of sample from which it derived. With aqueous samples, extraction volumes can actually be 1 litre (or sometimes less and in the range of 200–500 ml water), but with solid materials, the quantity analysed (and particularly with small specimens) can range between 5 g (sometimes less) and 50 g. The calculation, however, converts the data to represent (x) mg kg^{-1} .

Residue data can also be expressed as the total weight of pesticide detected in the sample and in some situations this may be a more useful form of data expression. For example, following a spray operation in a given area, it may be useful to look at pesticide deposition on the surface of leaves thought to be contaminated by spray or spray drift. In such a case, the total burden of pesticide (normally in μg) may be more important than the concentration of the contaminant expressed in mg kg^{-1} . Similarly the burden of pesticide in a recently contaminated vertebrate or invertebrate sample (where metabolism and distribution of residues in the body may not have occurred) may be more usefully expressed as the total weight of pesticide rather than in mg kg^{-1} .

SAMPLE SIZE AND THE LOWER LIMIT OF DETERMINATION

Despite the sensitivity of modern analytical equipment and whatever the expertise of the analyst, the sample size must be of a minimum quantity to allow effective analysis whilst retaining a portion of the sample for subsequent analysis (in cases of doubt over the original analysis or should an accident happen to the original analytical portion). This, however, is not always possible. A minimum sample size is also necessary to allow a reasonable *lower limit of determination* (LoD) to be achieved. This term is important and its derivation must be clear. The

LoD is the lowest residue that can be determined by the analytical procedure in use, as determined during laboratory validation of the analytical procedure. The magnitude of the LoD varies with a number of factors, including the nature of the pesticide but the most critical factor is the sample size. If the sample size is, e.g. 10 g, then the LoD for pesticide x in this sample will be 10 times lower than the LoD that could be achieved with a 1 g sample analysed for the same pesticide. This becomes important in environmental analysis where often, and because of the small sample sizes encountered, the whole sample is analysed. Because the samples vary in weight, in practice, a whole range of LoDs may be expected. This is often a source of confusion and the reasons for a range of LoDs is one of the commonest questions received by laboratories conducting environmental analysis.

Consideration of the implications of the LoD raises two key issues which should be considered in advance of the analysis and reviewed with the analytical laboratory.

- What is the appropriate LoD for the samples in question?
- Can the appropriate LoD actually be achieved knowing the likely sample sizes?

It is also worth bearing in mind that every analysis undertaken contains an element of error, despite all the precautions that are taken. This level of error is minimized by the laboratory and is generally kept within defined limits. With small samples, there is an increased risk of error, particularly where the final sample extract for analysis needs to be concentrated to very small volumes before analysis; errors can become magnified and data from small samples need to be considered with this in mind. The data are still meaningful but statistically, the confidence level is less.

RESIDUE CALCULATION

With 'solid' samples, the analytical laboratory will need to know how the residues are to be expressed, e.g. an assessment of the total pesticide deposit as a weight, in mg kg^{-1} and based on whole body weight, or based on the material dry weight or calculated as a residue in the lipid portion of the sample. If necessary the laboratory can provide all of this data but the analytical charge may be higher as this entails additional laboratory work and not just a recalculation of the basic data:

- for data expressed on a *wet weight* (fresh) basis, the weight of the sample on receipt is used
- for data expressed on sample *dry weight* (e.g. soil residue data are often expressed on a dry weight basis for ease of data comparison), the moisture content of the sample must be determined
- for data expressed as a residue *in the lipid*, the fat content of the material must be determined.

To determine moisture and fat content, portions of the sample or of the sample extract must be made available. In cases where the sample is particularly small, it may not always be possible to sacrifice material for these other tests, or material can be sacrificed at the risk of increased analytical error and a higher LoD (see above). This needs to be considered and, again, discussed with the analytical laboratory.

Where residues are originally calculated on a fresh weight basis and then re-calculated, using a factor, to allow for moisture or fat content, the figures can change quite dramatically, particularly where the factor is large. Although this procedure is common, an element of distortion of the values can sometimes occur, particularly where the residue is low and where the results have been rounded-up to one or two decimal places. This can inflate the residue to a level higher than that actually present and could create difficulties in data interpretation for the unwary. However, if the user of the data is aware of the potential problems that can arise, any misinterpretation of the data can be avoided.

A further problem to be aware of is that of summing residues where a pesticide may exist in isomeric form and where metabolites may also be present, or should be analysed for, with the residue being expressed as a total; the analysis of DDT is a good example.

DDT formulations contain the p'p and o'p isomers and both need to be determined. In addition, the two main metabolites – DDE and DDD (sometimes called TDE) – are also commonly determined. Both of these compounds can be produced from the p'p and o'p isomers of DDT and the analysis can include, therefore, six components (although some analysts tend to ignore o'p DDE and o'p DDD because their levels are usually insignificant).

The following example (Table 6.1) illustrates the problem when considering residues below the LoD. In this example, a moisture content of 35% and a lipid level of 9% is assumed.

A key question is should the total DDT value be the sum of the LoDs, the highest recorded LoD or a compromise value? A case could be made for each of these approaches and different authors' treatments of the subject vary. In most cases, it is probably best to present the individual data for each component.

Also, remember that the LoD value may already reflect a rounding-up (say the reported value of 0.02 reflected a calculated lower level or trace residue of 0.015 mg kg⁻¹, rounded-up to an agreed LoD, or lower reporting level of 0.02).

Table 6.1 Residues below the LoD

	Residue (mg kg ⁻¹) expressed on		
	wet weight	dry weight	in lipid
p'p DDT	<0.02	<0.03	0.22
o'p DDT	<0.02	<0.03	<0.22
p'p DDE	<0.01	<0.02	<0.11
o'p DDE	<0.01	<0.02	<0.11
p'p DDD	<0.02	<0.03	<0.22
o'p DDD	<0.02	<0.03	<0.22
Total DDT	<0.10	<0.16	<1.10

OTHER CONSIDERATIONS

The analyst needs to be sure that the moisture or lipid content of the sample has not changed unduly since collection in the field. If the moisture content has fallen, the determined residue value will be higher than its original level.

This is a real problem with certain samples, particularly when as explained earlier, moisture is a factor in the degradation of pesticides and the procedure recommended, e.g. leaf/vegetation samples, is to allow them to partially dry in the presence of adsorbent paper and silica gel. Calculation of the total pesticide deposit is not affected, but the expression of residues in mg kg⁻¹ will certainly be made more difficult. To get around this problem, the initial sample weight needs to be determined, either in the field using a pocket, portable balance or on return to base, and the observed weights provided to the analytical laboratory.

Tissue samples stored in formalin can also be affected. Although lipid content will not be affected by the formalin, the moisture content may be. Wherever possible, the fresh tissue weights should be recorded after sampling and provided to the laboratory. Sample dry weight can then be determined, allowing for calculation of the moisture content.

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FURTHER READING

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(This book is essential for the determination of chemical and physical properties of pesticides.)

EPA *Individual Methods for the Sampling and Analysis of Pesticides.* (Available from the Environmental Protection Agency of the United States of America.)

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