ELSEVIER

Journal of Chromatography A, 754 (1996) 463–478

JOURNAL OF CHROMATOGRAPHY A

Review

Identification of pesticide poisoning in wildlife

Peter Brown*, Andrew Charlton, Mary Cuthbert, Libby Barnett, Leigh Ross, Margaret Green, Liz Gillies, Kathryn Shaw, Mark Fletcher

CSL Wildlife Incident Unit, Central Science Laboratory, Sand Hutton, York YO4 1LZ, UK

Abstract

The Wildlife Incident Investigation Scheme investigates incidents of suspected poisoning of wildlife (also honey bees and companion animals) by pesticides in the United Kingdom. The approach to these investigations has evolved over the past 30 years. Field investigations, post-mortem examinations, toxicological data and experience of previous poisoning incidents assist in the selection and interpretation of appropriate chemical analyses. Several 'multi-residue' and several 'individual compound' analytical methods for pesticides in wildlife are currently in use; these are described.

Keywords: Reviews; Wildlife poisoning; Environmental analysis; Pesticides; Strychnine; Chloralose; Metaldehyde; Paraquat

Contents

*Corresponding author.

0021-9673/96/$15.00
PII S0021-9673(96)00311-1

1. Introduction

Pesticides are designed to have biological activity and may poison non-target species either directly or through accumulation in the environment. Before pesticides are brought into commercial use, most nations require that they are officially approved under a Registration Scheme that includes an assessment of the potential risk to wildlife and the environment. Even with the best of intentions, it is not possible to test every combination of pesticide, mode of use, habitat type and species under all weather conditions before a pesticide comes into commercial use. A scheme was set up to identify any unforeseen environmental problems from pesticide use in the UK; this post-registration surveillance is now known as the Wildlife Incident Investigation Scheme (WIIS) and forms part of the UK pesticides registration system [1]. The results of the WIIS assist in the validation and improvement of risk assessments [2]. During the early years of the scheme, it became apparent that much of the poisoning was deliberate [3,4]. Companion animals are frequently the victims of deliberate poisoning and negligent pesticide application; although not 'wildlife', they are included in the scheme. A recent publicity campaign has been designed to increase public awareness of the problem of deliberate poisoning of animals [5]. Where sufficient evidence can be obtained, the results of WIIS investigations are used in the enforcement of legislation covering pesticide use and the protection of wildlife and other animals. Honey bees are at particular risk from the use of pesticides and the WIIS includes them in its investigations [6]. The organisation and results of each year's investigations are published [7]. We now describe the methods currently used by the Central Science Laboratory (CSL) Wildlife Incident Unit to identify cases of pesticide poisoning in England and Wales.

2. General strategy

2.1. Non-chemical evidence

Not all birds, mammals and beneficial insects found dead are victims of pesticide poisoning. There are sometimes obvious clues; birds dead in a field immediately following pesticide application suggest accidental poisoning; birds and mammals dead in the vicinity of a pegged-out animal carcass suggest deliberate poisoning. In the second case, the poison could have been an agricultural chemical or a substance with no agricultural use. Sometimes it is clear that animals have been shot or hit by vehicles, but there remains a slight possibility that they became victims as they succumbed to a poison. Part-eaten bodies may have been killed by a predator, but may have been scavenged after death. The circumstances in which suspected poisoning victims are found may suggest possible events leading to the deaths, but it is easy to jump to incorrect conclusions [8]. The picture may be clarified by interviews with reliable witnesses. There may be records of pesticide applications, sightings of a bait being laid or descriptions of the type and duration of symptoms exhibited by the dying animals. There is usually more detailed information if the victim is a companion animal than if it is wild. Some reports, however, are based more on prejudice than on factual observations.

Symptoms of poisoning are not very specific and can vary between species, but may suggest the more likely groups of compounds. Time from suspected ingestion of the poison to onset of unusual behaviour and times to unconsciousness and death are particularly important. For example, some organophosphates can induce violent convulsions and kill within a few minutes, but with fluoroacetamide the onset of violent convulsions in mammals is delayed for perhaps 2 h. Chloralose is a narcotic that sends the

victim to sleep within a short time, but death occurs in small animals several hours later as body temperature cannot be maintained. Anti-coagulant rodenticides and paraquat act over a much longer time scale and death may occur several days after ingestion of the poison.

A post-mortem examination is essential as most animal deaths are not the result of pesticide poisoning. Traumatic death from road traffic accidents or from shotgun pellet injury may not be apparent from the outside of an animal. Many diseases give rise to changes to internal organs, which are obvious to a veterinary surgeon during post-mortem examination. Occasionally, effects such as internal bleeding resulting from anti-coagulant rodenticide poisoning may give a strong clue to the class of compound involved. Where no cause of death is obvious from post-mortem examination, poisoning must be suspected. In such cases, the identification of the contents of the stomach (or gizzard in birds) may provide a valuable clue to the nature of the poison. Although a post-mortem examination on beneficial insects, such as honey bees, may be of less value than in the case of vertebrates, it is possible to assess the extent of disease and parasite infestation in a colony and to identify pollen types carried by suspected poisoned bees.

2.2. Tissue and analytical method selection

Ingestion is the most common way that pesticides get into non-target organisms, but inhalation, absorption through the skin and injection are also possible. After ingestion, the compound (and/or breakdown products) must be absorbed into the bloodstream and distributed around the body. The compound may be metabolised to other products on the way to the site of action and it may be the original pesticide or a compound derived from it that does the damage. The effect may not be direct in that damage at one point in a chain of biochemical reactions may ultimately lead to a lethal effect at another point in the chain. All the while there are defence mechanisms trying to get rid of poisonous chemicals, either as water-soluble derivatives or by binding to large molecules or by storage in fat. The role of the analytical chemist is to identify the original poison and to try to demonstrate that this was responsible for the death of the animal.

To identify the pesticide responsible for a poisoning incident, the tissue of choice is the one with the largest residue of the original unchanged pesticide ('parent compound'). If this was ingested and death occurred within a few hours, then the stomach of mammals (or gizzard in birds) is the most likely place to find it. If the time to death is not known, but a pesticide is suspected that comes from a class of rapidly-acting compounds, the stomach is again selected. If the poison acts more slowly and the pesticide survives its journey through the gut, intestine contents or faeces may be appropriate. Alternatively, liver is a natural choice as this is where most of the detoxification occurs in vertebrates. From here, the more polar compounds move to the kidney before being expelled in the urine and less polar compounds are transported to be stored in fat. However, because the liver is so active, it may continue to break down pesticide residues after death and contains a wide variety of compounds that may interfere with analysis; small residues may be found for a longer time after death and more easily in the rather 'cleaner' muscle tissue. In honey bees, where exposure to pesticides may be by ingestion or by absorption through the body wall, it is convenient to analyse pooled whole bodies of bees that are found dead together, presumably from the same cause.

Suspected bait materials usually have the poison on their outer surface, but it may have been injected (e.g. into eggs or meat). In these samples, the amount present is usually very large (by residue analysis standards) and contamination of other samples must be avoided. The location of the poison on baits such as an animal carcass is not usually obvious, so it is important to find out which compounds might be involved before committing the sample to an extraction method which may jeopardise the possibility of further extractions by other methods.

If the pesticide itself cannot be identified, it may be identified from its breakdown products. Even if the pesticide is identified, we do not know if it was the cause of death. We can relate residues found to residues occurring in animals known to have died from poisoning by the same pesticide, or calculate that the total amount taken in is greater than a known lethal dose. If the mechanism of action is known, we

can look at the damage caused to vital processes (for example, enzyme-catalysed reactions) [9]. In some cases, microscopic examination of tissues will reveal extensive damage caused by poisoning [10].

2.3. Approach to chemical analysis

2.3.1. Multi-residue versus single compound analysis

In many cases of poisoning of wildlife and companion animals, there is no clear indication of the chemical compound involved. For each tissue to be analysed, a single analysis covering all toxic agricultural chemicals would seem the ideal approach. High resolution separation by GC or HPLC coupled to compound-specific mass spectrometric detection comes close to this ideal, but only the crudest of pre-instrument clean-up is possible. The instrument must be pre-programmed to recognise spectra from all candidate compounds in order to distinguish these from the overwhelming majority of co-extracted interferents. If only one compound is suspected, a clean-up specific to that compound may be used. Our approach is to analyse individual classes of pesticides (organophosphates, pyrethroids, etc.) by subjecting a tissue extract to a clean-up separation tailored to one class, then using a separation technique (GC or HPLC) and an element-selective detector (e.g. a flame photometric detector for organophosphates). Once a pesticide has been identified in this way, the identity may be confirmed, usually by GC-MS. Some of the toxic agricultural chemicals used for poisoning wildlife are not members of larger groups and single compound analyses are used for these.

2.3.2. Extraction strategies

To analyse pesticide residues in tissue samples, the pesticide normally must be isolated from the tissue. Vaporization methods such as sweep co-distillation [11] are useful in some circumstances, but solvent extraction is usually the most convenient method. Most tissue samples consist of a solid matrix of cells whose main constituent is water. Most pesticides are to some extent fat- or (organic solvent-) soluble and the problem is to extract them from their mainly aqueous environment. First, the tissue must be broken into small pieces to expose the maximum surface area to the extractant. Next, either use a water-miscible organic extraction solvent such as acetone, or remove the water with a drying agent and extract with a water-immiscible solvent (hexane, dichloromethane, diethyl ether, ethyl acetate). The actual extraction process may be assisted by maceration or agitation using a flask shaker or an ultrasonic bath or probe, followed by filtration. Alternatively, the finely divided sample may be extracted with an organic solvent in a Soxhlet (or similar) apparatus [12] or with supercritical fluid (usually carbon dioxide) in a supercritical fluid extraction ('SFE') apparatus [13]. The method and solvent of choice depends on the type of tissue and the range of pesticides to be extracted. Water-miscible solvents extract most pesticides but also many naturally occurring unwanted co-extractives. Hexane gives a much cleaner extract but may not fully extract even relatively low polarity compounds if they are strongly adsorbed on the surface of the dried solid. Diethyl ether is of convenient polarity to extract a wide range of pesticides without too many co-extractives and its volatility enables extracts to be concentrated with little pesticide loss, but it is highly flammable and forms explosive mixtures with air. For liquid samples (biological fluids, etc.), there are various designs of liquid–liquid extractors [14]. Alternatives include concentration by partition (or adsorption) on a chromatographic cartridge (trace enrichment by solid-phase extraction), partition using a water-absorbing solid matrix, or simply converting to a solid sample by addition of sufficient drying agent.

For samples where a pesticide is suspected to have been applied to the outer surface, the dry sample may be immersed whole in a beaker of solvent with occasional agitation or solvent may be repeatedly rinsed over the sample on a large tray with raised sides. Usually the extract is dried by passing through anhydrous sodium sulphate before analysis.

A small proportion of agricultural pesticides which poison wildlife are polar, water-soluble compounds. These are usually best extracted with water and the extract normally needs to be cleaned-up. Very specific detection methods (e.g. immunoassay methods) are suitable for analysis of some water extracts with little or no clean-up.

2.3.3. Clean-up strategies

Extracts of samples containing very large residues (e.g. some poisoned baits) may be suitable for

analysis after dilution without further clean-up. Extracts of stomach and gizzard contents containing lethal residues require different amounts of clean-up according to the analysis method. Extracts of poisoned honey bees may contain very small pesticide residues and always require a clean-up before analysis. A compound-specific clean-up is the most effective but is, by definition, unsuitable for multi-residue analysis.

Gel-permeation chromatography [15] is a means of separating large molecules from small ones and is therefore useful as a first clean-up for a wide range of pesticides which have a molecular weight less than 500. With automation of injection and fraction collection, this procedure is used on all honey bee extracts analysed by the Wildlife Incident Unit.

Column adsorption chromatography is a traditional means of cleaning up extracts for pesticide analysis [16]. The development of small cartridge columns (solid-phase extraction) has reduced analysis time [17] and the automation of the use of these enables some laboratories to cope with large numbers of similar analyses.

Partition between two solvents is particularly effective where low polarity pesticides are to be separated from more polar compounds and both are in solution in a small volume of a water-miscible solvent (such as acetone). For example, if hexane and water are added, the mixture is shaken and allowed to separate, the pesticides partition into the hexane layer leaving the more polar contaminants in the aqueous layer. Cartridges of reversed-phase (C_{18}) HPLC packings (another form of solid-phase extraction) enable crude versions of HPLC separations to be carried out as a clean-up step. Adsorption cartridges are best for cleaning up prior to reversed-phase HPLC, and vice-versa.

A further application of partition is to separate acidic compounds from neutral and basic ones, or basic compounds from neutral and acid compounds. The procedure relies on the 'organic' acid or base being soluble in an organic solvent (e.g. dichloromethane) but its salts being soluble in water. A change in pH of the aqueous phase followed by thorough shaking with the organic solvent will control the layer in which the acid or base is to be found.

A bonus of derivative formation (see below) is that it can also aid clean-up: a chromatographic clean-up prior to derivatisation lets through compounds of similar polarity to the underivatised analyte, but derivatisation usually changes the polarity of the analyte, providing scope for a second chromatographic clean-up.

For clean-up of individual water-soluble compounds in particular, molecules with compound-specific binding properties are becoming more widely used, particularly immobilised antibodies.

2.3.4. Derivative formation

Derivative formation is used for the following purposes:

1. to make a compound suitable for GC analysis, e.g. chloralose analysis [18]
2. to change the GC or LC retention time of a compound for confirmation purposes [19]
3. to improve sensitivity or selectivity of HPLC detection, e.g. production of fluorescent derivatives before or after HPLC separation as an alternative to UV absorbance [20]
4. to add a functional group detectable by a selective GC detector (usually unwise!)
5. to anchor a compound to a solid material, as is found in some immunoassays.

2.3.5. Instrumental analysis

For the analysis of relatively small pesticide residues in extracts containing co-extractives even after clean-up, chromatographic analytical techniques combine the advantages of separation with the possibility of using sensitive and selective detectors.

Gas chromatography is still the principal technique in pesticide residue analysis largely because of the sensitivity of a range of selective detectors (electron-capture, flame photometric, nitrogen–phosphorus, atomic emission, etc.). In particular, bench-top mass spectrometers now compete in sensitivity with the traditional detectors and offer the advantage of unequivocal identification of each compound present. Some analysts have abandoned other detectors, but because mass spectrometry (MS) is either insufficiently selective (total ion mode) or molecule-specific (selected ion mode with pre-set retention time window), selective GC detectors have a role in simplifying the chromatogram when looking for a small amount of an 'unknown'.

High performance liquid chromatography is a

useful alternative technique for compounds that degrade thermally (e.g. oxime carbamate insecticides) or are not very volatile (e.g. strychnine). The main problem is a lack of sensitivity and selectivity of detection. Fluorescence detection is particularly valuable here (even if derivative formation is needed to produce a fluorescent molecule). The confidence placed in results from a UV absorbance detector is increased by the use of a diode-array detector to obtain a spectral match. Recent improvements in LC–MS now make this an instrument for routine use in residue analysis.

Supercritical fluid chromatography is developing and will find application in certain specific areas of residue analysis, but is not used at present in WIIS work.

2.3.6. Approaches to confirmation

A chromatographic peak alone is insufficient evidence on which to implicate a product in poisoning of wildlife or to convict someone for illegal abuse of an agricultural chemical. The more selective the detector, the more convincing the evidence; but more is needed. An alternative technique (for example HPLC with UV absorbance detection to confirm results on GC with an element-selective detector) can be very convincing if quantitative agreement is obtained between the two methods. We normally use GC–MS to confirm compounds detected by GC.

2.3.7. Compound-specific reagents

Immunoassay techniques are usually compound-specific (but occasionally interference results in a false positive). Used as a screening analysis prior to a chromatographic analysis technique, immunoassay can rapidly eliminate negative samples and provide extra confidence in the positive chromatographic results.

3. Analytical methods

Where gas or liquid chromatographic analyses are mentioned, the conditions are given in separate tables of GC (Table 1) and LC (Table 2) conditions.

3.1. Multi-residue analysis methods

The compounds covered by each analysis are shown in a separate table (Table 3).

3.1.1. Organophosphates

Organophosphates usually act quickly (a few minutes to a few hours) and dead wildlife specimens are often found close to the source of the pesticide (for example recently sown treated seed or a deliberately laid bait). The most convenient material for analysis is the content of the stomach (or gizzard in birds). The basic conditions further down the gut may degrade organophosphates. The less polar and relatively stable compounds (e.g. carbophenothion) may be found at easily detectable levels (~1 mg kg^{-1}) in internal tissues [21], but the more polar or less stable organophosphates are not usually found in internal tissues.

Solvent extraction of the material (5 g) ground with sand (5 g) and a drying agent (anhydrous sodium sulphate, 25 g) is done by Soxhlet apparatus or by simple agitation in the solvent followed by filtration (diethyl ether and dichloromethane are convenient solvents). Treated seed samples may be similarly extracted without grinding and carcasses laid as baits may be extracted by carefully rinsing the whole of the outer surface with a solvent, then collecting the rinsings and filtering them through a drying agent. A typical 100 ml extract, without further clean-up, is suitable for gas chromatography with flame photometric detection (preferably a 2-channel instrument for both phosphorus and sulphur). The detection limit is around 0.01 $\mu g\ ml^{-1}$ for many organophosphates (0.2 mg kg^{-1} in a 5 g sample). Some compounds co-elute, so confirmation using a different GC column (e.g. DB225; J&W Scientific) or preferably by GC–MS (or by an alternative technique) is essential.

For detection of smaller residues or for 'dirtier' samples, a clean-up is required. For example, honey bees (5 g) are first ground and Soxhlet extracted with diethyl ether. Gel permeation chromatography of half the extract, concentrated and re-diluted in 5 ml hexane–ethyl acetate (1:1), on a 25 mm I.D. column containing 65 g Bio-beads S-X3 (Bio-Rad Labs.) with hexane–ethyl acetate (1:1) mobile phase at 5 ml min^{-1} elutes large molecules in the first 90 ml (to

Table 1
Gas chromatography conditions

Analysis	Column	Length (m)	Carrier gas flow	Injection	Column temp. (°C)	Gradient. to temp	Duration	Detector
Organo-	DB17	15	N_2	Direct	140		0.5 min	
phosphate			4.5 ml min^{-1}	200°C	140	10°C min^{-1} to 180°C		
					180	6°C min^{-1} to 270°C	10.5 min	FPD
Carbamate	DB1	15	He	Cool	50		0.5 min	
			10 ml min^{-1}	on-col.	50	20°C min^{-1} to 235°C	3.5 min	NPD
Organo-	DB1	30	He	Splitless	100		1.0 min	
chlorine			pressure	175°C	100	25°C min^{-1} to 225°C		
			7.2 p.s.i.		225	2.5°C min^{-1} to 265°C	3.0 min	ECD
Pyrethroid	DB1	30	He	Splitless	100		1.0 min	
			pressure	175°C	100	25°C min^{-1} to 225°C		
			7.2 p.s.i.		225	2°C min^{-1} to 275°C	9.0 min	ECD
Chloralose	DB1	30	He	Splitless	100		1.0 min	
(tris deriv)			6 ml min^{-1}	175°C	100	25°C min^{-1} to 225°C		
					225	2°C min^{-1} to 250°C	1.5 min	ECD
Metaldehyde	GS-Q	30	N_2	Splitless	110		1.0 min	
(acetaldehyde)			5 ml min^{-1}	220°C	110	5°C min^{-1} to 130°C	5.0 min	FID

All the above analyses are on 0.53 mm I.D. fused silica capillary columns with nitrogen make-up gas.
Where a pressure is quoted instead of a flow, this is a constant column head pressure (flow 5–10 ml min^{-1}). 1 p.s.i.=6894.76 Pa.
FPD=Flame photometric detection; NPD=nitrogen–phosphorus detection; ECD=electron-capture detection; FID=flame ionization detection.

GC–MS confirmation on 0.25 mm I.D. capillary with Finnigan ITD800 ion-trap detection (ITD) system:

OP, OC,	BPX-5	25	He	Splitless	60		1.0 min	
Pyrethroid			1 ml min^{-1}	220°C	60	25°C min^{-1} to 260°C	11.0 min	ITD
Carbamate		as above		150°C		as above		
Chloralose		as above		250°C		as above		
Strychnine		as above		270°C	260	isothermal	40.0 min	ITD
Aldicarb		as above		300°C	60		4.0 min	
as nitrile					60	25°C min^{-1} to 260°C	3.0 min	ITD

Columns: DB series and GS-Q are manufactured by J&W (Folsom, CA); BPX-5 by SGE (Ringwood, Australia). Injection volumes: 1 μl for ECD, NPD and ITD, 2 μl for FPD and 25 μl of vapour for metaldehyde analysis using FID. GC instruments: for OPs, Ai93 (Ai Cambridge Ltd., UK), for all others, HP5890 (Hewlett–Packard, Avondale, PA, USA).

waste) and the pesticides in a further 90 ml (collected). The cleaned extract is concentrated as necessary prior to GC analysis.

Evidence that the organophosphate actually caused the death of an animal may be obtained from brain acetylcholinesterase activity measurements [22], as long as the brain tissue is not decomposed and esterase data can be obtained for control animals of the same species. Activity less than 10% of that of the control indicates death from poisoning by an esterase inhibitor [23].

3.1.2. Carbamates

The choice of tissue and extraction method is the same as for organophosphates, so the same extract may be used for both analyses. Carbamates that are esters of N-methyl- (or N,N-dimethyl-) carbamic acid and a phenol may be analysed by GC with nitrogen–

Table 2
Liquid chromatography conditions

Analysis	Parameter	Conditions used
Carbamate	Column	150 mm×4.6 mm I.D. 5 μm C_{18} specifically for EPA method 531 (Pickering)
	Column temp.	42°C
	Flow rate	1 ml min^{-1}
	Mobile phase	18% methanol in water for 0.5 min, then linear gradient to 70% methanol in water over 28.5 min, then 70 to 100% methanol over 1 min; 100% methanol for 10 min.
	or | Column	250 mm×4.6 mm I.D. 5-phenyl column at 25°C
	| Flow rate	1 ml min^{-1}
	| Mobile phase	3% acetonitrile in water for 1 min, then linear gradient to 27% acetonitrile in water over 24 min, then to 37% acetonitrile over 5 min, then to 100% acetonitrile over 10 min; 100% acetonitrile for 5 min
	Injection volume	10 μl (if solvent is methanol; larger volumes if solvent is mainly water)
	Post-column reagents [see Ref. [27]]:	
	Hydrolysis	0.05 *M* NaOH in water, flow rate 0.3 ml min^{-1}, reactor 100°C
	Derivatisation	100 mg OPA in 10 methanol plus 2 g 'Thiofluor' dissolved in 950 ml pH 9.1 borate buffer, flow rate 0.3 ml min^{-1}, ambient temperature
	Detector	Fluorescence, excitation 330 nm, emission 465 nm (Perkin–Elmer LC240)
Rodenticide	Column	250 mm×4.6 mm I.D. 5 μm C_{18} (Spherisorb ODS-2, Anachem)
	Column temp.	Ambient (~23°C)
	Flow rate	0.8 ml min^{-1}
	Solvents	A=water+0.25% v/v acetic acid. B=methanol+0.25% acetic acid.
	Mobile phase	75% solvent A, 25% solvent B; linear gradient to 5% A, 95% B over 5 mins. then linear gradient to 100% B over 15 min; 100% B for 1 min.
	Injection volume	50 μl (solvent methanol)
	Post-column reagent:	
	To change pH	6% v/v solution of concentrated ammonia (aqueous sp. gr. 0.88) in water. Reagent enhances fluorescence. Compare analyses with and without.
	Detector	Fluorescence, excitation 310 nm, emission 390 nm (Perkin–Elmer LC240)
Strychnine	Column	150 mm×4.6 mm I.D. 3 μm C_8 (Spherisorb 3C8, HPLC Technology)
	Column temp.	Ambient (~23°C)
	Flow rate	0.6 ml min^{-1}
	Mobile phase	Isocratic ion-pair solvent: sodium octanesulphonate (2.16g) dissolved in water (700 ml)+acetonitrile (300 ml)+acetic acid (2.5 ml).
	Injection volume	50 μl [0.33% (v/v) 18 *M* sulphuric acid in water]
	Detector	UV absorbance 254 nm or preferably photo-diode array detector (Waters 996).
Metaldehyde (derivative)	Column	100 mm×4.6 mm I.D. 5 μm C_{18} (Spherisorb ODS-2, Phase Separations)
	Column temp.	Ambient (~23°C)
	Flow rate	0.8 ml min^{-1}
	Mobile phase	Isocratic: water–methanol (60:40, v/v).
	Injection volume	20 μl (solvent water)
	Detector	Fluorescence, excitation 380 nm, emission 450 nm (Shimadzu RF-535)
Paraquat	Column	250 mm×3.2 mm I.D. 5 μm PRP-1 resin (manufactured by Hamilton)
	Column temp.	Ambient (~23°C)
	Flow rate	0.8 ml min^{-1}
	Mobile phase	Isocratic ion-pair solvent: sodium octanesulphonate (3 g) dissolved in water (400 ml)+methanol (600 ml)+orthophosphoric acid (1.26 ml).
	Injection volume	100 μl [solvent 0.05% (w/v) sulphuric acid in water]
	Detector	UV absorbance 258 nm or preferably photo-diode array detector (Waters 996) To determine diquat in addition to paraquat, use UV absorbance at 286 nm

Except where otherwise stated, instruments are manufactured by Waters.

Table 3
Pesticides detected by multi-residue methods

ORGANOPHOSPHATES: Typical detection limit for 5 g sample by the standard procedure is 0.2 mg kg^{-1}

azinphos-methyl	dimethoate	fonofos	parathion	quinalphos
bromophos-methyl	disulfoton	heptenophos	phorate	triazophos
carbophenothion	ethion	iodofenphos	phosalone	vamidothion
chlorfenvinphos	etrimfos	malathion	phosmet	
demeton-S-methyl	famphur	mevinphos $E+Z$	pirimiphos-ethyl	
diazinon	fenitrothion	omethoate	pirimiphos-methyl	
dichlorvos	fenthion	oxydemeton-methyl	propetamphos	

Organophosphates, detection limits uncertain

azamethiphos	demephion	menazon	monocrotophos	thiometon
chlorpyrifos	dimefox	mephosfolan	morphothion	trichlorfon
chlorpyrifos-methyl	ethoprophos	methacrifos	naled	
coumaphos	fenchlorphos	methamidophos	phoxim	
cyanophos	formothion	methidathion	pyrazophos	

The method can also detect some other organophosphate pesticides

CARBAMATES (by GC): Typical detection limit for 5 g sample by the standard procedure is 0.2 mg kg^{-1}

aminocarb	carbaryl	carbosulfan	pirimicarb
bendiocarb	carbofuran	methiocarb	propoxur

Some other carbamates listed under 'Carbamates by LC' are also detectable by this GC method.
Examples: aldicarb (decomposes) methomyl
Methomyl and oxamyl may be measured by GC method as their oximes; aldicarb sulphone may be analysed by GC.
The compounds detectable by GC are also detectable by LC except aminocarb, pirimicarb (*N,N*-dimethylcarbamates) and carbosulfan (LC behaviour not fully evaluated).
Barbiturates may be detected during carbamate analysis by GC: pentobarbitone may be mistaken for aminocarb.

CARBAMATES (by LC) The detection limits for 5 g sample approx. 2 mg kg^{-1}, but depend on the nature of the sample.

aldicarb	bendiocarb	methiocarb	propoxur
aldicarb sulphone	carbaryl	methomyl	thiodicarb
aldicarb sulphoxide	carbofuran	oxamyl	thiofanox

A simple clean-up has been developed for aldicarb and that method is used together with GC and GC–MS to measure and confirm aldicarb in WIIS samples.

ORGANOCHLORINES: Typical detection limit for 5 g sample by the standard procedure is 0.4 mg kg^{-1}

aldrin	*o,p'*-DDT	β-endosulfan	γ-HCH (lindane)
trans-chlordane	*p,p'*-DDT	endrin	heptachlor
p,p'-DDD (*p,p'*-TDE)	dieldrin	α-HCH	heptachlor epoxide
p,p'-DDE	α-endosulfan	β-HCH	hexachlorobenzene

Organochlorines, detection limits uncertain

isobenzan (Telodrin)	methoxychlor	'methoxychlor DDE'

Organochlorine technical mixtures, difficult to quantify

chlordane	toxaphene (camphechlor)

The method can also detect polychlorinated biphenyls (PCBs)

PYRETHROIDS: Typical detection limits for 5 g sample are in the range 0.001 to 0.1 mg kg^{-1}

bifenthrin	cypermethrin	fenpropathrin	permethrin
cyfluthrin	α-cypermethrin	fenvalerate	tefluthrin
λ-cyhalothrin	deltamethrin	fluvalinate	tetramethrin

Other pyrethroids detectable (method not validated for these compounds)

S-bioallethrin	bioresmethrin	phenothrin

The procedure is also suitable for the determination of lindane (γ-HCH), an organochlorine pesticide. The method was designed for the analysis of honeybees, but may be applied to other samples.

ANTICOAGULANT RODENTICIDES: Typical detection limits for 4 g sample between 0.005 and 0.05 mg kg^{-1}

brodifacoum	coumatetralyl	flocoumafen
bromadiolone	difenacoum	warfarin

The method is not suitable for the analysis of anticoagulant rodenticides based on indanedione as these are not detectable by their fluorescence; an alternative method using a diode-array UV-visible absorbance detector is recommended for the analysis of chlorophacinone and diphacinone.

phosphorus detection (NPD) [24] if precautions are taken to minimise thermal decomposition. Carbamates that are esters formed from oximes mostly decompose under GC conditions and are better analysed by HPLC.

Prior to GC of phenol-carbamate esters, a clean-up is usually required. For particularly 'dirty' samples, gel permeation chromatography (GPC) may be used as a first or only clean-up. Alternatively, extract equivalent to 0.5 g of tissue is evaporated almost to dryness and re-dissolved in hexane (5 ml). This is applied to a pre-washed silica Sep-Pak cartridge (Waters) which is eluted sequentially with 10 ml portions of hexane, 10% diethyl ether in hexane and 65% diethyl ether in hexane. The first two eluates are discarded and the carbamates are found in the 65% ether in hexane eluate. For analyses of a single carbamate, the elution conditions can be adapted to obtain a better clean-up. The collected eluate is analysed by GC with cool on-column injection and NPD. Some barbiturates and organophosphates may also be detected. GC–MS using similar GC conditions, but with a narrower-bore capillary, is an alternative detection method and is used to confirm positive results obtained from GC–NPD.

For HPLC analysis of carbamates, although selective fluorescence detection is used, a clean-up (such as GPC) to remove compounds which interfere with the chromatographic separation is advisable. An alternative (or additional) clean-up involves evaporation of the sample extract (e.g. dissolved in diethyl ether), re-dissolving in 5 ml water (after addition of a drop of methanol), then application to a C_{18} Sep-Pak cartridge and elution with a solvent mixture appropriate to the range of carbamates to be analysed. HPLC of carbamates requires sensitive and selective detection. Following HPLC separation on a C_{18} column, the carbamates are hydrolysed by strong base at 100°C as they pass through an in-line reaction coil, then methylamine produced from N-methyl carbamates is allowed to react with *o*-phthalaldehyde (OPA) reagent and N,N-dimethyl-2-mercaptoethylamine (Thiofluor) to form a fluorescent derivative [25–27]. This is suitable for both phenol- and oxime-carbamates but not for those that are N,N-dimethyl carbamates because the hydrolysis product dimethylamine is unable to undergo the derivatisation reaction. Some of the carbamates commonly used in the UK co-elute on C_{18} columns intended for US Environmental Protection Agency (EPA) method 531, but alternative separation may be obtained using a 5-phenyl column.

3.1.3. Organochlorines

Acute poisoning by organochlorines is rare except where a compound of this class (typically endrin) is used deliberately to poison animals. Organochlorines accumulate in fat and may cause death if fat reserves are mobilised rapidly. Extracts of muscle tissue contain less co-extractives than those of liver tissue, but liver is usually selected for analysis because more is known about the significance of organochlorine residues in this organ. Where acute poisoning is suspected, additional analysis of stomach contents will show a large residue if the poison was ingested shortly before death. Extraction is conveniently by Soxhlet using diethyl ether as solvent as already described.

Clean-up is carried out using column chromatography on neutral alumina [28]; a portion of ether extract equivalent to 0.1 g of tissue, evaporated almost to dryness and dissolved in 1 ml of hexane, is applied to a 4 mm diameter mini-column of alumina (2 g, activity IV) and the organochlorines are eluted with more hexane until the elution volume is 25 ml. Analysis of this eluate by GC with electron-capture detection (ECD) or by sensitive GC–MS will detect lethal residues of organochlorine compounds (ranging from 0.5 to 5 mg kg^{-1} for endrin in liver, depending on species, to more than 100 mg kg^{-1} for residues of DDT and its metabolites in blackbird livers). Note that the cyclodiene pesticides (dieldrin, endrin, etc.) do not give easily identifiable positive ion mass spectra so negative ion chemical ionisation is preferred. To detect smaller 'background' residues, the cleaned extract may be concentrated by careful evaporation of the solvent (γ-HCH is easily lost) prior to GC analysis. Clean-up of a larger quantity of original extract, using a larger column, enables small residues to be measured [29].

3.1.4. Pyrethroids

Synthetic pyrethroid insecticides are of low toxicity to mammals and birds. Being insecticides, they are

very toxic to honey bees, but they are also claimed to have repellent properties. The analysis method that follows is intended primarily for the analysis of honey bees for synthetic pyrethroids together with the organochlorine compound γ-HCH.

A portion of extract of honey bees (equivalent to 1 g) in diethyl ether or ethyl acetate–hexane is evaporated and the residue re-dissolved in hexane (5 ml). The hexane is extracted with 3×5 ml portions of acetonitrile and the acetonitrile extracts combined. These are washed with hexane (15 ml) and the hexane washings discarded. The pyrethroids are then extracted back into hexane after addition of hexane and acetone (15 ml each) then water (15 ml) to the acetonitrile extract. The aqueous layer is further extracted with 2×15 ml hexane. The hexane extracts are combined, dried with anhydrous sodium sulphate, concentrated to 2 ml and put onto a column of Florisil (5 g, deactivated by addition of 2%, w/w, water), followed by 2×2 ml hexane washings of the container. After eluting unwanted material with hexane (16 ml) and hexane–diethyl ether (90:10) (10 ml), the pyrethroids are eluted in hexane–diethyl ether (35:65) (20 ml). The eluate collected is evaporated to 4 ml under nitrogen and analysed by gas chromatography. Confirmation is by GC–MS using an ion-trap detector.

3.1.5. Anticoagulant rodenticides

Anticoagulant rodenticides usually kill by interference with the blood-clotting mechanism [30], resulting in internal haemorrhage (large doses of some of these compounds can kill by their acute toxicity). Death usually occurs several days after ingestion of the rodenticide and the residues remaining are usually small (above or around 0.1 mg kg^{-1} in liver). Liver is the tissue of choice and the method described [31] is suitable for liver; the HPLC analysis may be applied to blood plasma with little preparation. The method has been used for rodenticide bait samples, but the extraction efficiency for some rodenticides from some grain-based bait samples is poor.

Liver (4 g) is ground with anhydrous sodium sulphate (40 g) and extracted with dichloromethane–acetone (70:30) in a screw-capped conical flask on a shaker for 2 h. The extract is filtered and made up to 100 ml with the same solvent mixture. A portion of extract equivalent to 0.4 g of tissue is transferred to a pre-washed neutral alumina Sep-Pak cartridge. The unwanted material is eluted with dichloromethane–acetone (70:30) then dichloromethane–acetone (25:75) (2 ml). The rodenticides are eluted with methanol–acetic acid (95:5, v/v), the eluate is collected and evaporated to dryness at 60°C under a stream of nitrogen. The rodenticides are re-dissolved in 0.5 ml of methanol for HPLC analysis. Blood samples are centrifuged to produce plasma, to 60 μl of which is added 240 μl of methanol in a 300 μl HPLC 'insert' vial inside a standard vial. This is centrifuged at 1500*g* for 10 min, to precipitate protein at the bottom of the insert vial, and the vials transferred to the HPLC for analysis.

The coumarin-based anticoagulants chromatograph well on C_{18} HPLC columns in the presence of a small amount of acetic acid, but they fluoresce strongly in basic solution. The HPLC method involves post-column addition of ammonia solution prior to fluorescence detection with excitation at 310 nm and emission at 390 nm [32].

The determination of chlorophacinone and diphacinone has been described by Hunter [33].

3.2. Single compound analysis methods

3.2.1. Strychnine

Although strychnine is a fast-acting poison, animals receiving a minimum lethal dose or subject to immediate veterinary intervention may survive for more than 2 days before death. Stomach contents have the highest concentration of strychnine if death was rapid, but residues are present in liver tissue over a longer time.

The extraction method is by Soxhlet apparatus with diethyl ether or by shaking the sample (after grinding with a drying agent) with dichloromethane followed by filtration. Because strychnine is an organic base, a suitable clean-up consists of evaporation of the organic solvent and re-dissolving the strychnine in 0.33% sulphuric acid as the sulphate, washing the aqueous acid with chloroform, then adding ammonia to convert the ionic strychnine salt to the non-ionic free base and extraction back into chloroform. After drying the chloroform, the extract

is suitable for HPLC analysis on a silica column. Better chromatography is obtained if the strychnine, transferred to aqueous acid solution, is chromatographed on a 3 μm C_8 HPLC column with sodium octanesulphonate ion pair reagent. A typical detection limit based on a 4 g sample is 0.2 mg kg^{-1}.

Further confirmation may be obtained by GC–MS. The peak shape is not very good and the results are qualitative rather than quantitative.

3.2.2. Chloralose

Chloralose is a narcotic compound formed from chloral hydrate and glucose. It immobilises its victims within minutes, but complete recovery or death may take several hours. It reduces the metabolic rate and small animals in particular die through loss of body heat. It is used under licence in the UK to control pigeons, gulls and mice [34]. Although residues may be present in the stomach at death, the residues in the kidneys tend to lie within a close range of values in animals that have died of poisoning, but are not detected in animals that have survived recent sub-lethal poisoning [18].

Chloralose itself is unsuitable for analysis by gas chromatography, but its trimethylsilyl ether derivative chromatographs well and the chlorine atom in the chloralose makes it suitable for detection by the sensitive and halogen-selective electron-capture detector.

Kidney tissue is Soxhlet-extracted with diethyl ether as previously described. A portion of evaporated extract equivalent to 1 g of tissue is dissolved in methanol (1 ml) and applied to a column of Sephadex LH-20 gel (prepared in methanol) supported on an acrylic sintered disc and occupying 9 ml of the barrel of a 10-ml glass syringe. Large molecules are eluted from the gel column to waste with 7 ml methanol (including the original 1 ml and a 1-ml rinse). Chloralose is eluted in a further 10 ml of methanol which is collected. An aliquot of this cleaned extract is evaporated completely to dryness and derivatised by addition of Trisil (100 μl; Pierce, Rockford, IL, USA) and heating to 80°C for 10 min [18]. The derivative is dissolved in hexane (3 ml) and water-soluble impurities are removed by shaking this with 8 ml water. A portion of the hexane layer (1 ml) is diluted to 10 ml with hexane and dried by addition of anhydrous sodium sulphate. A 5-ml aliquot of the diluted solution is cleaned-up on a pre-washed silica Sep-Pak cartridge as follows. The cartridge is attached to a 10-ml syringe barrel, the solution is applied to the cartridge through the barrel and pushed through to waste with gentle pressure using the syringe plunger. A further 5 ml of hexane is pushed through the cartridge and discarded, then the chloralose is eluted with 5 ml of 8% (v/v) diethyl ether in hexane which is collected and analysed by gas chromatography. A typical detection limit for a 4 g kidney sample is 0.8 mg kg^{-1}.

3.2.3. Metaldehyde

Metaldehyde is a cyclic polymer of 4 acetaldehyde molecules. It is only moderately toxic to birds and mammals, but the bran-based slug pellets in which it is used are readily eaten by animals such as dogs and cattle: The time from ingestion to death varies from a few hours to several days. Typically, a blue colouration from the dye in the pellets is present in the gut contents. A positive result from the analysis of gut contents indicates exposure and a residue of more than 50 mg kg^{-1} in liver tissue usually indicates that metaldehyde caused the death of the animal. The methods described are also suitable for the analysis of metaldehyde in slug pellets. Methods for the analysis of metaldehyde involve conversion of metaldehyde to acetaldehyde, which also occurs naturally and may be formed in decomposing tissue. It is necessary to distinguish between naturally-occurring acetaldehyde and that produced by reaction of metaldehyde with acid.

Stomach (or intestine) contents (1–4 g) or slug pellets (1 g) are ground with sand (5 g) and anhydrous sodium sulphate (25 g) and extracted with chloroform (75 ml) in a screw-capped bottle for 1 h on a flask shaker. The mixture is filtered, the filtrate is evaporated almost to dryness and then re-dissolved in methanol (10 ml) and the volume made up to 100 ml with methanol (15 ml) and water (75 ml). Liver samples (4 g) are extracted directly into methanol (30 ml then 20 ml rinse) by maceration. The homogenate is centrifuged (1500*g*) for 10 min and the supernatant made up to 100 ml with water.

For selective detection using HPLC analysis,

metaldehyde is converted to acetaldehyde by reaction with hydrochloric acid and the acetaldehyde is reacted with 1,3-cyclohexanedione in solution with ammonium acetate to form a fluorescent product [35]. To distinguish between naturally occurring acetaldehyde and that derived from metaldehyde, each sample is divided into 2 portions; acid is added to one (to convert metaldehyde to acetaldehyde) and water to the other, then they are heated at 60°C for 10 min. Cyclohexanedione reagent is added to both portions, together with water to the acid-reacted portion and hydrochloric acid to the other portion, then they are heated again at 60°C, this time for 45 min. The buffering effect of the sodium acetate prevents reaction between the acid and metaldehyde in the portion where metaldehyde remains. The products of the derivative formation reactions are analysed by HPLC. For each sample and standard, the response of the 'background acetaldehyde' portion is subtracted from that of the portion in which any metaldehyde present has also been converted to the derivative. The amount of metaldehyde present in the sample is calculated by reference to solutions of derivative prepared from standard solutions. A typical detection limit for a 4 g sample (2×acetaldehyde 'blank') is well below 20 mg kg^{-1}, but residues below 20 mg kg^{-1} are not considered significant.

An additional method, but which does not distinguish between natural acetaldehyde and metaldehyde, involves the conversion of metaldehyde to acetaldehyde in a sealed bottle and analysis of the head space by GC on a porous polymer column for acetaldehyde [35]. Approximately 1 g of sample (a smaller carefully weighed representative sample in the case of slug pellet samples) is heated with 3 *M* sulphuric acid (15 ml) in a septum-capped digestion bottle in a glycerol bath at 130°C for 10 min. A 25 μl aliquot of the head space in the bottle is taken in a pre-heated gas-tight syringe and injected onto a gas chromatograph with a 30 m×0.53 mm I.D. capillary column wall-coated with GS-Q divinylbenzene polymer (J&W). Acetaldehyde is detected with a retention time of approximately 5 min using a flame-ionisation detector. Measurement is by comparison with a similarly treated standard amount of metaldehyde and is semiquantitative even with the use of a dichloromethane internal standard.

3.2.4. Paraquat

Paraquat is a herbicide that is inactivated by binding to organic matter in soil and the residues present on plants present little hazard to wildlife [36]. It is, however, used at high concentrations to poison wildlife and domestic animals (contrary to UK legislation). Poisoned animals develop symptoms slowly and take several days to die; the extractable residues of paraquat at death are very small. Initial diagnosis of paraquat poisoning, based on congestion and characteristic lesions in lung tissue, identifies probable victims of paraquat poisoning. The highest residues of paraquat are reported to be in kidney tissue [37,38]. It is convenient to screen for paraquat using one of the commercial enzyme-linked immunosorbent assay (ELISA) test-kits; samples giving positive results can be further analysed using HPLC.

Kidney tissue (1–2 g) is weighed and macerated for 40 s at 1200 rpm in 12 ml of trichloroacetic acid (TCA). The homogenate is centrifuged at 1250*g* for 15 min. The supernatant is separated and the pellet rinsed with 6 ml TCA and centrifuged as before. The combined supernatants are adjusted to a volume of 20 ml with TCA. A 0.75-ml portion is taken and 0.25 ml of 2 *M* Tris buffer is added prior to ELISA according to the instructions on the commercial test-kit [39]. A typical detection limit based on a 2 g sample is 0.04 mg kg^{-1}.

From the remainder of the extract, 10 ml is extracted in a separating funnel with 3×20 ml diethyl ether (discarded) and remaining ether removed from the aqueous solution under a stream of nitrogen. A C_{18} Sep-Pak cartridge (Waters) is prepared by passing through it alkaline cetrimide solution [5 ml of a 0.05% (w/w) in a solution of 2 ml ammonia solution (sp.gr. 0.88) in 1000 ml water], water (5 ml), methanol (10 ml), water (5 ml), followed by alkaline sodium heptanesulphonate solution (10 ml of a solution of 2 g of the salt in 100 ml water containing 2 ml ammonia solution (sp.gr. 0.88). The aqueous extract is made alkaline and put through the cartridge. After washing the cartridge with water (3 ml) and methanol (3 ml), the paraquat is eluted with a acidified methanol (5 ml of a solution of 100 ml methanol + 2 ml conc. HCl). After evaporation to dryness, the residue is immediately re-dissolved in aqueous sulphuric acid [0.5 ml

of 0.05% (w/v) H_2SO_4 in water] for analysis by HPLC [40]. The detection limit depends on the sample, but typically 0.1 mg kg^{-1} based on a 2 g sample.

3.2.5. Others

In addition to the compounds mentioned, wildlife and companion animals are occasionally poisoned by other agricultural chemicals for which there are qualitative analyses in use. These include the herbicides sodium monochloroacetate and sodium chloride and the mammal poisons sodium cyanide and aluminium phosphide (used in rabbit control to release hydrogen cyanide and phosphine respectively) and fluoroacetamide, used as a rodenticide in sewers.

4. Interpretation of analysis results

Poisoning by agricultural chemicals affects a wide range of species in a variety of different circumstances. It is neither possible nor desirable to test the toxicity of a pesticide to every species with which it may come into contact. Likewise, it is not feasible to measure the residues of pesticides in the tissues of these species following both lethal and sub-lethal exposure. Interpretation of the residues of pesticides found in dead wildlife is therefore based on extrapolation from toxicity and residue data from other species, field and post-mortem evidence and wide experience of incidents covering a wide range of species and classes of chemical in different field situations.

If a residue in the stomach (or the total body burden) of a pesticide is larger than the estimated lethal dose, the compound found is likely to be the sole cause or a contributory cause of the animal's death. Supporting evidence from post-mortem examination adds extra confidence. The laying of poisoned baits (except under carefully specified conditions) is illegal in the UK. A dead animal found in the open with a large amount of a toxic chemical on it is evidence that an offence has been committed against UK laws.

Smaller residues are more difficult to interpret, particularly if the animal took a long time to die and was given veterinary treatment. In such cases there are often characteristic signs apparent at post-mortem examination. Again, the experience of incidents over many years enables a residue in a particular species to be correlated with death associated with a particular route of exposure to a pesticide. A residue of 10 mg kg^{-1} *p,p'*-DDE in a blackbird liver may be found as a high 'background' residue [41,42], but a residue of less than 0.1 mg kg^{-1} of an anticoagulant rodenticide in a barn owl liver may be accompanied by clinical symptoms of death from anticoagulant poisoning [43].

A negative result may not in all cases indicate the absence of the compound. Sometimes the suitable tissues are not available and the level of residue may be too low or the method may be unsuitable for the tissue that is analysed. If an analytical method is pushed far enough towards detection of small residues, interfering compounds will be detected, so care must be taken not to use a method to detect residues below the level at which their identity can be confirmed. For some samples, it may be necessary to quote a higher detection limit than usual because of the presence of interfering compounds. If pathological evidence points to a particular compound, but the residues expected are small, a more specific clean-up of the sample extract may permit more certain identification of that compound.

5. Conclusions

The Wildlife Incident Investigation Scheme in the UK suffers from a number of limitations. There are many biases in the reporting of animal deaths and therefore in their investigation. Companion animal deaths are often reported to a veterinary surgeon. Conspicuous and relatively rare bird deaths are much more likely to be reported than those of the common pigeon or sparrow. Small mammals may die unnoticed, concealed by undergrowth. The cause of death of an animal is not always obvious from a routine post-mortem examination and death from disease may be suspected as a possible poisoning case. The WIIS in the UK is limited to agricultural chemicals, so it does not usually identify cases of poisoning by natural toxins [8] and non-agricultural chemicals. There are also limitations imposed by

[37] C.W. Sharp, A. Ottolenghi and H.S. Posner, Toxicol. Appl. Pharmacol., 22 (1972) 241
[38] M.P. Quick, D.A. Dyson and A. Holliman, J. Forensic Sci. Soc., 30 (1990) 371.
[39] Anon., Millipore EnviroGard Paraquat Plate Kit Instruction Leaflet P31824, rev. B, Millipore Corp., Milford, MA, 1995.
[40] A. Jones, personal communication.
[41] P.J. Bunyan, P.I. Stanley, C.A. Blunden, G.L. Wardall, K.A. Tarrant, Pest Infestation Control Laboratory Report 1971–73, HMSO, London, 1975, p. 221.
[42] S. Bailey, P.J. Bunyan, D.M. Jennings, J.D. Norris, P.I. Stanley and J.H. Williams, Agro-Ecosystems, 1 (1974) 323.
[43] I. Wyllie, Pesticide Outlook, June 1995, p. 19.

cost and sample size on the extent of chemical analysis that is done.

Despite these limitations, the WIIS, using the methods described, identifies the cause of about 60% of incidents reported to it, and pesticides are found to be responsible for about half of these [7]. The Scheme benefits from a multi-disciplinary approach to investigations and, where possible, a multi-residue approach to chemical analyses. Although analyses are done in batches, the decisions on the course of the investigation and the interpretation of results are made for each incident on the basis of many years' experience of wildlife poisoning incidents, gained as the Scheme has evolved over the past 30 years.

Acknowledgments

The Wildlife Incident Investigation Scheme is administered by Pesticide Safety Directorate (a MAFF agency) on behalf of the UK Advisory Committee on Pesticides and mainly funded (from part of a levy on turnover) by the agricultural chemicals industry together with UK Agriculture Departments.

References

[1] M.R. Fletcher and R.C. Grave, Proceedings British Crop Protection Council: Pests and Diseases 1992, 2 (1992) 793.
[2] A. Hart and M. Clook, Seed Treatment: Progress and Prospects, British Crop Protection Council, monograph no. 57, BCPC, Croydon, 1994, p. 449.
[3] P.M. Brown, P.J. Bunyan and P.I. Stanley, J. Forensic Sci. Soc., 17 (1977) 211.
[4] A.R. Hardy, M.R. Fletcher and P.I. Stanley, State Vet. J., 40 (1986) 182.
[5] Anon., The Pesticide Register, Ministry of Agriculture, Fisheries and Food together with Health and Safety Executive, HMSO, London, issue 4, 1991, p. 1.
[6] M.R. Fletcher, P.W. Greig-Smith and J.H. Stevenson, Proceedings Fifth International Symposium on the Hazards of Pesticides to Bees, Plant Protection Service, Wageningen, The Netherlands, 1994, p. 139.
[7] M.R. Fletcher, K. Hunter and E.A. Barnett, Pesticide Poisoning of Animals 1994: Investigations of Suspected Incidents in the United Kingdom, Environmental Panel of the Advisory Committee on Pesticides, MAFF Publications, London, 1995.
[8] M.L. Clarke, D.G. Harvey and D.J. Humphreys, Veterinary Toxicol., 2nd ed., Bailliere Tindall, London, 1981, p. 13.
[9] P. Mineau (Editor), Chemicals in Agriculture, Vol. 2: Cholinesterase-inhibiting Insecticides: Their Impact on Wildlife and the Environment, Elsevier Science, Amsterdam, 1991.
[10] K.A. Tarrant, Environmental Effects of Pesticides, British Crop Protection Council, monograph no. 40, BCPC, Croydon, 1988, p.313.
[11] R.W. Storherr and R.R. Watts, J. Assoc. Off. Anal. Chem., 51 (1968) 662.
[12] M.C. Bowman, M. Beroza and D.B. Leuk, J. Agr. Food Chem., 16 (1968) 796.
[13] V. Janda, K.D. Bartle and A.A. Clifford, J. Chromatogr., 642 (1993) 283.
[14] F.G. Mann and B.C. Saunders, Practical Organic Chemistry, 4th ed., Longmans, Green and Co. Ltd., London, 1960, p. 35.
[15] R.C. Tindle and D.L. Stalling, Anal. Chem., 44 (1972) 1768.
[16] A.V. Holden and K. Marsden, J. Chromatogr., 44 (1969) 481.
[17] P.D. McDonald (Editor), Waters Sep-Pak Cartridge Applications Bibliography, 5th ed., Millipore Corp., Milford, MA, 1991.
[18] E.M. Odam, H.P. Wardall, S. Bailey and E. Findlay, Analyst (London), 109 (1984) 1335.
[19] A.J. Trim, P.M. Brown, P.J. Bunyan, E.M. Odam and P.I. Stanley, Analyst (London), 108 (1983) 33.
[20] H.A. Moye, S.J. Scherer and P.A. St. John, Anal. Lett., 10 (1977) 1049.
[21] G.A. Hamilton, K. Hunter, A.S. Ritchie, A.D. Ruthven, P.M. Brown and P.I. Stanley, Pestic. Sci., 7 (1976) 175.
[22] G.L. Ellman, K.D. Courtney, V. Andres and R.M. Featherstone, Biochem. Pharm., 7 (1961) 88.
[23] P.J. Bunyan, D.M. Jennings and A. Taylor, J. Agric. Food Chem., 16 (1968) 332.
[24] P.N. Kendrick, A.J. Trim, J.K. Atwal and P.M. Brown, Bull. Environ. Contam. Toxicol., 46 (1991) 654.
[25] R.T. Krause, J. Chromatogr. Sci., 16 (1978) 281.
[26] method 985.23, AOAC Official Methods of Analysis, AOAC International, Arlington, VA, 1990, p. 292
[27] Anon., User's Manual, PCX5100 Post-column Derivatization Instrument, Pickering Laboratories Inc., 1993.
[28] P.A. Greve and W.B.F. Grevenstuk, Meded. Rijksfac. Landbouwwet., Gent., 40 (1975) 1115.
[29] G.M. Telling, D.J. Sissons and H.W. Brinkman, J. Chromatogr., 137 (1977) 405.
[30] A.F. Pelfrene in W.J. Hayes Jr. and E.R. Laws, Jr. (Editors), Handbook of Pesticide Toxicology, Academic Press, San Diego, CA, 1991, Vol.3, p. 1290.
[31] A. Jones, personal communication.
[32] K. Hunter, J. Chromatogr., 270 (1983) 267 and 277 [also 321 (1985) 255].
[33] K. Hunter, J. Chromatogr., 299 (1984) 405.
[34] S. Forsen, B. Lindberg and B. Silvander, Acta Chem. Scand., 19 (1965) 359.
[35] K. Hunter, personal communication.
[36] J.T. Stevens and D.D. Sumner, in W.J. Hayes Jr. and E.R. Laws Jr. (Editors), Handbook of Pesticide Toxicology, Academic Press Inc., San Diego, CA, 1991, Vol.3, p. 1356.