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**THE OCCURRENCE AND  
DETECTION OF PESTICIDE  
RESIDUES IN UK GRAIN**

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THE OCCURRENCE AND DETECTION OF PESTICIDE RESIDUES IN UK GRAIN

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ABSTRACT

Regulations setting maximum residue levels for 64 pesticides in 39 commodities come into force in the UK during 1988 and responsibility for complying with these lies with the user of the pesticide. Users, including farmers, therefore need to be aware of residues likely to arise from the use of particular pesticides. This review summarizes the available data on the nature, detection and occurrence of organophosphorus, organochlorine, pyrethroid and fumigant residues in UK grain and assesses where further research and development work is required.

Enforcement of maximum residue levels requires suitable analytical methods which, because of the number of residues involved and the very low levels at which they may be found, are difficult and time-consuming. It is especially important to be certain of the identity of residues and this requires the use of sophisticated and expensive scientific equipment. The availability of simpler means of sample preparation, such as recently introduced equipment based on gel permeation chromatography, would be highly desirable and its application should be studied. Although adequate methods exist for the organochlorine and organophosphorus pesticides, there is a need for an improved method for the pyrethroids.

Few data exist on the pattern of pesticide residues found in UK cereals and most relate to the organophosphorus pesticides in wheat; these data do, however, show that it is very rare for residues to exceed the maximum residue levels. There are no data on pyrethroids, or on cereals other than wheat, and this gap in knowledge should be filled as a matter of priority.

Current understanding of the complex interactions between pesticides and natural components of cereal grains is limited. It is clear, however, that

residues become bound within the grain and thus are not identified by available analytical methods so that the pesticide applied cannot be completely accounted for. Also, the consequences of applying multiple treatments or mixtures of pesticides are difficult to evaluate. Further studies are therefore needed to reveal more about the nature and toxicity of degradation products of pesticides that accumulate within the grain following various treatment regimes.

UK statutory limits for pesticide residues only apply at present to raw cereals, not products such as flour and bread. International limits for cereal products do exist but studies have shown that because of losses during treatment, storage and processing, these are unlikely to be exceeded when pesticides are used at the approved application rate.

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## GLOSSARY OF TERMS

**Acceptable daily intake (ADI):** the amount of a chemical which can be consumed every day for an individual's entire lifetime in the practical certainty, on the basis of all the known facts, that no harm will result. The ADI is expressed as milligrams of the chemical per kilogram body weight of the consumer.

The ADI is based on the no-effect level (see below) in the most sensitive animal species or, if appropriate data are available, in man. It invariably includes a safety factor.

Studies from which no-effect levels and hence ADIs are derived are conducted with the technical chemical so that any toxicological effects of its impurities are included in the assessment. Account is also taken of metabolites which may influence the toxicity of the residue reaching the consumer.

**Chemical nomenclature:** individual pesticides are referred to by their trivial chemical names as listed in *The Pesticide Manual* seventh edition, 1983.

**Chromatography:** a group of techniques that permit the separation, isolation and identification of closely related components of complex mixtures by means of a continuous extraction process involving a moving gas or liquid and a stationary solid or liquid. Thus, gas-liquid chromatography involves a moving gas and a stationary liquid (coated onto a solid support), high performance liquid chromatography involves a moving liquid and a stationary solid or liquid (bonded onto a solid support) and thin-layer chromatography a moving liquid and a stationary solid coated onto a thin sheet of glass, metal or plastic.

**Fumigant:** a pesticide designed to kill insects by acting as a respiratory poison. This means fumigants are applied as gases or as liquids which readily vaporize to produce a toxic gas.

**Gel permeation chromatography:** a chromatographic technique in which separation and isolation are based, at least in part, on the shape and size of the different molecules in the sample.

**Good agricultural practice:** the officially recommended or authorized use of pesticides under practical conditions, which takes into account the minimum quantities necessary to achieve adequate control, and application in a manner so

as to leave a residue which is the smallest amount practicable.

**Insecticide:** a pesticide designed to kill insects by direct contact with, or ingestion of, a liquid or a solid.

**Limit of determination:** the limit of determination is the lowest concentration of a pesticide residue or contaminant that can be identified and measured in a specified food, agricultural commodity, or animal feed with an acceptable degree of certainty.

**Mass spectrometry:** a process in which a chemical is broken up into a family of charged particles whose mass distribution is useful for elucidating its identity.

**Maximum residue limit (MRL):** the maximum concentration of a pesticide residue likely to occur in or on a food commodity, either resulting from the use of the pesticide according to good agricultural practice directly or indirectly for the production and/or protection of the commodity concerned, or arising from environmental sources, including former agricultural uses. The MRL is expressed as milligrams of the residue per kilogram of the commodity unless otherwise stated.

**No-effect level:** the highest level of continual exposure to a chemical which causes no detectable adverse effect on morphology, functional capacity, growth, development or life span of individuals of the target species, which may be animal or human.

**Pesticide:** defined by the Food and Environment Protection Act 1985 as a substance that destroys organisms harmful to plants, undesired plants or harmful creatures.

**Pesticide residue:** any substance or substances present in food for humans or animals resulting from the use of a pesticide. (Note: a different definition of residue is employed in Chapter 5).

**Reporting limits:** preset limits above which detected residues are reported as positive.

## 1. INTRODUCTION

The opening chapter (2) on Control of Pesticides briefly reviews the development of controls in the UK. Reference is made to the publication of the EC Directive on cereals in which maximum residue limits are set for a number of pesticides and this has led to the introduction of UK statutory limits which come into force in 1988. The range of pesticides for which limits are set is given to illustrate the broad range for which cereals will have to be monitored.

The broad principles underlying the methods of analysis available for the determination of some of these pesticides are described in Chapter 3. Gas-liquid and high performance liquid chromatography are identified as the most versatile and sensitive methods and the particular values of individual detectors pointed out. Emphasis is given to the need for reliable data, an essential part of which is the unequivocal identification of any pesticide extracted from a sample of grain. Mass spectrometry has a particular value for this purpose.

Some of the methods referred to have been used to monitor residues in wheat (Chapter 4). In the UK these studies have been mainly carried out by FMBRA and the Working Party on Pesticide Residues. However, the total amount of data is relatively small with only about 600 samples of UK wheat having been analyzed since 1970. The majority of these contained organophosphorus insecticides but only in two cases did the residues approach or exceed the appropriate Codex maximum residue limit.

These studies have been primarily concerned with residues of the parent pesticide but this is only a part of a much more complex pattern of pesticide behaviour in grain and the interactions which can take place between different pesticides and between pesticides and grain components. This is dealt with in Chapter 5 which provides a wide-ranging account of the breakdown and metabolism studies which have sought to achieve a more comprehensive understanding of what happens to pesticides after they are added to grain. One finding of particular interest is that the apparent loss of pesticide observed soon after its addition to grain does not necessarily imply that the pesticide has been degraded. It has been shown in some instances that the pesticide has become 'bonded' to the tissues of the grain and is not extracted by the normal analytical procedures. The chemical processes within the grain by which pesticides are broken down lead to products (metabolites) which may or may not be readily extracted for analysis. Furthermore, the longer the pesticide is within the grain

the more difficult it is to extract. Whilst these studies provide valuable insight into the fate of pesticides in individual grains it is also necessary to be aware of how pesticides added to stored grain are affected by milling and baking and particularly how the pesticide is distributed in milling fractions. Studies which have been carried out are described in Chapter 6 and these showed that residues in white flour were about 30% of those in the whole grain. This apparent loss was in fact balanced by higher levels in the bran which were between 3 and 4 times those in the grain. When flour, brown or white, was baked into bread residue levels were reduced by about 50%. Fumigants were effectively removed by baking.

Notwithstanding the extensive studies which have been carried out and which are referred to in this review there are still significant gaps in the knowledge needed to achieve a comprehensive understanding of the consequences of adding toxic chemicals to stored grain. These gaps are identified in Chapter 7 and positive recommendations are made for further studies.



## 2. CONTROL OF PESTICIDES

In order to obtain a better perspective of the current procedures for the control of pesticides in the UK it is necessary briefly to consider the main international developments which have contributed to the present approach.

The Codex Alimentarius Commission (CAC) was established in 1962 to implement the Joint FAO/WHO Food Standards Programme. One purpose of the Programme is to protect the health of consumers and to ensure fair practices in the food trade. Codex is advised on matters relating to pesticides by the Codex Committee on Pesticide Residues (CCPR) at which Governments discuss MRLs for pesticides in commodities moving in international trade. In this way it is hoped to avoid serious inconsistencies in MRLs between countries. Also the FAO 'Panel of Experts on Pesticide Residues in Food and the Environment' and the WHO 'Expert Group on Pesticide Residues' hold joint meetings on pesticide residues (usually referred to as JMPR) to assess health hazards which may be posed by pesticide residues in foods. Toxicological data are evaluated with a view to establishing acceptable daily intakes (ADIs) for man.

MRLs however are agreed by CCPR on the basis of Good Agricultural Practice and lists of MRLs for a wide range of pesticide/food combinations are published from time to time. It must be emphasised that the MRL should not be considered as a measure of safety and this applies particularly to commodities such as cereals where any attempt to reconcile MRL and ADI must take into account degradation of the pesticide during storage of treated grain (Chapter 5) and losses of pesticide which can occur during processing (Chapter 6). Many countries which have established legislation to control pesticide residues in food have adopted the Codex MRLs as a basis for control. However, even though an MRL has been recommended by CAC it does not follow that it is appropriate to, or accepted by, all countries. National patterns of diet, agricultural practices and attitudes to the presence of pesticide residues in foodstuffs have resulted in the adoption of MRLs significantly different (and usually lower) from those recommended by CAC.

In 1986 the European Commission published a Directive (Anon., 1986a) in which limits were set for certain pesticides in cereals and the UK was obliged to implement these by the end of June 1988. It may be noted that certain pesticides (e.g., pirimiphos-methyl) used in the UK to control infestation in stored cereals are not included in the list and that limits are set for a large number of

pesticides not permitted in the UK for use on cereals, although some may be used on grain storage structures.

Until recently, control of pesticides in the UK had been supported by a voluntary agreement between Government Departments and the Agrochemical Industry (the Pesticide Safety Precaution Scheme). For reasons not relevant to this review, it was decided that this arrangement was no longer acceptable and that a statutory system would be introduced. The Food and Environment Protection Act was introduced in 1985 and provisions for the control of pesticides were made in Regulations under Part III of the Act. The Control of Pesticides Regulations 1986 set out the requirements for the storage, sale, supply and use of pesticides and the Pesticides (Maximum Residue Levels in Food) Regulations 1988 specify statutory MRLs for 64 pesticides on 39 commodities including raw cereals (wheat, rye, barley, oats, maize and rice). These MRLs come into force in two parts. Those which the UK was obliged to introduce under EEC Directive 86/362 and amendment 88/298 came into force on 29th July 1988. The remainder, which refer to MRLs specific to the UK, come into force on 31st December 1988. These MRLs additional to the EEC Directive refer to pesticides which have (a) been refused approval in the UK but may be used elsewhere, where the MRL is set at a level low enough to preclude use (generally the limit of determination) and (b) been consistently found in UK monitoring where the limits provide a check that good agricultural practice is being observed. Responsibility for complying with the MRLs lies with the user of the pesticide but, provided only approved substances are used according to their conditions of clearance, residue data (Chapter 4) indicate that there is no general cause for alarm within the agricultural industry.

In cereals, excluding rice, the MRLs (mg/kg) are as follows:-

ALDRIN and DIELDRIN	0.01
CAPTAFOL	0.05
CARBARYL	0.5
*CARBENDAZIM	0.5
CARBON DISULPHIDE	0.1
CARBON TETRACHLORIDE	0.1
CHLORDANE	0.02
*CHLORPYRIFOS METHYL	10
DDT (TOTAL)	0.05
DIAZINON	0.05

DICHLORVOS	2
ENDOSULFAN	0.1 (except maize, 0.2)
ENDRIN	0.01
ETHYLENE DIBROMIDE	0.05
*ETRIMFOS	10
*FENITROTHION	10
HEXACHLOROBENZENE	0.01
$\alpha$ + $\beta$ -HEXACHLORO CYCLOHEXANE (HCH)	0.02
$\gamma$ -HEXACHLORO CYCLOHEXANE (HCH)	0.1
HEPTACHLOR	0.01
HYDROGEN CYANIDE	15
HYDROGEN PHOSPHIDE	0.1
INORGANIC BROMIDE	50
*MALATHION	8
MERCURY COMPOUNDS	0.02
*METHACRIFOS	10
METHYL BROMIDE	0.1
PHOSPHAMIDON	0.05
*PIRIMIPHOS-METHYL	10
PYRETHRINS	3
TRICHLORPHON	0.1

A distinction can be drawn between those pesticides (indicated with an asterisk) which are approved for use on grain in the UK for which the MRL is set on the basis of Good Agricultural Practice and many of the others for which the MRL is set at the limit of determination.

### 3 METHODS OF ANALYSIS

Reliable data on the incidence and levels of pesticide residues are essential for recognition and control of potential food safety problems; establishment and enforcement of statutory or recommended limits therefore depend on the availability of suitable analytical methodology.

#### **Good analytical practice**

Residue analysis requires a high standard of analytical practice because of the sensitivity of the methods employed. Traces of contaminants such as greases and plasticizers in the final extracts can lead to serious interferences when working at this sensitivity and must be avoided. Thus, reagents and solvents need to be of high purity, plastic apparatus and stopcock grease should not be used, glassware should be scrupulously clean and careful clean-up of the grain extract should be performed.

It is also important to avoid losses of residues during storage and analysis of samples with particular reference to possible evaporation of volatile compounds during concentration of extracts. Analytical methods must be thoroughly evaluated before adoption and subjected to regular collaborative testing; particular attention needs to be given to recoveries, blanks, standard responses and the analysis of check samples.

#### **Limit of detection**

The limit of detection is defined as that concentration of a substance which gives rise to a measurement signal which is significantly different from the background signal. Limits of detection enable finite tolerances to be established but these may be lowered if more sensitive methods are devised or new toxicological data obtained. During the twenty years following the Second World War the limits of detection for pesticide residues had been lowered by a factor of 250 million (Zweig, 1970). Since then the emphasis has shifted towards obtaining more specific rather than more sensitive methods as it is often difficult to determine the toxicological significance of minute levels of pesticide residues (Zweig, 1978).

#### **Extraction and clean-up**

The first step in a pesticide analysis is efficient and selective extraction of the pesticide from the grain using an appropriate solvent. The extract invariably contains other substances which could interfere with the subsequent analysis so one or more clean-up stages are necessary. This general procedure is common

to all pesticide analyses (Williams, 1984) and is often the most time-consuming part of the analysis. Clean-up typically involves partition between immiscible solvents and column chromatography; nowadays disposable cartridges are commercially available which render the chromatographic clean-up more rapid and convenient (Young, 1984). The purified extract must now be concentrated to small volume by evaporation of the solvent without concurrent loss of volatile pesticides; special apparatus has been designed to accomplish this.

For analyses involving high performance liquid chromatography (HPLC), a totally automated method for extraction, clean-up and final analysis has been developed (Harvey & Zweig 1980). Automation of sample preparation has also been accomplished using gel permeation chromatography (Andersson & Ohlin, 1986).

#### Detection and determination

Pesticides tend to be analyzed in five distinct groups having common structural features or similar physical properties: organochlorine, organophosphorus, fumigants, carbamates and pyrethroids. The most widely used method for their analysis is gas-liquid chromatography (GLC) (Dickes & Nicholas, 1978; Young, 1984).

Chromatography separates the components in a mixture by virtue of their different affinities for two substances, one being stationary and the other mobile. When the stationary one is a liquid and the mobile a gas, the technique is called GLC. Fig. 1 shows the essential features of a gas-liquid chromatograph. The carrier gas is conveyed to the chromatograph from a cylinder at constant flow rate and a small aliquot of the sample extract is introduced through the injection port. The injection port is heated so that the sample is vaporized and the carrier gas conveys it towards the column. The column is enclosed in an oven at a temperature such that the sample remains vaporized during its passage through the column. The conventional type of column comprises a glass tube in the form of a spiral between 1 and 3m long packed with a finely divided solid which has been coated with a thin film of the liquid. Here the separation takes place by the column packing selectively retarding some components more than others so that the individual components emerge from it sequentially. A detector monitors the presence of these components in the gas stream by means of a suitable physical property which can be converted into an electrical signal. These signals are then amplified and displayed on a chart recorder in the form of a series of peaks plotted against time; such a plot is referred to as a chromatogram. The

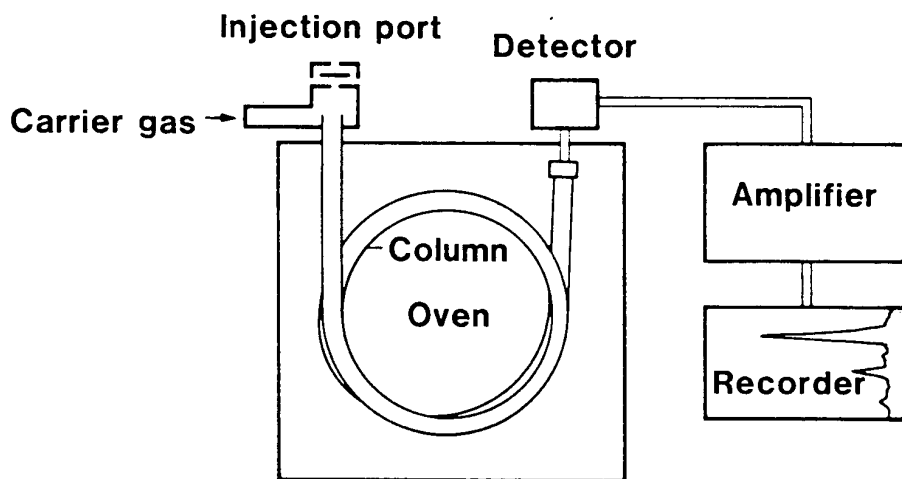


Fig. 1 Components of a gas-liquid chromatograph

MAFF Committee on Analytical Methods (CAM) has published reports on GLC methods for organophosphorus pesticides (CAM, 1973; CAM, 1980) and fumigants (CAM, 1974; CAM, 1976) in grain. CAM has also investigated a method for the simultaneous determination of organochlorine and organophosphorus residues (Becker, 1971) and found it to be satisfactory when applied to grain.

The capability of a GLC column to resolve components is partly limited by its length which in turn is determined by the back pressure experienced in maintaining reasonable carrier gas flow rates. In a packed column the stationary liquid is coated onto a porous solid support and the column length is typically 1.5m. The use of an open tubular (capillary) column in which the liquid is coated as a thin film on the inner wall allows column lengths of 25-100m with commensurate increase in resolution (Bottomley & Baker, 1984; Andersson & Ohlin, 1986).

The sensitivity of GLC in analysis of organochlorine compounds, fumigants and carbamates stems from the use of the electron capture detector, which, however, is not entirely specific. Recently, the resolving power of capillary GLC has been combined with mass spectrometry to produce a method which is both sensitive and unequivocal (Gilbert *et al*, 1985). For the organophosphorus pesticides, a phosphorus-specific detector is used, which is 1000 times less sensitive than the electron capture detector. Hence limits of detection for

organophosphorus pesticides are higher than for organochlorine pesticides.

HPLC (the principle of which is described in Part 2 of this review), has been used for the analysis of carbamates (Lawrence, 1976; Harvey & Zweig, 1980) and pyrethroids (Bottomley & Baker, 1984). However, HPLC is less sensitive and less specific than GLC and many analysts prefer to prepare volatile derivatives for GLC. Newer developments in HPLC of pesticides include attempts at improvement of sensitivity and specificity by employing electrochemical and fluorescence detection (Harvey & Zweig, 1980).

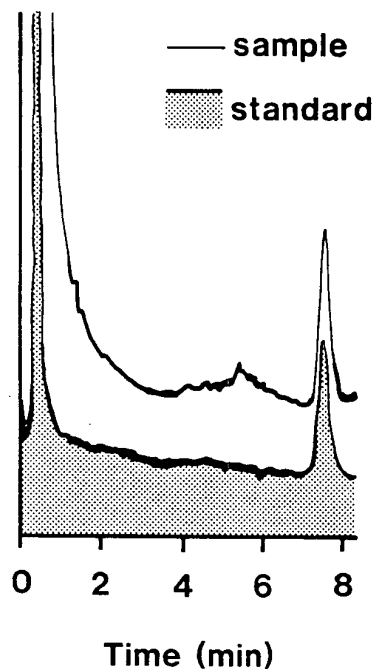
Thin-layer chromatography (TLC) - the principle of which is described in Part 2 of this review - has generally been regarded as a confirmatory method but the use of high performance TLC with *in situ* fluorimetry (Harvey & Zweig, 1980) may generate revived interest in TLC techniques.

Immunoassay techniques (Harvey & Zweig, 1980) appear to offer potential for more cost-effective screening if a number of samples are to be analyzed for a few pesticides but they are unlikely to replace established chromatographic methods for surveillance studies. There is, however, a need for rapid and inexpensive field tests of the dipstick type. Such tests are being developed in the United States and one example is a ticket for detecting gross contamination of grain with malathion and some other organophosphorus pesticides (Anon., 1986b). Unground wheat is shaken with water and one end of the ticket is then dipped into the water. The ticket is folded over to bring the end exposed to the grain extract into contact with the selective enzyme detector and a blue colour develops if no pesticide is present. This test can detect malathion if present at levels of 5 mg/kg or above which is sufficient to establish compliance with the UK statutory MRL of 8 mg/kg. None of the much smaller levels found in samples analyzed conventionally would be detected using the ticket and it is only applicable to two of the 33 pesticides for which UK statutory MRLs have been fixed. Nevertheless, more such tests will undoubtedly be developed in the future which will open up the possibility of farm-gate monitoring. In the meantime, the only methods available are the expensive and time-consuming laboratory tests previously described.

#### Confirmatory techniques

It is imperative for the generation of reliable data on the incidence and levels of pesticide residues that the identification of a residue be as unequivocal as possible. In GLC, identification of components is based on measurement of the

time elapsed between injection of a sample and peak maximum, the retention time.



*Fig. 2 Gas liquid chromatogram showing the presence of pirimiphos methyl in a sample compared with a standard*

The retention time is characteristic for each component though not uniquely so; it is quite possible for several compounds to have the same retention time under a given set of conditions. Nevertheless, with some knowledge of the sample and what it is likely to contain, it is possible to identify the components present by comparing their retention times with those of known compounds (Fig.2).

There are several accepted means of improving confidence in identification, the first of which is co-chromatography. This refers to the technique of adding a small amount of the suspected pesticide to the sample and repeating the chromatography. If the identification were correct the peak would simply increase in size, but if not, a new peak would appear on the chromatogram. The next method is to carry out GLC using two stationary liquids of widely differing properties when the probability of two separate compounds



having identical retention times is greatly diminished. Additionally, element-specific detectors confer enhanced confidence in identification. Alternatively, a completely different form of chromatography (e.g., TLC) may be used. Mass spectrometry, reviewed by Gilbert *et al* (1985) offers an unequivocal means of identification of residues and its use is recommended wherever possible. However, the equipment is very expensive and requires a high degree of technical knowledge to interpret the findings correctly; therefore it is not available to all residue analysis laboratories in the UK. The availability of lower cost bench top mass spectrometers may, however, help to alleviate this problem. Recent advances in Fourier transform infrared spectroscopy (Zweig, 1978) allow this to be used as an alternative to mass spectrometry as a confirmatory technique, although it is not unequivocal.

#### **Conclusion**

The analysis of pesticides in food is a complex and expensive process and the availability of simpler methods would be highly desirable. However, despite the emergence of new approaches in HPLC, TLC, Fourier transform infrared spectrometry, fluorimetry and immunoassay, GLC combined with mass spectrometry remains the only unequivocal method for ultratrace levels of residues.

#### 4 PESTICIDE RESIDUES IN UK WHEAT

The MAFF Panel on Residues of Pesticides in Foodstuffs, which became the Working Party on Pesticide Residues in 1977, has carried out extensive surveillance for residues in food and FMBRA has taken part in this work since 1970 by carrying out surveys on wheat and its products. These surveys have shown that despite the widespread use of pesticides during grain cultivation and storage, residues in wheat at the mill and products derived from it are low and do not give cause for concern. This section of the review summarizes the available data on pesticide residues in UK home-grown wheat, categorized by class of pesticide; there are no data on other home-grown cereals.

A computerised search of the literature has been carried out to determine the extent of published data on pesticide residues in UK cereals. Relevant data bases have been searched using Dialog covering 1972 to 1988. A preliminary search restricted to UK-specific data was not productive and a more comprehensive search was carried out without this restriction. The number of items abstracted was 547 and these have been checked to determine if any relevant papers had been overlooked. No references other than those listed in the review were found. The full listing from the search is provided with the report but as a separate item.

##### **Organochlorine pesticides**

The organochlorine pesticides are highly persistent in the environment and, when they do degrade, their residues are as toxic as, or even more toxic than, the original pesticide. For this reason they have declined in popularity and therefore have received less attention in surveys than the organophosphorus pesticides. Nevertheless, three separate surveys of UK wheat for organochlorine pesticide residues have been carried out. All of these were organized along similar lines which involved sampling from mills, geographically distributed around the country, with the co-operation of the National Association of British & Irish Millers. Hart and Willis (1973) found that all of 32 samples analyzed contained  $\gamma$ -HCH at levels in excess of 0.002mg/kg, although only one exceeded 0.02mg/kg. In 1978/79 a survey was designed by the Working Party to determine residues at an early stage of storage (October) and at a later stage (March/April). 38 of 84 samples analyzed in October and 33 of 71 samples analyzed in March/April contained  $\gamma$ -HCH in excess of 0.002mg/kg; other organochlorine pesticides were not detected (Bailey *et al*, 1982). It is not surprising there was no significant difference in the results obtained on the two occasions because of the

persistence of  $\gamma$ -HCH.

The sophistication of current analytical techniques enables the presence of minute quantities of residues to be detected. Since the significance of these quantities is not so readily determined, it is usual to adopt reporting limits. Current recommendations on the calculation of reporting limits (Keith *et al*, 1983) suggest on the basis of unpublished data obtained at FMBRA that the reporting limit for  $\gamma$ -HCH should be 0.01mg/kg. This is still at least an order of magnitude below both the Codex (0.5mg/kg) and EEC (0.1mg/kg) MRL for wheat. In the most recent work carried out at FMBRA (Osborne *et al*, 1988), only 6 out of 40 samples were found to contain lindane at this reporting limit and, again, other organochlorine pesticide residues were not detected.

#### Organophosphorus pesticides

Organophosphorus pesticides degrade rapidly in the presence of water and may remain in cereals after application from a few days to about twelve months. Because of a lack of methodology for breakdown products such as malaoxon, analysis has been concerned only with the parent pesticides.

Table 1  
*Results of surveys for organophosphorus pesticides  
in UK home-grown wheat*

Date	Number	Number of samples with detectable residues				
		P	C	M	E	F
1970/71	40	-	-	9	4	-
1978	84	0	0	1	0	0
1979	71	1	0	4	0	0
1982	139	53	6	0	0	5
1987/88	257	44	3	2	2	0

P	=	Pirimiphos-methyl
C	=	Chlorpyriphos-methyl
M	=	Malathion
F	=	Fenitrothion
E	=	Etrimphos

References: 1970/71 (Hart and Willis, 1973); 1978/79 (Bailey *et al*, 1982); 1982 (Anon, 1986 c); 1987/88 (Osborne *et al*, 1988).

The results of analysis of 591 samples of UK home-grown wheat which have been analyzed since 1970 are given in Table 1. The reporting limit has remained at 0.1 mg/kg for all the organophosphorus pesticides. The predominance of pirimiphos-methyl during the past six years is noticeable and reflects the increased use of this pesticide, superceding malathion in the UK as a grain protectant during storage, and its relatively greater persistence. The levels of residues found vary over a wide range but for the majority of samples these were only just above the reporting limit. With the single exception of a wheat from the 1982 survey found to contain 9.6 mg/kg of pirimiphos-methyl, all the levels found were at least an order of magnitude below the appropriate MRL.

#### **Volatile fumigants**

It might be assumed that fumigants such as carbon tetrachloride would not give rise to residues on account of their volatility. Modern analytical techniques have, however, revealed that minute residues do occur. In surveys carried out by the Working Party between 1978 and 1982 (Bailey *et al*, 1982; Anon, 1986c) a total of 294 home-grown wheats were analyzed for carbon tetrachloride, carbon disulphide, ethylene dichloride, ethylene dibromide and 1,1,2-trichloroethylene. Only carbon tetrachloride was detected, in 15% of the samples, although a single sample contained carbon tetrachloride (4 mg/kg) in admixture with ethylene dichloride (290 mg/kg) and 1,1,2-trichloroethylene (0.3 mg/kg). Even discounting this exceptional sample, levels of carbon tetrachloride were as high as 1 mg/kg which although well within the Codex guideline limit (50 mg/kg) would have exceeded the UK statutory MRL (0.1 mg/kg) were it in force at that time. However, Osborne *et al* (1988) found that none out of 28 samples examined recently at FMBRA contained carbon tetrachloride in excess of 0.01 mg/kg.

#### **Inorganic bromide**

The inorganic bromide ion is a normal constituent of the human diet and is naturally present in grain owing to its uptake from the soil. However, the use of methyl bromide as a fumigant gives rise to increased bromide levels in treated wheat. Bailey *et al* (1982) analyzed 16 samples of fumigated home-grown wheat for bromide content with the result that levels were found to range from 0.6 to 2.7 mg/kg compared with the MRL of 50 mg/kg.

### **Synthetic pyrethroids**

There are no data available on residues from pyrethroid insecticides in home-grown cereals.

### **Conclusion**

Less than 600 samples of UK home-grown wheat and none of other cereals have been analyzed for pesticide residues since 1970. The majority of these samples were examined for organophosphorus pesticides only. Although a significant number of the samples analyzed contained residues, in only two cases did these exceed or approach the appropriate MRL.

## 5. THE BREAKDOWN OF RESIDUES IN GRAIN AND INTERACTION BETWEEN DIFFERENT PESTICIDES OR BETWEEN PESTICIDES AND GRAIN COMPONENTS

The degradation and metabolic fate of pesticide residues in stored cereal grains (mainly wheat) has been the subject of a number of critical reviews (40, 41, 42, 44) and forms a major part of a recent book on Grain Protectants (Snelson, 1987). The sections of this book dealing with the use and properties of 23 pesticides (excluding fumigants) are unique in quoting data from residue and metabolism studies conducted by the manufacturers of these compounds for registration purposes under Codex Alimentarius arrangements. These studies have not been published in the open scientific press and have not therefore been subjected to the impartial scrutiny and refereeing inherent in normal presentation of research to the scientific community. Apart from this necessary proviso Snelson's book is the most valuable compendium yet produced on all aspects of grain protectants, and on residues and metabolism in particular.

### The occurrence of residues in grain

With insecticidal treatment of growing crops the residue at harvest is not wanted and is ideally nil. Data on pesticide residues present in harvested grain going into store are very few, but the amounts are popularly supposed to be negligible.

In applying a protectant to grain in store, however, there is deliberate contamination. The pesticide is required to persist for sufficient of the storage period to control insect and mite pests, but should gradually break down, so that residues are substantially reduced at time of sale for food processing or export.

The weathering and leaching effects which decrease residues in field crops do not apply in grain stores, and the relationship between dose applied and residue should therefore be more direct and predictable. Three stages pertain: (i) dose applied; (ii) dose achieved (immediate residue recovered by analysis at time of treatment); and (iii) aged residue (i.e., that residue persisting after a given storage period). The first is measured by the applicator diluting and applying the pesticide; the second and third are assessed chemically and by insect mortality, and are a measure of both success of the treatment and effectiveness of the pesticide.

Grain protectant treatments diminish by redistribution, evaporation and chemical or biochemical breakdown in store, or by screening, washing, milling, digestion and cooking operations during the processing of raw grains into animal or human food or beverages (Desmarchelier *et al.*, 1980, Wilkin and Fishwick, 1982).

The chemical breakdown is enhanced with increase in grain moisture (equilibrium relative humidity) and with temperature. (See Table 2).

*Table 2 Approximate time in store (months) to reach 50% of the applied dose*

Grain moisture:	12%		15%		18%	
Grain temperature (°C):	10	25	10	25	10	25
Insecticides:						
malathion	14	12	12	10	8	4
pirimiphos-methyl	>36	>36	>36	30	24	20
chlorpyrifos-methyl	>36	30	30	24	24	12

(Data from Rowlands (1986))

The implications of this are examined in the reviews by Rowlands and by Snelson cited above. Under most circumstances, protectants degrade in a predictable manner, and this has been developed by Desmarchelier *et al.*, (1980) into a useful set of mathematical formulae.

This present chapter is concerned mainly with the processes within the grain itself; but first there is need to define what constitutes a residue.

#### Breakdown of residues in grain

In the context of breakdown of pesticides in grain, "residue" is defined rather differently from its use in surveillance and efficacy studies. In the last two cases, the term residue usually refers to what is recoverable of the parent compound only, and often simply to that portion of the parent compound that is recovered by the extraction procedures of the recommended analytical methods, probably

some 70-90% at best. Since it is well established (Desmarchelier, 1986) that some days after application, the extraction of "aged" residues becomes increasingly difficult, it is easy to see that this concept of "residue", as a measure of the extractable and analyzable parent compound only, is rather simplistic. In breakdown/metabolism studies, "residue" is taken to mean those same amounts of recoverable parent compound, plus any metabolites or decomposition products of that compound that have occurred on the surface of, or within, the grain, plus any products formed within the grain by the parent compound or its metabolites reacting with natural constituents of the grain, such as fats, protein, waxes, cellulose etc., plus any amounts of the parent compound or metabolites that are not removed by conventional solvent-extraction techniques and which may be "bound" or held in some way as to be "unavailable". During storage, when metabolism, translocation and binding of pesticides are occurring in the grain, these are known as intermediate residues. When a state of equilibrium has been reached, or at the end of a defined storage period, these multi-component end-products remaining in the grain are known as the terminal residues. Studies on the breakdown of pesticides on grain during storage should endeavour to cover these many aspects, and it has to be said that very few such studies are undertaken. Those that have been, are critically reviewed in the papers by Rowlands (1967-86) and studies by the pesticide manufacturers in similar vein for registration are summarized in Snelson's book (1987).

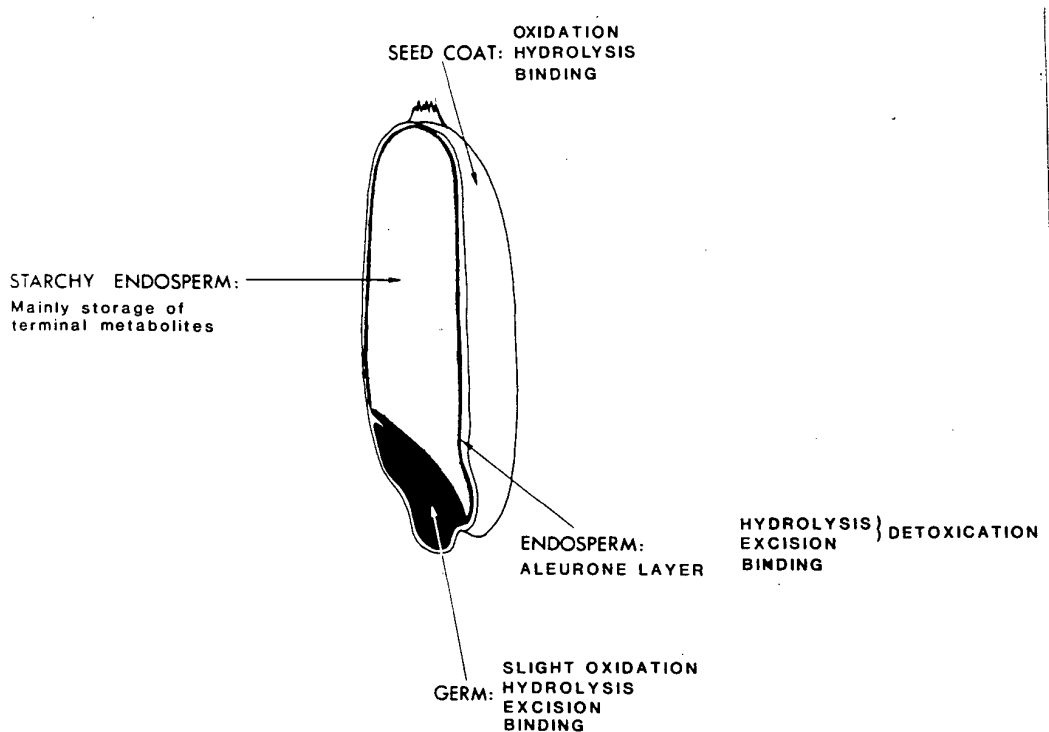
The majority of studies where sequential loss of protectant pesticide during storage is observed, content themselves with measuring the apparent loss of the parent compound, and even then there are serious misunderstandings about what is actually being reported. It is common to imply a loss of pesticide and to ascribe it to breakdown or to poor application, when it is simply not extracted. There are an increasing number of studies (notably from Australia) where the dose actually achieved on the grain at the time of application is not measured. Instead, the calculated application rate is assumed to have been achieved which masks the real degradation rate. If the calculated dose had not been obtained, then subsequent residues determined will tend to suggest that more degradation has taken place than is actually the case. Data on the doses achieved by farmers and storekeepers in practice are virtually non-existent, yet should be a matter for concern when perhaps 50-70% of the total weight of pesticide applied is not accounted for (Wilkin, 1985). In addition there is sufficient evidence of uneven dosing to cause concern. When a parcel of grain (exceeding 5,000 tonnes) was sampled at several points across the bulk, with each sample being the aggregate of samples taken at three depths, the results



varied from 0.3 to 7.4 mg/kg.

Factors which can contribute to uneven and inaccurate dosing of cereals and to a real or apparent loss include: poor stability of formulation, inadequate mixing of diluted emulsion, uneven flow-rate of grain or of the pesticide spray during treatment, spray drift (often onto neighbouring bins of untreated grain) or losses (primarily of dust formulations) during conveying of the treated grain. However from the standpoint of this chapter, there is a need to consider only the pesticide that reaches the grain, and into the grain tissues. Pesticide reaching the grain can be "lost" in several ways, but the most obvious loss (apart from frictional rubbing-off of deposit) is by rapid chemical breakdown of pesticide applied to grain coming warm and wet from the harvest field or direct from hot air driers.

#### Uptake and penetration of pesticide



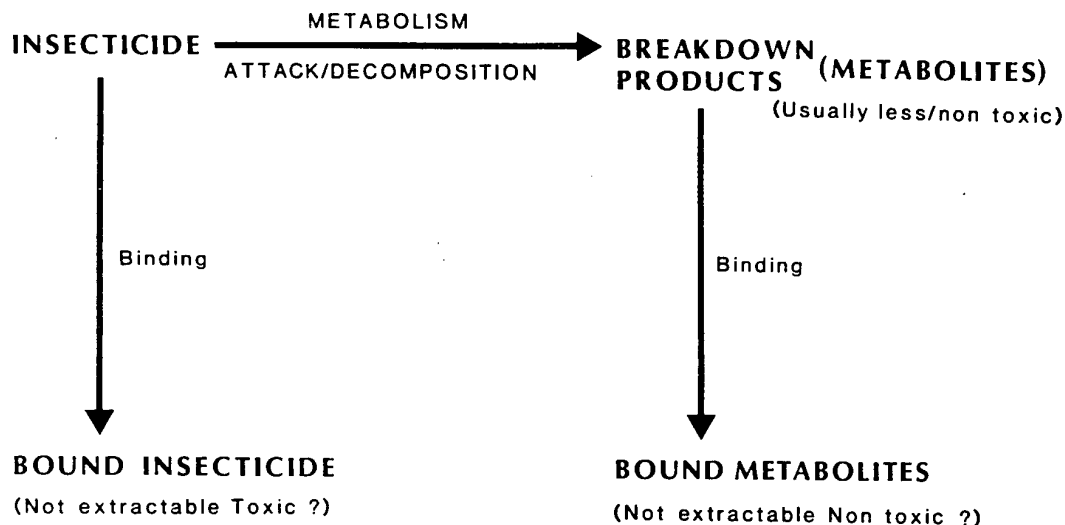
*Fig.3 Regions of insecticide metabolism in stored grains*

The rates at which insecticides penetrate into stored grain affect their intermediate metabolic fate and also the persistence of their residues since different

mechanisms of metabolism operate in different regions of the grain (Fig. 3). In addition to the grain *per se* the seedcoat contains a microflora: the so-called "field" fungi (surviving from growth of the seed) and the "storage" fungi (Christensen, 1985). The former do not develop in harvested grain and probably have little effect on the translocating pesticide; the latter develop readily at the higher moisture levels found in stored grain (>15%), and contain enzymes similar to those occurring in the grains and will undoubtedly play some role in the sequential degradation of pesticides present in the seed. It is difficult to separate the roles of grain and fungal enzymes experimentally, without drastically altering the nature of the grain, but Anderegg and Madisen (1983a) attempted, with some success, to assess the contribution of the microflora of maize and wheat in degrading malathion residues present in the grains.

Pesticides are normally applied in formulation, as emulsions or as dusts. The rate of uptake from different formulations varies considerably in the first few hours after treatment, but in laboratory-scale investigations the overall distribution in grain tissues (milling fractions) of pesticides and their metabolites changes very little after 7-14 days and on the tonne-scale appears to be stable after a month or so (Rowlands, 1986). Passage of sprayed-on pesticide through the seedcoat rapidly breaks the emulsion and causes partitioning of the ingredients. Uptake from dust is initially slower, more remaining on the grain surface to contact wandering insects, or to be lost by friction. Some uptake by the grain will be in the vapour phase, and indeed it may be that the intergranular vapour concentration of unabsorbed pesticide is an important aspect of pest control (Desmarchelier, 1986). Most uptake of vapour by grains seems to occur in the germ region and where formerly the grain was attached to the plant (Rowlands, 1971).

Nature of degradation (See Fig. 4)



*Fig. 4 Some routes of insecticide breakdown  
in stored grains*

Degradation involves several types of chemical reaction: (1) oxidation (combination with, or addition of oxygen to the molecule) which can be a toxicating process in the case of some organophosphorus insecticides, but is more usually a prelude to detoxication and greater decomposition. (2) Hydrolysis (decomposition involving water) which is almost invariably a detoxication process, and (3) excision reactions involving the removal of specific chemical groups, such as dechlorination or decarboxylation. A much lesser role is attributable to reduction (the opposite of oxidation) which normally takes place only in absence of air (oxygen) and is not of significance in pesticide breakdown in stored grain. Under most circumstances, therefore, it is safe to assume that once inside the grain, any alteration to the pesticide (breakdown) will be beneficial to the consumer in reducing the intrinsic toxicity. There are very, very few exceptions to this (Rowlands, 1971).

It can be seen from Fig. 3, that most regions of the grain, but particularly the aleurone layer of the endosperm, offer the prospect of hydrolytic attack, but that only the seedcoat and the germ have oxidation potential. Compounds that are slow to penetrate the seedcoat or germ to reach the starchy endosperm will therefore have greater opportunity than those that penetrate rapidly,

to be oxidized by the enzymes concerned in the ripening and hardening processes of the coat and present in the microflora and in the germ tissues. An apparent rapid loss of pesticide within a few hours of treatment will occur where a pesticide gets rapidly into the seedcoat but not through it. This can only be accepted as an explanation of the phenomenon when the conversion (metabolic) products of the pesticide can be identified and quantified (Rowlands, 1986). Such initial products may well be oxidative in nature (especially with freshly-harvested grain). Equally rapid breakdown may occur from rapid penetration through the seedcoat into the aleurone layer. The products would then be those from hydrolysis or excision.

Rowlands (1971) has described enzymes within the grain tissues encountered by penetrating insecticide in some detail. It is worth reiterating that the aleurone layer constitutes the most important tissue concerned in pesticide degradation and reaction. It is within this layer that much of the identifiable degradation within the grain occurs, and there is active transport between this layer of cells and both germ and endosperm. The starchy endosperm indeed forms a storage region for breakdown products that are not metabolized further (such as phenols from organophosphorus compounds like fenitrothion and bromophos), while the original pesticide remaining is subjected to other physiological processes.

Some of the products of these reactions and processes will be "free" or "available". That is to say the analytical chemist can easily extract, identify and quantify them without resorting to drastic methods. They will also be available to the consumer, be he mammal or insect. But the longer a pesticide is within the grain, the likelihood increases that the residue will become "bound" or in some way difficult to extract (Sampson, 1986).

#### **Ageing and other processes**

Many of the altering or attacking processes with which the penetrated pesticide can be involved, result in "binding" of the compound (or a metabolite) to cellular tissue in some form or another, or in dissolution by the cell contents as a result of conjugation. These processes need a little further definition.

By "ageing" of a pesticide within grain, is meant both a gradual loss of its effectiveness at killing insects, and an increased difficulty of detecting (or extracting) it with time. The loss of toxic effect may be due to alteration of the compound or to it being unavailable to insects because of penetration,

translocation or binding.

"Binding" implies that the pesticide is held by the tissues or structure of the grain. This might be a result of the biochemical process of conjugation (where the molecule becomes united to another - often larger - molecule, such as protein, amino-acid, starch or fat) or from some direct chemical link with structural material such as cellulose. Rowlands (1975) and Takimoto *et al*, (1978) have demonstrated release of bound insecticides or insecticide metabolites from grains by digesting with amylase, thus confirming the role of the starch in these reactions.

All these processes have been shown to occur with pesticides applied to grain, and some effort is at last being directed toward an understanding of unextractable or otherwise "bound" residues, in order to understand their nature and toxicological importance (Matthews, unpublished data). It may be that they can still affect pest or consumer if that organism digests the food in such a way as to release the toxic moiety. Work by Anderegg and Madisen (1983b) and Matthews (unpublished data) has suggested that such bound residues may delay development of insect pests inside grain without killing them.

A few recent studies of the breakdown of pesticides in stored grain have demonstrated (Table 3) that some 10-50% of the applied dose may be present in bound form as part of the terminal residue, and that this will probably have been wrongly attributed to loss or degradation since the recommended analytical procedures will not have extracted this portion.

*Table 3 Proportions of bound and free pesticides  
occurring during storage*

Pesticide on grain	Storage period months	% Free (extractable)		% Bound	Ref
		parent	metabolite		
chlorpyrifos	5	70	3	15	)
methyl	14	60	5	29	)
					) (a)
malathion	5	16	25	42	)
malathion	12	23	23	47	)
					) (b)
fenitrothion	12	26	68	6	)
pirimiphos	6	75	3	13	(c)
methyl	6	60	18	15	(d)

Refs: (a) Matthews (unpublished data); (b) Takimoto *et al* (1978)  
(c) Leahy & Curle (1982); (d) Rowlands (1981)

#### **Pesticide interactions with natural products**

Pesticides kill by causing physiological damage to the pest organisms, often by reacting with the protein of essential enzymes and rendering them ineffectual. Given that many pesticides react with protein, amino-acids, starches, sugars, celluloses and fats, it is not surprising that they also react with grain constituents such as cell walls, and with grain enzymes associated with those cells, such as amylases important in breadmaking and malting (Matthews, unpublished data). Pesticides will also inhibit those cereal enzymes that catalyze the oxidation or hydrolysis of the pesticides themselves (Rowlands, 1971). All of which makes for a very complex situation.

#### **Interaction with foreign compounds**

Foreign compounds other than the pesticide intentionally admixed may already be present in the grain as a result of pre-harvest treatment, anti-microbials, or another post-harvest treatment. Large parcels of grain being traded on the markets today, may well be treated prophylactically with the same or other insecticides each time

they enter a different store; thus leading to multiple or cumulative residues, the dangers of which are self-evident. It is not clear to what extent FEPA legislation will alleviate this problem, if at all.

The pesticides themselves may interact to produce an increased toxic hazard to the consumer (synergism), or to prolong the residual life of either or both components; usually by inhibiting the enzymes that normally degrade them. This topic has been thoroughly reviewed by Rowlands (1971) but little recent attention has been paid to such problems. At various times multiple admixture treatments are recommended or used to control a wider range of pests than usually cause problems in the UK, most commonly in Australia and notably with *R. dominica* (Bengston *et al*, 1983).

Nowadays such joint admixture frequently involves an organophosphorus compound and a pyrethroid. In the UK a mixture of malathion and lindane was formerly marketed for control where mites and insects were a problem, but the newer insecticides control both effectively without recourse to mixing. Malathion and dichlorvos mixtures have been marketed (in Europe) to provide rapid disinfestation without long-term residues (the dichlorvos component) and a protective effect in store (malathion). This too, has been superseded. In Australia for some years, dichlorvos treatments were used on export grain at the outgoing docks to kill off any live insects that had survived malathion treatment through being resistant. This practice has also ceased with the advent of more stable compounds. No account was ever taken of joint toxicity or synergistic effects in recommending these methods. In fact, as Rowlands (1970) showed, the interaction between dichlorvos and (say) malathion within the grain is complex and the mammalian toxicity of the joint residue considerably greater than the additive toxicity of the two components.

#### Fumigants

The fumigants likely to be found in grain in the UK were reviewed in Chapter 4.

During the course of fumigation, vapour is absorbed by grain and by the fabric and structure of buildings. As fumigants are often employed at concentrations approaching their saturated vapour pressure, sorption of vapour under such circumstances can be considerable. With volatile compounds such as methyl bromide or phosphine, sorption is much lower and will depend on many factors including the properties of the compounds used, ambient temperature, the commodity being treated, its moisture or lipid content and its physical condition.

However after treatment with any fumigant, varying amounts of the parent-fumigant will be held strongly by the commodity for a considerable period after removal of vapour from the surrounding air space. The rate at which this vapour is lost will depend on the volatility and reactivity of the fumigant, temperature and the properties and composition of the fumigated product.

Residues remaining after treatment may be classified as (a) unchanged fumigant, physically bound to the commodity (b) simple reaction products (c) those due to modification of constituents of the fumigated products such as protein amino acids and vitamins or (d) other compounds present in the original formulation either as impurities or added intentionally, e.g. as warning agents. The relative importance of each type of residue will depend on the fumigant, but chemical reactivity can result in undesirable effects of economic importance such as the occurrence of off odours, taints and loss in the viability of seeds.

The reaction of liquid fumigants with grain components is in most cases slow or non existent, e.g. no reaction of carbon tetrachloride, ethylene dichloride or 1,1,1-trichloroethane with food constituents has been reported. However, breakdown during steam distillation of carbon tetrachloride residues from grain was found with the production of chloroform (Scudamore & Heuser, 1973). Reduction in viability of moist seeds after treatment with carbon disulphide has been reported (Hinds, 1917). However the germination of wheat, barley, millet and rice was not affected at normal fumigation dosages (Kamel & Shahba, 1958), although when carbon disulphide was used in a mixture with carbon tetrachloride some effects on germination occurred (King *et al.*, 1960). Reaction of carbon disulphide with proteins and amino groups in cereals might be predicted but no studies on this have been published. Ethylene dibromide at ambient temperatures breaks down slowly to produce ethylene glycol and small amounts of inorganic bromide. Reaction with cereal constituents is considered to be insignificant under normal circumstances although ethylene glycol may result from reaction with methionine in cereal proteins (Bridges, 1956; Obomucki & Bondi, 1955).

Phosphine is relatively unreactive and the main reaction products have been shown to be non-toxic inorganic phosphorus compounds (Robinson & Bond, 1970; Disney & Fowler, 1972). The most reactive of the fumigants still used on cereals is methyl bromide. However, when used as recommended, problems are seldom encountered but overdosing or misuse can lead to excessive reaction which may have important economic consequences. These include production of



off-odours or taints, e.g. in bread baked from fumigated wheat and flour, reduction in the viability or germination of seeds, or off-odours due to reaction with building or packaging materials such as foam rubber. Studies (Winteringham *et al*, 1955) have shown that the off-odours which may be produced in bread are due to reaction with sulphur-containing protein amino acids such as methionine which may subsequently release volatile sulphur compounds. Fumigation with methyl bromide invariably leads to an increase in bromide content of the commodity. Concern over bromide intake in the total diet of the population has led to recommended maximum residue levels for this in a range of commodities.

### Conclusion

The fate of pesticide residues resulting from direct admixture with grain has been a "Cinderella" among research topics. Yet the processes and complex interactions outlined above are fundamental to evaluating the safety of the admixture technique. The consequences both in terms of possible toxicity or long-term chronic effects on the consumer - human or animal - and also for the food processing industry could be serious.

Considerable amounts of pesticide applied to grain are regarded as "lost" or degraded, without any confirmation of the fact, because the conventional analytical techniques do not extract and identify them. In most cases these residues are still present in the grain, as metabolites or in a bound form, and it is necessary that they be identified, quantified and their toxicity assessed.

## 6. THE DEGRADATION OF PESTICIDES DURING MILLING AND, THE DISTRIBUTION OF RESIDUES IN MILLING FRACTIONS AND IN BREAD

### Fumigants

Fumigants absorbed in wheat or other cereal grains will be lost by both volatilization and chemical degradation during milling or cooking processes. The few studies carried out on persistence of fumigant residues during processing suggest that little or no fumigant persists as the unchanged compound in finished products.

When wheat treated at commercial application rates containing carbon disulphide, carbon tetrachloride, ethylene dichloride or ethylene dibromide was milled, residues in all cases were found to be highest in the bran fraction. Depending on losses due to the volatility of the compounds the levels in the bran could be higher or lower than in the original wheat (Conroy *et al*, 1957). Similar results were obtained with carbon tetrachloride and ethylene dibromide in wheat (Jagielski *et al*, 1978) and with 1,1,1-trichloroethane in wheat (Goodship *et al*, 1982). When wholemeal or white bread was prepared from these milled wheat samples only traces of the unchanged parent fumigant could be detected. The level of ethylene dichloride in bread made from white flour from treated wheat was generally below 0.5mg/mg (FAO/WHO, 1980). Rapid cooking of rolled oats containing residues of four fumigants resulting from commercial treatments did not completely eliminate the residues, although the short cooking period of 1 minute removed 88% of carbon disulphide, 69% of carbon tetrachloride, 51% of ethylene dibromide and 49% of ethylene dichloride (Munsey *et al*, 1957). Bread baked from flour fumigated with excessive dosages of methyl bromide may have a foreign odour and if the bread is toasted an unpleasant off-flavour may be produced. Brown *et al*, (1961) reviewed a number of reports of this taint encountered under commercial conditions.

The significance of the very low levels of fumigants that could in theory be ingested by humans is not clear. However, in the U.K. wheat is only occasionally treated with fumigants and nowadays this is unlikely to be with anything other than methyl bromide, phosphine, carbon tetrachloride or ethylene dichloride.

## Insecticides

A considerable amount of data relating to degradation of pesticide has been presented to the Codex Alimentarius Commission/JMPR. This has been part of the larger body of data on which current MRLs and ADIs have been determined. Much of these data were not published, or at least not readily available, until the recent publication of Snelson (1987). In the UK, studies such as those described by Bullock (1974) give data on residues of pirimiphos-methyl in stored grain, bread, flour and milled products. However this was not published in the usual way and was available only on request. Largely because of the lack of data and the involvement of the Slough Laboratory in formulating advice on the use of these pesticides to control stored product pests, studies were undertaken (Wilkin & Fishwick, 1982) to examine pesticide residues in wheat, milled fractions and bread.

The work can be divided into three main areas:- preliminary studies on wholemeal bread and flour, studies on the fate of residues in various milling fractions and white and brown bread, and an investigation into the distribution of residues in milling fractions produced by commercial milling.

(i) Batches of 'Flanders' English milling wheat were treated with one of the six pesticides under test, using commercial emulsifiable concentrates diluted in water. The dosage and application rates used conformed to those specified on the manufacturers' labels and are given in Table 4, page 36. The treated grain was stored under ambient conditions for varying periods before being sent for milling and baking. Milling and baking were carried out by FMBRA on 5kg samples of treated and control grain using standard laboratory methods. The wholemeal bread and flour were returned to the Slough Laboratory for analysis of pesticide residues.

(ii) Twenty-five kg batches of 'Flanders' English milling wheat were treated with one of the five pesticides under test. The treated grain was aged for 4 weeks under ambient conditions before being sent for milling and baking at FMBRA. Separate portions of each batch of the grain were milled by two separate processes: Wholemeal flour, white flour and milling fractions were produced and some of each batch of flour was then baked into loaves. The loaves and flour fractions were sent to Slough for analysis.

(iii) A twenty-five tonne batch of 'Flanders' English milling wheat was treated with chlorpyrifos-methyl at the intended dose of 4.5 mg/kg using an aqueous spray made up from a commercial emulsifiable concentrate. The grain was treated during transfer between bins so that the process closely followed

commercial practice. The treated grain was then stored for 20 weeks under ambient conditions before being despatched to a flour mill by lorry.

At the mill the grain was held as a discrete parcel. The milling machinery was cleaned as far as possible of other grain and flour and then the treated grain was passed through the entire milling process. This can be broadly represented by 3 stages a) screening, b) break and reduction milling, c) separating and sieving of the machine flours. When milling of the treated grain commenced the machinery was allowed to flush for 2 hours before samples of grain, screened grain, screenings, white flour, bran, offal and germ were collected. The residues in these samples were determined in the same way as in (i) and (ii).

The results of experiments (i) and (ii) are given in Tables 5 and 6 (pages 37 & 38 respectively). The figures given for flour, bran and offal are means of two determinations carried out on separate sub-samples but in the case of bread a representative sample was taken from each of 3 loaves and analyzed. The results from experiment (iii), which are means of 2 determinations, are given in Table 7, page 39.

There was some duplication between experiments (i) and (ii) as both assessed residues in wholemeal flour and bread. Generally, there was good agreement between the results from the two experiments.

Milling treated grain into wholemeal flour resulted in little loss of pesticide so that on average more than 90% of the residue on the wheat was retained in the flour. The levels of residues in white flour were much less at about 30% of the original applied dose. However, this reduction was not caused by pesticide breakdown during milling since proportionately higher residues were found in the bran and offal. Hence, the total pesticide surviving milling was always about 90% but its distribution was determined by the fractionation of the grist after milling. The levels of pesticide found in bran and offal were about four times higher than those in samples of whole wheat.

The single trial at a commercial flour mill indicates that the results from laboratory milling may lead to an underestimate of that proportion of pesticide in the grain which remains in white flour following the milling of the grain under commercial conditions. The survival of pesticide in the other fractions is not directly comparable as the commercial process separated the germ from the bran

and offal. The results suggest that levels in germ may be five times that in the whole grain. The sophisticated grain cleaning process in operation at this mill did not substantially reduce pesticide levels in whole grain and hence would not be instrumental in reducing levels in milling fractions. Although the material removed as screenings contained residues 8 or 9 times higher than levels in whole grain, the weight of screenings was very small and would not account for a significant proportion of the pesticide originally present in the grain.

Some loss of pesticide occurred during baking and residue levels in bread were about 50-70% of those found in flour (Tables 5 and 6, pages 37-38) taking into account the difference in moisture content between bread and flour.

The differences found between separate pesticides were generally no larger than the differences between results for any particular pesticide observed in experiments (i) and (ii). The variations are more likely to be attributable to sampling and analytical error than to fundamental differences between the behaviour of the individual pesticides during cleaning, milling or baking. The pesticide methacrifos posed a particular problem in that after treatment of the grain at the correct application rate residue levels in the treated grain were significantly lower, even where analysis was undertaken shortly after treatment.

Table 4 shows the approved rates for the pesticides and the rates recommended by the manufacturers together with Codex MRLs for comparison. The residues in bread and milling fractions produced from wheat containing pesticide levels close to those recommended by the manufacturers exceeded Codex MRLs in certain cases although the amount by which they were exceeded was generally small. Taking into account losses during treatment and storage, these application rates are unlikely to result in MRLs being exceeded in cereal products.

The reference to Codex MRLs is because the EC Cereal Directive proposals for UK Statutory limits do not list MRLs for cereal products.

Table 4 Codex MRL's and recommended application rates (mg/kg) for organophosphorus insecticides on wheat

	Chlorpyrifos-methyl	Etrinfos	Fenitrothion	Malathion	Methacrifos	Pirimiphos-methyl
Approved application rate	5	5	6	10	5	4
Manufacturers recommended application rate	4.5	4.5	6	10	4.75	4
<hr/>						
Codex	10	10	10	8	10	10
MRL	2.0	2.0	1.0	2.0	2.0	2.0
Whole grain	10	10	10	8	10	10
White flour	2.0	2.0	1.0	2.0	2.0	2.0
Wholemeal flour	-	10	5.0	2.0	10	5
White bread	0.5	-	0.2	-	-	0.5
Wholemeal bread	2.0	-	-	-	-	1.0
Bran	20	20	20	20	20	20

Table 5 Experiment (i) Organophosphorus insecticide residues (mg/kg) in wheat flour and bread

	Chlorpyrifos- methyl	Etirimfos	Fenitrothion	Malathion	Methacrifos	Pirimiphos- methyl
Approved application rate	5	5	6	10	5	4
Actual application rate	3.7	5.0	6.8	8.2	2.6	3.4
Storage period (weeks)	4	36	4	4	24	36
Residues						
Aged wheat	3.8	4.4	3.3	5.4	1.4	3.1
Wholemeal flour	3.6	4.6	3.0	4.1	1.3	2.3
Wholemeal bread	1.5	2.6	1.6	1.6	0.5	1.2

Table 6 Experiment (ii) Organophosphorus insecticide residues (mg/kg) in wheat, wheat milling products and bread

	Chlorpyrifos- methyl	Etrinfos	Fenitrothion	Methacrifos	Pyrimphos- methyl
Approved application rate	5	5	6	5	4
Whole grain	4.3	4.2	4.2	1.0	3.7
White flour	1.1	1.3	1.2	0.3	0.9
Wholemeal flour	3.8	4.3	4.0	1.0	3.4
White bread	0.5	0.6	0.3	0.1	0.4
Wholemeal bread	2.2	2.1	2.2	0.5	1.7
Bran	14	15	15	4.0	14
Offal	15	16	19	3.7	10



Table 7 Experiment (iii) Residues of chlorpyrifos-methyl in milling fractions obtained by milling treated wheat at a commercial flour mill

	Chlorpyrifos- methyl (mg/kg)	Ratio of residue level to residue level in wheat
Wheat in holding bin	2.3	1.0
Wheat after first cleaning	2.3	1.0
Wheat after second cleaning	2.1	0.91
Screenings	20	8.7
White flour	1.1	0.48
Bran	7.6	3.3
Wheat germ	13.0	5.6
Wheatings (Ofals)	6.5	2.8

## **7. RECOMMENDATIONS FOR FURTHER STUDY**

### **1. Methods of analysis**

1.1 A range of analytical methods exists for organochlorine and organophosphorus pesticides but these are slow and labour intensive. Therefore, the use of automated techniques for sample preparation, such as gel permeation chromatography, should be investigated.

1.2 Methods for other classes of pesticide have not been studied so thoroughly. In particular, there is a need for an improved method for the synthetic pyrethroids.

1.3 Analytical procedures should be developed for those pesticide breakdown products, such as malaoxon, which are included in UK statutory maximum residue limits.

1.4 There is an urgent need for simple, cheap and rapid methods of the dipstick type.

### **2. Pesticide residues in UK cereals**

2.1 There are insufficient data on the full range of residues for which UK statutory maximum residue limits have been specified for cereals. In particular, data on fumigant residues in wheat are sparse and there are none for carbaryl. There is a need to acquire residue data for home-grown cereals other than wheat.

2.2 Surveillance needs to be undertaken to establish the levels of residues arising from the use of synthetic pyrethroids in grain stores.

### **3. The breakdown of pesticides on grain and interaction between pesticides and grain components**

More work should be undertaken on identifying and understanding:

3.1 The consequences of pesticide interaction with the natural products of cereal grains, particularly those with implications for the food industry and the consumer such as the possible effect of methyl bromide fumigation on the germination of barley.

3.2 The nature and toxicity of bound residues.

**3.3** The intermediate and terminal metabolic products of the pesticides that accumulate within the grain.

**3.4** The effect of multiple or cumulative treatments on the rate of breakdown of the pesticides involved and on the toxicity of residues.

**3.5** The interaction between admixed insecticide and:

- (a) residues from pre-harvest treatments
- (b) fumigants applied before or after admixture
- (c) natural products in the grain, other than those of direct commercial concern

**4. The degradation of pesticides during food processing**

Although conventional dry cleaning of wheat prior to milling did not result in a reduction in pesticide levels, there is a need to determine whether alternative procedures, such as wet cleaning techniques and scouring, would be more successful.

**5. Application of pesticides to grain**

Improved application equipment and procedures should be developed.

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