Committee on
TOXICITY

Committee on Toxicity of Chemicals in Food,
Consumer Products and the Environment

Risk Assessment of Mixtures of Pesticides and Similar Substances

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Chairman
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Chairman of the Working Group on Risk Assessment of Pesticides and similar substances:

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Appendices

1. Executive summary

1.1 This report of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment prepared at the request of the Food Standards Agency, considers the risk assessment of multiple residues of pesticides and veterinary medicines in food, and of multiple sources of exposure to these substances. A specially established working group, the membership of which is given in appendix 8, drafted the report. The terms of reference were:

- To assess the potential for multiple residues of pesticides and veterinary medicines in food to modify individual toxicity of chemicals in humans – the so-called "cocktail" effect.
- To evaluate what assumptions can be made about the toxicity of pesticides in combination.
- To consider the potential impact of combined exposure to pesticides and veterinary medicines by different routes.
- To formulate advice on the standard risk assessment procedures applicable to the safety evaluation of individual pesticides and veterinary medicines in the light of the above considerations.

1.2 The working group decided to use the terminology for combined actions of mixtures, of Cassee *et al*, as described in Chapters 7 and 10. It was considered to be the most practicable way of describing the various ways in which combined actions may occur. This divides combined action of toxic substances into non-interaction and interaction. Both are further subdivided (see table 1.1), non-interactive processes being divided into simple similar action, where the toxicological action of the components of the mixture are the same and simple dissimilar action, where they are different. Interactive processes are divided into potentiation, when the combined effect is greater than additive and antagonism where the combined effect is less than additive.

Table 1.1 Nomenclature used in this report for combined actions of components of mixtures (after Cassee *et al*).[1]

Concept	Term used in report	Synonym(s)	Effects observed
Non-interaction	Simple similar action	Simple joint action	Concentration/dose addition
	Simple dissimilar action	Simple independent action independent joint action	Effect/response addition
Interaction	Potentiation	Synergy, supra-additivity	Greater than additive effect
	Antagonism	Sub-additivity	Less than additive effect

The Structure of the Report

1.3 The introduction to the report (Chapter 2) describes in more detail than above the reasons for the establishment of the Working Group, its membership and expertise and its method of working. Chapter 3 discusses stakeholder concerns and particular groups of substances that give rise to disquiet. Chapter 4 discusses the current regulatory systems for the approval of pesticides and the marketing authorization of veterinary medicinal products in the United Kingdom (UK), and where the European Union (EU) impinges on these systems. The licensing of human pharmaceuticals and the regulation of animals feed additives is also briefly discussed. Although outside the remit of the report, occupational exposure to toxic substances is briefly discussed in the context of methods that are in place to deal with occupational exposure to mixtures of substances. The regulation of pesticides, veterinary medicinal products and (very briefly) human pharmaceuticals in the United States of America (USA) is also covered. Chapter 5 deals with exposure assessment and Chapter 6 with biological monitoring, a specific type of exposure assessment. Chapters 7 and 8 deal with the toxicology of mixtures. The theoretical concepts underlying combined actions of the constituents of mixtures is discussed, together with the experimental evidence underlying these concepts. Chapter 9 deals with probabilistic risk assessment, a tool for exposure assessment that may be needed if risk assessment is to take account of all sources of exposure as well as combined exposure to different pesticides.

The Committee's conclusions

General issues

1.4 The Committee recognizes that there is concern that the regulatory system for pesticides and veterinary products found in foods does not routinely address the toxic effects of different substances in combination.

1.5 There are a number of ways in which exposure to pesticide, veterinary and other chemical residues in food might theoretically result in unexpected toxicity and these have been considered in detail in this report. It was concluded that there is evidence for limited exposure of humans to multiple residues and that such exposure occurs at low levels. There are no substantiated accounts of adverse reactions

to such exposures except under laboratory conditions. Nevertheless, it was concluded that the nature and extent of combined exposure, together with the likelihood of any adverse effects, which might result, should be evaluated when carrying out risk assessment.

1.6 Groups of substances, which are of specific concern, include the anticholinesterase insecticides (organophosphates and carbamates) and certain groups of fungicides, as well as a broad range of endocrine disruptors.

1.7 As well as concern about specific types of combined action, the Committee was aware of disquiet about the "cocktail effect": that is the possibility that adverse effects that may arise from exposure to residues of many different pesticides and similar substances, not necessarily possessing toxicological similarity. Evidence of the occurrence and importance of such combined actions in humans remains limited.

1.8 It has been suggested that certain groups in the population, notably pregnant women and young children may be at higher risk from these effects than adults, the developing brain and endocrine systems of the fetus and of children being of particular concern. Moreover, young children have a high intake of food compared to adults on a body weight basis, so they are often the critical group in the population for risk assessment. In addition, there may be other sources of variability due to genetic or other factors.

1.9 Public interest groups have also emphasised the multiplicity of sources of human exposure to pesticides and veterinary drugs other than food. These include residues in water and home and garden use of pesticides and veterinary medicines.

Regulation of pesticides, veterinary medicines and human pharmaceuticals

1.10 It was clear to the Committee that the impact of combined exposure to multiple pesticides of either toxicologically different or similar groups is only rarely addressed by European regulatory authorities and combined exposure has only recently been considered in the USA. Moreover, the impact of multiple sources of exposure is not often considered. Many of the procedures for the regulation of pesticides and veterinary medicines, are being harmonised at the EU and international level. This means that many of conclusions of the Committee would have to be acted upon at EU level to be effective.

Evidence of exposure

1.11 The Committee considered that because of the nature of the pesticide and veterinary surveillance programmes, it was extremely difficult to assess the frequency with which residues, below or above legally enforceable maximum residue limits (MRLs) occur. The problem is that much surveillance is targeted at produce where residues are most likely to occur, and a random program of surveillance would be necessary to assess the frequency of residues, including multiple residues. Furthermore, data on exposure from sources other than food and water seem to be extremely scanty or non-existent.

1.12 Both biological monitoring and biological effect monitoring can be useful in validating exposure models and identifying internal and effective doses. Metabolites common to groups of compounds, such as the urinary excretion of alkylphosphates as biomarkers of exposure to organophosphates (OPs) can only be used with caution, since the toxicity of the parent compounds may vary markedly, whilst producing the same pattern of alkylphosphate excretion. Data show that organochlorines (OCs) are present in human breast milk, albeit at declining levels.

Modelling exposure

1.13 Current deterministic methods of exposure assessment are sometimes considered to be highly conservative and do not make use of all the available information on multiple sources of exposure, normal variation in dietary and other routes of exposure and exposure to more than one compound. The alternative is to utilize probabilistic methods, which use all the information available, including the distribution of intakes from all sources. These can be used for both aggregate risk assessment and for cumulative risk assessment where an assumption of additivity is made.

Toxicology of mixtures

1.14 Because of the complexity and variability of chemical mixtures that may occur in the environment, risk assessment of any toxic effects of chemical mixtures is extremely difficult. Most attention has been directed at toxic effects due to combined actions on biological systems at relatively high levels of exposure in laboratory experiments in laboratory animals or using *in vitro* systems.

1.15 Direct chemical reactions can occur between the components of a mixture: there are relatively few studies of these substances that have investigated such reactions.

1.16 Several studies claim to have identified synergistic interactions of some mixtures. However, for the most part, these studies have been inadequately designed and based on an incomplete understanding of the concepts involved, but a few well-designed studies have demonstrated the occurrence of both synergistic and antagonistic interactions, as well as additive effects in mixtures, usually at high concentrations or high experimental exposure levels, which are probably unrepresentative of exposure doses.

1.17 Some interactions may not be easy to predict, such as those that may occur at the transcriptional or transductional level of the genome.

1.18 The type of combined action or interaction found at clearly toxic effect levels may not predict what will happen at non-toxic effect levels, including levels only slightly lower than the lowest observed adverse effect levels (LOAELs).

1.19 In relation to most examples of possible human exposure to multiple residues, it will be important critically to evaluate whether any effects are likely to occur at low levels of exposure, such as those that will occur through food and water.

Implications for assessing potential health risks for humans exposed to pesticide mixtures

1.20 Studies *in vivo* with chemicals that exhibit the same target organ and the same mode of action have shown that the effects of mixtures of similarly acting toxicants show additivity (dose addition), which results from simple similar action. This is the case, over the whole dose range.

1.21 It is essential to know what happens at non-toxic-effect levels, including exposure levels just below the LOAEL, in order to assess the health risk for humans exposed to mixtures of pesticides, veterinary drugs and similar substances. Generally, when exposure levels of the chemicals within a mixture are in the range of the NOAELs, and the components of the mixture have different modes of toxic action, no additivity and no potentiating interactions are found, indicating the applicability of the basic concept of "simple dissimilar action", which suggests that adverse reactions would be unlikely.

1.22 Some studies (acute and subacute toxicity, genetic toxicity, carcinogenicity) have addressed the combined effect of mixtures of pesticides and in a few studies clear cases of potentiation were observed in animals exposed to levels of toxic substances showing adverse effects of individual compounds. However, direct extrapolation of these findings to much lower dose levels is not valid. Thus the probability of any health hazard due to additivity or potentiating interaction of mixtures of pesticides at (low) non-toxic doses of the individual chemicals is likely to be small, since the dose of pesticides to which humans are exposed is generally much lower than the NOAEL, at least through food.

1.23 Some endpoints that have been studied in animals or in *in vitro* systems are relevant to groups in the population believed to be at higher risk than the general population. Such endpoints include developmental toxicity studies, endocrine and neurotoxic effects and genotoxicity studies. On the basis of limited information it seems likely that the default assumptions in relation to mixtures in children and pregnant and nursing mothers, would be the same as for the rest of the population.

The Committee's recommendations

Regulatory

1.24 We *recommend* that the approval of pesticides used on crops, and authorization of similar compounds used in veterinary medicine should consider all sources of exposure.

1.25 We *recommend* that a scientific and systematic framework should be established to decide when it is appropriate to carry out combined risk assessments of exposures to more than one pesticide and/or veterinary medicine.

1.26 In the event that it is considered appropriate to carry out risk assessment of combined exposure, the default assumptions should be that chemicals with different toxic actions will act independently (simple dissimilar action), and those with the same toxic action will act additively (simple similar action). In the latter circumstances a toxic equivalency approach might be considered. In specific

instances the possibility of interaction, particularly potentiation, may have to be considered. In such circumstances adequate dose-response data will be essential in the interpretation of findings in relation to dietary intakes and other human exposures.

1.27 We *recommend* that the approval of pesticides and authorization of compounds used in veterinary medicine, should include more formal analysis, and possibly experimental investigation, of the potential for combined toxic action or interaction due to the addition of other substances to the formulations employed. This consideration should also include tank mixes of pesticides.

1.28 Analysis of all sources of exposure to pesticides and of concurrent exposure to more than one pesticide will require changes in the methods used for risk assessment, including, in some cases, the use of probabilistic exposure assessment. This will be contingent on changes in residue surveillance.

Surveillance

1.29 Dietary and food consumption surveys in the UK should continue to cover all social, age, and ethnic groups within the population. Consideration should be given as to whether additional groups need to be covered.

1.30 Aggregate exposure assessment will require acquisition of robust data on all pathways of exposure to pesticides and veterinary medicines and on sources of variation in such exposure.

1.31 We *recommend* that residue surveillance programmes should be modified in the light of the need for representative data for probabilistic exposure assessment. The effect of food processing and preparation on the bioavailability and chemical nature of residues should be further investigated.

Research

1.32 We *recommend* that methods be developed to provide valid and cost-effective biomarkers or other robust indicators of population exposure and systemic (body) burdens of mixtures of pesticides and relevant veterinary residues.

1.33 We *recommend* that valid markers be developed to enable the early and reliable detection of systemic responses and health effects arising from such exposures (biomarkers of effect).

1.34 This work should be extended to the characterisation of the possible variability in human responses to mixtures of pesticides and veterinary medicines.

1.35 We *recommend* that further work be undertaken, in suitable experimental systems, to characterise both the nature of, and dose-response relationships for, combined actions of pesticides, veterinary medicines and similar substances. Such studies should be performed at doses that include those potentially ingested by humans in the diet. Groups of pesticides having common targets of

toxicological action should be identified. Such work might include the identification of sites of action at a molecular level, to identify those groups of compounds that would be expected to show simple similar action. Studies of protein and/or RNA expression, using modern array technology, in relevant systems may be appropriate in some cases. These may be followed up by more detailed mechanistic studies of gene expression and/or enzyme or hormonal activity as necessary. Array technology (RNA and protein) may be appropriate in some cases, or enzyme or hormonal activity in others.

Public information

1.36 A central and accessible repository of information about all forms of human exposure to pesticides and similar substances should be established.

1.37 The extent and adequacy of the information available to the domestic user of pesticides and veterinary medicines requires review of its extent and ease of comprehension.

References

1. Cassee FR, Sühnel J, Groten P, Feron VJ. The toxicology of chemical mixtures. In: *General and Applied Toxicology* edited by Ballantyne B, Marrs TC, Syversen T. London, Macmillan Reference Limited, 1999, 303-320.

2. Introduction

2.1 This report of the Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) considers the effects of mixtures of pesticides and veterinary medicines and the implications of exposure to such mixtures for the risk assessments carried out during the approval processes for these substances.

2.2 Many pesticides and veterinary medicines contain only one active ingredient. However, some products may contain more than one active ingredient and more than one product may be used on a crop or food animal. Furthermore, in a meal individuals may consume produce that may have been treated with different pesticides or veterinary products. Over 350 active substances are approved as pesticides in the United Kingdom, while other compounds may be present in food of animal origin and imported food from other EU states and elsewhere. This means that food may contain residues of a number of different pesticides and veterinary medicines (often described as a cocktail) and termed by the United States Food Quality Protection Act (FQPA)[1] as "cumulative exposure". During the approval of both pesticides and veterinary medicines in the UK, active ingredients are generally assessed singly for their potential impact on human health and safety standards (the Acceptable Daily Intake [ADI] and for pesticides, the Acute Reference Dose [ARfD]) are set for individual chemicals. ADIs and ARfDs are also set by the Joint Meeting on Pesticide Residues (JMPR), a joint expert body of the World Health Organization and The Food and Agricultural Organization of the United Nations.

2.3 The justification given for considering individual active ingredients has been that they generally exert any harmful effects through different mechanisms. However, there are families of pesticides and veterinary medicines which work toxicologically through the same mechanism and, hence, it is possible that interactions between substances may result in a greater toxic effect than predicted during the approval process. Some research has been carried out on mixtures of chemicals but this has not to date been specifically reviewed in the UK for its applicability to mixtures of pesticides and veterinary medicines. Until this has been done, it cannot be judged whether the approach currently taken to risk assessment is sufficiently protective and based on sound toxicological principles.

2.4 In the UK, pesticides are recommended for approval by the Advisory Committee on Pesticides (ACP) and veterinary medicines by the Veterinary Products Committee (VPC) on the basis of data, including toxicological data, supplied by the company seeking approval or marketing authorisation.

2.5 Consumers have been concerned for some time about the possible implications of interactions between the components of mixtures of chemicals (the cocktail effect). Information on the occurrence of multiple pesticide residues in food has been published through work performed of the Working Party on Pesticide Residues (WPPR) and its successor the Pesticide Residues Committee (PRC), since 1988. However, in 1999, the outgoing Chairman of the WPPR drew attention to the fact that little is known about the toxicological interactions between pesticides and commented "that pesticide residues of the same class (for example, organophosphates) will be at least additive in their effects because they act by the same toxicological mechanism". The Food Standards Agency (FSA) had also carried out a telephone consultation of consumer groups. This highlighted concern about the "cocktail" effect, particularly when foods containing multiple residues were to be consumed by children.[2] The FSA was

also aware that some active ingredients might be used in both pesticides and veterinary medicines and felt that it was important to ensure that any consideration covered both types of products.

2.6 There were thus several reasons why the FSA asked the COT to establish a Working Group critically to review what is known about the science of mixtures and consider the implications for the risk assessment process. Further, the Working Group was to consider whether there is any scientific basis for consumer concerns about the occurrence of multiple residues of pesticides and veterinary residues and the resulting "cocktail" effect. The Working Group also considered the question of whether there was any fundamental difference in toxicological responses to mixtures of synthetic compounds as opposed to natural constituents of foods. The Working Group was also asked to consider exposure by mechanisms other than consumption of food containing pesticide and veterinary residues. These include consumption of drinking water, respiratory exposure to pesticides used in public hygiene, contact with pesticides applied to gardens, parks or in agricultural areas and veterinary products applied to pets. In addition, a few pesticidal active ingredients are used as human medicines. This consideration of all sources of exposure has termed "aggregate exposure" by the FQPA[1] and, again, is not routinely taken into account during the approval process. These considerations underpin the Terms of Reference given to the Working Group (set out below at 2.8) and its methods of working (set out below at 2.9-2.11).

2.7 The Working Group has compiled and reviewed information covering a number of areas. Its conclusions and recommendations are based on this information. The areas considered are:

- concerns which have been expressed by consumers and other stakeholders (Chapter 3).
- the current regulatory systems for pesticides and veterinary medicines in the European Union (EU), including the UK, and in the United States of America (USA). The effects of the FQPA in the USA, which mandated the consideration of all sources of exposure to pesticides and of exposure to more than one pesticide when carrying out risk assessments, have also been considered (Chapter 4).
- evidence of exposure from different routes and to multiple residues in food (Chapter 5).
- a technique which can contribute to knowledge about exposure (biomonitoring – Chapter 6).
- what is known about the toxicology of mixtures (Chapters 7 and 8).
- probabilistic modelling, a technique which may need to be adopted if consideration of all sources of exposure to pesticides and of exposure to more than one pesticide is deemed necessary during risk assessment (Chapter 9).

Full details of the literature and other sources of information considered by the Working Group are given in the bibliography: a comprehensive literature search on the toxicity of chemical mixtures, with special reference to pesticides, was carried out, and updated until January 2002. In addition, some later papers brought to the Working Group's attention have been cited. Details of submissions made by stakeholders are given in Appendix 7. The conclusions and recommendations of the Working Group are given in Chapters 10 and 11 respectively.

2.8 The Working Group had the following terms of reference:

- To assess the potential for multiple residues of pesticides and veterinary medicines in food to modify individual toxicity of chemicals in humans – the so-called cocktail effect.
- To evaluate what assumptions can be made about the toxicity of pesticides in combination.
- To consider the potential impact of combined exposure to pesticides and veterinary medicines by different routes.
- To formulate advice on the standard risk assessment procedures applicable to the safety evaluation of individual pesticides and veterinary medicines in the light of the above considerations.

Membership of the Working Group and its methods of working

2.9 The membership of the Working Group is given at Appendix 8. The Working Group was chaired by Professor Woods and included individuals with expertise in the areas of general medicine, paediatrics, toxicology, biostatistics, pathology and pharmacology. The Group also included two public interest members. The Working Group met first on 19 December 2000 and subsequently on seven other occasions. The draft report will be submitted to the COT at its meeting in April 2002.

2.10 The information that the Working Group considered comprised papers and reviews in the open scientific literature (largely accessed through PubMed) as well as reviews by official bodies and expert bodies. Information was also supplied by interested parties, in response to the announcement of the establishment of the Working Group and of an open meeting that took place on 17 April 2001 in London. In addition the draft report was the subject of an open consultation meeting at Norwich in February 2002, after which further material was received, and a further draft was prepared by the Working Group. This draft was considered by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment at its meeting on the 23rd April 2002. Some modifications were made in the light of members' comments.

2.11 The Working group was supported by a Secretariat provided by officials from the Food Standards Agency. Officials from the Department of Health, the Pesticides Safety Directorate, the Veterinary Medicines Directorate and the Health and Safety Executive were present at the meetings and contributed material to the report. The conclusions set out in the report are those of the members of the Working Group and have been endorsed by the COT. The opinions expressed in the report are independent of those of any other body.

Nomenclature

2.12 Throughout this document, English language International Organization for Standardization (ISO) names[3] are used for pesticides whenever possible. These are usually the same as the British Standards Institution common name.[4]

References

1. Food Quality Protection Act. US Public Law 104-70, August 3rd 1996.

2. FSA (2000). Food Standards Agency. Press Release, September 2000.

3. International Standard 1750. *Pesticides and other agrochemicals – common names.* Geneva: International Organization for Standardization, 1981.

4. *Pesticide Manual.* Twelfth edition. Farnham, England: British Crop Protection Council, 2000.

3. Stakeholder concerns

Introduction

3.1 Stakeholders are not simply consumers or end-users. Those who have an active interest in mixtures of pesticides and veterinary medicines include all those in the manufacture, supply, use and disposal of products, together with third parties and those who may be exposed at any stage either in consequence of professional or amateur use.

3.2 This chapter presents the concerns of stakeholders as expressed to Pesticide Action Network UK (PAN UK) and other public interest groups, orally or in writing, in various fora about specific pesticide active ingredients over which there may be mixture-related concerns, and also the situations in which use of those active ingredients may give rise to exposure.

Background

3.3 The UK Pesticides Safety Directorate (PSD) has stated that pesticide evaluations do not usually address the potential interactions when concurrent or sequential exposures occur to two or more pesticides having a similar mechanism of action.[1]

3.4 The interactive effects of mixtures has been known and indeed exploited for some time. Synergists[a] have been important in the formulations of pesticides (particularly the synthetic pyrethroids). Although the regulatory authority is aware of the content of formulations, the presence of synergists is not always disclosed on the product label or in published documents on the active substance. Assessment has, until recently, normally been limited to the effects of the synergist on the acute toxicity classification of the formulation.

3.5 Excipients in formulations can cause unwanted toxic effects: for example there were adverse reactions to some glyphosate formulations until the formulation was changed in the early 1990s.[2]

3.6 In the 1970s, a field example of an unexpected problem of mixtures occurred when red-legged partridges *(Alectoris rufa)* ingested seed treated with the fungicide prochloraz, and were then exposed to field treatments of the organophosphate (OP) cereal insecticide malathion. High mortality of the birds was observed due to the enhanced toxicity of the OP insecticide in combination with prochloraz.[3] These two pesticides have entirely different mechanisms of mammalian toxicity, the mechanism of the combined action being enzyme induction.

[a] See glossary for definition in this context.

Specific chemical concerns

Endocrine disrupting chemicals

3.7 Research has highlighted possible impacts of endocrine disrupting chemicals (EDCs) in recent years.[4] These concerns were investigated initially by biologists and ecotoxicologists, and particularly in the aquatic environment. It is suggested that EDCs may be responsible for adverse effects in wildlife including birth defects, reproductive failure and developmental abnormalities of the organs of reproduction, together with the increasing incidence of breast cancer in women (see Coleman[5]) and increasing incidence of testicular cancer in men. Stakeholders have also raised concerns about the decreasing sperm counts seen in some studies. Impacts on wildlife may serve as indicators of potential human health problems.

3.8 Many chemicals including some pesticides are linked with hormone disrupting effects. Regulators have published a number of lists of candidate pesticides. PAN UK has compiled these into the following comparative table.

Table 3.1 Lists produced by various bodies Of Endocrine Disrupting Pesticides, with UK usage status as of 1st January 2002.

		UK usage[a]		Status[c]				
Active ingredient	Type	Ag[b]	Non-ag[b]	EA[d]	Ger[e]	EU[f]	OSPAR[g]	WWF[h]
Acetochlor	Chloracetanilide herbicide	✗	✗			✓		✓
Alachlor	Chloracetanilide herbicide	✗	✗			✓		✓
Aldrin	Organochlorine insecticide	✗	✗	✓				✓
Amitraz	Insecticide	✓	✗		✓?			
Atrazine	Triazine herbicide	✓	✗	✓		✓	✓?	✓
Benomyl	Benzimidazole fungicide	✓	✗		✓?			✓
Beta-HCH	Organochlorine insecticide	✗	✗				✓?	✓
Camphechlor	Organochlorine insecticide	✗	✗			✓	✓?	✓
Carbendazim	Benzimidazole fungicide	✓	✓		✓?			
Carbofuran	Carbamate insecticide	0	✗		✓?			✓

		UK usage[a]			Status[c]			
Active ingredient	Type	Ag[b]	Non-ag[b]	EA[d]	Ger[e]	EU[f]	OSPAR[g]	WWF[h]
Chlordane	Organochlorine insecticide	✗	✗			✓	✓?	✓
Chlordecone	Organochlorine insecticide	✗	✗			✓	✓?	✓
Chlorpyrifos	Organophosphate insecticide	✓	✓		✓?			
DDT	Organochlorine insecticide	✗	✗	✓		✓	✓?	✓
Deltamethrin	Synthetic pyrethroid insecticide	✓	✓		✓?			✓
Demeton-S-methyl	Organophosphate insecticide	✗	✗	✓				
Dichlorvos	Organophosphate insecticide	✓	✓	✓				
Dicofol	Organochlorine acaricide	✓	✗				✓?	✓
Dieldrin	Organochlorine insecticide	✗	✗	✓			✓?	✓
Dimethoate	Organophosphate insecticide	✓	✗	✓	✓?			✓
Endosulfan	Organochlorine insecticide	✓	✗	✓			✓?	✓
Endrin	Organochlorine insecticide	✗	✗	✓				✓
Epoxyconazole	Triazole Fungicide	✓	✗		✓✓			
Fentin acetate	Organotin fungicide	✓	✗			✓		
Glyphosate	Organic phosphorus herbicide	✓	✗		✓?			
Hexachlorobenzene	Organochlorine fungicide	✗	✗			✓	✓?	✓
Lindane	Organochlorine insecticide	0	✗	✓		✓	✓?	✓
Linuron	Urea herbicide	✓	✗	✓		✓		✓
Maneb	Ethylene*bis*dithio carbamate fungicide	✓	✗			✓		✓

		UK usage[a]			Status[c]			
Active ingredient	Type	Ag[b]	Non-ag[b]	EA[d]	Ger[e]	EU[f]	OSPAR[g]	WWF[h]
Metam	Dithiocarbamate fungicide	✗	✗			✓		
Metiram	Ethylene*bis*dithio carbamate fungicide	✗	✗		✓✓			✓
Methoxychlor	Organochlorine insecticide	✗	✗				✓?	✓
Mirex	Organochlorine insecticide	✗	✗			✓		✓
Oxydemeton-methyl	Organophosphate insecticide	✗	✗		✓?			
Penconazole	Azole fungicide	✓	✗		✓?			
Permethrin	Synthetic pyrethroid insecticide	✓	✓	✓				✓
Prochloraz	Azole fungicide	✓	✗		✓?			
Procymidone	Dicarboximide fungicide	✗	✗		✓✓			
Prometryn	Triazine herbicide	✓	✗				✓?	
Propiconazole	Azole fungicide	✓	✓		✓?			
Simazine	Triazine herbicide	✓	✗	✓				✓
Thiram	Dimethyldithio - carbamate fungicide	✓	✗			✓		
Tributyltin	Organotin fungicide	✗	✓	✓		✓	✓?	✓
Triphenyltin	Organotin fungicide	✗	✗			✓		
Trichlorfon/ Metrifonate	Organophosphate insecticide and human pharmaceutical	✗	✗		✓?			
Tridemorph	Morpholine fungicide	0	✗		✓?			
Trifluralin	Aniline herbicide	✓	✗	✓				✓
Vinclozolin	Dicarboximide fungicide	✓	✗		✓✓	✓	✓?	✓
Zineb	Ethylene*bis*dithio carbamate fungicide	✓	✓			✓		✓

Notes

a. Pesticides marked ✓ in the usage column have UK uses. Those marked 0 in the usage column are no longer approved for sale but stocks may be used up over a limited period; ✗ means there is no improved UK use.

b. Ag, pesticide used in agriculture and/or horticulture. Non-ag, pesticide used for non-agricultural purposes.

c. ✓ = identified as Endocrine Disrupting Chemical (EDC) see definition below, ✓? = identified as potential EDC, ✓✓ = confirmed EDC.

d. **EA** – the UK Environment Agency's list of target EDCs, Strategy for Endocrine disrupting chemicals set out in Your Environment: Hormone Disrupting Substances at *http://www.environment-agency.gov.uk/yourenv/latest_issues/issues/main/?version=1. Last accessed 6 November 2001.*

e. **Ger** – *potential and confirmed EDCs by the German Federal Environment* Agency column, Pesticides suspected of endocrine-disrupting effects by the German Federal Environment Agency, ENDS Report 290, March 1999.

f. **EU** – considered as high concern EDC by the European Union, Commission moots priority list of endocrine chemicals, ENDS Report 306, July 2000.

g. **OSPAR** – identified as a potential EDC under Oslo and Paris Commission, Endocrine disrupting pesticide: Gwynne Lyons. Pesticides News 46, December 1999.

h. **WWF** – World Wide Fund for Nature list of pesticides reported to have reproductive and/or endocrine disrupting effects. NB: There are a number of other pesticides WWF suspect of being EDCs, but they are not listed if no other authority above cited them. For the full list see PN 46 p18.

3.9 Guidance is needed on issues such as exposure, persistence, potency and potential availability in the human body. The usage of some of those pesticides and veterinary medicines that have been identified as possible EDCs is considerable and users await advice on whether or what alternatives they should use.

Anticholinesterase Pesticides

3.10 Nearly all anticholinesterase pesticides (most OPs and some carbamates) have appreciable acute toxicity, often by virtue of their neurotoxicity. The anticholinesterases are currently under regulatory review. It may reasonably be expected that, as far as cholinesterase impacts are concerned, some exposures will be additive. Most of the anticholinesterase pesticides also have impacts on other enzyme systems.

3.11 There are particular groups that might be affected, including infants, children, pregnant and nursing women, and the elderly, who might be more sensitive to pesticides than the general population. In the case of the fetus, infants and children, the developing brain in the case of neurotoxic pesticides, and the developing reproductive system in the case of EDCs, may give the greatest cause for concern.[6,7] Moreover, children have a higher intake of food per kg body weight.

Dicarboximide Fungicides

3.12 The United States Environmental Protection Agency (USEPA) has published a memorandum setting out concerns over the common mechanisms of toxicity of some of the more widely-used fungicides of the dicarboximide group.[8] These include vinclozolin, procymidone and iprodione. Although procymidone has no UK uses, vinclozolin is widely used on oilseed rape and iprodione is also applied to many crops.

Benzimidazole Fungicides

3.13 Carbendazim, benomyl and thiophanate-methyl are systemic fungicides used to control a range of diseases in fruit, vegetables, mushrooms, field crops, ornamentals and turf. Benomyl and thiophanate-methyl are converted in soil and water at least in part to carbendazim.[9] Additionally thiophanate-methyl is metabolised in plants and animals partly to carbendazim. For the purposes of evaluating exposure, it may be that residues should be treated therefore as additive. All three are used widely on their own and in mixtures with other active ingredients.

Pesticides that do not share a common mechanism of action

3.14 Recent research has suggested that usage of the bipyridyl herbicide paraquat and the dithiocarbamate fungicide maneb may be risk factors for Parkinsonism, perhaps by additive impact on the dopamine system.[10] These pesticides do not share a common mechanism of action, and human health implications of the effects observed in animals is unclear. Paraquat and maneb are not formulated together in one product, but could be used sequentially on different crops.

Groups at risk within the population

3.15 Some sub-groups of the population may be more at risk of the adverse effects of pesticides than others. From the exposure point of view, young children have a greater intake of food than adults on a body weight basis and have different food intake patterns. Furthermore, chemicals may have permanent effects on developing systems in the fetus, neonate and young children which would transient in the adult. Such systems might include the endocrine system and the developing nervous system.

Exposure

Food residues

3.16 Levels for pesticides in food are established for individual pesticides in individual commodities, by way of Maximum Residue Levels (MRLs) (see chapter 4). The application of pesticides according to good agricultural practice should result in residues below MRLs and although MRLs are not safety limits *per se*, they are checked for compatibility with the acceptable daily intake (ADI) during the risk assessment process. For consumers, risks to health are calculated with reference to the ADI.

3.17 Not all pesticides leave residues in food. Thus, a large proportion of UK herbicide use is on railway lines. Furthermore, the pesticide may be used early in the season, before the crop is in the ground, to clear weeds (although there may still be a human or environmental health risk). Residues in food arise from use according to good agricultural practice. Residues may be left by the post-harvest use of pesticides for storage and/or transport. Residues may also arise from the overuse of a pesticide, or occasionally from the use of a pesticide that is not approved for use on that crop or that has no approved use in the UK.

3.18 The UK Pesticide Residues Committee (PRC) oversees a programme to monitor the UK food and drink supply for pesticide residues. In its report for the year 2000[11] the PRC notes that 2,304 samples were analysed for pesticides. Overall no residues were detected in 71% of samples. Residues below the MRL were found in 29% of the samples, and in 1% of the samples residues exceeded the MRL, or the sample contained a residue of a pesticide not approved in the UK. It is difficult to compare the figures on an annual basis as some of the commodity surveys are not random but targeted at produce where residues may be expected to be found.

3.19 Some pesticides that are used in other countries are not approved for use in the UK. It is clearly difficult to control what pesticides are used in countries outside the EU, on food that is then exported to the UK. Organic crops may be treated with a certain restricted number of pesticides: some of these also occur naturally in the soil and/or also degrade quickly, but some samples of organic food are included in the UK pesticide residue monitoring programme. The outgoing chair of the then Working Party on Pesticide Residues (now the PRC) Prof Ian Shaw noted in the 1999 Report that little is known about the toxicological interactions between pesticides and therefore we must turn our attention to foods more likely to contain multiple residues. It is likely that pesticide residues of the same class (eg OPs) will be at least additive in their effects because they act by the same toxicological mechanism.[12]

3.20 English winter lettuce, sweet oranges, apples and celery are the food items most likely to contain multiple residues (see Appendix 3).

3.21 The problem has been recently illustrated. During the course of the regulatory review of the OP insecticide dimethoate, it became apparent that although exposure from individual crop/pesticide combinations were acceptable, combined exposure from the totality of the diet including imports could potentially exceed acceptable levels.[13] Specifically if the total diet was considered, the ADI could be exceeded for toddlers for residues of dimethoate and its metabolite omethoate; and for infants by dimethoate residues.

3.22 There is no regulatory mechanism to decide which of a number of approvals or uses should be restricted or revoked and how to deal with imports that may contain residues at approved (ie within MRL) levels but which may add to the totality of dietary consumption.

3.23 The issue of residues in foods is complicated by the phenomenon of variability: application of pesticides by good agricultural practice can produce a large variation in the residues found in neighbouring apples or carrots or other small fruit and vegetables. It is possible for the average residue in a comminuted sample of 10 items to be well below the MRL, but for there to be an individual apple or carrot in the batch containing high residues. Residue surveillance needs to be targeted on food safety as well as the application of good agricultural practice, and to be supported with appropriate sample sizes, to account for this phenomenon.

3.24 A further complication is that consumer safety is generally based on the ADI or lifetime safe dose. It is now recognised that particular pesticides (including a number of OPs) may cause harm following a day's consumption and for these chemicals an Acute Reference Dose (ARfD) has been established; in such cases an additional risk assessment, this time of acute exposure, is undertaken.

3.25 A survey of the most commonly found pesticide residues in food across the EU and Norway[14] showed that of the 12 most commonly found residues, 5 (iprodione, procymidone, benomyl group, endosulfan and vinclozolin) were possible EDCs. It is not yet clear how biologically available these residues might be.

Occupational exposures

3.26 Agricultural and horticultural pesticides were more than 84% of the total market by sales of £466 million in 2000, and 18,213 tonnes were sold.[15] It is estimated that about 90% of the occupational risk from pesticides occurs during the mixing, and also the loading of application equipment.[16]

3.27 How common are pesticide mixtures in practice? In the field, pesticides are frequently applied as mixtures, or 'tank-mixes'. The regulatory process specifies that a pesticide may only be used for the purpose or purposes and under the conditions of use for which an approval has been granted. There are tank mixes approved by PSD, but assessments relate to crop safety rather than operator safety. There are specific restrictions relating to the tank mixing of OPs. Crop pests do not arrive one species at a time, nor do they announce their coming. A farmer may therefore spray for a number of different weed, insect or disease pests on the same occasion. Depending on the treatment history, advice may be to use a combination of different pesticides to minimise the development of pest resistance. The result may be a mixture of different chemicals in a tank mix in order to satisfy these varying requirements for control.

3.28 The Pesticide Usage Survey Group (PUSG) has estimated that for arable crops in 1998, excluding all adjuvants, 8,859,181 ha were treated with single product sprays and 11,326,636 ha treated with tank-mixes of 2 or more pesticide products, representing some 56% of all sprays applied to arable crops.[17] Arable crops represent about 92% of all spray applications across all areas of agriculture and horticulture. Because the number of potential permutations of tank-mixes is huge, even the most popular are actually used on a small area in comparison to the total area treated with tank-mixes. Even the most popular mix represented only 3.3% of the total area treated with tank-mixes. Some of the most widely used combinations are shown in table 3.2.

Table 3.2 Frequently used tank mixes of pesticides in the UK. These data are from the Pesticides Usage Survey Group and relate to 1998 data, the latest available.[17]

Mix	Formulations in mix	Area treated	% of all tank-mixes
3-way	Cypermethrin + diflufenican/isoproturon+ isoproturon	381,653 ha	3.3
2-way	Chlorothalonil + epoxyconazole	156,387 ha	1.4
2-way	Carbendazim + tebuconazole	119,771 ha	1.1
3-way	Cypermethrin + isoproturon + trifluralin	112,980 ha	1.0
2-way	Diflufenican/isoproturon + isoproturon	105,950 ha	0.9
2-way	Cypermethrin + isoproturon	93,719 ha	0.8
2-way	Azoxystrobin + epoxyconazole	71,882 ha	0.6
2-way	Fluroxypyr + metsulfuron-methyl	63,022 ha	0.6
2-way	Chlormequat + trinexapac-ethyl	61,455 ha	0.5
2-way	Chlorothalonil + cyproconazole	59,686 ha	0.5

3.29 Farmers regularly use a number of different active ingredients. These may be formulated into one product, or as indicated above, several products may be tank-mixed for application. Applications are made, of course, not only to one crop at a time. The farm enterprise may be growing several different crops – cereals, fruit, vegetables or more specialised produce. Workers may find themselves using pesticides sequentially, and at some times of the year, day after day. Evidence is available about the lack of washing, indoor ventilation and other facilities.

3.30 Examples of treatment histories of commonly consumed UK grown fruit and vegetables are set out in Appendix 2.

3.31 Within the general category of agricultural use, priority areas of concern should be identified. These are likely to be fruit and vegetable growing, where a high-value crop receives many pesticide applications and damage prior to marketing can be costly. These crops may often be grown in glasshouses, which can increase the exposure risk for workers. Flowers are another sensitive crop often grown indoors and grain storage pesticide application is done in a confined space. Farmers are not as a matter of law required to keep records of applications but the UK Pesticide Usage Survey Group carries out surveys[a] of different agricultural sectors from time to time. The surveys set out crop areas treated, pesticide active ingredients used by weight, volume and combination and give comparative data where available.

[a] Pesticide usage survey group, DEFRA, Central Science Laboratory, York.

Home and garden exposures

3.32 The market for home and garden and amenity use is almost £34.6 million a year and results in the application of 4,306 tonnes of pesticide active ingredients (although 2,738 of this is ferrous sulfate based).[15] For UK homes and gardens, a range of about 600 pesticide products are available with about 100 active pesticide ingredients. However, little more is known about how pesticides and veterinary medicines are used in the home and garden, the resulting exposure of young children, or the public's understanding regarding pesticide toxicity and how this influences the way they use them, all of which may be important risk factors.[18] Furthermore, little is known of the pesticide content of home-grown vegetables.

3.33 Pesticides are the fourth major cause of poisoning incidents in the UK, after pharmaceuticals, household products (which may include some pesticides) and industrial chemicals. Many of these poisonings are deliberate. Poisons Centres in 1987 (the most recent figures available) estimated that about 5,500 acute pesticide poisoning incidents occurred in England and Wales each year, of which about 700 resulted in hospitalisation. 91.2% of poisonings occur in the home compared with only 4.4% in the workplace.[19]

3.34 In London, most of the roads, parks and playing fields are regularly sprayed with herbicides. One fifth of ground cover in the Greater London area consists of private gardens and allotments, according to the London Ecology Unit's analysis of aerial photographs.[20] Thus garden pesticide usage in urban areas is likely to be high. As well as in gardens, pesticides are used in homes for wood preservation and as insecticidal sprays, while animal flea treatments, and headlice treatments for children frequently contain active ingredients identical to those used in pesticides (see below).

Head lice medicines

3.35 A number of products, containing active ingredients which are the same as pesticides, are used as medicines. By law, products can be sold over the counter to the public in a pharmacy, provided a pharmacist is available to advise; or such products can be dispensed by a pharmacist pursuant to a GP's prescription. The UK market in conventional pesticide medicines was worth £29.7 million in 1997/98[21] for a total of approximately 10 million doses "the best indication there is of the number of cases".[22]

3.36 Ibarra[22] reports that four active ingredients are currently used for headlice treatments: malathion (an OP), phenothrin and permethrin (synthetic pyrethroids) and carbaryl (a carbamate). Head lice resistance and cross-resistance to chemical treatment is a problem. It is also reported that although the products are licensed for single applications, professionals frequently recommend multiple applications. PAN UK has also come across cases of repeat applications in a family (2-4 times per week over a considerable period) to cure infestations.

Consumer goods

3.37 Current pesticide legislation applies to pesticides and pesticidal products, but not to manufactured products that may contain pesticides. Particular examples are the treatment of carpets with mothproofing and agents to kill dust mites – the pesticide active ingredient may be permethrin or tributyltin (TBT). Other textile items may be similarly treated, including duvets. Paints frequently contain a fungicide such as carbendazim to protect the integrity of the paint.

3.38 Virtually everyone will be subject to a regular, low-level background exposure to a number of pesticide active ingredients (see Chapter 2). Consumers, particularly those who may be sensitive to particular chemicals will find it difficult to know how to avoid such exposures.

Timber treatments

3.39 Somewhere in the region of 50,000 – 150,000 remedial timber treatments are carried out in British homes each year. Homes change ownership frequently, and many surveyors and mortgage lenders recommend treatments enthusiastically. Formerly many treatments were very long-lasting. Older organochlorine pesticides such as lindane, and pentachlorophenol were used for treatments. These have been replaced by a variety of pesticides including synthetic pyrethroids (cypermethrin, permethrin), organotin compounds, OPs such as pirimiphos-methyl, and acypetacs-zinc and others. There have been long standing concerns that many have been unnecessary and may have been unprofessionally carried out, with exposure consequences for occupiers. There is currently no system for recording chemical wood preservation treatments, so that many buildings have had repeat treatments. Occupiers may be subject to continual background exposures.

Implications

3.40 Users, both professional and amateur, and consumers need to know that the mixtures and sequential exposures they experience are not damaging either for their health or the environment. This chapter highlights some of the more well known active ingredient types and combinations of concern, and some of the situations where multiple or sequential exposure is common. Where toxicity data can supply answers, changes in approvals may be necessary. Users can make informed choices by accessing current information available on http://www.pesticides.gov.uk.

3.41 This work will also have direct relevance to patterns of pesticide exposure elsewhere. Workers and consumers in many developing countries are exposed to older and more hazardous products which are frequently used inappropriately or under unsuitable conditions. Issues and concerns that this group can highlight may help inform other agencies and contribute to reducing pesticide poisonings in the Third World.

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4. Current regulation of pesticides and veterinary medicines in the United Kingdom and the United States of America

Regulation of pesticides and veterinary medicines in the UK

4.1 The general principles whereby pesticides and veterinary medicines are regulated are similar, but there are some differences in detail, so that these two groups of substances are considered separately in this chapter. Both regulatory systems require pre-marketing authorisation before sale or use, and some degree of post-marketing surveillance takes place. These systems are discussed below, as is briefly, the regulation of human pharmaceuticals.

Pesticides

Introduction

4.2 Pesticides is a general term, which comprises chemical and biological products used to kill or control pests. Pesticides include products designed to act as insecticides, herbicides, fungicides, and rodenticides as well as animal and bird repellents. Food storage treatments, plant growth regulators, anti-fouling products for boats and wood preservatives all also fall within the definition of pesticides.

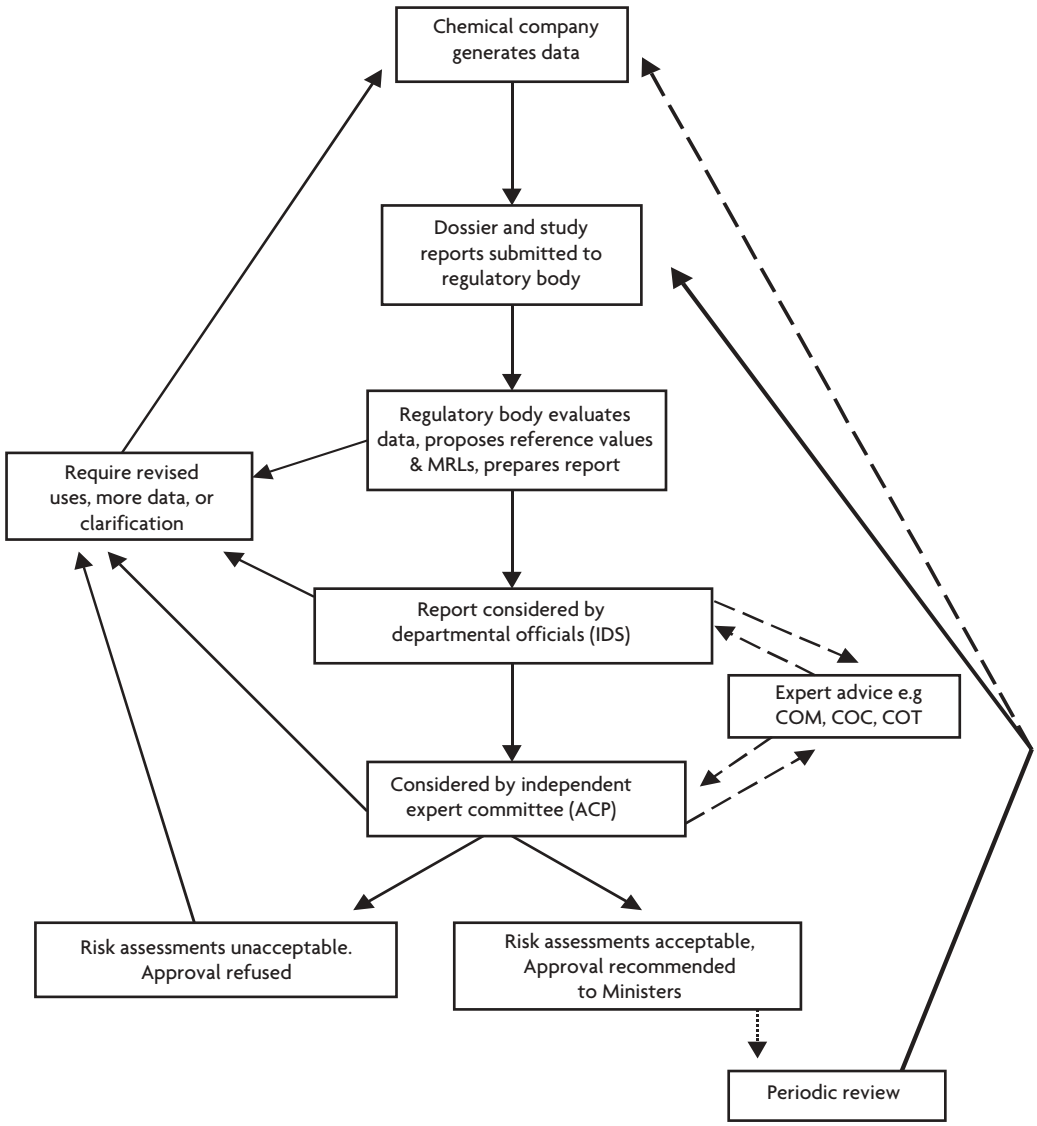
4.3 Under the current UK system for approval of pesticides products are classified as agricultural or non-agricultural pesticides according to the purpose for which they are used. Agricultural pesticides include those used in agriculture, horticulture, private gardens and forestry as well as weedkillers for use in and around watercourses, lakes and for use on non-crop land such as roads and railways. Non-agricultural pesticides (also called biocides) include those used in wood preservation, as masonry biocides, as public hygiene/nuisance insecticides and as anti-fouling products on boats. The Pesticides Safety Directorate (PSD), an agency of the Department for Food and Rural Affairs (DEFRA) is responsible for operating the system for regulating agricultural pesticides, whereas that for non-agricultural pesticides is the responsibility of the Health and Safety Executive (HSE). A distinction is also made between professional and amateur use. Professional products may only be used by people as part of their work and they must be competent in their use. Amateur products are those that may be used by the general public.

4.4 At present there are two parallel systems for the approval of pesticides in the UK: a domestic system and a European system.

4.5 The UK system has evolved since the 1940s. It currently involves various government agencies and departments and their Ministers as well as the devolved administrations in Scotland, Wales and Northern Ireland. The Advisory Committee on Pesticides (ACP), which comprises independent experts and independent lay members, advises Ministers after carrying out risk assessments. The ACP is supported by a number of panels, including a Medical and Toxicology Panel that advises on a range of issues associated with human risk assessments. The Department of Health/Food Standard Agency Committees on Toxicity of Chemicals in Food Consumer Products and the Environment, Mutagenicity

and Carcinogenicity provide advice on specific issues. The main laws that apply in this area are the Food and Environment Protection Act 1985 (FEPA)[1] and the Control of Pesticides Regulations 1986 (as amended) (COPR)[2] (see Fig 4.1).

Fig 4.1 Flow diagram of the approvals process for pesticides in the UK



4.6 The UK system is gradually being replaced by a second system in which a major part of the scientific evaluation is organised by the European Commission, according to Directive 91/414/EEC.[3] Under the European system, the active ingredients in pesticides are assessed by a committee of member states

and if they are shown to be acceptable they are entered on a list of such substances (known as Annex 1). Contentious cases can be referred to the independent Scientific Committee on Pesticides (SCP). Once an active ingredient has been listed in this way, applications can be made to have products that contain it approved in individual Member States for specified uses. In responding to such an application, the government concerned would be expected to draw upon the scientific assessment that had already been agreed. Directive 91/414 has been enacted in the UK by the Plant Protection Products Regulations 1995 (as amended).[4] It will however, be some years before the process is complete, and in the meantime the national and European systems continue to work in parallel.

4.7 A broadly similar system is being established for non-agricultural pesticides (biocides), under Directive 98/8/EC[5].

The approvals process for new pesticides

4.8 Before a new pesticide can be approved for sale and use, evidence is required of its efficacy and that it will not pose an unacceptable risk to human health or to wildlife. To this end, companies seeking approval for a new product are required to submit an extensive package of scientific data. The components of the package can vary according to the nature of the pesticide and the uses to which it will be put, but as far as possible they are standardised. One of the main components of a data package addresses the potential toxicity to humans.

4.9 To assess the risks to humans, a number of processes are performed sequentially. Firstly the toxic properties of the active substance are identified and the dose-response relationships determined. Then acceptable levels of exposure are calculated. These acceptable levels are then compared with estimates of likely exposures. Currently assessments are normally based on assessing an individual pesticide active substance in isolation. Approval will not be granted if the available data do not show the overall risk assessment to be acceptable.

Potential toxicity in humans

4.10 Data on potential toxicity are required for the active ingredient, the formulated product as sold, and also any important metabolites of the active ingredient to which humans might be exposed. These data are derived largely from tests in laboratory animals, but care is taken to ensure that all use of laboratory animals is the minimum strictly necessary and appropriate animal welfare standards are applied. To ensure the quality of the data and to aid acceptance by all regulatory authorities, studies are expected to be performed to accepted international guidelines and be compliant with the principles of Good Laboratory Practice (GLP). If reliable information can be obtained by other means, such as extrapolation from information on related compounds, these should be used in preference to animal studies. Where information on the health of exposed humans is available (e.g. of workers involved in their manufacture and testing), this is taken into account. The information required to address the potential human toxicity covers:

- How the active ingredient is absorbed, metabolised and excreted in mammals;
- The acute toxicity of a single high dose of the active ingredient and of the product by various routes of exposure. The potential of the active ingredient and product to irritate the skin or eyes or to cause skin allergies (sensitisation);
- The toxicity and carcinogenic potential of the active ingredient when administered over periods of several weeks to a lifetime;
- The genotoxic potential of the active ingredient;
- The potential for the active ingredient to affect reproduction or to impair the development of the fetus or neonate;
- Further tests may be required if there is a need to understand specific effects better, for example on particular organ systems such as the nervous (eg neurotoxicity studies or delayed neurotoxicity studies), immune, or endocrine systems.

4.11 On the basis of these data, a decision is made as to whether the product requires labelling as a hazard (eg. irritant, harmful, toxic). In addition, acceptable levels of exposure may be derived from the no observed adverse effect levels (NOAELs) for any ill-effects that might occur. A NOAEL is the highest dose in an investigation that does not cause adverse effects. In the case of anticholinesterases (OPs and carbamates) an adverse finding is generally taken to be a greater than 20% depression in either erythrocyte or brain acetylcholinesterase activity. Some regulatory bodies have ignored depression in red cell acetylcholinesterase, where brain cholinesterase has been unaffected (for further discussion of this issue see FAO/WHO[6] and Marrs[7]). A depression in plasma cholinesterase activity is generally not considered adverse (see also 4.39 below).

4.12 Three key acceptable exposure levels (sometimes called reference doses) will normally be derived for an agricultural pesticide:

- Acceptable daily intake (ADI)

This is the mean amount of a chemical which can be consumed every day for a lifetime in the practical certainty, on the basis of all known facts, that no harm will result. It is expressed in milligrammes of the chemical per kilogramme bodyweight of the consumer (mg/kg bw). The starting point for the derivation of the ADI is usually the lowest relevant NOAEL that has been observed in toxicity studies. This is then divided by an assessment factor (also known as a safety factor or uncertainty factor) to allow for the possibility that animals may be less sensitive than humans and also to account for possible variation in sensitivity between individuals. The assessment factor is normally 100 (a factor of 10 for animal to human sensitivity and a factor of 10 for variation within the human population) but it can vary depending on the available data. A factor of less than 100 may be used

when there are appropriate human data or a larger factor may be used for compounds producing severe effects or as an interim measure when there is additional uncertainty surrounding an aspect of the data package. The studies from which NOAELs and hence ADIs are derived take into account any impurities in the pesticide active ingredient as manufactured, and also any toxic metabolites formed in the body.

- Acute reference dose (ARfD)

The definition of the ARfD is similar to that of the ADI, but it relates to the amount of a chemical that can be taken in at one meal or on one day. It is normally derived by applying an appropriate assessment factor to the lowest relevant NOAEL in studies that have assessed effects following short-term exposure or end-points such as developmental toxicity that may be affected by a single dose at a critical time.

- Acceptable operator exposure level (AOEL)

This is intended to define a level of daily exposure that would not cause adverse effects in operators who work with a pesticide regularly over a period of days, weeks or months. Depending on the pattern of usage of the pesticide, it may be appropriate to define a short-term AOEL (i.e. for exposures over several weeks or on a seasonal basis), long-term AOEL (i.e. for repeated exposures over the course of a year) or both. AOELs are derived in a manner analogous to the ADI. Because operators are normally exposed primarily via the skin some AOELs are based on an appropriate study that used dermal exposures. AOELs are used in risk management procedures and are not formal occupational exposure limits; moreover compliance with AOELs is not normally monitored. However, use of the protective equipment recommended on the label should result in exposures, averaged over a season of use that are below the AOEL. Insofar as operator protection is concerned the Control of Pesticides Regulations[2] are enforced by the Health and Safety Executive (HSE). Under the Control of Pesticides Regulations, a Certificate of Competence is required for those using agricultural pesticides, except for those born before the 31st January 1964, or who are working under supervision.

Estimating exposures

Dietary intakes

4.13 In assessing the risks from residues of a pesticide in foods, it is necessary to identify and take account of all foodstuffs in which significant residues might occur, including those resulting from the use of other products that contain the same active ingredient. If the use of a pesticide produces significant concentrations of toxic metabolites in food the acceptability of exposure to each of these metabolites is also assessed. To check whether the proposed use of a pesticide might cause unacceptable dietary exposures, an estimate is made of the maximum likely intake that an individual would be expected to incur over a prolonged period. Also, if the pesticide has toxic effects that could arise from a single dose, an estimate is made of the maximum likely dietary exposure that could occur in a single day or

from a large portion of that food. These estimates are based on the distribution of measured residues of the pesticide in foods derived (directly or indirectly) from treated crops, and data on the national patterns of consumption for different foods from surveys commissioned by the former Ministry of Agriculture, Fisheries and Food (MAFF). Separate calculations are carried out for dietary exposures in a number of different consumer groups, including infants, toddlers, children and adults to check that the particular dietary characteristics of all age groups are covered. Initial estimates are currently performed using a set of conservative assumptions and produce point (deterministic) estimates representing a realistic worst case.

4.14 In determining the likely long term exposure (termed a national estimate of dietary intake [NEDI]), the median residue level from trials performed following application of the pesticide according to the highest approved application rate and shortest pre-harvest interval, is used. This figure is multiplied by the mean daily consumption for a high level consumer (97.5 centile) and corrected for the body weight of the consumers in the survey. This process is performed for all commodities treated with the pesticide. The results are summed to give a value in mg/kg bw, which can be compared with the ADI. Because the long-term assessment is based on an average lifetime exposure, it is considered reasonable to choose the median residue value from composite samples as occasional exposures to high residues (within the confines of any acute dietary intake assessment) should be balanced with exposures on other occasions to products containing nil or low residues. For the acute intake estimate the highest residue found in trials (often the same as the maximum residue level (MRL[a] – see below) is multiplied by a variability factor of (1 – 10) and by the daily consumption for a high level consumer (97.5 centile) and corrected for body weight. This also gives a value in mg/kg bw and is compared with the ARfD. The variability factor is included in the acute assessment because the residues data are normally based on a composite sample and it has been shown that residues in individual items can be several times higher than the composite value. If appropriate data are available, it is possible to refine these estimates to account for factors such as changes in the levels of residue following processing or cooking.

4.15 Similar processes for assessing dietary intakes of pesticides are used by international organisations such as the EU and Codex Alimentarius Commission. The only significant difference is that the food consumption data are not specific to the UK.

Exposures to operators, other workers and bystanders

4.16 The other circumstance in which human exposure to pesticides commonly occurs is in the course of their application or through contact with crops or other materials that have been treated with pesticides, in ways other than through the diet. Most often, the uptake of pesticides from such sources will be by absorption through the skin but exposure by inhalation is also possible when products are sprayed or the active ingredient vaporises. Estimating the profile of exposure in operators, other workers and bystanders is complex and must take into account many factors. These include the physical form of the pesticide formulation, the way in which the pesticide is used, tasks performed on treated crops and the nature of any personal protective equipment such as gloves or a face mask. Other factors include the extent to which the pesticide penetrates the skin and patterns and extent of use.

[a] MRL is an acronym for maximum residue level in the case of pesticides and maximum residue limit in the case of veterinary products.

4.17 Initial estimates of exposure normally use a predictive model appropriate to the nature of the formulation and its pattern of use. The models are based on representative measurements from real operations, and have a tendency to err on the side of over-estimating exposures. The models include the ability to correct for protection afforded by specified protective equipment and the degree of penetration through the skin. The models generate exposure values in mg/kg body weight and these are compared with the AOEL.

4.18 In some instances workers using a particular pesticide will have had their actual exposures monitored during their work[a]. If such data exist they will be used in preference to the mathematical models.

Checks on existing pesticides

4.19 Once a pesticide has been approved, it is important to check that its use does not give rise to problems that were not foreseen when the approval was granted. To this end, several systems are in place to monitor pesticide usage in the UK and the occurrence of possible adverse effects.

- Monitoring of both home-produced and imported food for pesticide residues is carried out under the direction of the Pesticides Residues Committee (PRC), which has replaced the Working Party on Pesticide Residues (WPPR). The PRC includes expert and lay representation, both groups playing a role in directing the surveillance programme.

- The HSE Pesticide Incidents Appraisal Panel (PIAP) aims to consider all incidents investigated by HSE and local authorities in which the use of pesticides may have affected a person's health.

- Epidemiological studies of the possibility of pesticidal effects on populations are regularly published in the scientific literature, and to ensure that this information is given proper consideration, a system for reviewing the published literature has been established. If a problem is identified that could have implications for the regulation of pesticides, this is brought to the attention of the ACP and when appropriate, action is taken.

- There is a requirement for approval holders to notify any adverse findings.

4.20 Over the years the regulation of pesticides has become progressively more precautionary and now much more evidence is sought that human exposures will be acceptable than was customary in the past. It follows that the scientific data supporting older products are often less extensive than would now be required for a new approval, and it is therefore necessary to bring the data packages up to modern standards. This is achieved by programmes reviewing older compounds.

[a] Actually measured, as opposed to modelled.

Maximum Residue Levels (MRLs)

4.21 The dietary intake estimates described above rely on data generated in field trials. Another use of the trials data is to generate a legally enforceable limit known as the MRL. MRLs are generated by a statistical treatment of the residue data, and normally approximate to the highest residue seen in the trials. MRLs are not safety limits, and exposure to residues in excess of an MRL does not necessarily imply a risk to health. MRLs are defined as the maximum concentration of pesticide residue (expressed as milligrams of residue per kilogram of food/feeding stuff) legally permitted in or on food commodities and animal feeds. These are based on Good Agricultural Practice (GAP) and are intended primarily as a check that GAP is being followed and to assist international trade in treated produce. Formerly MRLs were set domestically under the Pesticides (Maximum Residue Levels in Food) Regulations.[8] Nowadays, most MRLs in the UK legislation are those that have been agreed at the EU level. Though MRLs are not formally used in pesticide risk assessments in the UK, in general consumption by individuals at the 97.5th centile of crops containing pesticides at the MRL should not give rise to breaches of the ADI.

4.22 MRLs are also set on a world-wide basis by the Codex Committee on Pesticide Residues (CCPR). Some Codex MRLs are higher than EU ones, but under the World Trade Organisation agreement on Sanitary and Phytosanitary barriers to trade[9] it is not possible to stop the import of foodstuff complying with Codex MRLs unless an unacceptable risk to health can be demonstrated. Such instances should be rare, as risk assessments are performed before Codex MRLs are agreed. These assessments are similar to those performed in the UK, with reference doses set by the WHO/FAO Joint Meeting on Pesticide Residues (JMPR) and food consumption data chosen to cover typical diets over a number of regions of the world.

Assessment of exposures to combinations of pesticides

4.23 Current assessments of pesticides concentrate on the acceptability of an individual active substance in isolation. The potential for the toxicity of an active substance to be altered by other components of the formulation or other residues on a crop is not investigated in as much detail as the properties of the individual active substance.

4.24 The acute toxicity of the formulation as sold must be addressed. This may be done either by testing the formulation or by a calculation based on the properties of its constituents. If data show the acute toxicity of the formulation to be significantly different from that expected based on its individual constituents this will be investigated further. It is unusual for repeat dose oral studies to be performed on a formulation therefore any interactions that alter the toxicity profile of the active ingredient following repeated exposure are unlikely to be identified.

4.25 Formulations may contain more than one active substance. When assessing formulations containing 2 or more active substances with a common mechanism of action or similar toxicity profiles the potential for interaction is currently considered. These assessments are performed on a case-by-case basis. Normally this assumes simple additive effects and involves summing the contributions relative to the applicable reference dose for each individual active substance. In some instances, additional information has been requested from companies. Such assessments were not performed routinely in the past, when the majority of the mixed formulations currently on the market were originally registered.

4.26 There are no generic restrictions on an operator applying sequentially 2 or more compounds with a similar mechanism of action. Similarly, there are few restrictions on applying two pesticides concurrently. If a pesticide formulation specifically indicates that it can be used in combination with another formulation, a limited assessment of the combination may be performed. The only specific restrictions are those on the mixing of two or more anticholinesterase compounds in the same spray tank.

4.27 Current UK and international assessments of pesticides do not routinely take any specific account of the risk to consumers from the potential for interaction of residues of different pesticides or to operators from the potential effects of simultaneous or sequential exposure to different active substances. In specific cases, where a range of compounds degrade to a common toxic metabolite and residue, a group ADI has been set – e.g. ethylene*bis*dithiocarbamates (EBDCs) are assessed in terms of ethylene thiourea (ETU). The basic assumptions underlying these procedures are that exposures will be significantly below the NOAELs in animals, making significant interactions unlikely and that the assessment factors address the potential for simple interaction.

Baby Foods

4.28 Processed cereal-based foods and baby foods for infants and young children are regulated under Directives 96/5/EC and 1999/39/EC.[10,11] These directives provide for a general MRL of 0.01 mg/kg for any individual pesticide in processed cereal-based foods and baby foods.

Veterinary Medicines

Introduction

4.29 Veterinary Medicinal Products (VMPs) include products that are used on food-producing animals (including poultry and fish) and, as with pesticides used on crops, such products may leave residues in meat, milk and dairy products, eggs, fish and honey. Some products, particularly ectoparasiticides (such as sheep dips) may contain active ingredients that are also used in pesticide formulations. It should also be noted that VMPs for pet animals (such as flea sprays and collars) may bring about non-food exposure of the public to some of these active ingredients.

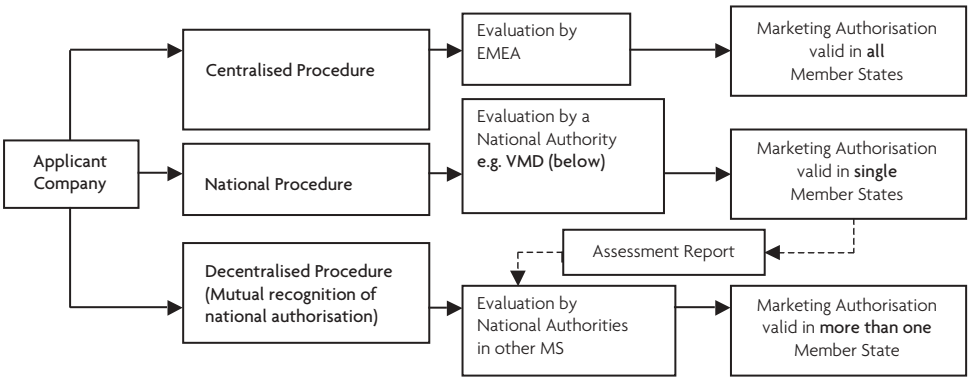
4.30 Regulation of veterinary medicines dates from the enactment of the Medicines Act in 1968.[12] This Act covered drugs used in humans as well as those used on animals. The Veterinary Products Committee (VPC-see below) was established under section 4 of the Medicines Act and the Medicines Commission, to which appeals against the decisions of the VPC may be made, was established under section 2 of the Act. The Licensing Authority was defined as UK health and agriculture ministers. In the Act, a medicinal product was defined as a product used for a medicinal purpose, the latter expression being defined as *inter alia* for the treatment or prevention of a disease.

4.31 The VPC is the statutory independent expert committee that advises the Licensing Authority on the safety, quality and efficacy of VMPs marketed in the UK to which any provision of the Medicines Act (1968)[12] or EU legislation is applicable. The VPC has two main sub-committees; the Medical and Scientific Panel advises on research and effects of OP sheep dips, and the Appraisal Panel on Human Suspected Adverse Reactions to VMPs considers reported human reactions The Veterinary Residues Committee provides independent advice to VMD and FSA on the scope, operation and interpretation of the statutory and non-statutory residues surveillance programmes. Government departments and/or agencies other than DEFRA (principally the FSA, the Department of Health, the Environment Agency, the Health and Safety Executive and representatives of the devolved administrations) have input through the Scientific Secretariat to the VPC, or directly with VMD on specific issues. As with pesticides, the Department of Health/Food Standard Agency Committees on Toxicity of Chemicals in Food, Consumer Products and the Environment, Mutagenicity and Carcinogenicity provide advice on specific issues.

4.32 An EU system for the evaluation and approval of certain VMPs (e.g. those containing novel active ingredients or produced using recombinant DNA technology) has been established to provide a centralised procedure of authorisation. The EU centralised system, which is operated by the European Medicines Evaluation Agency (EMEA), in London, was established under Council Regulation EEC 2309/93.[13] Under this regulation the Committee for Veterinary Medicinal Products (CVMP) is the expert scientific committee responsible for preparing the opinion of the EMEA on any scientific matters relating to the evaluation of VMPs. It consists of two members nominated by each European Member State and two non-voting members from participating EFTA States (Iceland and Norway). The CVMP is advised on matters relating to human safety by the Safety Working Party consisting of one delegate (CVMP member or European Expert) from each EU and EFTA state plus additional European Experts as required by the agenda. The centralised procedure is binding on all member states. There is also a decentralised (mutual recognition) procedure where member states may, at the authorisation holder's request, recognise another member state's authorisation and disputes between member states on a particular authorisation can be referred for binding arbitration to the CVMP (see Fig 4.2).

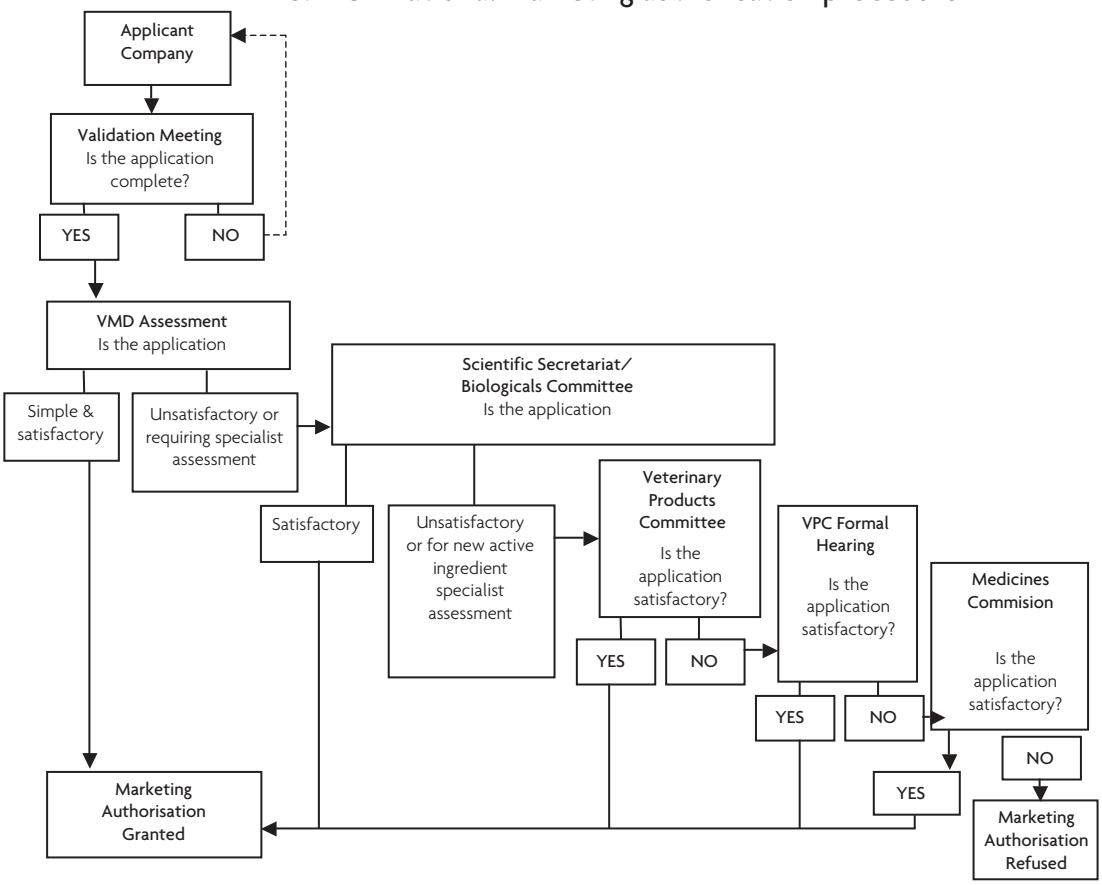
Fig 4.2 Flow diagram for the marketing authorisation process for veterinary medicinal products

a. Routes to approval of veterinary medicinal products in Europe



MRLs must be determined for new active ingredients before new products can be authorised. This is a Centralised Procedure

b. UK national marketing authorisation procedure



The approvals process for new veterinary medicines

4.33 Before a new veterinary medicine can be granted a marketing authorisation, evidence for safety, quality and efficacy must be provided. In the context of food-producing animals safety for the target species, humans using the VMP (on animals) and consumers of edible tissues/products from treated animals are considered.

4.34 As with pesticides, it is necessary to identify the toxic properties of the active ingredients, to determine dose response relationships and to determine acceptable levels of exposure. Individual assessment of veterinary medicines is the norm, and interactions with other veterinary medicines or with pesticides is not usually assessed.

Human toxicity

4.35 As with pesticides the potential for human toxicity is largely determined on the basis of *in vitro* and laboratory animal studies. The major risks to consumers are considered to be potential chronic (lifetime) low dose toxic effects including carcinogenicity and reproductive effects. Pharmacological effects can also be important. Effects on the normal gut microflora are considered for antimicrobial substances. In the case of user safety the major risks are related to exposure to the VMP during/after treatment. The data required would usually comprise:

- pharmacodynamics;
- absorption, distribution, excretion and metabolism;
- acute toxicity of a single dose;
- chronic oral toxicity and carcinogenicity;
- genotoxicity;
- reproductive toxicity;
- safety studies in target species.
- Other data: these may include immunotoxicity, dermal, inhalational, ocular, neurotoxicity or microbiological studies. In the case of anticholinesterase compounds such as OPs, studies for delayed neurotoxicity are required. Human data (if available) will be used.

Some attention is also paid to the environmental effects of the VMP.

4.36 To consider properly consumer safety, a full dossier of data would normally be required. For user safety, a more selected data set depending on the inherent properties of the VMP and the route and extent of exposure would be acceptable.

4.37 Acceptable levels of exposure are derived from NOAELs for any ill-effects that are observed in the above studies, the NOAEL being the highest dose that does not cause adverse effects. In certain circumstances where a NOAEL cannot be established, a lowest adverse effect level (LOAEL) may be used with an additional assessment factor.

Consumer safety assessment

4.38 This involves assessment of the risk for consumers of edible tissues (muscle, liver, kidney, fat/skin) and/or other edible products (milk/dairy products, eggs and honey) from food-producing animals (cattle, sheep, pigs, poultry, fish, bees, etc.). Acceptable daily intakes (ADIs) and maximum residue limits (MRLs) have to be determined for all substances (active ingredients and pharmacologically active excipients) used in VMPs within the EU. Assessments are conducted at a European level by the CVMP. ADIs have no statutory standing, but are used to determine the statutory MRLs. The MRLs and residue depletion data are used to determine withdrawal periods for products containing these substances, during which treated animals may not be slaughtered for food, or produce collected. Withdrawal periods may be set at national or European levels.

4.39 The key reference dose for residues of veterinary medicines is the acceptable daily intake. This is the amount of the residue (active substance and/or metabolite) which may be consumed every day for a lifetime in the practical certainty, on the basis of all the known facts, that adverse effects will not result. For some veterinary medicines pharmacological and microbiological ADIs may also be needed as well as toxicological ADIs, and the overall ADI and MRLs for the substance will be based on the lowest of these three endpoints.

- Pharmacological ADIs:

A pharmacological ADI would be established, for substances where the pharmacodynamic activity of a substance is likely to be of greater biological significance than other endpoints, e.g., tranquillisers, hormones, prostaglandins. They are based on the most relevant NOAEL/LOAEL for the primary (i.e. the intended therapeutic activity) and secondary (other) pharmacodynamic effects of the substance.

- Toxicological ADIs:

A toxicological ADI should be established, based on the most appropriate NOAEL/LOAEL in the most sensitive species. ADIs are not established for genotoxic compounds due to the assumed non-threshold nature of the effects, and these compounds would generally not be permitted in products intended for food species. In the case of non-genotoxic carcinogens if a mechanism can be identified for any carcinogenic effect observed an ADI could be determined. This could be based on the NOAEL

for the most sensitive indicator of that mechanism with a standard 100-fold safety factor. If the mechanism is unclear, a greater assessment factor may be used. In the case of aneugens, an ADI may be established if a threshold for aneugenic effects can be demonstrated that is greater than concentrations likely to reach target tissues in humans exposed to residues. A positive result in a delayed neurotoxicity study would not permit an ADI to be established. For anticholinesterase compounds, significant inhibition of plasma cholinesterase without overt toxicity and no depression of erythrocytic or brain cholinesterase is generally regarded as an indicator of exposure rather than toxicity. A greater than 20% depression in erythrocyte or brain cholinesterase is generally considered adverse (see also 4.11 above).

Pharmacological and toxicological ADIs are determined using the 10 x 10 assessment factor approach, plus where necessary, an additional factor of 2-10 depending on the quality of the data and the nature and severity of the critical effect.

- Microbiological ADIs:

For substances with microbiological activity, a microbiological ADI must be established. The intake of ingested residues should not result in perturbation of the normal human gut microflora. In the EU, this ADI is usually established from determination *in vitro* of minimum inhibitory concentration (MIC) studies on a range of microorganisms representative of the flora of the distal human gut. An ADI is calculated using appropriate safety factors to take into account the range and variability of the MIC data available, evidence of microbial resistance and extrapolation from *in vitro* to *in vivo* conditions.

4.40 Unlike pesticides, ARfDs are not established for active substances used in VMPs. The MRLs are determined from the ADI so that the theoretical maximum daily intake should not exceed the ADI when VMPs containing the substance are used in accordance with the authorised conditions of use.

Maximum residue limits

4.41 Maximum residue limits[a] for veterinary medicines are, statutory and set across the EU,[14] but unlike those for pesticides, they are health-based limits derived directly from the ADI. In establishing ADIs and MRLs, due consideration is always given to the opinions of other international bodies (such as the Codex Alimentarius Committee on Veterinary Residues and the FAO/WHO Joint Expert Committee on Food Additives) to ensure a harmonised approach whenever possible. As with pesticides, since the SPS agreement,[9] it is not possible to stop the import of products from outside the EU that comply with Codex MRLs, unless an unacceptable health risk can be demonstrated.

4.42 Based on pharmacokinetic and tissue residues studies in the target species, and taking into account the ratio of the analytical marker residue to total residues, the distribution of the residues of the substance between the various edible tissues and products can be determined. Account is also taken of the pattern of residue depletion of the substance in the target animal and possible persistence of residues in specific organs such as the liver or kidneys, or at the injection site. Based on a standard

[a] MRL is an acronym for maximum residue levels in the case of pesticides and maximum residue limit in the case of veterinary residues (see appendix 1)

food basket, MRLs can be allocated to the different items in such proportions that residue intake of a 60 kg adult should not exceed the ADI.

4.43 This food basket was defined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and it represents a high level of daily intake, adding further conservatism to the risk assessment:

100	g	liver
50	g	kidney (10 g for poultry)
300	g	muscle (or muscle and skin in natural proportions for fish)
50	g	fat (or fat/skin for pigs and poultry- 90 g for poultry)
1.5	l	milk
100	g	eggs
20	g	honey

4.44 In the case of substances that also have pesticidal uses, the proportion of the ADI allocated to veterinary residues would generally not exceed 45%. Normally, residue data must be provided for, and MRLs established separately, for each species in which the substance is intended to be used. In the case of minor food species, e.g. deer, rabbits or game birds, there are reduced data requirements that allow extrapolation from major food species such as cattle, sheep or poultry. A validated analytical method suitable for routine monitoring purposes must be available. This would normally be expected to have a limit of quantification at least 50% lower than the MRLs in the target tissues. The MRLs may be further reduced, but never increased, to take into account the capabilities of the analytical method and the likely level of residue that may be expected to remain after use of the substance in accordance with good veterinary practice.

Withdrawal periods

4.45 Once MRLs have been established and published for substances, withdrawal periods can be determined for products that contain them, based on residue depletion data in target tissues. The withdrawal period should ensure that under the authorised usage conditions, residues are below the determined MRLs at the time of slaughter or collection. During the withdrawal period, animals may not be sent for slaughter and their products may not be used for human consumption. Once MRLs have been established for a substance by the CVMP, it can be entered into one of the four Annexes of Council Regulation (EEC) 2377/90:[14]

Annex I Full ADI established and MRLs determined
Substances have a full ADI and MRLs have been established for the intended target species. A validated analytical method is available for determination of withdrawal periods, and to allow routine surveillance for residues monitoring.

Annex II No MRLs necessary
This annex contains substances that are normal components of the animal's or human diet or where levels of use are toxicologically insignificant e.g. homeopathic remedies. It may also include substances where it was not possible to establish an ADI, but pharmacokinetic data or intended usage indicates no significant consumer exposure, e.g. no NOEL could be identified for the most sensitive toxicological endpoint, but residues studies show no detectable residues in the target tissue, within hours of treatment.

Annex III Provisional MRLs
This annex is for substances where a full ADI has been established but there are still minor outstanding issues relating to residues data, or the analytical method. A time limit is specified for the provision of additional data.

Annex IV No ADI. Must not be used in food species
This annex is for substances where it has been determined that residues of the substance, at whatever limit, constitute a risk to the consumer.

4.46 A substance may be entered into more than one annex for use in different species, e.g. Annexes I and II, or I and III. Annex entries (particularly Annex II) may contain other provisions limiting their use e.g., topical, intravenous or intramammary use only, or set a limit on the concentration of the substance permitted in formulations.

User Safety Assessment

4.47 User safety assessment considers the risks to those who may be exposed to VMPs during, or after treatment. This includes veterinary surgeons/nurses, farmers and farm workers, kennel/stable workers, animal handlers, pet owners and children who may come into contact with not only food-producing species, but also companion animals (pets, working/sporting animals, ornamental fish and birds, and other exotic species).

Risks

4.48 The most significant user safety risks are; accidental ingestion, self-injection, dermal, ocular, inhalational exposure and are often acute effects. Hypersensitivity reactions can be a problem with repeated exposure to sensitising chemicals. Multiple, repeated and/or frequent exposures are possible from occupational use e.g., veterinary staff and farm workers. Intimate and/or prolonged exposure is possible in the case of pet owners, particularly children.

Safety data

4.49 Acute oral, parenteral, dermal, and/or inhalational toxicity, dermal and ocular irritancy, and sensitisation data are normally required. Basic genotoxicity data are usually sought, but are specified in the legislation only if the active substance is either new to VMPs and/or for use in food-producing species. Data on chronic and reproductive toxicity are not mandatory for user safety assessment, but would be required if deemed necessary by the nature of the active ingredient(s) and the type, likely extent and frequency of exposure or a combination of these considerations.

Risk Assessment/Management

4.50 The primary legislation controlling the protection of workers is the Control of Substances Hazardous to Health Regulations known as COSHH.[15] COSSH mandates employers, where hazardous substances are used at work, to carry out an assessment of the health risks involved. Risk assessment involves the identification of the toxicological endpoints relevant to user safety dependent on likely exposure from administration of the product and contact with treated animals. There is no provision for setting Acceptable Operator Exposure Levels (AOELs) for VMPs under EU legislation. It may be necessary, on a case-by-case basis, for applicants to provide evidence that user exposure will not exceed levels considered to be acceptable from the risk assessment. Where exposure to VMPs occurs in an occupational setting (veterinary surgeries, feed manufacturers, etc), the user may need to conduct some form of workplace exposure risk assessment that may involve consideration of AOELs for components of the VMP.

4.51 User safety is generally managed by avoidance of unnecessary exposure with advice on safe storage, handling and disposal of the product. Product literature should include directions on safe administration, advice on suitable protective clothing, and advice on removal of contamination. Engineering controls are used to avoid exposure during the incorporation of medicated pre-mixes into feed. Appropriate dosing devices may be required; multi-dose syringes, shielded needles, oral-dosing pumps, coated tablets, single dose applicators and sachets. In some cases, warnings to seek medical attention and advice to doctors may be included in the literature. In the case of sensitising agents, users may be advised to avoid contact when a known sensitivity/allergy exists. Operator training may also be required, e.g., certification of competence for sheep dipping operatives.[16]

Animal Feed Additives

4.52 A wide variety of substances including antibacterials, antiprotozoal agents, fungistats, vermicides, inorganic chemicals and novel protein sources are used in animal feedstuffs for a variety of purposes, including to prevent infection, to preserve the feedstuff, to increase growth, and to alter the physiology of the animals. Many of these substances are pharmacologically active. In the EU, they are controlled under Council Directive 70/524/EEC.[17] The safety and efficacy of animal feed materials under their recommended conditions of use are evaluated by the Standing Committee on Animal Nutrition, which advises the European Commission. Each Member State has representatives on the Standing Committee. In the UK, the representatives are employees of the VMD, for pharmacologically

active substances, and of the FSA, for all other substances. The European Commission routinely refers specific questions on scientific aspects of submissions, including the potential to produce adverse effects on consumers of animal products, to the Scientific Committee on Animal Nutrition (SCAN). When the European Food Safety Authority (EFSA) is established these procedures will be replaced by a new legislative framework, with the EFSA taking over the responsibilities of the Commission and setting up new committees of its own.

International Harmonisation of Regulation of Veterinary Medicines

4.53 The role of Codex in relation to setting of MRLs for trade purposes has been discussed above. Additionally the International Harmonisation of Veterinary Medicinal Products (VICH) is currently attempting to harmonise the technical requirements for the registration of veterinary medicinal products. VICH is a tripartite agreement between the EU, the USA and Japan, with other countries including Australia and New Zealand as observers. Working groups are currently developing common guidelines for the assessment of the quality, safety and efficacy of veterinary medicinal products.

Conclusion

4.54 The approvals system for pesticides and that for marketing authorisation of VMPs, is broadly similar but a major difference is that there is a statutory appeals system from decisions of the VPC to the Medicines Commission. There are two further differences:-

- although MRLs for pesticides and veterinary medicines are statutory limits, pesticidal MRLs are not safety based, whereas those for veterinary medicines are.
- the veterinary medicine marketing authorization system has moved further towards a pan-EU system than has that for pesticides approvals.

Human medicines

4.55 A few products regulated as human medicinal products contain active ingredients that are similar or, in some cases, the same as substances used as pesticides or VMPs. These include OPs, such as malathion used as a head louse treatment, metrifonate/trichlorfon used in infestation with *Schistosoma haematobium* and ecothiopate iodide used in the treatment of glaucoma. Azole fungicides are used in human medicine and as agricultural fungicides, sometimes being given different names in the two roles. Thus the pesticide with the ISO name imazalil,[18] is used as a human pharmaceutical under the name enilconazole. In nearly all developed countries, human medicines are subject to a prior authorization system of regulation. The UK system, established under the Medicines Act (1968)[12] and run by the Medicines Control Agency has been largely superseded by procedures established under EEC regulations.[13] These regulations established procedure for authorization and supervision of human medicines across the EC and established the EMEA London. The basis for risk assessment of human pharmaceuticals is different from that for pesticides and human exposure to veterinary medicines as it

is based upon balancing risk, including toxicological risk, against expected benefit. Combined exposure with pesticides and other substances is not normally considered.

Mixed exposures in the workplace

4.56 The focus of this report is exposure to pesticides and similar compounds through food and to a lesser extent, water and in the home. However, procedures are already in place to cover mixed exposures to air-borne toxicants in the work place and these are briefly discussed below.

4.57 Many pesticides are substances hazardous to health and therefore covered by the requirements of the Control of Substances Hazardous to Health Regulations (COSHH).[19] Under COSHH the HSE sets two types of occupational exposure limits for hazardous substances at work: maximum exposure limits (MELs) and occupational exposure standards (OESs). Both types of limits are concentrations of hazardous substances in the air averaged over a specified period of time referred to as a time-weighted average (TWA). A MEL is set for substances which may cause the most serious health effects and for which safe levels of exposure cannot be determined or it is not reasonably practicable to control exposure to a safe level. An OES is set at a level at which there is no indication of risk to health of workers exposed by inhalation day after day.

4.58 The majority of the exposure limits are for single compounds, however, a few of the limits relate to substances commonly encountered as complex mixtures eg welding fumes. As workers are frequently subject to a variety of mixed exposures the HSE has published basic guidance on an approach for the assessment of mixed exposures and the application of exposure limits in these circumstances.[20]

4.59 Where mixed exposures occur, the first requirement is to ensure adequate control of exposure for each individual substance. If available, limits for specific mixtures should only be used where they are applicable and in addition to any relevant individual limits. In most cases, close examination of the toxicological data is required to determine which of the main types of interaction (if any) are likely for the particular combination of substances concerned; the various types have to be considered in the following order:

- Synergistic substances: Although known cases of synergism and potentiation are considerably less common than the other types of behaviour in mixed exposures, they are the most serious in their effects and require the most strict control. They are also the most difficult to assess and whenever there is reason to suspect such an interaction, specialist advice will be required.

- Additive substances: Where there is reason to believe that the effects of the constituents are additive, and where the exposure limits are based on the same health effects the mixed exposure is assessed by means of the formula:

C1/L1 + C2/L2 + C3/L3..<1

where C1, C2 etc are the TWA concentrations of constituents in air and L1, L2 etc are the corresponding exposure limits.

The use of this formula is only applicable where the additive substances have been assigned OESs and L1, L2 etc relate to the same reference period in the list of approved OESs. Where the sum of the C/L fractions does not exceed 1, the exposure is considered not to exceed the notional exposure limits. If one of the constituents has been assigned a MEL, then the additive effect has to be taken into account in deciding the extent to which it is reasonably practicable to further reduce exposure.

- Independent substances: Where no synergistic or additive effects are known or considered likely, the constituents can be regarded as acting independently. It is then sufficient to ensure compliance with each of the exposure limits individually.

These methods are used in occupational health in other countries (e.g. the USA).

4.60 It is important to note that factors complicating the assessment and control of exposure to individual substances will also affect cases of mixed exposures and require special consideration. These include:

- exposure to a substance for which there is no established limit (as would be the case for most pesticides) or for which a MEL has been set,
- exposure to and absorption through the skin (particularly important in the case of pesticides as the skin can often be the predominant route of exposure) and
- the relevance of factors such as alcohol, medication and smoking.

In each of these circumstances specialist advice will be required.

Regulatory controls in the United States

Pesticides

4.61 The regulation of pesticides in the United States of America (USA) is, in general terms similar to that in the UK. The responsible agency is the United States Environmental Protection Agency (USEPA). It should be noted that there are some differences in the way toxicity data are evaluated in the USA compared to the UK and the term reference dose is used instead of ADI.

4.62 The Food Quality Protection Act (FQPA) of 1996[21] mandated the consideration of all sources of pesticide exposure when carrying out risk assessments and also the consideration of the effects of combined exposure to different pesticides. This act introduced the terms aggregate and cumulative risk assessment to the pesticide regulatory framework in the USA. Aggregate risk assessment considers exposure to a single chemical from multiple sources, specifically food, drinking-water and residential-use sources.[22] Cumulative risk assessment covers concurrent exposure to multiple chemicals with the same mechanism of toxicity.[23] For risk assessment, the analogy of the risk cup is used. The size of the risk cup is defined using toxicology data, as the acceptable exposure level, from all sources, to all chemicals in the same cumulative assessment group (CAG), i.e. pesticides deemed to share a common mechanism of toxicity.

4.63 The concepts of aggregate and cumulative risk assessment are straightforward and readily defined but the practicalities are considerably more complex. When the FQPA was enacted in 1996, cumulative and aggregate risk assessments had not been carried out in practice. The passing of the act initiated a considerable degree of policy-making in order to define the scientific and regulatory details of implementing aggregate and cumulative risk assessment.[24,25] In 2002 the USA is still some distance from full practical implementation of FQPA, but some progress has been made at the science policy level. All regulatory actions must be accompanied by an FQPA risk assessment. In practice this means a single chemical assessment, with the emphasis on exposure via food and water residues. Residential uses are considered, but many problems in this area remain to be resolved. Regulatory cumulative risk assessments have not yet been carried out for any group of substances, although simplified draft examples have been produced for review in science policy fora.[23,26] Methodologies and software for estimating aggregate and cumulative exposure are still under development. Crucially, cumulative-risk based regulatory decision-making for the first priority substances, OPs, has yet to be initiated.[27] These areas are considered in a little more detail below.

Food residues

4.64 Single chemical risk assessment related to residues in food was well established before enactment of the FQPA. Even here cumulative risk assessment has generated difficulties. Regulatory field residue studies are based on single chemicals, and so are perceived to be of less value when considering exposure to multiple chemicals.[23] Consequently, there has been an increased emphasis on data where each pesticide in the cumulative assessment group is monitored simultaneously.[28] Existing monitoring data is rarely comprehensive in terms of the chemicals under study so specific market-based surveys are sometimes performed. Monitoring data typically shows residues that are far lower than data from field trials conducted at the highest level of good agricultural practice (GAP). This fact underpins a reluctance to perform risk assessments with a mixture of conventional field trials and market-basket data.[23] The increased emphasis on expensive, multiple-residue food monitoring data assumes that the most sensitive factor in a cumulative exposure assessment is the co-occurrence of multiple residues in a single food item. This may be inappropriate if, for example, co-occurrence of multiple residues in different foods consumed at the same meal, or on the same day, proves ultimately to be more important in practice.

4.65 Residues resulting from veterinary uses and uses in food handling establishments are also considered, in addition to residues resulting from agricultural uses. However, these additional sources of food residues are rarely significant contributors to overall exposure.

Drinking-water residues

4.66 For acute exposure to residues, surface-water will tend to be the worst-case source of drinking-water. For chronic exposure, either ground-water or surface-water might represent the worst case. In both examples very conservative models are used to estimate exposure to single chemicals, which often results in water dominating an aggregate risk assessment.[29,30] This often simply reflects the fact that there are better data on food residues than on residues in water. The options for refining the conservative surface-water exposure estimates are expensive monitoring programs, but acceptable generic study designs remain to be agreed.[31] The role to be played by existing monitoring programs is also a matter of debate, especially for acute exposure. One of the main issues is whether extremely frequent monitoring is needed to capture peaks of exposure, other whether less intensive monitoring of more watersheds can achieve the same end on a statistical basis.

4.67 In the case of exposure to multiple chemicals there is no specific procedure for estimating co-occurrence of residues in water. Even if monitoring data are collected, it is currently unclear how this should be used in an aggregate or cumulative exposure assessment.

Residential uses

4.68 There are a number USEPA Residential Standard Operating Procedures (SOPs) that are used to estimate exposure from residential use scenarios.[32] These include the exposure of the person applying the pesticide (except where this is a professional applicator) and post-application exposure in and around the home. Occupational exposures are excluded from consideration. Each of these is essentially a relatively simple calculation, with a number of input parameters. However, in many cases measurements are not available. Instead conservative default values are used. In these circumstances, the aggregate exposure assessment for pesticides with residential and crop uses will usually be dominated by the residential exposure estimates.[23] A number of studies are underway to refine some of these conservative assumptions. It remains unclear how possible exposure to multiple pesticide residues in the home may be taken into account.

Estimating aggregate and cumulative exposure

4.69 Models had been developed prior to the enactment of FQPA to assist in the estimation of human exposure to individual chemicals from individual exposure sources, i.e. food, water, and from residential uses. Two new probabilistic models are being developed to handle the whole range of aggregate and cumulative exposure, CARES[33] and LifeLine[34] (see also Chapter 9). Whether either or both will ultimately be accepted for regulatory use remains a subject for debate. In their current form these models are applicable only to the USA.

4.70 Currently, residential and drinking-water exposure assessments tend to be far more conservative than those based on diet resulting in a reluctance to include them together in a single exposure assessment.[23] For multiple chemicals in a cumulative exposure assessment, each with exposure from multiple sources, an assessment on this basis could be so conservative as to be meaningless.

4.71 The USEPA has recently published its first assessment of cumulative toxicity (for OPs).[35]

Regulatory implementation

4.72 As in the UK and EU, the entire basis of the US registration process is the assessment of single pesticide active ingredients and their formulated products. The FQPA risk cup presents some major problems in this context, if the cup overflows, *i.e.* if there is not an acceptable safety margin for the group of pesticides being considered. It has proved extremely difficult both administratively and legally for the USEPA to address this challenge. For example, for the USEPA selectively to cancel product registrations, or to allocate fractions of the risk cup to different registrants, would contravene US Anti-Trust Law (analogous to EU and UK Competition Law).

Veterinary medicines

4.73 Compounds that are regulated as veterinary medicines in the EU, including the UK are regulated by three different agencies in the USA. Biological veterinary products (vaccines, sera and toxins) are dealt with by the United States Department of Agriculture (USDA), while ectoparasiticides are regulated by the USEPA under the Federal Insecticide, Fungicide and Rodenticide Act,[36] sometimes known as FIFRA (see also USEPA).[37] Such products include sheep and cattle dips, pour-on formulations for farm animals, ear tags for cattle and products to treat fleas on pet cats and dogs. Other compounds such as antimicrobial drugs, anthlemintics, anti-inflamatories, analgesics and anesthetics and anti-fungal drugs are regulated by the Center for Veterinary Medicines which is part of the Food and Drug Administration. The toxicity data required for ectoparasiticides and for other veterinary pharmaceuticals are similar to those required in the UK.

4.74 Residue surveillance for veterinary residues, including those derived from ectoparasiticides, is carried out by the Food and Safety Inspection Service of the USDA. Raw meat containing residues above the tolerance (equivalent to the MRL) is considered to be adulterated (see Woodward).[38]

Human medicines

4.75 In the United States, human drugs are regulated by the Food and Drug Administration under the Food Drug and Cosmetic Act[39] as amended. See review by Davies and Watson.[40]

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5. Evidence of dietary exposure

Introduction

5.1 There are a number of difficulties in estimating exposure of human populations to pesticides and veterinary drugs. In theory such estimation can be carried out in several ways. Firstly, by sampling food for pesticide or veterinary residues; here with knowledge of food intake patterns, pesticide intake may be calculated. This process is the main concern of this chapter. Secondly, biomarkers of exposure may be used, such as organochlorines (OCs) in fat or breast milk; these enable an assessment of exposure of women, fetuses and suckling infants to such compounds. However, for most pesticides there is no appropriate biomarker and some biomarkers are common to more than one compound, with different toxicities. Thirdly, biological effect monitoring can be carried out, but this is generally far too insensitive for the amounts of pesticide or veterinary medicine found as food residues (biomarkers and biological effect monitoring are discussed in chapter 6). Fourthly, exposure may be modelled: this approach is frequently used for assessing operator exposure and draws on factors such as skin penetration, use or non-use of protective equipment and volatility or particle size in the case of exposure by inhalation; in any case modelling requires validation at some stage.

5.2 This chapter describes the available evidence of multiple exposure to residues of pesticides and veterinary medicines. The evidence presented in this chapter is a summary of what is known about multiple occurrence of residues in foods and in the diet. It is apparent that co-occurrence of residues of different pesticides and veterinary medicines in the diet does exist and therefore this is an issue that should be addressed from the point of view of a toxicological risk assessment. However the evidence on occurrence does not come from random surveys conducted with the aim of looking at multiple occurrence. Therefore it is not possible to deduce the true frequency of multiple occurrence of pesticide residues in the food supply from the available evidence.

5.3 It is important to investigate whether the multiple occurrence of residues in the diet compromises the risk assessment that is currently carried out for individual substances as part of the approvals process. Current practice for individual substances considers both acute intake (a whole day) and long term, chronic exposure (the daily average over the duration of the dietary survey). It should be recognised that the potential for the "cocktail" effect will depend on what is eaten in a whole meal or in a whole day. For chemicals that are prone to bioaccumulate (for example the OCs) then longer term multiple exposures may be relevant.

5.4 It is important to consider how cumulative exposure assessment work could be developed in the UK. When considering the "cocktail" effect it is relevant to discuss potential methods for cumulative exposure assessment since it is the potential for different chemicals with similar toxic effects to act together that is the main issue. Aggregate exposure assessment which combines exposure from different pathways such as food, air and water is important in considering the total personal exposure to a given chemical, but this subject is also relevant to the "cocktail" effect when there is exposure to more than one chemical (which have a similar mode of action) from different routes.

5.5 The model in use in the UK closest to a cumulative exposure assessment is the Toxic Equivalency Factor (TEF) used to equate the toxicity of the different dioxin congeners and dioxin-like PCBs. However, they are a special case as every sample is analysed for all specified congeners, so there is a full congener profile for each of the samples analysed.

5.6 Information on food consumption for estimating exposure comes from a number of sources: the National Diet and Nutrition Surveys (which have been carried out for adults, young people, the elderly and toddlers[1-4]), the National Food Survey, the Total Diet Study and other specific surveys for groups such as infants and vegetarians. These surveys have been carried out by the Food Standards Agency (FSA) and previously by MAFF over the last 17 years. The FSA carries out dietary surveys to provide information on food consumption patterns of the UK population. This consumption data can be combined with data on concentrations of chemicals in food (e.g. pesticides) from surveillance in order to estimate the exposure of these chemicals in the diet. These estimates underpin the FSA's risk assessment work on food safety and food quality and provide information on the nutritional adequacy of the UK diet.

5.7 Information about the various sources of food consumption data is published in more detail elsewhere[5,6]. Raw data in electronic form is available on the Data Archive website. The Data Archive is the largest collection of research data in the social sciences and humanities in the UK. It was set up in 1967 to collect and preserve machine-readable data relating to social and economic affairs from academic, commercial and governmental sources, and to make these data available for secondary analysis. The Archive (http://www.data-archive.ac.uk/) charges a small handling fee for dispatching data.

5.8 It should be noted that if a surveillance programme reports a zero result for a given analysis then this does not mean that the analyte is necessarily absent but it may be present below the reporting limit (RL), at a low level. The reporting limit for veterinary residues is usually 50% of the MRL. This means if the residue is detected at 49% of the MRL it would be reported as zero. In comparison, the RL for pesticides is the lowest calibrated level employed during analysis to detect residues and is usually substantially lower than the MRL.

Evidence of exposure to multiple pesticide residues in the diet

5.9 The information on multiple exposure to pesticides is obtained from three main sources:

- The pesticides surveillance programme in which food commodities are analysed for a range of pesticides in each year.
- Analyses of pesticides in the Total Diet Survey which measure levels in the main components of the diet.
- Data on biomonitoring from certain human adipose tissue and breast milk, which gives evidence of the accumulation of pesticides within the body (see chapter 6).

The pesticide surveillance programme

A pesticides residue surveillance programme has been undertaken in the United Kingdom (UK) for many years, formerly under the aegis of the Working Party on Pesticide Residues (WPPR). The programme to monitor the UK food supply for pesticides residues is now overseen by the Pesticides Residue Committee (PRC). The purpose of the programme is threefold:

- To back up the statutory approvals process for pesticides by checking that no unexpected residues are occurring;
- To ensure that residues do not exceed statutory maximum residue levels (MRLs)
- To check that human dietary intakes of residues are within acceptable daily intakes (ADIs) and acute reference doses (ARfDs).

5.11 The range of pesticides, which may be used in agriculture and food production, either in this country or abroad, is very wide. About 350 active substances are currently approved for use as agricultural pesticides in the UK and over 800 are approved in one or more European Union (EU) States. If account is taken of old chemicals such as DDT, which are now banned in the EU but may persist in the environment, potentially around 1,000 different chemicals might be looked for. In addition, the range of foodstuffs now available to UK consumers throughout the year is very broad. To make the most of resources the programme takes the form of surveys, which cover different commodities in turn; these are known as rolling programmes. Thus the programme is varied from year to year (see 5.17). Pesticide residue surveillance does not only comprise rolling surveys; there are also specially targeted surveys to look at persistent problems such as UK-grown winter lettuce and imported sweet peppers. Additionally, surveys of three dietary staples, bread, milk and potatoes, are undertaken each year.

5.12 The PRC surveillance monitoring programme is designed as a shopping basket survey, to reflect what any UK household might buy at its local supermarket or retail outlet. The surveys are not random and therefore cannot reflect the overall prevalence of residues in food and some of the surveys specifically target commodities where residues are expected to be found. Random surveys would need to be larger, and given a fixed budget. This would result in fewer commodities being sampled each year and less exceedences being detected for a given amount of money.

5.13 In recent years the total sample numbers analysed under the surveillance programme have been around 2,200-2,500 and the number of analyses has been about 80,000. Sample numbers for individual surveys have ranged from 150-200 samples for the dietary staples and between 48-72 samples for most other commodities with a minimum of 24 samples for the more minor commodities, such as nut butters.

5.14 For the 2001 programme the total sample numbers were increased to about 4,000. The programme allows about 30-40 surveys of different commodities to be carried out.

5.15 For risk assessment purposes the criteria which have been applied are a minimum of 50 data points for minor crops and 200 for a major crop (based on the World Health Organisation guidelines).

5.16 The basic programme consists of the following elements:

- Surveys of three dietary staples – bread, milk and potatoes – which are undertaken each year.
- Surveys of the main food groups – fruit and vegetables, cereals and cereal products and animal products – which generally vary from year to year.
- Surveys, usually of certain fruit and vegetables, conducted as part of a wider harmonised EU programme.
- Miscellaneous and special surveys; miscellaneous surveys include those on animal feeding stuffs, or processed foods such as baby foods or fast foods such as burgers; special surveys are generally those which are set up at short notice to address new issues which need to be investigated quickly.

5.17 With the exception of the dietary staples which are tested each year, all other commodities are tested as part of the rolling programme. The frequency with which individual commodities should be monitored has been decided in the past based on factors such as dietary importance, especially for infants and toddlers, the potential for residues to occur in terms of concentration and frequency, and evidence for MRL exceedances. The intake of pesticide residues by toddlers is higher for most commodities than any other population sub-group on a body weight basis, so this group receives particular attention.

5.18 The choice of pesticides to be sought is primarily influenced by:

- Pesticide use
- Potential for residues based on use pattern and the physico-chemical properties of the pesticide
- Analytical capabilities
- Toxicological profile of the pesticide
- Existence of MRLs
- Information available on likely problems, such as evidence of residues from earlier surveys or other information

5.19 A range of sources of information is used to prioritise the surveillance programme, including:

- data from previous monitoring in the UK and elsewhere,
- from Rapid Alerts: this is an EU system organised by the European Commission that notifies Member States when pesticide residues in foodstuffs of plant origin are found that are of significant health concern,
- Pesticide Usage Surveys – information on the use of pesticides in England and Wales is collected by the Pesticide Usage Survey Group (PUSG) based at the Central Science Laboratory, an Agency of the Department of the Environment, Food and Rural Affairs (DEFRA). The group collects data on pesticides used on arable crops, vegetables, glasshouse crops, soft fruit, top fruit, fodder and forage, stored fruit, vegetables and grain. Other groups collect data on pesticide usage in Scotland and Northern Ireland. Survey reports for Great Britain are produced by the Central Science Laboratory using the data from England, Wales and Scotland. The survey reports provide accurate information concerning regional and national pesticide usage including: the total treated area; proportion of crops treated; methods and timing of application. The data collected provide essential information for determining government policy concerning control of pesticides especially during the reviews of approvals. More information on the work of the Group and summaries of pesticide survey results can be found within the Central Science Laboratories website on www.csl.gov.uk/prodserv.
- pesticide registration data,
- UK and Codex MRLs and other information including contributions from members of the PRC.

5.20 Information on pesticide use in a large range of countries across the world is available commercially. This might provide valuable data to allow greater targeting of the pesticides sought, particularly for crops imported from outside the EU. Generally the emphasis is on insecticides, fungicides and post harvest treatments since these have the greatest potential for producing residues. For most surveys it is not worthwhile analysing for herbicides since very few herbicidal uses actually result in detectable residues.

5.21 A range of approximately 80-100 pesticides can be reliably looked for using a multi-residue method, but analysis for other pesticides needs to be carried out separately, a procedure which is relatively expensive. Furthermore, the lower the RLs at which pesticides are sought, the more expensive is the procedure.

5.22 The cost of the programme in 2000/2001 (which corresponds with the 2000 sampling year) was £1.7 million. 60% of this comes from a levy on the sales of pesticides and the balance of 40% from the Government.

5.23 Most samples tested are collected from retail outlets. In 2000 a total of 2,304 samples were collected over the year from monthly purchases in 12 cities throughout the United Kingdom (UK) (increased to about 4,000 for 2001 – see 5.14).

5.24 Detailed results of the PRC surveillance programme are published in quarterly bulletins on the PRC website to allow industry the opportunity to respond more quickly to any findings and also to make the results available to the wider audience. An Annual Report draws together the surveillance results for pesticides for that year and describes the work to be undertaken during the following year. The report and the quarterly results can be found on the PRC website at http://www.pesticides.gov.uk/committees/PRC/prc.htm. More information on the monitoring programme, analytical methods and quality assurance is available on the PSD website at http://www.pesticides.gov.uk.

5.25 In addition to the national monitoring programme, the UK participates in an EU-wide co-ordinated monitoring programme. The aim of the community programmes is to ensure compliance with residues legislation and to enable estimation of the actual exposure to pesticides from the diet. The EU monitoring programme is also a rolling programme. By the end of 2003 it will have covered all major pesticide-food commodity combinations. The latest community report of pesticide residues surveillance covers the year 1999 and was published in July 2001. This report, along with further information on EU monitoring can be found on the European Commission's website for food safety at http://europa.eu.int/comm/food/index_en.html.

5.26 The UK Food Industry also undertakes its own monitoring for pesticide residues. A number of retailers submit their data to PSD and this information is included in the PRC Annual Reports. Some retailers now also publish their own surveillance data on their websites.

Data on occurrence of multiple pesticide residues from the surveillance programme

5.27 The Central Science Laboratory undertook a short project to examine which pesticides found in food surveillance are likely to occur as "cocktails" (i.e. in combination) of residues in food, using UK residues monitoring data gathered under the WPPR/PRC monitoring programme over the years 1997 to 2001.

5.28 All of the data collected from 1997-2001 (that arose from samples from retail outlets up to the first quarter of 2001) were used to perform the analysis. Some of the surveys were targeted to particular problems so are likely to present a worst-case scenario of the level of pesticides found in the particular foods. However, the aim of the project was to produce a picture from what is known about pesticide occurrence using all the available data and so these surveys were included.

5.29 Firstly, pesticides were ranked according to their worst-case frequency of overall occurrence in each commodity (See Table A3.1 – Appendix 3). Secondly, pesticides were ranked according to worst-case frequency of occurrence as multiple residues (See Table A3.2 – Appendix 3).

5.30 Worst-case[a] frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits (RL) being adopted, in different surveys of the same commodity. They were also adopted because apparent changes in the residues profile from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the programme is not designed to be representative.

5.31 To avoid prejudging the parameters required for interaction between residues in the diet, no attempt was made to differentiate pesticides having similar end-points of toxicity or to assess consumer intakes (by mass or concentration) of the pesticides. It would be possible to identify acutely toxic pesticides in the tables, if required, but it was wished not to prejudge acute effects involving different end-points.

5.32 For Table A3.1 total numbers of residues and samples were entered into Excel spreadsheets and, where multiple surveys of the same commodity had been conducted, the worst-case frequencies for all pesticides were taken. For Table A3.2, a similar exercise was performed but utilising results only where two or more pesticides had occurred in the same sample. For both tables, all commodities were considered individually. Inorganic bromide data for multiple residues (i.e. Table A3.2) include only results exceeding 10 mg/kg (the highest level at which bromide is normally present naturally), whereas the overall frequencies (Table A3.1) include all results.

5.33 The results presented in the tables involved making some simple assumptions but it is important to recognise that the source data also have many limitations. In particular, the residues surveys were too small to permit a sound statistical design.

5.34 It should be noted that in the source data, residues data for bananas, oranges and soft citrus fruits are based on the whole fruit, including the skin/peel. This is because an important objective of the residues monitoring is to support good agricultural practice (GAP) and to check compliance with statutory MRLs and, for this purpose, whole fruit must be analysed. For assessment of consumer exposure, it is necessary to use so-called processing factors, which for example account for residues in the part of the banana usually eaten rather than the peel, to adjust the results. However, processing factors are only used in clearly defined circumstances when it is agreed that the process will always take place. Where there are concerns that not all consumers will eat a food in the normal manner then definitions of edible portion need to be considered before processing factors are incorporated into the risk assessment.

5.35 Table A3.1 ranks the pesticides found in each food commodity in order of most frequently found. Table A3.2 ranks the frequency of pesticides found in combination with at least one other pesticide. This table does not give information on the number of different pesticides found in an individual sample but such information is published in the relevant WPPR/PRC reports.

[a] The worst-case means the year with the highest frequency of the particular pesticide.

5.36 Notwithstanding the imperfections in the source data and calculations used, the frequency rankings of pesticides in Tables A3.1 and A3.2 provide a sufficiently sound basis for making decisions on the most important or likely pesticide residue combinations to be studied for possible additive, potentiating or antagonistic effects. For example, in the case of apples 43% of samples surveyed contained the pesticide diphenylamine (Table A3.1). From Table A3.2, it can be seen that 39% of samples contained diphenylamine in combination with at least one other pesticide. The next most frequently found pesticide in apples was carbendazim (in 39% of apple samples, from Table A3.1). Carbendazim was found in combination with at least one other pesticide in 39% of samples (from Table A3.2). Consequently, it is reasonable to make the assumption that an apple is more likely to contain diphenylamine and carbendazim in combination than two pesticides ranked at the bottom of the tables. It should be noted that the figures quoted in the tables represent worst case, that is they represent the worst year's figure in the sampling period 1997-2000.

5.37 In addition, a further table (Table A3.3 Appendix 3) was prepared from work by Central Science Laboratory that shows the number of times particular combinations of residues occurred in different years and shows what the most common combinations of residues are. Unlike Tables A3.1 and A3.2, this table provides detailed information on the actual combinations that were detected.

5.38 It should be noted that it may be necessary to read from more than one row of the table to find out how many times a given combination of say, 2 pesticides occurs. This is because a combination of 2, 3 or 4 or more pesticides is each listed as a separate row. A particular combination of 2 pesticides can be present in a sample with 3 or more pesticides. In 1997, for example, 12 out of 72 apple samples were found to contain residues of the pesticides diphenylamine and carbendazim (2 samples contained just these pesticides, a further 10 samples contained diphenylamine and carbendazim in combination with at least one other pesticide). There were also 12 occurrences of diphenylamine and thiabendazole (5 samples with these alone and a further 7 samples containing these two pesticides in combination with at least one other pesticide). It should be noted if a particular combination was not detected in a particular year, it does not necessarily indicate absence of this combination as one or all of the pesticides in the combination may not have been sought in the surveys(s).

5.39 Highlighted entries in Table A3.3 represent organophosphorus (OP) and carbamate compounds. Sections of the table indicated by (OP/C) beneath the year are surveys that were restricted to OPs and carbamate compounds.

5.40 The data presented here provide a good starting point for estimating the likelihood of particular combinations of pesticides in certain foods. It can be reasonably concluded that fruit and vegetables are more likely to contain multiple residues than cereals and produce of animal origin. Consideration needs to be given to performing statistically valid surveys of high frequency residues in the most important contributory components of the diet.

Data on occurrence of multiple pesticide residues from total diet studies (TDS)

5.41 The Total Diet Study (TDS) provides a means of assessing the general levels of exposure of consumers to pesticide residues from the diet as a whole. TDS samples have been analysed for pesticide residues at intervals of approximately 5 years. First initiated in 1966, the TDS was extensively re-organised in 1981[7] and the types and quantities of foods in the 'total diet' are updated annually to reflect changing eating habits.[8]

5.42 Food samples are purchased from a variety of retail outlets in randomly selected towns throughout the UK over a 12-month period. The commodities, purchased from 119 categories, are divided into 20 groups of similar foods (food groups) and prepared as if for eating.[7,8] Foods, for example, which would not be eaten raw are cooked, in line with normal household practice. The relative proportions of foods within each food group reflect their importance in the average UK household diet and are based on annually updated data from the National Food Survey (NFS), which primarily surveys food purchased by households. Consumption is calculated on the basis of a household member and is irrespective of age. For foods not included in this survey, trade volume statistics are used. Foods are grouped so that commodities known to be susceptible to contamination (e.g. offals and fish) are kept separate, as are foods consumed in large quantities (e.g. bread, potatoes and milk).[7,8]

5.43 It is important to note that a total diet study is different to routine surveillance conducted by the WPPR/PRC. Unlike the TDS, the majority of WPPR/PRC surveillance is undertaken on separate, raw commodities.[9]

5.44 The last pesticides TDS was undertaken in 1996-97 and is reported in the 1996 Annual Report of the WPPR.[9] Results of previous pesticides total diet studies are described in Food Surveillance Papers[10-12] and other WPPR reports.[13]

5.45 Analysis of the 1996-97 pesticides TDS departed from earlier methods. Results were analysed by calculating National Estimated Daily Intakes (NEDIs) for a range of population groups (adults, school children and infants), based on the high level (97.5th percentile) consumption of individual commodities (derived from National Diet and Nutrition Survey and other related data)[1,14,15] and the highest residues found. The use of this method makes assumptions as to the likely or possible source of the pesticide residues in the food groups. These assumptions are not considered appropriate for estimating chronic exposure from the total diet. The TDS is not intended to provide clear information on exposure from particular commodities.[9] The work carried out by the WPPR on the 1996-97 TDS results is thus not re-produced here. Instead, for the purposes of this chapter, the 1996-97 data are re-analysed using the TDS methodology that has been applied previously.[7, 10-12] This combines the consumption estimates for each TDS food group (also derived from the NFS) with the pesticide residues data from each food group to estimate dietary exposure for a member of an average UK household. In this way, account is taken of both the consumption of the various foods making up the general diet and the concentrations of the pesticide residues in these foods. The method provides a population exposure estimate. The 1996-97 results are then compared with previous results from the 1984-85 TDS[11] and the 1989-90 TDS[12,13], derived using the same methodology.

1996-97 Pesticides Total Diet Study

5.46 In the 1996-97 TDS, 20 different food groups were analysed covering fruit and vegetables, animal products, cereals and beverages. The study involved 24 sets of total diet samples obtained between February 1996 and January 1997 (Personal Communication from the Institute of Food Research, Norwich, 12th February 2002). The pesticides sought in the food groups were selected on the basis of whether or not residues were likely to be present. Therefore, not all individual groups were analysed for the presence of all residues. A summary of the residues found in the samples is given in Table 5.1, along with consumption estimates for the TDS food groups. In general, the profile of the residues found in the 1996-97 TDS samples was consistent with previous monitoring. In the majority of the samples DDT residues were present as p,p'.-DDE which is consistent with residues arising from environmental contamination. A further discussion on the residues found in the 1996-97 TDS is provided in the 1996-97 WPPR Report.[9]

5.47 Average intakes of pesticides (population exposure estimates) calculated for the 1996-97 TDS using the consumption estimates for the food group is given in Table 5.2, along with comparison figures for previous years. The results show that the average dietary exposure to pesticide residues is very low; all calculated intakes are well below respective Acceptable Daily Intakes (ADIs) where set. The results give some indication of potential combinations of residues in the diet. Note that all figures are lower bound values. This means that for samples in which a pesticide was not detected because the concentration was below the RL, it has been assumed that the concentration is equal to zero (and not the RL). The result is that exposures may be underestimated to some extent. There are also difficulties associated with comparing population exposure estimates to ADIs. Population exposure estimates invariably result in an underestimate of real consumption.

5.48 It should be noted that TDSs are employed to study exposures to a range of contaminants.[20-23] In many of these cases exposure estimates are calculated using more advanced methodology than has, so far, been applied to pesticides. In the studies examined[22-23] two types of dietary exposure assessment are applied concurrently. The first type is a population exposure estimate similar to that applied to pesticide total diet studies in the past and carried out in this chapter in respect of the re-analysis of the 1996-97 TDS. The major difference is in the use of an upper bound mean concentration (where the concentration of a substance not detected is set equal to the RL). The lower bound mean concentration is the only figure to have so far been used for pesticide total diet studies.

5.49 The second type of dietary exposure assessment to be carried out is an adult consumer exposure assessment.[22-23] It estimates exposure for average and high level (97.5th percentile) adult consumers using the upper bound mean contaminant concentrations and consumption data on each food group derived from the National Diet and Nutrition Survey[1]. This method could also be extended to other population groups. This method has, so far, not been applied to pesticides.

Table 5.1 Summary of pesticide residues in the 20 food groups of the Total Diet Study, 1996-97[a]. 24 total diets obtained between February 1996 and January 1997 (Personal Communication from the Institute of Food Research, Norwich, 12th February 2002) were analysed for a range of pesticide residues. Reporting limits, ranges and means are given. Data on individual samples have been obtained from the Final Reports of the Laboratory of the Government Chemist to the WPPR.[16]

Food group	Estimated Average Consumption[a] (kg/person/day)	Residues found	Reporting limit (RL)[b] (mg/kg)	Range[c] (mg/kg)	No. of samples with residues (24 diets)	Mean residue level[d] (mg/kg)
Bread	0.108	chlorpyrifos-methyl	0.005	NF – 0.01	3	0.001
		pirimiphos-methyl	0.005	NF – 0.06	22	0.02
Miscellaneous Cereal	0.101	chlorpyrifos-methyl	0.005	NF – 0.009	3	0.0008
		etrimfos	0.005	NF – 0.006	1	<0.0005
		lindane (γ-HCH)	0.002	NF – 0.01	1	<0.0005
		phosphamidon total	0.01	NF – 0.01	1	0.0005
		Pirimiphos-methyl	0.005	NF – 0.04	20	0.01
Carcass Meat[e]	0.023	p,p' DDE	–	NF – 0.07	8	0.009
		DDT total	0.01	NF – 0.07	8	0.009
		Propetamphos	0.02	NF – 0.04	2	0.003
Offal	0.001	p,p' DDE	–	NF – 0.02	6	0.002
		DDT total	0.002	NF – 0.02	6	0.002
		Pentachlorophenol	0.01	NF – 0.03	3	0.003
Meat Products[e]	0.046	p,p' DDE	–	NF – 0.003	2	<0.0005
		p,p' DDT	–	NF – 0.009	2	0.0007
		DDT total	0.01	NF – 0.01	2	0.0009
		Beta HCH	0.01	NF – 0.01	1	<0.0005
Poultry	0.018	p,p' DDE		NF – 0.003	3	<0.0005
		DDT total	0.002	NF – 0.003	3	<0.0005
		Beta HCH	0.002	NF – 0.002	1	<0.0005
		Lindane (γ-HCH)	0.002	NF – 0.002	2	<0.0005
Fish and fish Products	0.014	p,p' DDE	–	NF – 0.003	7	0.0006
		p,p' TDE	–	NF – 0.001	5	<0.0005
		p,p' DDT	–	NF – 0.001	1	<0.0005
		DDT total	0.002	NF – 0.004	7	0.0009
		Tecnazene	0.001	NF – 0.01	3	0.001
		Dieldrin	0.002	NF – 0.003	1	<0.0005
		TCA	0.001	NF – 0.003	3	<0.0005
Oils and Fats	0.028	p,p' DDE	–	NF – 0.02	8	0.003
		DDT total	0.005	NF – 0.02	8	0.003
Eggs	0.014	p,p' DDE	–	NF – 0.012	1	0.0005
		p,p' TDE	–	NF – 0.004	1	<0.0005
		o,p' DDT	–	NF – 0.0002	1	<0.0005
		p,p' DDT	–	NF – 0.018	1	0.0008
		DDT total	0.002	NF – 0.03	1	0.0014
Sugars and Preserves	0.064	Bromopropylate	0.005	NF – 0.01	1	<0.0005
		Lindane (γ-HCH)	0.005	NF – 0.007	7	0.002
		Imazalil	0.02	NF – 0.2	1	0.008
		Thiabendazole	0.05	NF – 0.05	1	0.002

Food group	Estimated Average Consumption[a] (kg/person/day)	Residues found	Reporting limit (RL)[b] (mg/kg)	Range[c] (mg/kg)	No. of samples with residues (24 diets)	Mean residue level[d] (mg/kg)
Green Vegetables	0.035	cypermethrin	0.005	NF – 0.02	1	0.0008
		Vinclozolin	0.005	NF – 0.02	4	0.002
Potato	0.127	chlorpropham	0.005	NF – 0.5	11	0.08
		Maleic hydrazide	0.5	NF – 5.4	2	0.4
		Tecnazene	0.001	NF – 0.03	4	0.002
		TCA[f]	0.001	NF – 0.02	1	<0.0005
		TCTA[g]	0.001	NF – 0.002	1	<0.0005
		Thiabendazole	0.05	NF – 0.4	2	0.02
Other Vegetables	0.076	cypermethrin	0.005	NF – 0.009	1	<0.0005
		iprodione	0.005	NF – 0.2	5	0.02
		maleic hydrazide	0.5	NF – 1.3	2	0.1
		procymidone	0.005	NF – 0.03	7	0.005
		triazophos	0.005	NF – 0.006	1	<0.0005
		vinclozolin	0.005	NF – 0.007	1	<0.0005
Canned Vegetables	0.034	none detected	–	–	–	–
Fresh Fruit	0.067	bromopropylate	0.005	NF – 0.02	2	0.001
		bupirimate	0.005	NF – 0.01	1	<0.0005
		carbendazim	0.1	NF – 0.1	1	0.004
		chlorpyrifos	0.005	NF – 0.08	5	0.008
		dimethoate	0.01	NF – 0.04	3	0.003
		iprodione	0.005	NF – 0.6	2	0.06
		metalaxyl	0.005	NF – 0.06	3	0.005
		parathion	0.005	NF – 0.01	1	<0.0005
		phosalone	0.005	NF – 0.2	3	0.01
		propargite	0.005	NF – 0.5	6	0.03
		thiabendazole	0.05	NF – 0.1	4	0.01
Fruit Products	0.043	bromopropylate	0.005	NF – 0.01	1	<0.0005
		carbaryl	0.005	NF – 0.01	1	<0.0005
		carbendazim	0.1	NF – 0.4	2	0.02
		iprodione	0.005	NF – 0.02	4	0.003
		metalaxyl	0.005	NF – 0.03	1	0.001
		propargite	0.005	NF – 0.03	3	0.003
		thiabendazole	0.05	NF – 0.1	1	0.004
Beverages	0.878	carbendazim	0.1	NF – 0.6	1	0.03
		thiabendazole	0.05	NF – 0.4	1	0.02
Milk	0.286	lindane (γ-HCH)	0.0004	NF – 0.003	3	<0.0005

Food group	Estimated Average Consumption[a] (kg/person/day)	Residues found	Reporting limit (RL)[b] (mg/kg)	Range[c] (mg/kg)	No. of samples with residues (24 diets)	Mean residue level[d] (mg/kg)
Dairy Products[e]	0.060	p,p' DDE	–	NF – 0.065	8	0.009
		p,p' DDT	–	NF – 0.004	1	<0.0005
		DDT total	0.01	NF – 0.07	8	0.009
		lindane (γ-HCH)	0.01	NF – 0.02	6	0.003
Nuts	0.002	alpha HCH	0.005	NF – 0.03	1	0.001

Notes

a. 1996 data are based on an average of the available data from three previous years of the National Food Survey (NFS), 1992-1994[17-19]. It includes the change in weight due to preparation and cooking. The same NFS data are also used to calculate the relative proportion of each food in the food group. Note that though the samples were obtained over 1996-97, only 1996 consumption estimates have been applied in the calculations.

b. Reporting limits depend on the compound and the group examined. In cases were the food commodities were cooked prior to analysis and where the cooking process may lead to a reduction in residues, the reporting limits were reduced in order that lower level residues could be detected.[9]

c. NF not found

d. The mean residue level is the mean of the 24 samples in the food group. Levels below the RL are taken as 0 (lower bound value).

e. Residues are expressed on a fat basis, all other results are expressed on a whole product basis. For the purposes of Table 5.2, residues expressed on a fat weight basis are converted to a whole product basis using the average fat content of the 24 samples in the food group.

f. TCA 2,3,5,6-tetrachloroaniline (tecnazene metabolite)

g. TCTA 2,3,5,6-tetrachlorothioanisole (tecnazene metabolite)

Table 5.2 Calculated mean intakes of pesticide residues from the Total Diet Studies for 1984-1985,[11] 1989-90[12] and 1996-97 and current Acceptable Daily Intakes. The mean intakes of pesticide residues are calculated by multiplying the mean residue level of each pesticide found in each food group by the estimated consumption of the food group (based on purchase data from the appropriate years of the National Food Survey). The pesticides sought in each study were not necessarily approved. Some approved pesticides found in 1996-97 may no longer hold current approval.

Pesticide		Intakes[a, b] (µg/person/day)			Acceptable Daily Intake (ADI)[c]		Intake as % of ADI for a 60 kg adult (1996-97)
					For 60 kg adult		
	class	1984-85 25 diets	1989-90 26 diets	1996-97 24 diets	(µg/person/day)	(µg/kg bw/day)	
Hexachlorobenzene	OC	<0.1	0.2	NC	No ADI		–
α-HCH	OC	0.3	<0.1	<0.1	60	1	<0.1
β-HCH	OC	0.5	<0.1	<0.1	6	0.1	0.1
lindane (γ-HCH)	OC	0.5	0.3	0.2	60	1	0.4
Dieldrin	OC	0.5	<0.1	<0.1	6	0.1	<0.1
o,p' DDT	OC	ND	ND	<0.1	–	–	–
p,p' DDE	OC	0.5	0.1	0.3	–	–	–
p,p' DDT	OC	ND	ND	<0.1	–	–	–
p,p' TDE	OC	ND	ND	<0.1	–	–	–
DDT total	OC	0.5	0.1	0.3	600[f]	10[f]	<0.1
Chlorpyrifos	OP	ND	ND	0.5	600	10	0.1
Chlorpyrifos-methyl	OP	NC	0.6	0.2	600	10	<0.1
Dimethoate	OP	ND	ND	0.2	120	2	0.1
Etrimfos	OP	NC	0.2	<0.1	180	3	<0.1
Parathion	OP	ND	ND	<0.1	240	4	<0.1
Phosalone	OP	ND	ND	0.7	1200	20	0.1
Phosphamidon total	OP	ND	ND	<0.1	30	0.5	0.2
Pirimiphos-methyl	OP	1.8	3.0	3.3	1800	30	0.2
Propetamphos	OP	ND	ND	<0.1	No ADI		–
Triazophos	OP	ND	ND	<0.1	60	1	<0.1
Malathion	OP	0.1	<0.1	NC	18000	300	NC
Bromopropylate	other	ND	ND	0.1	1800	30	<0.1
Bupirimate	other	ND	ND	<0.1	No ADI		–

Pesticide		Intakes[a, b] (µg/person/day)			Acceptable Daily Intake (ADI)[c]		Intake as % of ADI for a 60 kg adult (1996-97)
	class	1984-85 25 diets	1989-90 26 diets	1996-97 24 diets	For 60 kg adult (µg/person/day)	(µg/kg bw/day)	
Carbaryl	other	ND	ND	<0.1	480	8	<0.1
Carbendazim	other	ND	ND	23.1	1800	30	1.3
Chlorpropham	other	NC	7.1	10.2	1800	30	0.6
Cypermethrin	other	ND	ND	0.1	3000	50	<0.1
Imazalil	other	ND	ND	0.5	1800	30	<0.1
Iprodione	other	ND	ND	5.3	3600	60	0.1
Maleic hydrazide	other	ND	ND	53.1	18000	300	0.3
Metalaxyl	other	ND	ND	0.4	1800	30	<0.1
Pentachlorophenol	other	3.2	<0.1	<0.1	No ADI		–
Procymidone	other	ND	ND	0.4	6000	100	<0.1
Propargite	other	ND	ND	1.9	600	10	0.3
Tecnazene	other	4.3	3.3	0.2	1200	20	<0.1
TCA[d]	other	ND	ND	<0.1	1200	20[g]	<0.1
TCTA[e]	other	ND	ND	<0.1	1200	20[g]	<0.1
Thiabendazole	other	ND	ND	19.7	6000	100	0.3
Vinclozolin	other	ND	ND	0.1	600	10	<0.1

Notes

a. NC not calculated since no residues were found in the samples above the RL
b. ND no data (residues generally not sought)
c. Joint Expert Meeting on Pesticide Residues (JMPR) ADI (as of 2001)
d. TCA 2,3,5,6-tetrachloroaniline (tecnazene metabolite)
e. TCTA 2,3,5,6-tetrachlorothioanisole (tecnazene metabolite)
f. DDT has a provisional daily tolerable intake, not an ADI
g. ADI is for tecnazene

Evidence of exposure to pesticide residues in drinking water

5.50 Pesticide residues in water arise from both agricultural and non-agricultural uses. Contamination events from production or transport of pesticides are rare. Monitoring of pesticide residues in public or private water supplies is the responsibility of the Drinking Water Inspectorate in England and Wales, the Water Services Unit on behalf of the Scottish Executive for Scotland, and the Drinking Water Inspectorate of the Northern Ireland Department of the Environment. The PRC however monitors bottled water and other drinks as part of its surveillance programme.

5.51 The 1980 EC Drinking Water Directive 80/778/EEC[24] set a standard of 0.1μg/l for each pesticide in drinking water. As this concentration was equivalent to the detection limits of analytical methods available at the time, this standard was effectively a zero concentration. The Directive also set a standard for total pesticides of 0.5μg/l. These limits were included in the national drinking water regulations. The standards set in 1980 were maintained in the 1998 revision of the Directive (98/83/EC[25]) and will remain in the new national drinking water regulations. In addition to these standards, the Water Supply (Water Quality) England and Wales Regulations 2000 introduce a specific limit of 0.03μg/l for each of the organochlorines (OCs), aldrin, dieldrin, heptachlor and heptachlor epoxide. This regulation and the revised directive will be implemented by the end of 2003.

5.52 The Chief Inspector for the Drinking Water Inspectorate reports the results of testing each year to the Secretary of State for Environment, Food and Rural Affairs and to the First Secretary of the National Assembly for Wales. As a result of enforcement action over the last ten years, water companies have introduced extensive water treatment processes to remove pesticides from drinking water. In 2000, 99.99% of over 606,900 regulatory analyses for pesticides in drinking water in England and Wales met the standards. Seven individual pesticides were detected above this level, in 45 samples. In every instance, the concentrations found corresponded to exposures far smaller than those known to be harmful or likely to affect health. Further details may be found at:
http://www.dwi.gov.uk
http://www.scotland.gov.uk/library3/environment/dwq00-00.asp
http://www.ehsni.gov.uk/EnvironProtect/

Evidence of exposure to multiple veterinary medicine residues in the diet

5.53 The evidence presented on veterinary medicines comes from the data obtained in the veterinary medicines surveillance programmes.

The veterinary medicines surveillance programmes

Statutory Residue Surveillance

5.54 The UK's statutory National Surveillance Scheme fulfils our obligations under an EU Directive to monitor whether veterinary residues are passing into meat and animal products for human consumption in unacceptable concentrations. Other EU legislation lays down the sampling frequency and levels.

5.55 The programme in 2000 covered red meat, poultry, salmon, trout, eggs, wild and farmed game and milk. Under the National Surveillance Scheme a specific percentage of the total UK samples are allocated to Northern Ireland each year. The collection of these samples is undertaken by staff of the Department of Agriculture and Rural Development in Northern Ireland (DARDNI). All analyses are performed, as appropriate, by DARDNI's Veterinary Sciences Division or Food Sciences Division laboratories. DARDNI Veterinary Officers undertake follow-up investigations on positive samples. In Great Britain, the State Veterinary Service (SVS) undertakes on-farm sampling for red meat, poultry and milk. The Meat Hygiene Service carries out sampling for red meat and poultry in slaughterhouses. Samples of trout and salmon are collected by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) in England and Wales and the Fisheries Research Services (FRS) in Scotland. The SVS, CEFAS and FRS undertake investigative action on positive results. Egg Marketing Inspectors have responsibility for collecting eggs (for England & Wales) along with Egg Marketing Officers (for Scotland). The SVS follow-up any residue positive eggs. All samples are analysed by the Laboratory of the Government Chemist (LGC).

5.56 The EU Directive governing surveillance covers red meat, poultry, salmon and trout, eggs, milk, wild and farmed game, rabbit and honey. However, owing to the limited production of rabbit in the UK and the diversity of honey production, surveillance for these products is carried out under the Non-Statutory programme. The numbers of samples to be collected and analysed are a fixed proportion of the numbers of animals and animal products which the Member State forecasts will be slaughtered or produced based on figures from the preceding year.

5.57 The poultry and red meat plans for 2001 were based on the forecasts of final production for 2000 taken from the Ministry's December figures from the Meat and Livestock Quarterly Review. The figures for milk and eggs were taken from data provided by Ministry of Agriculture, Fisheries and Food's (MAFF) Statistics Division. The 2001 salmon plan was based on 1999 figures published in the Scottish Fish Farms' Annual Production Survey 1999. The trout plan was based on figures provided by MAFF's Fisheries Department. No figures for game production are currently available and sample numbers were therefore based on the minimum required.

5.58 The Foot and Mouth Disease outbreak in 2001 severely affected the collection of on-farm samples of poultry, red meat and milk. Sampling of poultry and red meat was increased in slaughterhouses to offset the shortfalls of on-farm sampling and alternative arrangements were made for the collection of milk from dairy farms and from retail outlets. As such, sampling of commodities in this year could not be carried out to the original plan, as stated below.

5.59 The sampling plan for the 2001 survey involved collecting samples of red meat and poultry from farms and slaughterhouses throughout the year. Sample collection for milk and eggs started in April and the collection of farmed fish samples followed the pattern of fish disease inspection visits by the Fish Health Inspectorate starting in February. A proportion of samples was planned to be collected seasonally; for example cattle feed samples in the autumn and winter quarters. Samples would also be batched within individual months wherever possible to ensure economic batch sizes for individual analyses. For the 2001 UK national plan, individual substances within these groupings were determined by the Veterinary Medicines Directorate (VMD) in consultation with the Advisory Group on Veterinary Residues with specialist input into the Group by experts from the FSA, MAFF and DARDNI.

5.60 Approximately 38,000 samples were due to be collected during 2001 in the UK. This was a decrease on the number of samples in the 2000 plan, and in particular reflected the reduction in the number of pigs and sheep slaughtered in 2000. Overall, about 38,200 analyses, covering 160 substances, were planned on samples from Great Britain in 2001. Sample numbers for 2001 have been increased for those matrix/analyte combinations where positive samples were detected in 2000. As in previous years, targeted sampling is carried out for individual species/analyte combinations.

Non-statutory Residue Surveillance

5.61 The national statutory surveillance programme is complemented and supplemented by a non-statutory programme, which extends to analyte/matrix combinations not covered by the statutory programme. Sampling is carried out from April to December. However, the Veterinary Residues Committee (VRC), the successor to the Advisory Group on Veterinary Residues, is currently reviewing the sampling programme and has agreed in principle to carry out the sampling over 12 months and not just April to December as at present.

5.62 The 2000 plan consisted of the most popular preparations of meat and animal products, including imported produce. The majority of samples were subjected to a range of analyses in order to obtain the maximum possible information on residues, whilst seeking to make the programme cost effective.

5.63 Sample collection in 2000 was undertaken by 3 different agencies. The British Marketing Research Bureau purchased samples at retail outlets under contract to the Veterinary Medicines Directorate (VMD). Around 28% of the sampling plan was provided by major retailers under the Retail Co-ordination of Veterinary Residues Testing Scheme. In addition samples of imported raw meat were collected at border inspection posts (the control points for imports). All the samples were analysed by the Central Science Laboratory in York. Retailers were informed by the VMD of any positive samples purchased from their stores and results were also provided to members of the Retail Co-ordination of Veterinary Residues Testing Scheme. MAFF's Animal Health International Trade Division were informed of positive samples collected at the border inspection posts. In addition, where products were imported, the SVS contacted the Chief Veterinary Officer in the country concerned.

5.64 In the non-statutory programme the aim is to target matrices to cater for the average consumer based on the most popular preparations of meat and animal products, according to the national average consumption figures. The programme also occasionally targets commodities that do not make sufficient contribution to the diet of most consumers to warrant long-term inclusion in the main programme but nevertheless should not be omitted completely.

5.65 The general principles followed and the factors which are taken into account in setting up the surveillance programme include the importance of the commodity in the diet and the extent of the use of the particular veterinary medicine. Use is also made of monitoring data, e.g. The Dietary Consumption of Meat and Meat Products by the UK Population and intelligence from other sources. The programme also includes analyte/matrix combinations as requested by the VRC's predecessor, the Advisory Group on Veterinary Residues.

5.66 The 2001 non-statutory plan, as with each non-statutory programme, is composed of some matrices which are routinely sampled because they:

- form a popular part of the diet, e.g. ham
- have been previously requested by the Advisory Group on Veterinary Residues, e.g. prawns
- are statutory matrices, which it would not be cost efficient to sample under the statutory scheme, (honey and rabbit), bearing in mind that the costs are recovered from the industry.

5.67 Results of the VRC surveillance are reported in the VMD publication 'MAVIS online' which is updated monthly, and in an Annual Report. The 2001 Annual Report will be the first published by the VRC. Reports can be found on the VMD website at http://www.vet-residues-committee.gov.uk

Data on the occurrence of multiple residues in veterinary medicines

5.68 There are few data available on multiple veterinary medicine residues since there is not often analysis for more than one residue on one sample. The data that are available from surveillance are summarised in table 5.3. There are some data on antibiotics from the statutory scheme.

Table 5.3 Data from recent annual reports on veterinary residues[26-31] on the occurrence of multiple veterinary residues

Year	Statutory Programme	Non-Statutory Programme
1995	1 pig kidney sample contained residues of penicillin G, chlortetracycline, oxytetracycline and sulfonamide 1 pig kidney sample contained residues of streptomycin and chlortetracycline	3 samples of calf kidney contained residues of chlortetracycline and oxytetracycline 1 cattle kidney sample contained residues of oxytetracycline and sulfadimidine
1996	None	None
1997	None	None
1998	None	None
1999	None	1 bacon sample contained residues of sulfadimidine and sulphmethoxypyridazine
2000	1 sample of imported turkey muscle contained residues of sulphadimethoxine and chlortetracycline 1 sample of quail eggs contained enrofloxacin and dimetridazole	None

Estimation of dietary intake using food consumption data and residues data

5.69 The FSA commissions large and detailed dietary surveys of individuals to provide information on food consumption patterns in the UK population. This information can be combined with data on concentrations of chemicals in food (e.g. pesticides) in order to estimate the amounts of these chemicals in the diet. Since the FSA was established, it has managed the National Diet and Nutrition Surveys which are carried out at approximately 3-yearly intervals in a rolling programme. Each survey examines a nationally representative sample drawn from a given age group of the population and includes up to 2500 individuals. Information on food consumption inside and outside the home, nutritional status, socio-economic, demographic and lifestyle characteristics are gathered in the surveys. Food consumption is recorded in great detail and allows the examination of the eating patterns and nutrient intakes of individual consumers over 4 or 7 days. The first National Diet and Nutrition Survey was conducted in 1986/7 and surveyed British adults aged 16-64 years. The National Diet and Nutrition Survey programme has subsequently surveyed young children (1½-4½ years), young people (4-18 years) and people aged over 65 years. Fieldwork is underway for an updated adults survey for people aged 19-64 years. Additional dietary studies for schoolchildren, infants and vegetarians have also been conducted.

5.70 Estimates of dietary exposure to pesticides and veterinary medicines need to be made in order to ensure that levels of residues in food do not lead to exceedance of acceptable daily intakes (ADIs) derived from toxicological studies. At its most basic level, if an estimate of long term (chronic) exposure to a residue is less than the ADI, or if short term (acute) exposure is less than an Acute Reference Dose (ARfD), then the risk to consumers is considered acceptable.

5.71 PSD publishes a Data Requirements Handbook for companies which contains a chapter on the estimation of dietary intakes. This guide provides details of how PSD carries out exposure assessments (both chronic and acute) (see Chapter 4).

Estimating cumulative and aggregate exposure

Aggregate Exposure

5.72 An estimate of aggregate exposure assesses exposure to one chemical from all sources, for example, total exposure for someone living near to an industrial site from food, air, water and soil.

5.73 To calculate aggregate exposure to pesticides, veterinary medicines and similar substances it would be necessary to take an approach that involves several Government Departments. For pesticides and veterinary medicines, although food is an important source of exposure, residential exposure may be equally if not more significant. The route of exposure will include oral, dermal and inhalation.

5.74 The use of data from several Government Departments and model development in this forum would allow assessment of the total environmental exposure to a given chemical. Sources of data in the UK on non-food pathways such as domestic exposure would need to be reviewed to determine their suitability for use in UK aggregate models. The FSA has initiated an exercise (through the Interdepartmental Secretariat) to ascertain what information Government Departments have on exposure. The Interdepartmental Group on the Health Risks of Chemicals (IGHRC) may provide a suitable forum.

Cumulative Exposure

5.75 Cumulative assessment estimates exposure to multiple chemicals on the basis of whether they have a common mechanism of action.

5.76 Work would need to continue establishing groups of chemicals with related toxicology that would lend themselves to cumulative exposure assessment. Methods must be agreed about how best to combine exposure from each group of chemicals in a meaningful way. Co-ordination would be needed with relevant bodies to establish a picture of all the different chemicals (and their respective amounts) to which a person may be exposed.

Probabilistic modelling as a tool for assessing exposure

5.77 The probabilistic modelling of exposure offers an opportunity to combine multiple data sets for exposure and chemical residues in a realistic manner. It also allows the assessment to take account of inherent variability of all system components and their relative importance. The use of probabilistic modelling is, in fact, absolutely vital to achieve meaningful cumulative and aggregate modelling (see Chapter 9).

5.78 The current methods primarily used for estimating exposure to pesticides and veterinary medicines are deterministic (i.e. based on and producing single data points).

5.79 For pesticides, the current deterministic approach is to compare the chronic and acute exposure for a high level (97.5th percentile) consumer with the ADI or acute reference dose (respectively). This assessment is made for several age ranges (adults, young people, toddlers and infants) based on data collected on the GB diet in the National Diet and Nutrition Survey. For chronic exposure it is considered that high level consumption is unlikely to continue over a prolonged period and occasional excursions over the ADI are not considered significant. For acute exposure, ideally there should be no excursions over the ARfD.

5.80 In the case of veterinary medicines, risk assessments are currently based on the same single food basket for a 60kg adult used to set MRLs. Therefore, the risk assessment for veterinary medicines is very simple and easy to carry out. However, this does mean that it is not the best approach for all age groups.

5.81 In contrast to the deterministic approach, a probabilistic method generates a distribution of possible exposure values that would require regulatory decisions to be based on the probability of a given level of intake occurring (see Chapter 9).

5.82 Guidance has been produced by the US Environmental Protection Agency (USEPA) on carrying out probabilistic modelling. It is possible that the USEPA guidelines could be modified and adapted for the UK.

5.83 If it is possible to consider some of the models that are being used then it may be possible to decide on what criteria must be adhered to in models before probabilistic methods are considered acceptable.

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6. Biomonitoring and biological effect monitoring

Introduction

6.1 Biomonitoring and biological effect monitoring are techniques that help quantify the exposure of individuals to chemicals and the effects of those chemicals, respectively. These techniques are widely used in industrial settings to ensure that exposure of workers to hazardous chemicals is at or below acceptable levels, and the techniques can also be used to quantify exposure in other situations.

Biomonitoring

6.2 Biomonitoring is usually defined as the use of biomarkers to determine the extent of exposure of individuals in a human population to a particular chemical. In this context a biomarker is the compound to which someone is exposed, or a metabolite of that compound, or some other product directly derived from the exposure, which can be used to measure exposure to the compound of interest.

6.3 Biomarkers may be measured in blood, urine, fat, hair or some other accessible biofluid or tissue. In industrial biomonitoring, urine samples are often used, as collection is a non-invasive technique. Organochlorines (OCs) have been measured in human breast milk or fat; the former reflect the mobilization of OC residues from human fat *post partum* and also show the potential for exposure of infants, and both reflect exposure to OCs over a long period. For some chemicals, including many pesticides, there may be no satisfactory biomarker of exposure.

6.4 It is clear that an accurate measurement of the amount of a chemical to which a human is exposed is crucial in any study of the effects of chemicals on the human population. Although measurements of a chemical substance in the ambient air, water supply or food may be used to give estimates of potential exposure, more precise measurements of actual exposure and uptake are ideally required for any proper study of toxicity. This is the case particularly when there is exposure to mixtures of chemicals, such as pesticides, perhaps from different sources. Indeed estimates of exposure based on lifestyle and dietary information may be very inaccurate. For example a study in the United States of America (USA) compared estimates of aggregate exposure to chlorpyrifos "under a flea treatment scenario" with actual biomonitoring results. The United States Environmental Protection Agency (USEPA) estimates were 60-fold higher for adults and 27-fold higher for children than the actual values.[1]

6.5 In some cases measurement of an active metabolite(s) may be more useful than measurement of the parent compound, and this level is sometimes termed the internal dose. Often, this is the dose to which the target organ is exposed. If the metabolite is the product of a reactive metabolite such as a glutathione conjugate or DNA or protein adduct it may be indicative of metabolic pathways involved in the toxicity. The level of this metabolite is termed the effective dose and can relate more closely to the toxicity. However for simple determination of exposure, measurement of the parent compound may be a better biomarker, as the level of any metabolite will also be affected by the activity of the enzymes involved.

6.6 When using a metabolite as a quantitative indicator of exposure it is important to be aware that various factors can affect the proportion of compound being metabolised by a particular route and therefore the amount of the metabolite appearing in the blood or urine. For example, chemicals other than the compound in question may induce or inhibit cytochrome P450, which is involved in the metabolism of many chemicals including organophosphorus (OP) pesticides. Furthermore, if the hydrolytic pathways, important in the detoxification of OP pesticides, are inhibited, this can increase the toxicity[2] yet excretion of dialkylphosphate metabolites (often used as biomarkers of exposure) may be lowered. Also, genetic factors and age may influence metabolism. For example, the elimination of drugs may be lower in neonates and young children compared to adults.[3] The enzymes which can hydrolyse OPs (A esterases) show a ten-fold variation in activity in humans and also the main enzyme involved exhibits a genetic polymorphism, such that 40-50% of the population in the UK has low activity towards paraoxon.[4,5] Thus although measurement of a single metabolite may indicate exposure has occurred, using it for precise exposure quantitation may not always be appropriate or possible. Measurement of the parent compound and the major metabolites is preferable.

6.7 Many OP insecticides have similarities of structure and consequently have common metabolites such as alkyl phosphates.[6] Similarly, some synthetic pyrethroid pesticides have common metabolites such as phenoxybenzyl alcohol and substituted cyclopropyl derivatives. This allows measurement of several of such metabolites to be used to estimate exposure to any pesticide in the group producing them.[7] Moreover, it was found that measurement of dialkylphosphate metabolites in urine samples from various groups of exposed workers was more sensitive than inhibition of acetylcholinesterase as an indicator of exposure.[6,8] Also measurement of these common metabolites allows estimation of total exposure to a mixture of similar pesticides and this approach has been followed in situations where exposure to mixtures may occur.[9] However, this approach does not give sufficient information about exposure levels for the individual pesticide and therefore could not be used for studies on combined effects of pesticides, including interactions such as potentiation or antagonism. It is clear that in order to establish if interactions between pesticides occur in humans at food or environmental exposure levels, knowledge of levels of exposure to individual pesticides is essential.

6.8 Studies have been carried out in experimental animals given known doses of individual pesticides to investigate combined actions of mixtures (see chapter 8). However, a comprehensive search of the literature has not revealed any studies in which biomonitoring has been used to measure the levels of individual pesticides in human subjects (volunteer or field studies) exposed to mixtures of pesticides to study the combined actions of components.

6.9 In one study[9], dimethylphosphate metabolites were measured in the urine of children of pesticide applicators. Dimethylphosphate (DMP), dimethylthiophosphate (DMTP) and dimethyldithiophosphate (DMDTP) were measured. Several different pesticides had been used by the applicators including azinphos-methyl, chlorpyrifos and phosmet. The method of Nutley and Cocker[6] was used to measure the metabolites. However, in this study there were no data on inhibition of acetylcholinesterase in the children exposed. It was also notable that the control, supposedly non-exposed, children had measurable levels of the metabolites in their urine. It was noted by the authors that although some

pesticides have common metabolites (dialkylphosphates) the potency of the individual OPs may be very different, even with a common mechanism of action. A further study on children from Washington State, in which urinary alkyl phosphates were measured, found increased levels in children whose parents used pesticides in the garden but no relationship with pet treatments or residential use.[10] In a study carried out in Germany on people who moved into ex-USA Forces' housing that it was believed had been treated with chlorpyrifos, household dust was measured for chlorpyrifos and alkyl phosphates were measured in the urine of subjects living in the housing. Levels of alkyl phosphates were no higher for any age group in those households where chlorpyrifos was found in household dust. Elevations in urinary alkyl phosphates were not found in adults, when compared with reference values for the German population.[11] In a study in Italy carried out in children, increased excretion of alkyl phosphates was associated with pest control operations carried out inside or outside the home in the preceding month.[12]

6.10 In these examples DMTP measurements are not a direct indication of toxic potential of the mixture. Furthermore there may be toxic effects, particularly sub-chronic and chronic ones which are not due to the common mechanism of action, typically associated with the group (in this case OPs). However, there seems to be a paucity of information on such non-acute effects. Therefore, although data generated from measurement of common metabolites (eg dialkylphosphate metabolites) following exposure to a mixture of pesticides could be used for risk assessment where the individual pesticides have a common mechanism of toxicity, the data needs to be interpreted with caution for the following reasons:

- factors that affect metabolising enzymes, such as age and genetic polymorphisms, can affect the relative proportions of metabolites and rate of metabolism and might therefore affect exposure estimates;
- one component may induce or inhibit the metabolism of another;
- the measurement of a common metabolite(s) may indicate total exposure but does not indicate the proportion of exposure attributable to the individual components;
- levels of the common metabolites may not relate directly to toxicity as some groups of compounds are more potent than others despite sharing the same metabolite(s). Such groups include the OPs and synthetic pyrethroids.

6.11 For the proper investigation of interactions (eg potentiation) between pesticides in human populations, measurement of the exposures to each component of the mixture would be necessary together with measurement of toxic responses. Although measurement of a common metabolite(s) would indicate total exposure to similar pesticides, and the response to that total exposure could be measured, this would not allow the nature of any combined action to be demonstrated as the particular concentrations of the pesticides involved would not be known. Few studies have been carried out, in human populations, using more than one pesticide-specific metabolite. In one such study, specific urinary metabolites of four pesticides were measured, namely 1-naphthol, atrazine mercapturate,

malathion dicarboxylic acid, and 3, 5, 6-trichloro-2-pyridinol, metabolites of carbaryl, atrazine, malathion and chlorpyrifos respectively, in children exposed to pesticides in Minnesota. However, this study was intended as a study of pesticide exposure and there is no information on the clinical status of the subjects and thus no conclusion on interactions can be drawn. Interestingly, levels of many metabolites were not significantly higher in households reporting high pesticide usage.[13]

Biological effect monitoring

6.12 A biomarker of effect is a characteristic that can be objectively measured and evaluated as an indicator of normal biological or pathological processes, or toxicological responses to a chemical exposure. The most reliable biomarkers of effect are mechanistically based. Measurement of such biomarkers forms the basis of biological effect monitoring. Some biomarkers can be used as surrogate endpoints. These can substitute for a clinical endpoint, and should be able to predict clinical outcome.

6.13 Biological effect monitoring could be an important component of a study of interactive effects of pesticides and related compounds. To be effective, the biomarker should reflect a response (e.g. inhibition of acetylcholinesterase) that is common to several components of a mixture (e.g. OPs). This may be a more meaningful parameter than the measurement of a metabolite common to compounds of differing potencies. Biomarkers of effect in current use lack sensitivity, for example alkyl phosphates can be detected in the urine of individuals after exposure to amounts of OP well below those causing depression of acetylcholinesterase activity, although this may also reflect the concentration-effect relationship that exists for such compounds (see Moretto and Lotti for a review).[14]

6.14 Sensitive biomarkers of effect offer considerable potential for use in studies of individuals exposed to low levels of pesticides. They would also be invaluable as a bridge between studies in experimental animals and in humans and between those in cultured cells and in the intact organism. Much effort is now being devoted to the application of modern biological methods, including transcriptomics, proteomics, metabonomics and non- or minimally-invasive imaging, to identify and develop effective biomarkers of effect. Examples of the application of this approach have been published recently.[15,16] Any accessible biofluid or tissue can be used for biomarker assessment. Techniques now available offer high sensitivity and are applicable to a broad range of endpoints. However, for use in studies of pesticide interactions, it will be important to establish the mechanistic relationship between biomarkers of effect identified in this way and biological responses of concern. Adequate validation, demonstrating their reproducibility and reliability, will be necessary before adoption for widespread use in the study of the toxicology of mixtures.

Biomonitoring data in relation to pesticide residues in food and other sources

6.15 The Working Party on Pesticide Residues (WPPR) Annual Reports have reported data on OC residues in human breast milk and fat since 1963. Data on human milk are given in Fig 6.1 and on human fat in Fig 6.2. Analysis of milk and adipose tissue was carried out to study the bioaccumulation of OCs in humans. Because OCs are fat-soluble, they have the potential to accumulate in adipose tissue and they also

tend to persist in the environment. They represent worst case exposure in terms of residues accumulating in tissues and hence biomonitoring represents a useful estimate of long-term exposure. These pesticides were used for about thirty years in the UK although all, with the exception of lindane, have not been approved in the UK for over a decade. Approval for lindane was revoked in the European Union (EU) at the end of December 2000.

6.16 Figs 6.1 and 6.2 show that the mean concentration of 1,1'-(2,2-dichloroethenylidene)-bis(4-chlorobenzene) (p,p'-DDE), DDT, dieldrin, ß-hexachlorocyclohexane (ß-HCH) and hexachlorobenzene has been decreasing since the early eighties, when most OCs were banned in the UK. Overall, dieldrin and DDT (as measured by p,p'-DDE and DDT) levels have declined since the first biomonitoring surveys carried out by the WPPR. ß-HCH was the predominant HCH isomer detected; its levels in human milk have declined steadily over the last few decades, yet no corresponding reduction was seen in fat till the 1980s. In the most recent biomonitoring surveys, lindane was detected in only 3% of human fat and 2% of human milk samples (not shown), generally at very low levels.

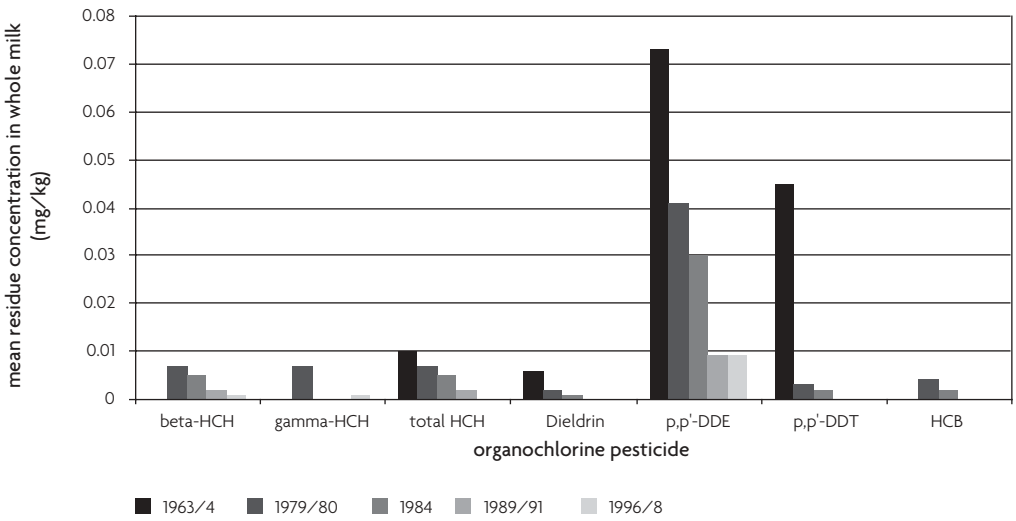
6.17 It should be noted that the simultaneous presence of different OCs in breast milk does not imply that the mother has been exposed to these chemicals from the same source.

6.18 For reasons already stated, OC residues are actively sought in human tissues to monitor the body's retention of pesticides resulting from environmental and dietary exposure. In the latest human milk survey, a range of the most frequently occurring and/or fat soluble OPs was sought for the first time. No residues were detected at or above their reporting limits.

Conclusion

6.19 Biological monitoring and biological effect monitoring have been little used to study combined effects of multiple pesticides. Measurement of pesticides or their metabolites in asymptomatic populations provides no information on combined effects of pesticides, even if parent compounds or specific metabolites are measured in biological fluids. Much more frequently group-specific metabolites are measured, as with OPs, and these are difficult to relate to the toxicity of specific pesticides. On the other hand studies such as that of Adgate and colleagues[13] and the human milk and fat surveys[17-21] show that simultaneous exposure to more than one pesticide clearly occurs. A further limitation of biomonotoring is that strategies for biomonitoring of exposure are still strongly influenced by availability of suitable biomarkers and, for many pesticides, there are none. The alternative of biological effect monitoring may be more promising for the study of combined effects, when new techniques become more widely available. Present methods of biological effect monitoring are rather insensitive.

Fig 6.1 Mean concentrations of organochlorines (OCs) in human milk 1963-1998

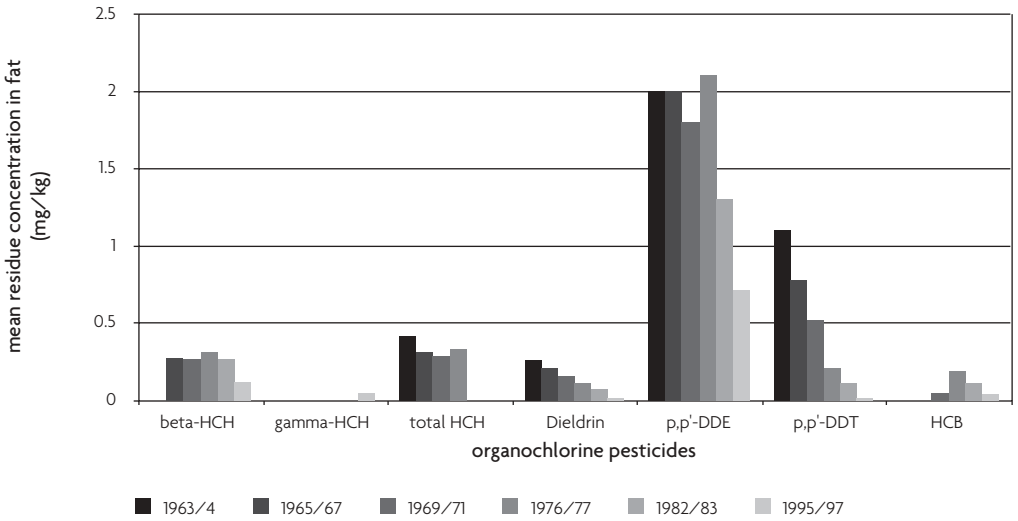


Human milk samples were obtained from hospitals across the UK. Five sets of breast milk samples have been analysed at approximately five yearly intervals since 1963 (data from the WPPR[17-21]).

Notes

1. Hexachlorocyclohexane (HCH) isomers in human milk:
   (a) Residue concentrations for individual isomers of HCH (alpha, beta and gamma [lindane]) were not reported in 1963/4.
   (b) Total HCH residues were not reported in 1996/8.
   (c) Alpha-HCH was also not reported in 1979/80 but sought in all the following surveys (1984, 1989/91, 1996/8) where it was not detected at or above the reporting limit.
   (d) Gamma-HCH was not detected in milk in 1984 and the mean residue concentration in 1989/91 survey was calculated to be <0.001mg/kg (the reporting limit).
2. DDT in human milk:
   (a) DDT is measured as the sum of p,p'-DDT, o,p'-DDT, p,p'-DDE and p,p'-TDE (DDD). Metabolites p,p'-DDE and p,p'-DDT were analysed in the human milk biomonitoring surveys.
   (b) The mean p,p'-DDT residue level was calculated to be <0.001mg/kg (the reporting limit) in 1989/91 and 1996/8 milk biomonitoring surveys.
3. Dieldrin in human milk was sought in all surveys. The mean residue concentration in 1989/91 and 1996/8 was calculated to be <0.001mg/kg (the reporting limit).
4. HCB in human milk was not reported in 1963/4 and it was calculated to be <0.001mg/kg (the reporting limit) in 1989/91 and 1996/8.

Fig 6.2 Mean concentrations of organochlorines (OCs) in human fat 1963-1997



Human fat samples were obtained at autopsy in UK subjects over 5 years old. Six studies of human fat have been carried out since 1963. (data from the WPPR[17-21]).

Notes

1. Hexachlorocyclohexane (HCH) isomers in fat:
   (a) Residue concentrations for individual isomers of HCH (alpha, beta and gamma [lindane]) were not reported in 1963/4 for human fat.
   (b) Total HCH residues were not reported in the last two surveys in 1982/3 and 1995/7.
   (c) Alpha-HCH and gamma-HCH were not reported in any survey except the most recent (1995/7). Alpha-HCH was not detected at or above the reporting limit.
2. DDT is measured as the sum of p,p'-DDT, o,p'-DDT, p,p'-DDE and p,p'-TDE (DDD). Metabolites p,p'-DDE and p,p'-DDT were analysed and detected in all the human fat biomonitoring surveys.
3. Dieldrin in human fat was sought in all surveys.
4. HCB was not reported in human fat in 1963/4 and 1965/7 but sought in the following surveys.

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7. Toxicology of mixtures – concepts and models

Introduction

7.1 The vast majority of toxicological studies of chemicals have been concerned with the evaluation of exposures to single compounds.[1] In practice, humans are exposed to complex and variable mixtures of chemicals, which may act independently as in a single exposure, but may also interact to modulate the effects of the mixture as a whole and components therein. Risk assessment of real life exposures is thus much more difficult than that of exposure to single agents. In assessing such risks from a public health perspective, it is necessary to assess whether chemicals in a mixture interact to produce an increased or different overall response as compared with the sum of the responses of the individual chemicals present in the mixture, or whether the overall effect is simply a summation of the expected effect of each chemical[1] – i.e., it is necessary to determine whether the toxic effect of a mixture will be one of additivity of dose or effect of the individual components, or supra-/infra-additivity.

7.2 This chapter discusses current knowledge of the toxicology of chemical mixtures, including methods and general terminology used in the field of mixture toxicology together with potential mechanisms by which interactions between chemicals within a mixture may occur. It is clear that, because of the complexity and variability of chemical mixtures that may occur in the environment, risk assessment of their potential toxic effects is an extremely difficult task. Although there are many reports that describe the toxic effects of mixtures, relatively few studies have adequately investigated the nature of interactions that may be occurring between the constituents within a mixture and deviations from additivity. It is probable that no single approach is suitable for application to the risk assessment of all chemical mixtures, but rather that specific problems will need to be addressed by the development of appropriate strategies. Approaches which have been most successful to date are those which are able to incorporate mechanistic data.

Basic concepts of mixture toxicology

Terminology

7.3 In principle, to assess interactions, one can make use of mechanistic or empirical models. Empirical, or descriptive, means that only information on doses or concentrations and effects is available, including dose-response relationship. On the other hand, mechanistic indicates that additional information on the sequence of reaction steps is available and quantitative parameters are known. For example, the joint action of multiple chemicals at a target receptor or enzyme can be described mechanistically, by Michaelis-Menten kinetics. In general, this will result in a phenomenon called competitive agonism and the ultimate combined effect may be less than expected on the basis of effect-addition because of competition for the same receptor or active site. In fact, this type of interaction can also be considered as a special case of similar joint action (dose-additivity – see below). A basic problem with this approach is that in most combination studies there is a gap in available information that prevents the application of such models. Currently empirical models play the dominant role (see review by Groten *et al*).[2]

7.4 Cassee *et al*[3] noted that there is widespread disagreement over the terminology and definitions used in the description of interactions of mixture components. They observed that a commonly used approach is to calculate the expected combined response (in the absence of interaction) from the results for single agents, and that any deviations between observed and expected response are termed an interaction. These authors recommended that, in order to avoid confusion, the terminology used to describe various interactive effects should be clearly defined. To this end, the International Study Group on Combination Effects had attempted to implement uniform definitions for descriptions such as synergism, potentiation and antagonism, and for standard methodologies to assess such effects.[3]

7.5 The description of the toxicological action of mixture constituents is based on three fundamental concepts, as originally described by Bliss (1939) (cited by Cassee *et al*).[3] These three concepts are described briefly in the following paragraphs. In reality, several principles of joint action may occur at the same time (partial dose and/or effect additivity), especially when mixtures consist of more than two compounds and when several target sites are involved. Hazards of mixtures cannot be characterised without the use of appropriate test designs and clear, testable hypotheses to explore joint action.

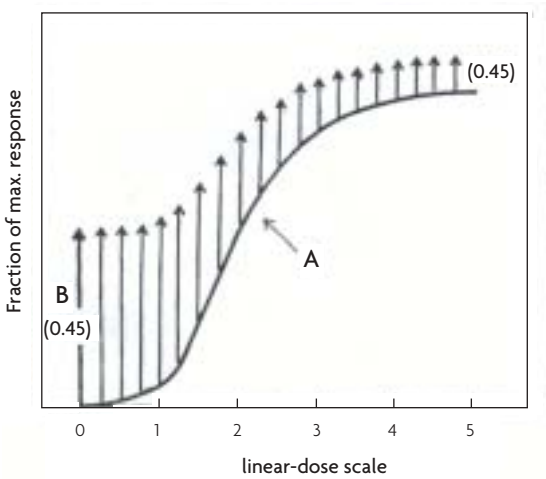
Simple similar action (simple joint action, concentration/dose addition).

7.6 Simple similar action (simple similar action, or simple joint action) results in dose additivity. It should be used as the starting point if the chemicals in the mixture act in the same way, by the same mechanism(s), and differ only in their potencies. Under simple joint action, the effect (or response) for the mixture is obtained by summing the doses of the individual compounds, after adjustment for differences in their potencies. Mathematically, if R(x) is the dose-response function of two similarly acting compounds A and B, the response for a mixture with standardised dose x_A of A and x_B of B is $R(x_A + x_B)$ (see Figs 7.1 and 7.2).

Simple dissimilar action (simple independent action, independent joint action, effect/response addition)

7.7 The other starting point for a reliable analysis of additivity is response addition (or effect addition or simple dissimilar action, or independent joint action). In this case, it is assumed that the modes of action and possibly the nature and site of action differ among the chemicals in the mixture which exert their individual effects, but do not modulate the effect of other constituents of the mixture. Effect addition is defined by the summation of the effects of each compound in the mixture, i.e. for the previous mixture of A and B, by $R(x_A) + R(x_B)$. The term response addition is more appropriately used to describe the proportion of responders in a population rather than their average effect. This applies if each individual of the population has a certain tolerance to each chemical of the mixture and exhibits a response only if the concentration exceeds the tolerance dose. If two compounds A

Fig 7.1 Importance of dose-response characterisation in determining deviation from additivity



Effect of a fixed concentration of component B on the dose-response curve for A; dose vs fraction of response (from Cassee *et al*[4] reproduced by kind permission of the authors and CRC press).

and B show response addition, the proportion of responders in the mixture is equal to $R(x_A) + R(x_B) - R(x_A)R(x_B)$, as those who respond to constituent A, will not be able also to respond to constituent B; this would be true, for example, of death. This assumes there is no correlation in individuals in the studied population between susceptability to A and susceptability to B: it is possible to propose other situations:

- There could be complete positive correlation in susceptability of individuals to components of the mixture, in which case the proportion of individuals responding would be determined by the more toxic component of the mixture.

- There could be complete negative correlation; here the individuals most susceptible to one component in a mixture are least susceptible to another. In this case the percentage responding to the mixture will be equal to the sum of the percentages responding to each of the components.

These relationships have been most widely studied in respect of death as a study outcome and with binary mixtures. When other outcomes are considered, it would be possible to have individuals who respond to both constituents. Additionally, with complex mixtures there might be very complex relationships in susceptibility to the various components of a mixture.

Fig 7.2 Simple similar action: with several components

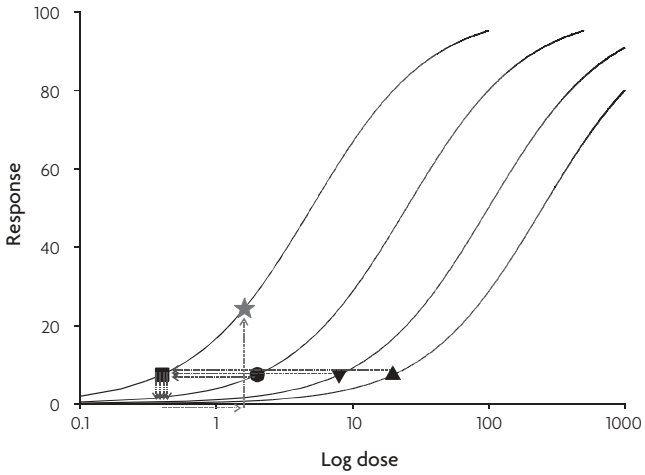


Illustration of concept of simple similar action, with simultaneous exposure to several different compounds acting by the same mechanism. The response to a mixture of four compounds acting on the same receptor with different affinities is illustrated. The response to each compound alone is less than 10%. The response to the mixture is over 20%

Interaction (e.g., synergism, potentiation, supra-additivity, antagonism, sub-additivity, inhibition)

7.8 Apart from the concepts of simple similar action and simple dissimilar action, any situation that deviates from either of these models may be defined as an interaction. The overall effect may be one that is either stronger or weaker than that predicted by an additivity model; it should be noted that in this situation the term interaction is used conceptually to describe deviation from an additive model. The mechanism underlying the interaction may be at the chemical, physico-chemical or biological level, for example interactions between two chemicals in a mixture/formulation, or interactions in either the toxicokinetic or toxicodynamic phase. Mechanistic aspects are discussed later in this chapter.

7.9 In order for a meaningful analysis to be carried out of any potentially additive or interactive effects of compounds within a mixture, it is first necessary to establish dose-response curves for each of the individual components on its own. In the absence of such information, it is not possible to predict the effect of a single compound at doses outside the range for which the shape of the curve is known and the theoretical additive effects of the compound in combination with any other mixture component cannot be predicted. The absence of adequate dose-response characterisation is a major problem in the interpretation of much of the published literature on the effects produced by mixtures of pesticides.

7.10 The importance of dose-response characterisation in determining deviation from additivity is illustrated by the effect of a fixed concentration of constituent B on the dose-response curve for

constituent A (Fig 7.1). In the absence of an interaction, when working in the region of the dose-response curve, which approximates to linear between 2 and 4 in Fig. 7.1, the effects of B will be additive with the effects of A. However, consumer exposure almost always occurs in the non-linear region of the curve, < 1 in Fig. 7.1. Here, even in the absence of any interaction, the effects of B may contribute disproportionately to the response, depending where on the dose-response curve the sum of A and B appears. In the absence of information on the shape of the dose-response curve for both A and B, it is impossible to predict what the expected response would be to any combination of the two compounds, even without any interaction. The matter is further complicated in that in biological systems, for many effects, there is a threshold below which a response cannot be detected. In such cases, addition of the action of A and B can result in a detectable reponse where, separately no reponse was detectable; this may be falsely described as potentiation.

Experimental design

7.11 For complete analysis of the effects of a mixture in comparison with the separate effects of each of its components, it is first necessary to ascertain all the components of the mixture. Identification of toxicological and other relevant data (e.g. mechanism of action) regarding each of the constituents may be of use. However, experimental studies comparing the effect of the mixture with that of the individual components (and establishing a dose-response curve for each component) are required in order to evaluate effects of the mixture and potential interactive effects of components. Studies would preferably evaluate effects at both high (i.e. effective) and low (i.e. realistic) doses. In reality, the major hindrance to experimental studies of mixtures is that the number of test groups required to evaluate all possible interactions of components within the mixture is likely to be prohibitively large. The aim in the design of such studies is to plan experiments which minimise as much as possible the number of test groups, whilst maximising the amount of information obtained with respect to individual mixture components and combinations.

7.12 In practice, two basic methodological strategies exist to study the toxicology of mixtures. Component interaction analysis (bottom-up approach) can be applied to the analysis of simple mixtures with a small number of constituents and where the composition is clearly known. In the absence of specific knowledge of the composition of a mixture, or where there are numerous components (a complex mixture), whole mixture analysis may be more appropriate. However, such studies cannot define the extent of true interactions between components of the complex mixture without data on fractions of the mixture.

Component-interaction analysis

7.13 In the case of where a mixture comprises a small number of (for example, two) defined components, such that sufficient experimental data can be obtained, a number of mathematical modelling approaches can be used to evaluate the data, for example isobolographic and response-surface analyses. Physiologically-based toxicokinetic (PBTK) modelling, by which data from a variety of sources including animal studies, *in vitro* enzyme kinetic studies are used to predict *in vivo* human toxicokinetics, may be useful for modelling interactions in the toxicokinetic phase. For a description of these models see

Cassee *et al*[3] and Groten *et al*[2]. Methods such as isobolographic analysis and response-surface analysis are limited to mixtures of compounds with the same target (i.e., with potential interactions in the toxicodynamic phase) (For a description of these modelling methods, see Cassee *et al*).[3,4]

7.14 In cases where a mixture consists of more than two components, the numbers of possible experimental groups required to evaluate component interactions increase exponentially with the number of compounds in the mixture. In this situation a number of statistical designs are available to evaluate the effects of mixtures compared with their individual components, e.g., ray, composite and factorial designs (for descritptions of these modelling methods see Cassee *et al*,[4] Groten *et al*,[2] Tajima *et al*[5] and references therein). Another approach may be to analyse a mixture for departure from additivity, rather than for specific interactions, comparing dose-response information for individual components to the observed responses induced by specific combinations of interest.[6]

Whole mixture analysis

7.15 Where mixtures are too complex to be studied using a bottom-up approach, an alternative approach of whole mixture analysis may be appropriate. A simple method of carrying out such a study is to evaluate the effects of the mixture and of all individual constituents at one dose level without testing all possible combinations of components (requiring n + 1 test groups, where n is the number of components in the mixture); this method is most frequently used in the study of mixtures of pesticides. However, as dose-response relationships are not characterised, this strategy does not allow for the evaluation of potential interactive effects between mixture components. It has been suggested that this approach may be appropriate for primary screening for adverse effects of a mixture.[4]

7.16 Whilst it may not be possible to determine whether there is an interaction between any of the components of a complex mixture, it should be possible to identify NOAELs for all critical effects. Hence, as long as exposure is at a suitable margin below these NOAELs, consumers will be protected from the effects of any potential interactions that might occur within the mixture, providing there is an adequate margin of safety. This is particularly so if the ratios of compounds in the mixture remain relatively constant.

Risk assessment of mixtures

7.17 In terms of hazard identification the testing of all possible (complex) mixtures of chemicals existing in the real world or of all possible combinations of chemicals in simple mixtures at different dose levels is virtually impossible. Moreover, even if toxicity data on individual compounds were available, one is still faced with the problem of extrapolation of findings obtained at relatively high exposure concentrations in laboratory animals to man exposed to much lower concentrations. This means that exposure data either on the mixture of choice or on each of the individual compounds will be necessary for extrapolation. This problem is one of the key issues in the assessment of possible health risks from disinfection by-products and contaminants in drinking water,[7,8] and led to the development of the hazard index (HI) and toxic equivalency factor (TEF) approaches.

Hazard Index (HI) and the dose additivity concept

7.18 In spite of the necessity of carrying out simple test case studies with chemical mixtures, generic methods are clearly needed for their risk assessment of mixtures. One approach to estimating the risk posed by exposure to a mixture is the Hazard Index (HI) as put forward in the United States Environmental Protection Agency (USEPA) mixture guidelines[9] and derived from well-established techniques. In this approach, the hazard quotients (ratio of exposure level to a defined limit exposure value) are calculated for individual compounds and the quotients for each compound in the mixture are than summed. It will often be impossible to obtain sufficient and adequate toxicological information on all of the components of a mixture to make these calculations. Data on the nature of any interaction are not taken into account in this approach, and in certain cases the risk of joint action will be incorrectly estimated. This approach is particularly applicable when components act by simple dissimilar action.

7.19 A different approach, originally published by Mumtaz and Durkin in 1992,[10] takes into account both synergistic and antagonistic interactions in the derivation of the HI. In this approach a weight-of-evidence classification is followed to estimate the joint actions (additivity, antagonism and synergism) for binary mixtures of chemicals based on information about the individual compounds. In this procedure, several weighting factors are used, including the mechanistic understanding of the binary interactions, the demonstration of toxicity, and additional factors, i.e. modifiers of interactions, such as route of exposure, *in vitro* data. The better the data set on the individual compounds is, the more precise the joint action can be predicted. The HI method can be regarded as a general first assessment of the risk of joint action. The weight of evidence method should be used as a follow-up in those cases where priority mixtures have been established. In order to show its usefulness in future risk assessment, the weight of evidence method has to be validated first with experimental studies as illustrated by Mumtaz *et al*.[11]

Toxic equivalancy factor (TEF) approach

7.20 Another strategy to assess the hazard of mixed exposure is the TEF approach, which was developed for environmental contaminants, where it is widely recognised that structurally related chemicals may exhibit similar toxicity, and therefore may show joint actions. For example, the contaminants, polychlorinated dibenzo-*p*-dioxins (PCDDs), widely distributed in the environment, share common toxicity mediated by actions on the aryl hydrocarbon (Ah), receptor. To allow for combined intakes, the different congeners have been allocated TEFs related to their potency at the Ah receptor. The total combined intake of PCDDs is calculated as the sum of the concentration of each individual congener multiplied by its TEF, and the sum total is compared with the tolerable daily intake for the congener used as the referent (2,3,7,8-tetrachlorodibenzo-*p*-dioxin). The summation of [concentration x TEF] for each congener assumes joint action leading to full dose-additivity. However, joint action could also result in partial additivity when a congener with a lower potency (or more importantly, a lower efficacy) displaces one with higher potency/efficacy. This type of uncertainty and others (nonadditive interactions, differences in shape of the dose-response curve, and species responsiveness) have been

reviewed extensively and have indicated that the TEF concept is still the most suitable method for the hazard assessment for mixtures of halogenated aromatic hydrocarbons with dioxin-like properties.[12] Some of the dioxin-like compounds known to be Ah receptor agonists also exhibit estrogenic and antiestrogenic activities and considerable attention is currently being paid to assessing the human health risk of these estrogen-like responses. Besides these industrial compounds, food contains compounds such as bioflavonoids and indol-3 carbinol that also have estrogenic activity. A mass balance of dietary levels of industrial and natural estrogens, coupled with their estimated estrogenic potencies, indicates that the dietary contribution of mixtures of estrogenic industrial compounds is much lower than the daily intake of estrogenic flavonoids.[13,14]

Other approaches

7.21 The situation with food components is different from that discussed above for structurally and biologically related compounds like the PCDDs and estrogens. Nevertheless, for some food components such as food additives and pesticides, regulatory bodies acknowledge that some such compounds might share common properties, and such compound are allocated a group acceptable daily intake (ADI). A group ADI may be allocated when each member of the group is metabolised to a common metabolite, the activity of which determines the toxicity profile and hence the NOAEL. Also, compounds would be considered as a class when they showed a common effect, or a common mechanism/mode of action, despite not sharing a common metabolite. In such circumstances, the NOAEL and ADI could be based on any member of the group (using molar equivalents of the parent compound or the toxic metabolite formed). The ADI would then apply to all members of the group, and the total combined intake should not exceed the ADI (dose-additivity). Examples of compounds with a group ADI are polyols, allyl esters and some pesticides. The ADI applies to the total combined intake of all compounds. In some cases, this may be unavoidable for analytical reasons.

7.22 Another strategy to assess the risk of mixed exposure was recently adopted by the International Life Sciences Institute-Europe Acceptable Daily Intake Task Force which evaluated the possibility of interactions occurring between the 350 food additives currently approved in the European Union (EU).[15] The strategy chosen was to identify those interactions that theoretically could be of a health concern, based on similar criteria to those used by the joint expert committee on food additives (JECFA) to establish group ADI values, but without the criterion of close structural or metabolic similarities. In total, 65 additives were identified with numerical ADI values. To analyse this list of additives further the principle was accepted that joint actions and/or interactions would be most likely between compounds that shared a common target organ, and produced similar adverse effects at doses above the NOAEL. The toxicology data for the additives considered were further assessed to determine which of these might share a common effect profile on the target organ (e.g. liver or kidney) and also in light of their possibility to show toxicokinetic interactions. This further analysis revealed that possible joint actions could not be excluded for four additives in the liver, three in the kidneys and four in the thyroid. The value of this approach remains to be demonstrated.

7.23 A similar type of approach has been proposed by the USEPA Office of Pesticides Programs (OPP)[16] for the cumulative assessment of risk posed by exposure to multiple pesticides by multiple pathways (including food, drinking water and air). To undertake a cumulative risk assessment for a set of pesticides that have common mechanism of toxicity, OPP follows a procedure in which pesticides are identified that belong to a Common Mechanism Group (CMG) for which scientifically reliable data demonstrate a common toxic effect by a common mechanism of action. OPP will perform cumulative risk assessment using conventional risk assessment, viz. hazard assessment, dose-response evaluation, exposure characterization and risk characterization. Steps 1 and 2 will be carried out by using a weight-of-evidence approach to determine the toxic endpoint that occurs through a common mechanism for the chemicals in a CMG and by establishing a common measure of toxic potency. In the case of organophosphates (OPs) the cumulative risk was established on the basis of dose additivity. For the last two steps, OPP will estimate exposure and risk for the dietary (food), residential/non-occupational and drinking water pathways. It is recognized that, for the time being, the only extensive exposure data are for dietary intakes and that initially it will be difficult to obtain cumulative totals for all the sources of potential exposure (OPP, 2000).[16] A further consideration is that a substantial amount of work is necessary to determine whether particular pesticides fall into a CMG.

7.24 All methods (hazard index, equivalency factor, common target organ toxicity) are used in conjunction with information on the exposure data and margins of safety, to estimate the health concern of the components in the mixture. The theoretical considerations in hazard characterization of the mixture should be verified by simple case studies.

Mechanisms and causes of interactions

7.25 The precise mechanisms of interactions between constituents of mixtures, in most cases, are not known. However, in general terms, any interaction could occur at the chemical-chemical, the pharmacokinetic and/or the toxicodynamic level. These types of interaction are outlined below with examples. Where possible, proposed mechanisms for interactions between components of mixtures containing pesticides or related chemicals have been included.

Chemical-chemical interaction

7.26 Components of a mixture react together directly to form (an)other compound(s) that possess(es) a higher or lower toxicity. An example of such a reaction is that which occurs between nitrites and amines to form carcinogenic nitrosamines. An example of a chemical-chemical interaction resulting in decreased toxicity would be that between cobalt edetate and cyanide to produce a complex less acutely toxic than the individual components.[17] No examples of this type of interaction involving only pesticides have been identified. However, the proposed mechanism by which certain dithiocarbamates increase the uptake of lead (and some other metals) into the brain involves the formation of a lipophilic lead-dithiocarbamate complex which, when compared to inorganic lead, has a higher retention and higher capacity to penetrate the blood brain-barrier and bind to lipid-rich brain tissue components.[18] However, neither the stability of the lead complex within the brain nor its neurotoxicity

as a complex is known. It has been suggested that the teratogenicity of the dithiocarbonate, maneb may involve a similar mechanism[19] in that maneb complexes with zinc, thus depriving zinc requiring tissues, including embryonic tissue, of this essential metal.

Toxicokinetic interactions

7.27 Prior to the approval of a pesticide, studies of its absorption, distribution, metabolism and excretion (ADME studies) are performed, which provide knowledge on the fate of the compound in the body, and its potential for accumulation. The term toxicokinetics relates to the movement of the chemical around the body, and involves the data from ADME studies, plus any data on the concentration-time curve for the chemical and its metabolites in plasma and tissues. For some pesticides the biological activity, including potential toxicity, is due to the pesticide itself (the parent compound), whereas in other cases the activity may be due to a metabolite. Toxicokinetic interactions can increase or decrease the amount of the active chemical delivered to its site of action, and therefore could represent an underlying mechanism for any of the different effects discussed earlier.

7.28 Environmental chemicals, such as pesticide residues, are handled in the body by non-specific pathways that are found in all mammalian species, and that have evolved to eliminate the vast number of non-nutrient chemicals that enter the body every day in the air, food and drinking water. Toxicokinetic interactions occur when chemicals share aspects of their absorption, distribution or elimination, but do not require the chemicals to share a common mechanism of action. Non-nutrient chemicals usually undergo passive absorption from the gut lumen and elimination in the urine either unchanged, or as metabolites produced by enzyme systems such as cytochrome P450 and glucuronyl transferase. Toxicokinetic interactions would be of concern if one chemical were to increase the internal dose of the active form (parent compound or metabolite) of another chemical. Alteration of the metabolism of a chemical would require enzyme induction, enzyme inhibition or saturation of the enzyme by the presence of both substrates. Alteration of the absorption, distribution or renal or biliary clearance of a chemical would often require interference with the normal physiology of these processes.

7.29 The absorption of most non-nutrient chemicals from the gastro-intestinal tract is by passive diffusion of the unionised, lipid-soluble form. Some non-nutrients, such as essential minerals, may be absorbed by specific carrier systems so that their absorption is controlled; however, this would not occur for pesticides, and the majority of the ingested dose would be absorbed from the gut. The intestinal wall contains high concentrations of P-glycoprotein, which acts to transport certain lipid-soluble compounds from the cells of the intestinal wall into the gut lumen. In consequence, this transporter will decrease the extent of absorption of such ingested compounds, and in theory could reduce the absorption of pesticides present in the diet. The lumen and wall of the gastro-intestinal tract, and the liver are important sites of metabolism, and metabolism during the absorption process (first-pass or pre-systemic metabolism) may limit the amount of the ingested compound that reaches the systemic circulation in the unchanged form. The consequences of first-pass metabolism depend on whether the activity/toxicity resides in the parent compound or one of its metabolites. First-pass metabolism in the gastro-intestinal tract or liver would reduce toxicity after oral exposure compared to other routes

if the parent compound is the active form, but the converse may be true if metabolism produces the active and potentially toxic chemical form. Absorption across the skin is an important route of exposure to pesticides; only a fraction of the applied dose would cross the permeability barrier of the stratum corneum, however that fraction would not be subject to extensive metabolism within the skin and most would enter the systemic circulation. With the exception of first-pass metabolism, all of the processes involved in the absorption of pesticides involve passive diffusion. Passive diffusion across the skin could be affected by the presence of solvents that affect the partitioning of the compound between the applied formulation and the skin, and by compounds that disrupt the architecture of the stratum corneum.

7.30 Distribution is the reversible movement of the compound between the blood and the body tissues. For non-nutrient chemicals this is normally by simple passive diffusion down a concentration gradient. The extent of distribution depends on the affinity of the blood and tissues for the compound. Non-nutrient chemicals may partition into lipids, and also undergo reversible binding to proteins in blood and tissues. The blood vessels supplying certain organs, particularly the brain, allow only lipid-soluble compounds to diffuse freely into the organ, while water-soluble compounds enter only slowly or not at all. These characteristics have given rise to the concept of the blood:brain barrier, which is due to tight inter-cellular junctions between endothelial cells, plus a reduction in membrane pores, high activities of certain metabolising enzymes such as mono-amine oxidase, and carrier proteins including P-glycoprotein which serves to transport compounds back into the blood system. Thus, lipid-soluble pesticides are more likely than water-soluble pesticides to produce central nervous system effects, whereas water-soluble pesticides would affect peripheral nerves to a greater extent than central nerves.

7.31 The metabolism of non-nutrients (foreign compound metabolism) normally involves enzymes and processes that show low specificity, but high capacity. A single enzyme may be responsible for the simultaneous metabolism of more than one chemical in a mixture, and in reality this is the normal situation because the enzymes are simultaneously metabolising large numbers of plant non-nutrients, environmental chemicals, medicines etc. Different pesticides in a mixture could theoretically interact, such that one interfered with the elimination of another; for example if one pesticide were to saturate the enzyme responsible for the metabolism of both that pesticide and another pesticide to which the individual was simultaneously exposed. An additional type of interaction could occur when a chemical alters the amounts of an enzyme available to metabolise other substrates. The majority of enzymes involved in the elimination of foreign compounds are expressed constitutively, and the activities in the liver and other organs are controlled genetically. However, the administration of certain compounds, including some medicines and plant products, such as flavones, and isoflavones, can either increase the amounts of enzyme present in the cell (enzyme induction) or interact with the enzyme to inhibit it. In consequence, enzymes involved in the elimination of foreign chemicals represent an important site for potential toxicokinetic interactions. The potential impact of inhibition of metabolism of a pesticide on its toxicity is illustrated by a marked increase (70-fold) in the toxicity of malathion due to the presence of an impurity (isomalathion) in a formulation. This resulted in inhibition of the enzyme carboxylesterase, which catalyses a major route of detoxication.[20]

7.32 The urine and bile are important routes of elimination of non-volatile foreign compounds. Lipid-soluble compounds are not eliminated to a significant extent by these routes, and such chemicals will undergo metabolism (see above) to produce a water-soluble excretory product. The processes mainly involve passive diffusion, although active transporters are responsible for the elimination of some compounds and metabolites.

7.33 Assessment of the potential for toxicokinetic interactions in humans exposed to mixtures of pesticides needs to consider a spectrum of different exposure scenarios, the extremes of which may be represented by:

- Multiple low-level exposure, such as exposure via the food to residues of different pesticides, each of which is at low levels (close to or below its MRL) and results in intakes of each pesticide below its ADI.

- High-level exposure, such as might occur following occupational exposure to high levels of one or more pesticides, possibly combined with low level exposure, for example to residues in food.

7.34 Each of these will result in different concentrations being delivered to possible sites for interaction. In addition the time course for exposure needs to be considered, because some possible interactions do not need coincidental intakes or exposures. Interactions could take the form of simple competition for a common process or pathway, without a change in the activity of that process; alternatively, one component of a mixture could produce a change in the activity of the pathway either by activation or induction, which would increase the total activity, or by inhibition, which would decrease the total activity.

Simple interactions due to the coincidental presence of two substrates competing for the same process

7.35 The processes of absorption, distribution and excretion are usually by simple diffusion. Diffusion would not be affected by the presence of more than one compound (at the concentrations that would be relevant to human exposures to pesticides): each compound would diffuse down its concentration gradient as if the other compounds were not present. The diffusivity through a biological matrix could be affected by the presence of a second chemical, but only if it altered the nature of the biological matrix or the physico-chemical properties of the mixture. This type of chemical-chemical interaction could theoretically occur between a pesticide and a solvent or surfactant, and could be relevant to certain exposure scenarios, for example dermal absorption following exposure to sheep-dip concentrate compared with diluted preparations. Diffusivity through a biological matrix may be an important modulator of the toxicity of pesticide residues present in food by reducing the rate of absorption compared with oral administration of the same amount of pesticide in a solvent.

7.36 Interactions could arise when compounds share a common pathway that is catalysed by a protein, and processes such as active transport and metabolism are possible sites for toxicokinetic interactions between pesticides. Such processes are first-order with respect to substrate at low concentrations, which means that the rates of absorption, metabolism and excretion are independent of dose. Therefore *in vivo* toxicokinetic parameters that represent such processes, for example clearance and half-life, are constant at low concentrations. Because of the availability of excess unoccupied protein (enzyme) sites, the toxicokinetics of a low concentration of one chemical would not be affected by the presence of low concentrations of a number of different compounds. However, this situation would not obtain at higher substrate concentrations, when there was not an excess of available enzyme active sites. At high concentrations the available enzyme active sites become increasingly occupied with substrate and the enzyme system becomes saturated. If one substrate were to saturate the enzyme, then the rate of elimination of that substrate and any other substrates for the same enzyme would be reduced (until the concentrations of all substrates no longer saturated the enzyme active sites). Saturation of metabolism is an example of a toxicokinetic interaction that displays simple dose-additivity, because saturation is simply a function of the combined concentrations of all substrates in relation to their affinity constants (Km values).

7.37 Saturation of metabolism or elimination is dose-dependent, and is sometimes found in high-dose animal feeding studies. Saturation of metabolism or elimination would be unlikely in humans exposed via pesticide residues in food, because the MRL would give intakes below the ADI and the ADI is normally a dose 100-fold lower than the no-observed-adverse-effect level identified in animal studies. No such interaction would be expected, even in the unlikely event that an individual simultaneously consumed food containing multiple pesticide residues, each of which was close to or below the MRL. In contrast, saturation of metabolism could occur following high-dose exposure, for example occupational exposure, and this would lead to transient excessive blood and tissue concentrations of the substrate(s) for the saturated process. The toxicological consequences of this would depend on whether the substrate for the saturated process was active or inactive, and whether the concentrations of each pesticide alone or the combination (if they shared a common toxicodynamic mechanism/mode of action) was sufficient to elicit a response. In contrast to other types of toxicokinetic interaction, simple saturation of metabolism would have only a limited impact with respect to the substrate and would be of short duration, but saturation of one pathway could result in increased utilisation of alternate pathways, which might enhance toxicity.

Interactions in which the toxicokinetics of one pesticide are altered due to a change in the amount of enzyme activity, produced by either concurrent or prior exposure to a second pesticide

7.38 Exposure to one compound could affect the fate of a second compound either by enzyme induction or by enzyme inhibition. Enzyme induction gives rise to a greater concentration of the enzyme within the cell, and can be due to increased transcription of the mRNA, stabilisation of the mRNA, or stabilisation of the enzyme protein. The elevated enzyme activity typically persists for a period of 2-3 weeks after the cessation of high-dose exposure. Enzyme induction usually requires exposure for a period of a few days to doses that are above the NOAEL, although for some pesticides simple induction of

cytochrome P450 and adaptive liver enlargement would not be considered as adverse effects, and therefore may occur at doses less than the NOAEL. There are two main types of enzyme inhibition, reversible inhibition, in which the enzyme activity returns to normal when the inhibitor is eliminated from the body, and irreversible inhibition, which represents a permanent change in the enzyme and which persists after the removal of the inhibitor. Reversible inhibition usually requires simultaneous exposure to both the substrate and the inhibitor, which has to be at a sufficiently high concentration or dosage. Irreversible inhibition could occur following a single acute high-level exposure to an irreversible enzyme inhibitor, such as certain organophosphates (OPs). The extent of irreversible inhibition would decrease with time, as new enzyme was synthesised; the duration of inhibition after a single high-dose exposure would depend on the rate of synthesis of new enzyme. Multiple low-level exposures to irreversible inhibitors, each of which was too low to produce a measurable effect, could also produce enzyme inhibition, providing that the combined doses were sufficient to produce a response (dose-addition), and that the rate of increase in enzyme inhibition exceeded the rate of synthesis of new enzyme during the period of multiple exposures. Prolonged inhibition could also arise from depletion of enzyme co-factors, but often this would require an intake that saturated its own elimination.

7.39 Enzyme induction would be unlikely in humans exposed via pesticide residues in food, because the intakes would be much lower than any intake associated with enzyme induction and hepatic changes identified in animal studies. Therefore even combined exposure to multiple residues of pesticides that shared the same enzyme-inducing properties would not result in a significant toxicokinetic interaction. The inhibition of serine containing enzymes, such as acetylcholine esterase, is used to define the no-observed-adverse effect level for OPs. Therefore, simultaneous exposure to multiple residues of OPs in food would be unlikely to result in significant persistent enzyme inhibition, unless the combined intake was sufficient to erode the safety margin used to establish the ADI. A possible exception to this may be the irreversible inhibition of cytochrome P450 during the oxidation of P=S phosphorothioate OPs, because this inhibition may be detectable at lower doses than those that inhibit acetylcholineesterase.

7.40 Interpretation of the toxicological consequences of an interaction involving enzyme induction or inhibition is not simple, and would depend on the biological effects of the substrate and of the metabolite. In some cases the parent compound is the active form and an enhanced response would arise from inhibition of metabolism. In contrast, enzyme induction can result in enhanced toxicity when this is due to a toxic metabolite, for example the oxidative metabolism of P=S phosphorothioate OP pesticides to their corresponding active oxon (P=O) metabolites. Toxicokinetic interactions may show dose-addition, response-addition or synergism. Toxicokinetic interactions could give rise to synergism when assessed in relation to the administered external dose, but not if the comparison were made on a biomarker of internal dose, such as the plasma concentration. For example, a chemical that induces or inhibits enzyme activity may not share a common toxicity with the compound that has its metabolism altered, so that the enhanced response for the combination cannot be explained by external dose-addition, while the magnitude of the response to the combination can be predicted directly from the increased plasma concentration. This type of interaction may arise with combinations of pesticides and adjuvants (synergists[a]), such as piperonyl butoxide, which are added to pesticide formulations specifically to alter the metabolism in the target organism in order to enhance toxicity and lower usage levels. Comparison of the plasma concentrations of the toxic compound (or

[a] For definition in this context see glossary.

its circulating active form) when given with the inducing or inhibiting chemical, with the plasma concentration-response relationship for the single chemical, would reveal whether the interaction was due simply to a change in the internal dose and circulating concentrations of the active compound (toxicokinetics), or was due to toxicodynamic potentiation.

Toxicodynamic interactions

7.41 Toxicodynamic interactions are those that occur at the cellular receptor/functional target level, either through one component interfering with another's binding to a receptor site or through one components binding to a receptor resulting in action that alters the toxic actions of another component.

7.42 Examples of the first type include the antagonistic effects of oxygen on carbon monoxide, atropine on cholinesterase inhibitors and naloxone on morphine. While it seems conceivable that one compound could increase the intrinsic activity of another by modification of the receptor site in a way that is analogous to the effect of allosteric modulators of regulatory enzymes, such interactions have not been demonstrated with environmental compounds.

7.43 Generally, the effect of combined action of two components at the same site of primary action is unlikely to result in potentiation. Competition for the receptor will usually result in summation of effects or antagonism, such as illustrated by the effects of mixtures of phytoestrogens and other estrogenic compounds of varying potency. In simple terms, an antagonist negatively regulates the activity of an agonist. A partial agonist, on the other hand, exhibits properties of both agonist and antagonist. At low concentrations and/or in the absence of other functional ligands, a partial agonist will act as an agonist. Some weak agonists may function as antagonists by occupying receptors in the presence of a more potent agonist. When significant numbers of receptors are occupied by low potency ligands, in the presence of a low concentration of a higher potency ligand, they will appear to exert an antagonistic effect. However, in actuality they will still be functioning as agonists, in that they will be inducing a response. Factors affecting lower affinity ligand/receptor occupancy in the presence of higher affinity ligands include the relative concentrations of each ligand and their relative affinity for the receptor and the kinetics of ligand/receptor association and dissociation.

7.44 Pharmacodynamic interactions could also result from two or more components of a mixture acting at different receptor/target sites resulting in effects that are either synergistic or opposite. Functional antagonism is illustrated by effects of histamine and norepinephrine on vasodilation and blood pressure and the anticonvulsive action of barbiturates in relation to compounds that cause convulsions.

7.45 Examples of functional potentiation also exist. Drugs that affect clotting in different ways, e.g. warfarin by competition with vitamin K and aspirin by inhibiting platelet thromboxane A_2 synthase, increase the risk of bleeding, in this way. Diuretics that lower potassium concentration, e.g. frusemide, can enhance the cardiotoxicity of cardiac glycosides. It should be noted that these interactions occur only at pharmacologically active doses of the drugs involved.

7.46 Dynamic interactions at the transcriptional or transductional level have, to date, been little investigated. Efficient detection of these types of interaction is likely to require studies at the molecular level, utilising for example, genomic, proteomic or metabonomic methodology.

7.47 Potentiation may also occur when two or more components act at different receptor/target sites. For example, inhibition of one or more of the various DNA repair pathways by a mixture component may result in more than expected DNA damage caused by another component that is genotoxic. Conversely, the actions of components that, for instance, act as antioxidants may be DNA protective, and result in the neutralisation of free radicals and other reactive oxygen species (ROS) that may be generated due to the presence of another component.

7.48 Several compounds induce in some tissues an increment or a reduction in glutathione levels. Since glutathione provides a first line defence against the toxicity of many toxins, modulation of cellular levels of glutathione by one component of a mixture may alter the toxicity of another component.

7.49 The production of harmful xenobiotic radicals or other reactive species during the oxidative metabolism of one component of a mixture may be accelerated as a result of redox mediation by another component and thus may result in enhanced toxicity. An example of such a reaction has been demonstrated *in vitro* where the chlorpromazine cation radical (and those of other phenothiazines) generated by lipoxygenase triggered a rapid oxygenation of benzidine to the toxic intermediate benzidine diimine.[21]

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8. Toxicology of mixtures-experimental evidence

Introduction

8.1 This chapter comprises a summary of relevant experimental data in the field of mixture toxicology. In general, the studies described here are limited to those pertaining to mixtures that contain pesticides. However, some studies involving other chemical mixtures are included, for example, where they demonstrate useful approaches to the study of mixture toxicology. For the most part, the studies included here are limited to those that have investigated the acute, sub-acute or chronic toxic effects of simultaneous combined exposures and the examination of dynamic rather than metabolic or toxicokinetic endpoints. As was discussed in the previous chapter, the design of many studies on mixtures precludes drawing conclusions on what type of combined action is present.

Experimental studies

Acute effects

8.2 There are many data in the literature concerning the modulation of acute or subacute toxic effects of chemicals, including pesticides, by prior administration of other chemicals, the latter often well-known inducers or inhibitors of hepatic drug metabolising enzymes. It would be neither practical nor useful to describe all of these studies here. However, for illustrative purposes only, a small number of studies are outlined below where both pre-treatment and treatment was with pesticides.

8.3 Williams and Casterline[1] studied the interactive effects of aldrin, chlordane, piperonyl butoxide and carbanolate in Osborne-Mendel rats. Groups of animals (n = 10) received either aldrin (50 mg/kg [2/5 LD_{50}]) or chlordane (300 mg/kg [1/2 LD_{50}], i.p.), on day 1 with or without subsequent administration of piperonyl butoxide (500 mg/kg [1/15 LD_{50}], p.o.) on days 2, 3, 6 and 7. A further group of animals received piperonyl butoxide treatment only. Vehicle control animals received corn oil. Four hours after the administration of piperonyl butoxide or corn oil on day 7, all animals received carbanolate (63.2 mg/kg, p.o.) at a dose that was twice the LD_{50}. Chlordane and aldrin reduced the toxicity of carbanolate, primarily by induction of detoxification. Repeated administration of piperonyl butoxide blocked the protective effects that these afforded against carbanolate toxicity, by inhibiting detoxification. The acute toxicity of carbanolate following treatment with piperonyl butoxide alone was no different to that seen in animals pre-treated with corn oil.

8.4 Gaughan *et al*[2] studied the interactive effects of organophosphate (OP) pesticides and carbamates on the acute toxicity of malathion and fenvalerate in mice (strain not specified). Mice were treated intraperitoneally with profenofos, sulprofos, EPN, S,S,S-tributyl phosphorotrithioate (DEF), monocrotophos, azinphos-methyl, parathion-methyl, acephate, carbaryl, methomyl or chlordimeform, at doses of 4 or 25 mg/kg, and 1 hour later with the pesticides malathion or fenvalerate. Mortality was recorded 24 hours later. LD_{50} values for fenvalerate and malathion were decreased in mice pre-treated with EPN, profenofos, and DEF. The LD_{50} for malathion was also decreased in mice pre-treated with

sulprofos. These data illustrate the modulation of the acute toxicity of pesticides by prior exposure to relatively large doses of other pesticides, most likely through effects on their metabolism.

8.5 In contrast, Takahashi *et al*[3] investigated the modulating effects of a single low dose (1/20 or 1/24 of the LD_{50} dose) of one of three P=S type OP insecticides (cyanophos, fenitrothion, and malathion) or a P=O type OP insecticide (dichlorvos) on the acute toxicities (oral LD_{50} values) of the N-methylcarbamate insecticides, fenobucarb, xylylcarb, metolcarb, carbaryl and 3,5-dimethylphenyl N-methylcarbamate in male ICR mice. Animals (10 per group) were dosed with a methylcarbamate, 0, 2, 4, 24, 48 or 72 hours after receiving the organophosphate. Animals treated with OP chemicals alone exhibited no overt signs of toxicity or effects related to anti-acetylcholinesterase (AChE) activity. Anti-AChE related effects following treatment with the N-methylcarbamates were not altered by concurrent or subsequent treatment with OP compounds. However, the acute toxicities of the N-methylcarbamate compounds were potentiated. The degree of potentiation differed according to the treatment combination. The acute toxicity of fenobucarb was markedly potentiated by each of the P=S compounds. Maximal potentiation was observed with simultaneous malathion treatment and 0.5 – 4 hour pretreatments with cyanophos and fenitrothion (cyanophos decreased the LD_{50} >10-fold; fenitrothion decreased the LD_{50} >5-fold). In contrast, fenobucarb toxicity was not significantly affected by the P=O compound, dichlorvos. Potentiation of fenobucarb acute toxicity was associated with increased levels of fenobucarb found in plasma. These effects are most likely a consequence of changes in the respective rates of activation and detoxification.

8.6 Johnston[4] has reviewed the interactive effects of pesticides on serum butyryl cholinesterase (BuChE) and brain AChE in birds. Studies have shown that red-legged partridges (*Alectoris rufa* cross) pre-treated with prochloraz were more sensitive to the effects of the OP malathion than controls. For example, a dose of 90 mg/kg p.o., prochloraz produced greater inhibition at 1, 4 and 24 hours following oral administration of 50 mg/kg malathion, compared to vehicle controls. This was due to induction of malathion bioactivation.

8.7 Ensenbach and Nagel[5] assessed the acute and chronic toxicity of 3,4-dichloroaniline and lindane, both individually and as a mixture, in zebrafish (*Danio rerio*). Zebrafish were exposed to lindane (0.04, 0.06, 0.08, 0.10, 0.12 or 0.14 mg/l) or 3,4-dichloroaniline (4, 6, 8, 10, 11 or 12 mg/l) or mixtures of lindane/3,4-dicholoroaniline (0.02/1, 0.04/2, 0.06/4, 0.08/6, 0.1/8 mg/l), in tap or in river water. LC_{50} values for 96 hour exposure in tap and river water, respectively, were 8.5 and 9.8 mg/l for 3,4-dichloroaniline and 0.10 and 0.11 mg/l for lindane. The 96 hour LC_{50} values for the compounds as a binary mixture in tap water were approximately half those observed for individual chemicals (4.5 mg/l for 3,4-dichloroaniline, 0.057 mg/l for lindane). The authors thus concluded that the combined toxicity of this mixture could be characterised as additive. This was presumably a consequence primarily of the induction of bioactivation by lindane.

8.8 The usefulness of acute study data is limited with regards to the prediction of the effects at low exposures. The metabolic pathways and receptors involved are likely to be saturated. Furthermore, the critical target for acute toxic effects may not be the same as those at lower levels of exposure.

However, the mechanisms by which toxicities of compounds are modulated may have relevance to the interactions that may occur during exposure to mixtures.

Effects on the respiratory system

8.9 Very few reports were identified regarding toxic effects on the respiratory system of mixtures containing pesticides.

8.10 Model studies carried out by Cassee and colleagues[6,7] have evaluated the respiratory effects of binary and ternary mixtures of the vapours of three aldehydes (formaldehyde, acetaldehyde and acrolein) in rats). These studies, which are further described below, established that the three compounds probably cause sensory irritation by a mechanism of competitive agonism for a common receptor, rather than showing complete dose-additive effects. Evaluation of the toxic effects of mixtures of these compounds on the nasal epithelium showed possible potentiating effects at high doses (i.e. doses at which the individual compounds exerted observable toxicity). Conversely, combinations at lower doses (around the no observable adverse effect concentrations [NOAECs]) suggested a simple dissimilar action of the mixture, with toxic effects approximately equal to those observed with the single most toxic component. These experiments thus demonstrate that models of component interaction within a mixture may not necessarily extrapolate between different doses, and that interactive effects seen at relatively high doses may not be applicable when doses around the NOAEC are present.

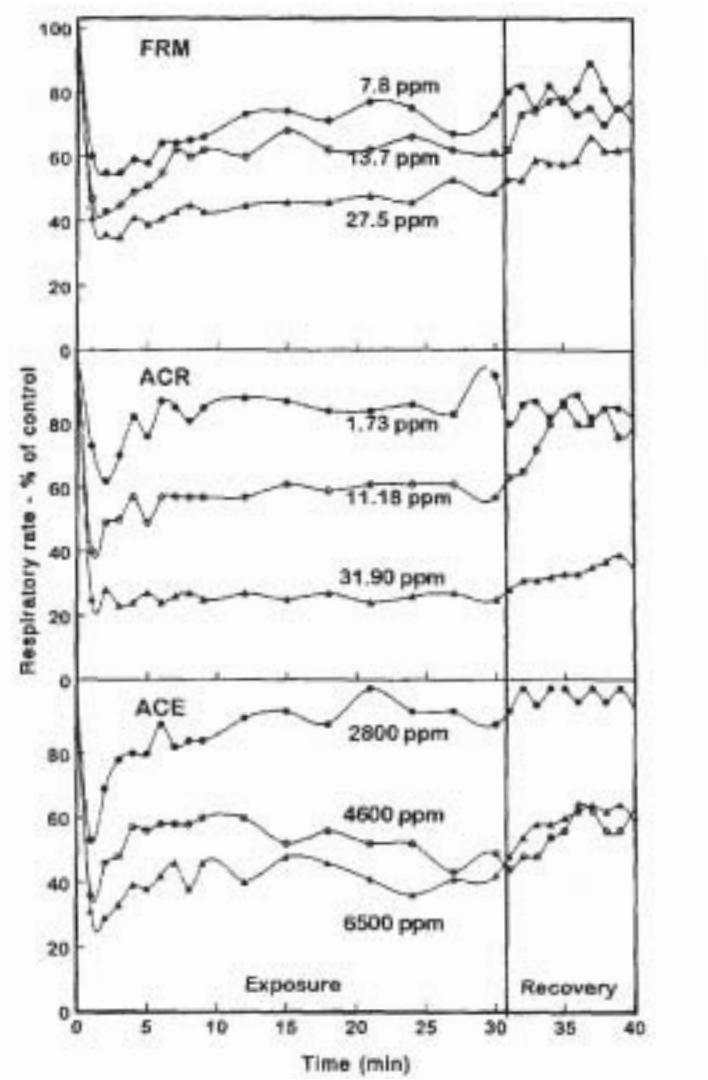
Sensory irritation

8.11 Studies in animals have demonstrated that exposure to irritant levels of various individual aldehydes results in a concentration-dependent respiratory rate depression via direct stimulation of the trigeminal nerve endings in the nasal mucosa. This effect is commonly used as a measure of the extent of sensory irritation associated with specific levels of a particular vapour. It is assumed that many sensory irritants act on the same target receptor of the trigeminal nerve (similar joint action), and thus a mixture of irritants may, potentially, result in additive dose effects. Alternatively, competition for the receptor may be expected to result in overall effects which are less than the sum total expected by dose addition.

8.12 Cassee *et al*[6] studied the irritant effects, in male Wistar rats, of formaldehyde, acrolein and acetaldehyde vapours, individually, or as mixtures. In initial, single-compound studies, carried out to establish the concentration necessary to decrease respiratory rate by 50% (RD_{50} values) and concentration-effects curves for individual compounds, groups of 4 animals were exposed to test vapour concentrations of 7.8, 13.7 or 27.5 ppm of formaldehyde; 1.73, 11.2, or 31.90 ppm acrolein; 2800, 4600, or 6500 ppm acetaldehyde. For mixed vapour exposures, groups of 4 animals were exposed to test vapours at concentrations which were calculated to result in a decreased respiratory rate between 10 and 35% for each of the compounds. Various combinations of formaldehyde, acrolein and/or acetaldehyde were tested, within the following concentration ranges; 0.86-2.58 ppm formaldehyde; 0-1.29 ppm acrolein; 1310-2637 ppm acetaldehyde. Respiratory rates of each animal were assessed over a 50-min test period (10 min acclimatisation; 30 min exposure; 10 min recovery).

Measured decreased respiratory rates were then compared with those predicted for each mixture by model of either additive or competitive agonism for the trigeminal nerve receptor. The results of individual exposures to each compound are represented in Fig 8.1.

Fig 8.1 Effect of aldehydes on respiratory rate.I.

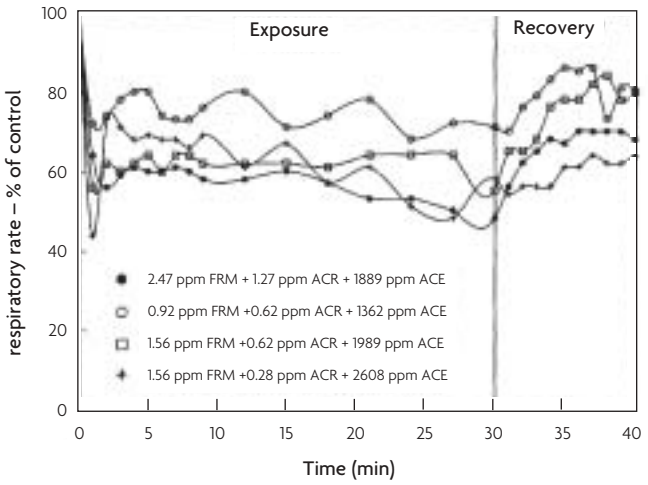


Time effect relationships showing the decrease in respiratory rate on and after exposure to three different aldehydes. Each represents the average of four rates. FRM formaldehyde; ACR acrolein; ACE acetaldehyde (from Cassee *et al*, with kind permission of Springer-Verlag).[6]

8.13 With formaldehyde exposure an initial, rapid decreased respiratory rate was followed by desensitisation (recovery during exposure) during the next few minutes, and partial recovery occurred during the 10-min post exposure period. As a result of acrolein exposure, rats responded with an initial fast decreased respiratory rate, followed by marked desensitisation at lower levels, although desensitisation did not occur in animals exposed to the highest concentration (31.9 ppm). Partial or full recovery occurred during the 10-min post-exposure period. Exposure to acetaldehyde resulted in an initial rapid decreased respiratory rate during the first few minutes of exposure, followed by desensitisation during the next few minutes. After around 15 min, a further, more gradual, decreased respiratory rate was observed, especially at the higher exposure levels. Partial or full recovery occurred during the 10-min post-exposure period. The authors suggested that the second, more gradual decreased respiratory rate, which occurred with acetaldehyde, but not formaldehyde or acrolein, might be caused by the development of pulmonary irritation, or as a result of other factors such as tissue damage or systemic effects.

8.14 Time-effect responses to mixtures of formaldehyde, acrolein and acetaldehyde vapours were very similar in all cases (Fig 8.2).

Fig 8.2 Effect of aldehyde mixtures on respiratory rates.II.



Examples of time-effect relationships of mixtures of aldehydes in rats exposed for 30 min to formaldehyde (FRM), acrolein (ACR) and acetaldehyde (ACE) and a subsequent 10 min period to clean air. Each line represents data from four rats (from Cassee *et al*, with kind permission of Springer-Verlag).[6]

8.15 A maximum decreased respiratory rate was observed within 3 min after the start of the exposure to the test mixture, with subsequent recovery to a plateau for the decreased respiratory rate after approximately 10 min. Values then remained nearly stable, or showed a further decrease during the remainder of the 10 min post-exposure period. Both partial and full recovery was observed. In all cases, mean observed decreased respiratory rates with exposure to mixtures were greater than those predicted for each compound separately at the concentration tested, but lower than the calculated value assuming summation of effects. However, the mean observed decreased respiratory rates correlated well with those predicted by a competition model for stimulation of the receptor, except when high acetaldehyde concentrations were present, in which case observed effects were stronger than those predicted by the competition model (see Table 8.1).

Table 8.1 Sensory irritation of mixtures of formaldehyde (FRM), acrolein (ACR), and acetaldehyde (ACE) with the lowest observed breathing frequencies taken within 0.3 min of the exposure period (from Cassee *et al*)[6].

Group	Exposure concentrations (ppm)			Expected effect upon single compound exposures[a]			Expected DBF[b] using effect addition	Expected DBF[b] using competitive agonism[c]	Observed DBF[b]
	FRM	ACR	ACE	D_{FRM}	D_{ACR}	D_{ACE}	$D_{FRM}+D_{ACR}+D_{ACE}$	$D_{FRM.ACR.ACE}$	D_{OBS}
1	0.86	0.63	1326	9.9	7.3	17.4	34.5	29.0	25.0
2	0.93	0.59	2592	10.5	6.8	32.2	49.6	40.9	24.4
3	0.95	0.28	1929	10.7	3.4	24.6	38.7	33.2	36.1
4	0.95	1.29	1986	10.7	13.7	25.3	49.7	39.2	26.3
5	1.36	0.62	2001	14.5	7.2	25.5	47.1	37.9	40.1
6	1.52	1.24	1341	15.9	13.2	17.6	46.6	36.2	43.2
7	1.56	0	2013	16.2	0.0	25.6	41.8	35.7	42.0
8	1.58	0.62	1956	16.3	7.2	24.9	48.4	38.5	52.0
9	1.58	0.62	1956	16.3	7.2	24.9	48.4	38.5	55.0
10	1.59	0.27	1981	16.4	3.3	25.2	44.9	37.0	38.8
11	1.59	0.59	1463	16.4	6.8	19.0	42.3	34.2	29.6
12	1.59	0.68	2044	16.4	7.8	26.0	50.2	39.5	49.7
13	1.59	1.24	2637	16.4	13.2	32.7	62.4	46.6	29.9
14	1.66	0.27	2575	17.0	3.3	32.0	52.3	42.3	37.4
15	2.38	0.59	2606	22.2	6.8	32.4	61.4	46.5	37.3

Group	Exposure concentrations (ppm)			Expected effect upon single compound exposures[a]			Expected DBF[b] using effect addition	Expected DBF[b] using competitive agonism[c]	Observed DBF[b]
	FRM	ACR	ACE	D_{FRM}	D_{ACR}	D_{ACE}	$D_{FRM}+D_{ACR}+D_{ACE}$	$D_{FRM.ACR.ACE}$	D_{OBS}
16	2.45	0.27	1388	22.7	3.3	18.1	44.1	36.1	35.7
17	2.45	1.29	1986	22.7	13.7	25.3	61.7	44.9	40.2
18	2.46	0.28	1310	22.7	3.4	17.2	43.3	35.5	39.5
19	2.50	0.63	1342	23.0	7.3	17.6	47.8	37.6	29.8
20	2.58	0.28	2060	23.5	3.4	26.2	53.0	41.9	46.0
				Overall mean:			48.4	38.6	37.9*
				Standard deviation:			7.3	4.4	8.7

Notes

a Expressed as percentage decrease of the breathing frequency (DFT) from control values

b DBF is decreased breathing frequency (respiratory rate)

c Calculated according to equation 4 (Materials and methods) using D_{max} and K values indicated in Table 1

* Significantly different from the expected DBF using effect addition (t-test).

8.16 Previous studies have also suggested that sensory irritation, as a result of exposure to aldehyde vapour mixtures, occurs via competitive agonism for a common receptor. Babiuk *et al*[8] showed that formaldehyde pretreatment resulted in cross-tolerance for acetaldehyde and acrolein, whilst Kane and Alarie[9] reported competition between formaldehyde and acrolein in studies in which mice were exposed to mixtures of the two compounds. Cassee *et al*[6] suggested that their results, which were in agreement with those of Kane and Alarie,[9] supported the conclusion that the commonly used additivity rule may not be applied for the prediction of decreased respiratory rates of mixtures of sensory irritants (although at concentrations much lower than the RD_{50}, a competition model would result in similar results to those predicted by dose-addition of equal doses of each compound). Overall, these authors concluded that "the degree of sensory irritation of a mixture of irritant aldehydes is stronger than that of the individual aldehydes but less than that of the sum of the individual irritant potencies, which is basically a result of competition for a common receptor." Co-stimulation by these compounds may lead to common desensitisation of the receptor.

Respiratory tract changes

8.17 Cassee and colleagues also carried out studies to evaluate the potential additive or interactive effects of inhalation exposure to mixtures of aldehydes on biochemical, histopathological and proliferative changes of the nasal epithelium in animal models.[7] Groups of male Wistar rats were exposed to either single aldehydes, or combinations of formaldehyde (1.0, 3.2 or 6.4 ppm), acetaldehyde (750 or 1500 ppm) and/or acrolein (0.25, 0.67 or 1.40 ppm) vapours, for 1 or 3 days (6 h/day).

8.18 Single-aldehyde exposures, for 3 days, resulted in regional differences in histopathological changes associated with the different compounds. Formaldehyde (≥ 3.2 ppm) and acrolein (all doses) produced adverse effects on the respiratory epithelium of the nose, whilst acetaldehyde (all doses) affected the olfactory part in some animals, inducing changes described as minor and of doubtful toxicological significance. These observations were consistent with the findings of other studies.

8.19 Three-day exposure to a mixture containing the highest dose of each compound resulted in changes in both the respiratory and olfactory parts of the nose, which were more severe than those seen after exposure to the individual compounds at the same concentrations. The authors concluded that these findings were indicative of (at least partially) additive effects for formaldehyde and acrolein, and also that acrolein and/or formaldehyde might potentiate the adverse effects of acetaldehyde on the olfactory epithelium. Conversely, low-dose mixtures (1.0 ppm formaldehyde + 0.25 ppm acrolein; 1.0 ppm formaldehyde + 750 ppm acetaldehyde + 0.25 ppm acrolein) (levels considered by the authors to be around the minimum observed effect concentration [MOEC]) showed changes which were very similar in site, type, degree and incidence to those produced by 0.25 ppm acrolein alone, indicating no dose-additive actions or potentiating interactions. Thus, although the high dose mixture showed more severe effects than those of the individual compounds, neither effect addition nor potentiating interactions occurred when concentrations were NOAECs or when one component was present at the lowest obserable adverse effect concentration (LOAEC). At exposure levels of chemicals in the mixture in the range of NOAECs, the results indicated the applicability of the basic concept of simple dissimilar action, whereby the most hazardous chemical in the mixture (in this case, acrolein) provides the NOAEC for the mixture.

8.20 The authors of this study concluded that their findings "suggest that combined exposure to these aldehydes with the same target organ (nose), and exerting the same type of adverse effect (nasal irritation/cytotoxicity), but with partly different target sites (different regions of nasal mucosa), is not associated with increased hazard compared to exposure to the individual chemicals, provided the exposure levels are around or lower than NOAECs." These data clearly demonstrate that the combined actions of mixture components observed at toxic effect levels are not necessarily helpful in predicting what will happen at no toxic effect levels, including levels only slightly lower than the LOAEC.

Immunological effects

8.21 Schlesinger and colleagues[10] carried out a study to assess the responses resulting from acute inhalation exposures to graded concentrations of sulfuric acid in combination with graded concentrations of ozone. Groups of male New Zealand white rabbits were exposed for periods of 3 hours to target concentrations of sulfuric acid of 50, 75 or 125 $\mu g/m^3$, ozone of 0.1, 0.3 or 0.6 ppm or mixtures of each concentration of sulfuric acid with each concentration of ozone.[a] Control animals were exposed to an atmosphere of nebulised distilled water. Following exposure, animals were killed, and lungs were

[a] The authors note that ambient air ozone levels >0.3 ppm are frequently encountered in the USA, whilst peak levels of sulfuric acid have been estimated at 75 $\mu g/m^3$, with longer averages about one third of this value, and that concentrations of the two chemicals often peak simultaneously.

lavaged. Recovered cells were assayed for immune cell types, cell viability, phagocytic activity of pulmonary macrophages, production of superoxide anion radical by stimulated and unstimulated macrophages, and measurement of the cytotoxicity of tumour necrosis factor (TNF) elicited from stimulated macrophages. Acellular lavage fluid was analysed for lactate dehydrogenase and two selected prostanoids, known to mediate biological activities in the respiratory tract. Interaction was considered to occur when the response to the mixture did not equal the sum of the responses to the individual pollutants.

8.22 Exposures had no significant effects on lavage fluid lactate dehydrogenase or prostaglandin levels, nor on the numbers, viability or types of immune cells recovered by lavage.

8.23 Phagocytic activity of macrophages was significantly depressed following exposures to 75 and 125 $\mu g/m^3$ sulfuric acid and following exposures at all concentrations of ozone. Exposure to mixtures containing these concentrations also resulted in depressed activity as compared with controls. The authors noted that significant antagonistic interactions were associated with exposure to all acid/ozone atmosphere combinations, and that the magnitude of these interactions was observed to be independent of the concentration of either pollutant in the mixture.

8.24 Superoxide production by stimulated macrophages was significantly depressed following exposure to 75 and 125 $\mu g/m^3$ sulfuric acid, whilst no effects were observed with ozone. Exposure to mixtures also resulted in no significant change in superoxide production as compared to controls. Interaction analysis showed significant antagonistic interactions of mixtures of 75 or 125 $\mu g/m^3$ sulfuric acid with 0.1 or 0.3 ppm ozone. The authors reported that at each of these concentrations of ozone, the magnitude of the interaction appeared to increase as the concentration of sulfuric acid in the mixture increased.

8.25 Analysis of TNF-induced cytotoxicity showed that exposure to sulfuric acid at 75 and 125 $\mu g/m^3$ resulted in depression of activity, whilst exposure to ozone had no significant effect. Exposures to all mixtures containing 75 $\mu g/m^3$ sulfuric acid resulted in depressed TNF-induced cytotoxicity compared with that of controls. Conversely, exposure to mixtures of 125 $\mu g/m^3$ sulfuric acid with 0.3 or 0.6 ppm ozone resulted in enhanced TNF-induced cytotoxicity. The authors noted that interaction analysis showed significant synergistic interaction following exposure to 125 $\mu g/m^3$ sulfuric acid in combination with either 0.3 or 0.6 ppm ozone.

8.26 The authors of the study concluded that, depending upon the endpoint assessed, both antagonism and synergy were observed, and that furthermore, the magnitude of interaction was not necessarily dependent on the exposure concentrations of the pollutant within the mixture. It should be noted that the authors' interpretation of interaction as "considered to occur when the effects of combined exposure were either significantly greater or less than additive" is not necessarily in agreement with the more rigorously applied definition of this concept, as described in Chapter 7. The authors also noted that the mechanisms underlying the interactive responses observed were not established, making it difficult to explain these interactive responses.

Effects on dermal absorption and toxicity

8.27 A number of studies have used mechanistically defined chemical mixtures to investigate the effects of complex mixtures on absorption and/or toxicity of chemicals via the dermal route. Although mixtures of pesticides were not studied, these data may be useful in that they reflect potential interactions that could occur. Many of the interactions arose from solvent-mediated perturbation of the permeability barrier of the stratum corneum.

8.28 Using acetone or dimethylsulfoxide (DMSO) (80% in water) as a vehicle, percutaneous absorption and cutaneous disposition of radiolabelled parathion were studied using an isolated perfused porcine skin model.[11] The single and combined effects of a surfactant, sodium lauryl sulfate, a rubifacient, methyl nicotinate and a reducing agent, stannous chloride ($SnCl_2$) on percutaneous absorption and cutaneous disposition of parathion were also investigated. A full 2 x 4 factorial design was used to assess treatment-related effects and potential interactions. Greater amounts of radioactivity were absorbed when DMSO was used as vehicle as compared to acetone. Furthermore, an earlier flux time was observed with DMSO and peak flux was lower with acetone. The absorption flux rate profiles with DMSO showed a steady increase whereas double peak profiles were observed with acetone. Sodium lauryl sulfate enhanced parathion absorption with both vehicles. The presence of methyl nicotinate with either vehicle blunted absorption rate curves but did not significantly alter total absorption. $SnCl_2$ blocked parathion absorption and increased the residue level found on the skin surface. Several other more complex interactions were also noted when components were applied together.

8.29 Baynes and colleagues[12] used a similar system to investigate the absorption of a marker compound (benzidine) included as a component of 10 binary, ternary, quaternary or quintuple mixtures, consisting of the marker compound itself, a solvent vehicle (acetone, or DMSO; 80% in water), a surfactant (0 or 10% sodium lauryl sulfate), a vasodilator (0 or 180 μg methyl nicotinate) and a reducing agent (0 or 2% stannous chloride, $SnCl_2$). Findings were similar to those of Qiao *et al* (1996) in that absorption was dependent upon the vehicle used. Compared with acetone, DMSO enhanced the dermal penetration of benzidine in most mixtures. Sodium lauryl sulfate + methyl nicotinate was also found to have an enhancing effect. $SnCl_2$ alone resulted in the inhibition of benzidine absorption, irrespective of the solvent used. It was suggested that chemical-chemical interactions between benzidine and $SnCl_2$ inhibit benzidine absorption and chemical-biological interactions between methyl nicotinate + sodium lauryl sulfate and skin enhance benzidine absorption.

8.30 Using the same model, Baynes *et al*[13] went on to test the absorption and dermal toxicity of a total of 16 binary, ternary, quaternary or quintuple mixtures, consisting of a marker chemical direct red 28 (DR28 – present in each mixture), a solvent (80% acetone or DMSO in water – one of which was present in each mixture), a surfactant (0 or 10 % sodium lauryl sulfate), a vasodilator (0 or 180 μg methyl nicotinate) and a reducing agent (0 or 2% $SnCl_2$). None of the mixtures caused severe dermal toxicity. However, minor alterations (intra- and inter-cellular epidermal oedema) were observed on light microscopy, particularly when DMSO was included instead of acetone. The presence of sodium lauryl sulfate caused alteration in the stratum corneum. Increased histochemical staining for acid

phosphatase in the stratum basale was associated with sodium lauryl sulfate or sodium lauryl sulfate + methyl nicotinate in DMSO. DMSO mixtures containing sodium lauryl sulfate + methyl nicotinate, sodium lauryl sulfate + $SnCl_2$ or sodium lauryl sulfate + methyl nicotinate + $SnCl_2$ showed significant transepidermal water loss. The authors suggested that the various complex interactions that occur in these mixtures, especially those containing sodium lauryl sulfate, differentially alter the epidermal barrier.

8.31 The studies described above provide little or no information on deviation from additivity of the effects of the mixtures tested and the data probably represent toxicokinetic interactions simply affecting the extent of chemical absorption.

Neurotoxicity

8.32 Abou-Donia *et al*[14] studied the neurotoxicity produced in Leghorn hens (n = 5 per group) by sub-chronic (90 days followed by a 30 day observation period) dermal application of n-hexane (2.5 mmol/kg), methyl n-butyl ketone (1.0 mmol/kg), 2,5-hexanediol (1.0 mmol/kg) or 2,5-hexanedione (1.0 mmol/kg), either alone or in combination with the OP, EPN (1 mg/kg) either at the same site (EPN dissolved in one of the above compounds) or at a different site (EPN dissolved in 0.1 ml acetone). Hens treated with EPN developed severe ataxia. Concurrent dermal application of EPN with n-hexane or 2,5-hexanediol, regardless of site of application, resulted in an effect described by the authors as additive. The effect of simultaneous dermal application of EPN and methyl n-butyl ketone at different sites were also described as additive. However, when applied at the same site, the neurotoxic effect was said to be potentiated. No histological changes were seen at the end of the observation period with any single treatment. However, in some hens, binary treatments of EPN and one of the other compounds resulted in histopathological changes that were characteristics of EPN neurotoxicity. The study design precluded any conclusion on the nature of the combined effects observed.

8.33 Oskarsson and Lind[15] investigated the effects of the dithiocarbamates, thiram and disulfiram[a] (0.1 mmol/kg), and diethyldithiocarbamate (DEDTC, 0.2 mmol/kg) and sodium dimethyldithiocarbamate (DMDTC, 0.2 mmol/kg), administered by oral gavage twice weekly, on levels of lead found in the blood and brain tissue of male Sprague Dawley rats (n = 5 per group) exposed to lead acetate (12 mM) in the drinking water for 6 weeks. Estimated intake of lead was approximately 60 mg/day. One control group of animals received 0.3 ml 0.5% gelatine in place of dithiocarbamate treatment. Further control groups received similar dithiocarbamate treatments but were given 12 mM sodium acetate in the drinking water. In a second set of experiments, treatment with dithiocarbamate, either by gavage (DEDTC or thiram, 0.1 mmol/kg/day, 5 day/week x 2 weeks, in 0.3 ml 0.5% gelatine) or by i.p. injection (DEDTC, 0.5 mmol/kg/day x 4 days, in 0.3 ml 0.5% gelatine) was performed after the cessation of 6 weeks lead treatment in the drinking water.

8.34 In rats that received lead plus thiram, disulfiram, DEDTC or DMDTC concurrently, lead levels in blood and brain were significantly increased (maximally 3-fold and 4-fold, respectively) compared to rats that received lead treatment alone. In this context, disulfiram was the most effective agent. The levels of

[a] Disulfiram is the tetraethyl homologue of thiram.

lead achieved in the brains of these rats were similar to those thought to cause serious CNS damage in humans. In rats that were treated with DEDTC or thiram by gavage after cessation of lead treatment, there was no increase in blood lead concentrations but levels of lead in the brain tissue of animals treated with thiram were increased by nearly 3-fold. Concentrations of lead were increased in both blood and brain in rats treated with DEDTC i.p. after cessation of lead treatment. The authors did not mention any overt signs of toxicity in any of the study groups and there was no histopathological examination of brain tissue.

8.35 This study suggested that combined treatments of lead and dithiocarbamate/thiram derivatives could result in an increased uptake of lead into the brain that was not necessarily reflected by any increase in lead concentration in blood. It is worth noting that similar effects have been found regarding the uptake of other metals (mercury, nickel, cadmium, copper, zinc) into the brains of rodents treated with similar dithiocarbamate compounds.[16-25] However, the levels of lead employed here were relatively high and the study cannot predict the outcome at lower exposures to either dithiocarbamate agents and/or lead. Consequently the toxicological significance of this interaction is not clear at levels of exposure likely to be encountered by humans. In addition, data on metals may not be relevant to the toxicokinetics of organic pesticides.

8.36 Behavioural changes and corresponding neurochemical alterations associated with exposure to pesticides, either individually or in combination, were investigated in food-restricted weanling male Sprague Dawley rats.[26] Aldicarb, metribuzin and methomyl were administered via the drinking water at the following concentrations: aldicarb alone (10 ppb); metribuzin alone (10,000 ppb); a high dose binary mixture of aldicarb + metribuzin (10 ppb + 10,000 ppb); a low dose binary mixture of aldicarb + metribuzin (1 ppb + 1000 ppb); a ternary mixture of aldicarb + methomyl + metribuzin (1 ppb + 1000 ppb + 100 ppb) for 90 days. Control animals received distilled water only. The number of animals per group was 4-6. During the exposure period, animals were trained to run a T-maze and tested for spatial discrimination reversal. At the end of the experiment, animals were killed. The cortex, hippocampus and neostriatum regions of the brain were assayed for the neurotransmitters, dopamine, acetylcholine and serotonin. Relative to the control group, animals treated with metribuzin took significantly longer to learn the first reversal. The same animals were found to have significantly lower acetylcholine/choline ratios in the hippocampus. However, the biological significance of this was uncertain. Subsequent reversals were learned at rates similar to the control group. None of the other treatments had any apparent effect on learning ability. Animals treated with the mixture of the three chemicals were found to have consistently slower speeds in maze running than either the controls or any other treatment group. These animals also had altered choline levels in their neostriata. No comment was made regarding the possible interaction between metribuzin and either of the other pesticides. The design of the experiment probably would preclude this in any case. It was noted that exposure to compounds individually and collectively resulted in reduction in spleen plaque-forming cells, lymphocytes and white blood cells, parameters of immune function. No significant differences between the groups were indicated.

8.37 Abou-Donia *et al*[27] investigated the neurotoxic effects in hens (*Gallus gallus* domesticus, 18 months old, n = 5 per group) resulting from individual or simultaneous exposure to the nerve agent prophylaxis, pyridostigmine bromide 5 mg/kg/day p.o. in water, the insect repellent DEET, 500 mg/kg/day s.c. neat and the insecticide permethrin, 500 mg/kg/day s.c. in corn oil, 5 days/week for 2 months. A preliminary dose-ranging study had established that the dose levels selected resulted in what was termed minimal toxicity. Animals treated with pyridostigmine bromide developed transient mild signs of cholinergic toxicity, characterised by decreased activity and slight diarrhoea. Animals treated with DEET developed a rapid shallow breathing and showed a tendency towards temporary inactivity after dosing. Birds in this group also exhibited a significant reduction in terminal body weight. Birds treated with permethrin developed no clinical signs of toxicity. Some DEET and some permethrin-treated birds showed minor neuropathological changes that comprised a small increase in the frequency of enlarged axons. At termination, pyridostigmine bromide-treated birds exhibited a marked inhibition of plasma butyrylcholinesterase (BuChE) activity, activity being 17% of control values. Birds exposed to DEET exhibited a lesser inhibition of plasma BuChE activity (activity was 83% of controls) and permethrin did not affect BuChE activity. Brain AChE was not affected by treatment with any individual compound. Neurotoxic effects (clinical signs, locomotor dysfunction, tremor) were enhanced in birds treated with the binary combinations (pyridostigmine bromide/permethrin, pyridostigmine bromide/DEET or permethrin/DEET), compared to those observed with each of the compounds alone and deaths (1/5, 2/5 and 2/5, respectively) occurred in each group. Significant reductions in terminal body weights were also observed in the pyridostigmine bromide/DEET and permethrin/DEET groups. Histopathological changes were not seen in the nervous systems from the birds treated with the pyridostigmine bromide/permethrin binary mixture. Mild neuropathological changes (slightly increased frequency of enlarged axons) were observed in 2/5 animals treated with permethrin/DEET and mild to moderate changes (significantly increased frequency and degree of axon enlargement) were observed in all animals treated with pyridostigmine bromide/DEET. Pyridostigmine bromide/DEET decreased plasma BuChE activity to 8% of control values, pyridostigmine bromide/permethrin to 20% of activity in the controls and DEET/permethrin to 83%. Neurotoxicity was further enhanced in animals treated with all three compounds concurrently. Brain acetylcholinesterase activity was not depressed by any of the binary treatment regimes. Mortality was 4/5 in the birds treated with the ternary mixture. Neuropathological changes varied from mild to severe in this group. Plasma BuChE was inhibited to 26% of control activity with the ternary mixture, but, again, brain AChE remained unaffected. Mean rank value (combined rank for clinical signs, locomotor dysfunction, tremor, neuropathology) was used to quantify and compare the neurotoxic effects of the various treatments. The mean rank value for the control group was significantly less than the values for all treated groups except for the group treated with permethrin alone. Hens treated with two compounds, pyridostigmine bromide/DEET, pyridostigmine bromide/permethrin and DEET/permethrin, had mean rank values significantly higher than for the single treatments, except for pyridostigmine bromide/permethrin which was not significantly different from DEET alone. Treatments with all three compounds together had a mean rank value that was higher than any single or two compound treatments.

8.38 The authors suggested these findings indicated increased neurotoxicity when individual chemicals were combined with pyridostigmine bromide. They further suggested that this may be attributed to pyridostigmine bromide-induced carbamylation of esterases leading to decreased hydrolysis of DEET and permethrin, and consequential increase in their effective concentrations (see chapter 7). However, the design of the study used precludes assessment of any interaction that may have occurred between the constituents of the binary and ternary combinations, the doses employed being the same as those used when the components were given by themselves. Furthermore, the dose level of permethrin employed in this study was far in excess of that which would likely to be produced by residues in food. In addition, the neurotoxicity produced by permethrin when given by parenteral administration, is not usually observed when exposure to the chemical is by the oral route.

8.39 Thiruchelvam *et al*[28,29] carried out studies to assess the potential involvement of combined exposure to the herbicide paraquat and the ethylene*bis*dithiocarbamate fungicide, maneb in the aetiology of idiopathic Parkinson's disease. The authors reported that previous studies had shown equivocal results in associating paraquat exposure with Parkinson's disease, whilst dithiocarbamate fungicides have been shown to potentiate the neurotoxicity of paraquat-like compounds *in vivo*.

8.40 Thiruchelvam *et al*[28] evaluated the effects of paraquat dichloride (5 or 10 mg/kg bw) and/or maneb (15 or 30 mg/kg bw) given once weekly for a total of 4 weeks, by i.p. injection, to male C57BL/6 mice. Assessed endpoints were effects on locomotor activity, density of tyrosine hydroxylase (TH) positive neurons, levels of dopamine and metabolites and dopamine turnover. The authors noted that decreases in motor activity immediately following injections were observed more consistently with combined exposures of maneb/paraquat. Levels of dopamine and metabolites and dopamine turnover were slightly increased immediately post-injection by combined exposures compared to maneb alone. In addition, significant reductions in TH immunoreactivity, measured 3 days after the last injection, were detected in the dorsal striatum of animals given combined treatments, but not those treated with single compounds. The authors concluded that these results demonstrated potentiating effects on nigrostriatal dopamine systems of combined exposures to paraquat and maneb.

8.41 Thiruchelvam *et al*[29] described similar experiments in which male C57BL/6 mice were treated with single compounds (10 mg/kg bw paraquat, 30 mg/kg maneb) or a combination (10 mg/kg bw paraquat + 30 mg/kg bw maneb), twice weekly by i.p. injection for 6 weeks. The authors reported that maneb, but not paraquat, reduced motor activity immediately after treatment, and this effect was potentiated by combined paraquat/maneb treatment. As treatments progressed, only the combined paraquat/maneb group showed a failure of motor activity levels to recover within 24 hours. Paraquat/maneb in combination, but neither singly, reduced TH and dopamine transporter immunoreactivity in the dorsal striatum, but not the nucleus accumbens. Reactive gliosis occurred only in response to combined paraquat/maneb in dorsal-medial but not ventral striatum. TH immunoreactivity and cell counts were significantly reduced only by the mixture of paraquat and maneb and not by the pesticides alone in the substantia nigra, while no treatment produced significant effects on TH immunoreactivity and cell counts in the ventral tegmental area. The authors suggested that the combination of paraquat/maneb showed synergistic effects, preferentially expressed in the

nigrostriatal dopamine system, suggesting that such mixtures could play a role in the aetiology of Parkinson's disease. The study was not designed appropriately to investigate potentiation and the results could have reflected dose-additivity.

8.42 Rebert *et al*[30] examined the effect, in Long Evans rats, of combinations of organic solvents, known to be individually ototoxic. Animals were exposed by inhalation (8 hours/day x 5 consecutive days) to pairs of solvents; trichloroethylene + toluene, mixed xylenes + trichloroethylene, mixed xylenes + chlorobenzene and chlorobenzene + toluene, using complementary proportions of isoeffective concentrations of the solvents alone (dose-response curves for each individual compound were determined shortly prior to the interaction studies; interaction studies employed one control group and 5 exposure groups for each combination; n = 8 – 9 per dose group). Effects on hearing were assessed by brainstem-evoked response audiometry. Data analysis employed an isobolic approach. Observed effects were predicted by a linear dose-addition model, indicating additive rather than synergistic or antagonistic responses. Similar results were observed with combinations of styrene and trichloroethylene.[31] The outcome implies these solvents act through a common or similar mechanism (possibly causing damage to the outer hairs of the cochlea through the disruption of ATPase in the cellular membranes that are differentially distributed in the inner and outer hair cells).

8.43 Nylén and colleagues[32-34] have reported the synergistic loss of auditory sensitivity in rats simultaneously exposed to toluene and n-hexane or xylene and n-hexane by inhalation. In contrast, antagonisms were reported for effects concerning nerve conduction velocity and/or action potential amplitudes in the auditory pathway, the visual pathways and peripheral nerve. Also, severe testicular atrophy induced by n-hexane alone was alleviated by co-treatment with toluene or xylene. However, in these studies, exposures used only a single concentration (1000 ppm) for each constituent, individually or combined. Earlier, Pryor and Rebert (1992)[35] had shown that while the ototoxic compound toluene (1200 ppm) greatly reduced peripheral neuropathy caused by the non-ototoxic hexane (4000 ppm), hexane-induced abnormalities in central components of the brainstem response were much less reduced in the presence of toluene. Furthermore, there was no reciprocal effect of hexane on the motor syndrome and hearing loss caused by toluene.

8.44 In a study reported in abstract carried out in murine NB2a neuroblastoma cells, it was reported that a greater than additive effect was produced by two OPs (phosmet and pirimiphos-methyl) on NB2a neuroblastoma cells as measured by reduction in neurite outgrowth.[36]

8.45 In another study carried out in murine NB2a neuroblastoma cells, cells were exposed to pyrethrins, piperonyl butoxide, diazinon, mineral spirit and odourless mineral spirit, a commercial formulation comprising these five components and, additionally, chlorpyrifos. The cells were also exposed to mixtures of pairs of the compounds at various ratios. The length of the neurites produced was measured, toxicity being assessed by the reduction in length of neurites compared to controls. Synergy was reported between chlorpyrifos and pyrethrins and between chlorpyrifos and one of the solvents. The effects of other combinations were did not differ from those expected on the assumption of concentration additivity.[37]

Nephrotoxicity

8.46 Jonker *et al*[38] compared the acute (24 hour) nephrotoxicity of mercuric chloride, potassium dichromate, *d*-limonene and hexachloro-1,3-butadiene (HCB) administered simultaneously to male Wistar rats (12 weeks old; n = 5 per group) with the effects of each chemical administered alone. Although these chemicals have the same target organ, they are thought to have different modes of action. Dose levels for each of the chemicals, administered alone or in combination, corresponded to the lowest observed nephrotoxic effect level (LONEL) and to the no observed nephrotoxic effect level (NONEL) established for each individual chemical in a preliminary range-finding study. Mercuric chloride and potassium dichromate were administered as neutralised aqueous solutions (1 ml/kg) by s.c. injection and *d*-limonene and HCB were administered by oral gavage (o.g.) in corn oil (10 ml/kg). The vehicle control group was dosed with both water (s.c.) and corn oil (o.g.). When the combination was administered at the LONEL, some adverse effects were reported to be less severe than would be predicted by addition (e.g. a less severe increase in urinary γ-glutamyl transferase activity). However, synergy was reported for other effects (e.g. increased severity of renal necrosis; a more marked increase in urinary lysozyme, lactate dehydrogenase, alkaline phosphatase and N-acetyl-β-glucosaminidase and in blood plasma creatine and urea), although no supporting data were provided to allow any independent substantiation of this claim. Approximately additive responses were reported for other effects, for example, the effects on the urinary excretion of glucose and protein. However, when the combination was administered at the NONEL, there were no signs of impaired renal function or evidence of renal damage. This suggested an absence of either dose additivity or potentiating interaction at sub-effective levels of the individual toxicants. A similar conclusion was drawn from a further study by Jonker *et al*[39,40] in which Wistar rats (10 weeks old, n = 5 /treatment group for each sex, n = 10 /control group for each sex) were fed proximal tubule nephrotoxins, HCB, mercuric chloride, *d*-limonene and lysinoalanine, either alone or in combination, for 4 weeks. Again, although these compounds have the same target (the tubular epithelial cells), they are thought to have dissimilar modes of action. Each chemical was given alone at a dose that corresponded to its LONEL and NONEL established in a preliminary range-finding study. The combination was given at the LONEL, the NONEL and one quarter the NONEL of each individual chemical. The individual treatments in males caused a slight depression in growth at the LONEL, but not at the NONEL. Treatment with the combination appeared to exacerbate this effect in that a slight and a severe growth retardation effect were observed at the NONEL and at the LONEL, respectively. In contrast, growth retardation in females resulting from the administration of HCB at the LONEL was not aggravated by the combined treatment. Nephrotoxic effects in male rats at the LONEL (decreased renal concentrating ability and moderate histopathologic changes in the kidney and increases in kidney weight and number of epithelial cells found in the urine) were more severe in those that received the combination than in those fed single nephrotoxins. A slight but statistically significant increase in relative kidney weight was observed in male rats receiving the combination at the NONEL and this may have suggested exacerbation of nephrotoxicity by the combination at this dose level. However, this possibility was dismissed by the authors on the grounds that relative kidney weights were also increased, although not statistically so, in animals treated with HCB alone or mercuric chloride alone at their respective NONELs. Similarly, an increase in epithelial cells found in the urine of animals receiving the

combination at the NONEL was not considered an exacerbation of toxicity since, contrary to expectation from the range-finding study, a similar change had occurred in males receiving *d*-limonene at the NONEL. In females, no renal changes induced by the combination were more severe than those observed with the individual chemicals. No effects were observed in any animals fed the combination at one quarter of the NONEL.

8.47 Overall, it was concluded that simultaneous exposure to these four nephrotoxins at their individual NONELs did not constitute an obvious increase in hazard whereas at the LONEL there was clear evidence that renal toxicity was enhanced in males, but not in females. Furthermore, the authors[41] suggested that, in this case, the NONEL of the mixture is determined by the NONEL of the chemical with the smallest margin between its actual level in the mixture and its true nephrotoxic effect level, assuming that that the actual level of each component of the mixture is lower than its true no nephrotoxic effect level.

8.48 In a subsequent study by the same group of workers,[40] the dose addition assumption was tested using a selection of mechanistically similar nephrotoxins tetrachloroethylene, trichloroethylene, HCB and 1,1,2-trichloro-3,3,3-trifluoropropene. These compounds were administered daily in corn oil (10 ml/kg) by oral gavage to female rats (n = 10/group) for 32 days, either alone at the LONEL and NONEL (= LONEL/4), or in a quaternary combination at the NONEL and the LONEL/2, or four combinations of three chemicals at the LONEL/3. Controls received the vehicle alone. Treatment with the individual chemicals at the LONEL resulted in increases in relative kidney weight. Similar increases were observed following combined treatments at the NONEL or the LONEL/3. On the basis of this endpoint alone, it was concluded that the renal toxicity of the mixtures corresponded to the effect that would be expected on the basis of the additivity assumption. Other endpoints for nephrotoxicity observed with the individual treatments were not or were minimally affected on combined exposure.

8.49 It should be noted in the above studies, that although prior dose ranging studies were carried out to ascertain dose responses and NONELs and LONELs, these data were published elsewhere (see Mumtaz *et al).*[42]

Haematotoxicity

8.50 The published studies of haematotoxic interactions involving organic chemicals, such as those present in commercial products and petroleum preparations, have been reviewed by Krishnan and Pelekis.[43] The studies were limited to investigations of the modulation of haematotoxicity of benzene, dichloromethane and dimethylalanines during co-exposure with other chemicals (generally inducers, inhibitors or substrates of CYP2E1), none of which are herbicides or pesticides.

8.51 Many studies have investigated the additive effects of mixtures of dioxins and polychlorinated biphenyls. For example, van Birgelen *et al*[44] have investigated the effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD – 15, 25, 50, 300, 1000 ng/kg/day), 2,2',4,4',5, 5'-hexachlorobiphenyl (PCB 153 – 0.5, 2.0, 6.0 mg/kg/day), 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156 – 80, 365 or 700 μg/kg/day), 3,3'4,4',

5-pentachlorbiphenyl (PCB 126 – 0.5, 3 or 10 μg/kg/day) given singly or in various binary combinations with TCDD (0, 25 or 300 ng/kg/day) on hepatic porphyrin accumulation when included in the diets of female Sprague Dawley rats (n = 8 – 9/group) for 13 weeks. A dose-dependent increase (maximally a 2-fold increase over control values) in hepatic porphyrin occurred after administration of TCDD (lowest observable effect level [LOEL] equivalent to 50 ng/kg body weight/day), PCB 126 (LOEL 3.0 μg/kg body weight/day) or PCB 156 (LOEL 365 μg/kg body weight/day). The authors reported relative potencies (no observable adverse effect level [NOAEL] and lowest observable adverse effect levels [LOAELs] relative to those for TCDD) were 0.015 – 0.06 for PCB 126 and 0.0001 to 0.0003 for PCB 156. PCB 153 alone, at doses ≤ 6.0 mg/kg body weight/day, did not result in hepatic porphyrin accumulation. Co-administration of PCB 153 (2 or 6 mg/kg/day) and TCDD (300 ng/kg/day) revealed what was described as a strong synergistic effect (porphyrin accumulation up to 500-fold that seen with TCDD treatment alone and 800-fold control levels).

Carcinogenicity

8.52 Because pesticides that are believed to be genotoxic *in vivo* are not normally approved for use,[a] few carcinogenicity studies have been performed to test combination treatments. Where data exist, relatively few studies include concurrent data regarding the effects of the individual constituents alone. In many cases, short-term models of carcinogenicity have been used, particularly when studying large numbers of dose groups. How predictive these models are of tumorigenic potential for different classes of chemical is open to question.

8.53 Ito *et al*[45-47] investigated the carcinogenic potential of pesticide combinations at low doses. The test protocol adopted a short-term initiation/promotion model of carcinogenesis with glutathione S-transferase (GST) (placental or pi form) positive (GSTp+ve) hepatocyte foci as a preneoplastic endpoint marker. After tumour initiation with diethylnitrosamine (DEN) and a two-thirds partial hepatectomy, young adult rats received the test chemicals in their diets for 6 weeks. Frequency and size of GSTp+ve foci were examined in the livers of animals following termination. A combination of twenty pesticides was tested (19 OPs and one organochlorine compound), administered at 1 x the acceptable daily intake (ADI) and 100 x ADI for each individual chemical. At 1 x the ADI level, there was no enhancement of the development of preneoplastic lesions initiated by DEN. At 100 x the ADI, both the number and the area of the lesions was increased. According to Ito *et al*[46] the combination effects observed at the higher (100 x ADI) dose suggested several of the pesticides were acting as tumour promoters in the liver. It is also worth noting that several constituents were Ames test positive. Also, similar enhancing effects had been demonstrated previously for methidathion and malathion, both of which were included in the present study.

8.54 In a second study from the same group,[46,47] using a medium-term multiorgan protocol of 28 weeks (in which tumours were initiated by five known potent carcinogens in combination), the carcinogenicity of a mixture of 40 pesticides (high volume compounds) and another of 20 pesticides (suspected carcinogens) was investigated at the constituents' respective ADIs. There was no enhancement of carcinogenesis.

[a] except for spindle inhibitors, where there is a clear biological basis for the expectation of a threshold.

8.55 While these studies provide reassurance in the fact that no enhancement of observed effects is observed at low levels of exposure, positive effects were seen at multiples of the ADI dose. However, these studies provide little or no information regarding the nature of any interactions that may have occurred. The following paragraphs describe some other studies that have investigated the nature of interactions within mixtures that contain chemicals other than pesticides.

8.56 To determine whether combinations of two carcinogens with the same target can act synergistically, the long-term dietary effects of hepatocarcinogens, cycad flour, lasiocarpine, aflatoxin and dipentylnitrosamine (DPN) were studied in pair-wise combinations in male and female F344 rats.[48] Each of the six possible pairs was studied in a 4 x 4 factorial study design (including a zero and 3 non-zero dose groups for each agent). The maximum dose level used for each individual chemical was high enough to cause tumours in a large proportion of animals without producing toxicity sufficient to reduce survival. Other doses were equally spaced on a log scale. Data were analysed by traditional methods and methods specifically designed to determine the additivity index and test for deviation from simple additivity (for parameters of time to death, tumorigenicity, intercurrent mortality/occult tumours). No chemical was found to antagonise the effects of any other. Some chemicals were reported to act synergistically, for example cycad flour and lasiocarpine, when the low dose or the mid dose of cycad flour was combined with the mid-dose or the high dose of lasiocarpine either where the endpoint was taken as time of death or time to death with malignant liver tumour. Lasiocarpine and DPN were reported to act synergistically when the endpoint was taken as time to death or time to death with a liver tumour. Cycad flour and DPN were reported to act synergistically when the endpoint was taken as time to death. Findings in male and female animals were generally in agreement. Although clear excess toxicity was seen with some of the mixtures, the study design precludes clear definition of the type of combined toxicity seen.

8.57 A further study reported by the same group investigated the outcome of exposure to combinations of carcinogens that independently act on different organ systems.[49] Four carcinogens, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), N-butanol-butylnitrosamine (NBBN), nitrilotriacetic acid and DPN were studied in pair-wise combinations in F344 rats, again using a 4 x 4 factorial design. Data were analysed by methods specifically designed to determine the additivity index and test for deviation from simple additivity. Antagonism was reported for some mixtures containing nitriloacetic acid. Other combinations were found not to interact. Findings in male and female animals were generally in agreement.

8.58 Potential synergism among five heterocyclic amines was investigated in a short-term initiation/promotion model of carcinogenesis with glutathione S-transferase (placental form) positive (GSTp+ve) hepatocyte foci as a preneoplastic endpoint marker.[50] Separate groups (n = 15 – 18 per group) were treated with a combination of the five chemicals or each chemical individually at the following dose levels incorporated in the diet: 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2, 500 ppm); 2-amino-6-methyldipyridol[1,2-*a*:3',2'-*d*]-imadazole (Glu-P-1, 500 ppm); 2-amino-3-methyl-9H-pyrido[2,3b]indole (MeAαC, 800 ppm); 2-amino-9H-pyrido[2,3-*b*]indole (AαC, 800 ppm); 2-amino-1-methyl-6-phenylimadazol[4,5-*b*]pyridine (PhIP, 400 ppm), and at 1/5 and 1/25 these levels. With the exception of PhIP, all chemicals individually at the highest dose increased the numbers and areas of

GSTp+ve foci. Data were analysed for additive or synergistic effects using a test for linear statistical inference, assuming dose-linearity in response. Combined treatment at the 1/5 dose level, but not at 1/25 level, resulted in what the authors described as a synergistic enhancement of foci parameters in that the numbers and areas of foci were significantly increased above the sums of the individual data. However, the individual dose-response relationships were not characterised in sufficient detail to support this conclusion.

8.59 Using a similar experimental protocol, separate groups of animals were treated with carcinogenic doses of 3-amino-1,4-dimethyl 5H-pyrido[4,3-b]indole (Trp-P-1, 150 ppm), 2-aminodipyrido[1,2-a:3',2'-d]imidazole (Glu-P-2, 500 ppm), 2-amino-3,8-dimethylimidazo[4,5-f]quinoline (MeIQ, 300 ppm), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ, 300 ppm) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx, 400 ppm) and 1/5 and 1/25 these dose levels. Further groups received the chemicals in combination at 1/5 and 1/25 the individual carcinogenic dose levels.[51,52] All chemicals significantly increased GSTp+ve foci numbers at the highest dose levels. Trp-P-1, MeIQ and IQ also exerted a positive influence at the 1/5 dose level. With the exception of Glu-P-2, similar results were obtained at the highest dose level regarding foci area. An increase was also observed with MeIQ at the 1/5 dose level. The authors suggested that the data were consistent with the occurrence of both additive and synergistic effects (as determined by modified Yoshimura's *t*-test) in animals treated with the combination at both the 1/5 and 1/25 dose levels. However, the results may have simply represented dose-addition.

8.60 Similarly, combination effects of 10 heterocyclic amines were investigated at the following doses: Trp-P-2 (500 ppm); Glu-P-1(500 ppm); MeAαC (800 ppm); AαC (800 ppm); PhIP (400 ppm); Trp-P-1 (150 ppm); Glu-P-2 (500 ppm); MeIQ (300 ppm); IQ (300 ppm) and MeIQx (400 ppm), and at 1/10th and at 1/100 of these dose levels.[53] Chemicals were tested individually at the same dose levels. The authors claimed that synergism was observed at the 1/10 dose level but not at the 1/100th. However, the results may have simply represented dose-addition.

8.61 PhIP, Glu-P-1, Glu-P-2, IQ and MeIQ were also tested individually and in combination in a medium term (28 week) multi-organ model of carcinogenesis (involving initiation with 5 potent carcinogens) in male F344 rats at levels of 300, 300, 600, 300 and 200 ppm, respectively and at 1/5th and 1/25th these levels in the diet.[52,54] The combination was reported to act synergistically in relation to the multiplicity of adenocarcinomas in the small intestine and multiplicity of Zymbal gland tumours at the 1/25 but not at the 1/5 dose level, thus failing to demonstrate a dose response. Furthermore, none of these findings were statistically significant. The results may have simply represented dose-addition.

8.62 The carcinogenic potential of low dietary levels of antioxidants known to target the rodent forestomach or glandular stomach, either alone or in combination, was investigated in a long term feeding study and in a medium (28 week) term multi-organ model, the latter involving pre-initiation with several potent carcinogens.[55] In the long-term test, butylated hydroxyanisole (BHA, 0.4%), caffeic acid (0.4%), sesamol (0.4%), 4-methoxyphenol (4-MP) (0.4%) and catechol (0.16%), alone or in combination, were fed to male F344 rats (n = 30-31/group) for up to 104 weeks. Slight increases in

forestomach papillomas (3-16% incidence compared to 0 % incidence with the basal diet) were observed in all groups except the BHA group. Incidence in the group receiving the combination was 43%. Enhanced multiplicity of papillomas in the combination group was interpreted as evidence for a synergistic interaction. Furthermore, this was the only group in which carcinoma of the stomach was observed albeit only in a single animal. The incidence of papillary or nodular hyperplasia was reported as less than additive, with the response in the combination group being similar to the response observed with caffeic acid alone. However, the nature of the combined effect, particularly whether any potentiation was present cannot be assessed from these data as only a single dose level was tested.

8.63 In the medium-term test, BHA, caffeic acid, sesamol and 4-MP were administered at doses of 0.4% or 0.08% and catechol at doses of 0.16% or 0.032%, individually or as a high or a low dose combination (n = 15/group). Incidences of forestomach papillomas and papillary or nodular hyperplasia were increased in each of the high dose groups, significantly so in the caffeic acid and 4-MP groups. The incidence of forestomach papillomas was also significantly increased in the catechol high dose group. The effects of the high dose combination were less than would be predicted by dose addition. Compared to the spontaneous incidence, there was a tendency for a reduction in numbers of carcinomas and adenocarcinomas of the large intestine with each individual antioxidant treatment. However, this reduction became significant with the combination treatment. In the low dose groups, the incidence of forestomach papillomas was increased only in those animals receiving the combination.

8.64 Nesnow *et al*[56] studied the binary, ternary, quaternary and quintuple interactive effects of a five component mixture of environmental polyaromatic hydrocarbons (PAHs) on the development of lung tumours in male A/J mice. Individual dose-response data (lung adenoma score following a single i.p. administration 8 months prior to termination) were obtained for benzo[*a*]pyprene, benzo[*b*]fluoranthene, dibenz[*a,h*]anthracene, 5-methylchrysene and cyclopenta[*c,d*]pyrene. From these data, quintuple mixture doses were selected, based on survival, range of dose response and predicted tumour yield. The ratios of chemicals within the mixture were design to be representative of the ratios found in the air or from combustion samples. A 2^5 factorial study design (32 groups, n = 20 per group) was employed, incorporating high and low dose level groups. This scheme allowed the calculation of five PAH dose parameters, 10 binary interaction parameters, ten ternary interaction parameters, five quaternary interaction parameters and one quintuple interaction parameter. Comparison of observed lung adenoma score with that expected from additivity identified a greater than additive response at the low dose and a less than additive response at the high dose. Less than additive interactions were observed under most mixture conditions and binary interactions were dominated by the inhibitory effect of dibenz[*a,h*]anthracene. Surface response analysis (using response addition) predicted the observed lung tumorigenic responses of quintuple mixtures. Data suggested that interactions between PAHs do occur but to a limited extent.

8.65 Further to this, an analysis of the binary carcinogen interaction literature that encompasses multiple species, organs and routes of administration has identified both greater than and less than additive effects for PAHs, depending on target tissue, species and route of administration.[57] That is to say that the occurrence of interactions may be dependent on the mixture, dose, individual target, experimental model employed and/or the route of administration.

Reproductive and developmental toxicology

8.66 In principle, development might be affected by toxic actions occurring during prenatal life, when genotypic sex is manifested phenotypically and development of the genital tract commences, or during postnatal life when sexual maturity occurs, males and females mate, the fertilised ovum undergoes implantation and intra-uterine development, and finally the young are born and continue the cycle of development for the next generation. Toxicity might affect any of the anatomical structures underlying these stages or the complex genetic, endocrinological, immunological and metabolic mechanisms and functions required for their proper performance.

8.67 There is no basic distinction between the nature of toxicity affecting these processes and that manifested as effects on a target organ during adult life. The key difference is that as development is a continuing process and physiological events and anatomical structures may only be present for a limited period, it may only be possible for a particular harmful action to occur during a vulnerable period. The counterpart of that limited opportunity for a toxic action at one stage of development is that damage to the precursor of an adult tissue or function may result in permanent consequences because regeneration or repair are not feasible.

8.68 Although not a fundamental difference, the complexity of the physiological mechanisms underlying successful reproduction and development, which rely on precise integration of a range of hormones and other signals, means that disorders of function and of integration must always be considered, as well as more conventional metabolic and pathological types of toxicity.

8.69 There is considerable experimental and much clinical evidence that toxic actions at any stage of development follow the same dose-response relationships as do those associated with conventional target organ toxicity. There is a difference in the effect of time of exposure during development, because of the possibilities of a limited period of susceptibility and of long-term consequences that may not be manifested until sexual maturation of the subsequent generation. It is also possible for effects in the mother to influence postnatal development of her offspring by affecting, for example, the quantity and quality of milk produced, changes in the level of immunoglobulins and other factors in her milk, and abnormalities in behaviour during upbringing of the young.

8.70 As the same types of dose-response relationships are followed, it would be anticipated that the same basic possibilities of dose-additive and effect-additive toxicity, as well as potentiation and antagonism might occur following exposure to a mixture of toxicants.

8.71 Studies, such as those of Heindel *et al*[58,59] have investigated the reproductive and developmental toxicity of complex mixtures that represent contamination in the field (the reports cited above were specifically concerned with contaminants in ground water sources). However, the aim of these studies was not to assess the nature of any interaction that might occur between the constituents of the mixture and consequently there was no need for concurrent investigation of the effects of the individual components. As a result, these studies provide no information with regard to the nature of

toxicological interactions specifically due to the chemical mixtures. However, no adverse effects were found in rats treated with dose levels representative of 100 times the median pesticide contamination encountered in Iowa and California.

8.72 There have been a few studies of the adverse effects of far less complex mixtures and their individual components on reproduction and development that shed some light on the nature of toxicological interactions that occur. However, much of the work has been carried out in aquatic and avian species, and its direct relevance to humans is therefore uncertain.

Studies of teratogenicity in aquatic species

8.73 Seven chemicals (semicarbazide, ß-aminopropionitrile, isoniazid, penicillamine, valproic acid, butyric acid, hydroxyurea), all known to be teratogenic to frog embryos, were tested, using a modified FETAX[a] test, individually or as 5 binary mixtures (semicarbazide + ß-aminopropionitrile, semicarbazide + isoniazid, semicarbazide + penicillamine, valproic acid + butyric acid, isoniazid + hydroxyurea, in proportions 3:1, 1:1 and 1:3, over 5 concentrations giving a linear range of 0-100% malformations). Weibull functions were used to model the concentration-response relationships. Data were consistent with additive concentration effects of the mixtures.[60]

8.74 Similarly, the joint action of all-*trans* retinoic acid and valproic acid was investigated at concentrations known to induce malformations in *Xenopus laevis* embryos.[61] Mixtures of all-trans retinoic acid and valproic acid (in ratios of 3:1, 1:1 and 1:3) were tested over 5 concentrations designed to give a linear response range from 0 – 100%. In addition, 5 concentrations of each individual component alone were tested. Testing was performed in triplicate. All malformation types were included. The EC_{50} values for all-*trans* retinoic acid and valproic acid alone were 0.0061 mg/l and 39.5 mg/l, respectively. Isobolic analysis suggested that the response to mixtures of all-*trans* retinoic acid/valproic acid was response additive, indicating dissimilarity of toxic action. This joint action was not changed by analysis of specific malformation types.

8.75 Mizell and Romig[62] investigated TCDD, toluene, benzene, and mixtures of these chemicals for their ability to adversely affect the embryonic development of zebrafish (*Danio rerio*) following exposure during the late gastrula stage by dechorionation and microinjection into the perivitelline space. Exposure to TCDD (0.0001 – 1 ppm), toluene (0.0001 – 100 ppm) or benzene (0.0001 – 10 ppm) alone adversely affected embryonic cardiovascular development and survival in a dose-dependent manner. There were some additional abnormalities associated with exposure to toluene or benzene. Embryos exposed to 50:50 TCDD/benzene (0.0001 ppm – 1 ppm) or 50:50 benzene/toluene (0.0001 – 10 ppm) mixtures developed cardiovascular and other defects similar in nature to those observed following exposure to the individual chemicals. However, the chemical combinations were reported to act synergistically in that they were more toxic (in terms of cardiovascular defect development and mortality) than either component alone (the LC_{50} values for the TCDD/benzene and the toluene/benzene mixtures were 0.01 ppm and 0.05 ppm, respectively, compared to LC_{50} values of 0.1, 0.05 and 0.1 ppm, respectively, for exposure to TCDD, benzene and toluene alone).

[a] Acronym for Fetal Teratogenesis Assay – Xenopus.

8.76 Faust *et al*[63] reported a study using 18 triazine herbicides (including a number never or no longer used in the UK) on reproduction of the fresh water alga, *Scendesmus vacuolatus*, in the context of contamination of surface waters. The authors reported that the concept of concentration addition accurately described the toxicity of triazine mixture, even for components calculated to be contributing effects that were, alone, not statistically significant. This finding is unsurprising in view of the similarity of herbicidal action of this group of compounds, namely the inhibition of photosynthesis.

Studies of teratogenicity in birds

8.77 Varga *et al*[64] reported the individual and combined effects of the insecticide Sumithion 50 EC (50% fenitrothion) and the herbicide Fusilade S (12.5% fluazifop-P-butyl) in a study of embryotoxicity and teratogenicity in pheasant (*Phasianus colchicus mongolicus et torquatus*) eggs. Eggs were injected with 0.1 ml volumes of various concentrations of Sumithion 50 EC or Fusilade S (0.033, 0.33, 3.33% and 0.1, 1.0, 10.0%, respectively) or mixtures (Sumithion 50 EC + Fusilade S; 0.33% + 0.1%, 0.33% + 1.0%, 0.33% + 10%) of the two chemicals, into the air space on day 12 of incubation. Pathological examination was on day 23 of incubation. Group size was from 13-24. Sumithion 50 EC caused a dose-related decrease in embryo body mass and high embryo mortality at the top dose. Developmental abnormalities were increased at all dose levels, but their frequency was not dose-dependent. Fusilade S significantly decreased body mass at the mid-dose level but caused 100% mortality at the top dose. Malformations were observed only at the low dose. Combined treatment resulted in an apparent moderation by Sumithion 50 EC of the high dose mortality effects of Fusilade S, although it should be noted that a similar moderating effect of the combination was not observed at the mid dose of Fusilade S, where the percentage of live embryos was actually decreased relative to the effects of Fusilade S alone. However, no skeletal malformations were observed in embryos receiving Fusilade S and Sumithion 50 EC together whereas such malformations were seen with Sumithion 50 EC alone.

Studies of teratogenicity in laboratory rodents

8.78 Narotsky *et al*[65] investigated the combined effects of three compounds, trichloroethylene (TCE), di(2-ethylhexyl) phthalate (DEHP) and heptachlor on the fetal development of F344 rats. Using five dose levels in a full five factorial study design, pregnant females (n = 7-12/group) were treated with mixtures (in corn oil) by oral gavage on gestation days (GD) 6-15. Four dose levels were selected on the basis of a preliminary dose-ranging study performed for each chemical individually. Linear regression was used to predict the dose causing a maternal body weight deficit of 2g on GD 6-8. The high dose levels (dose level 4) in the 5 x 5 x 5 study – represented the lower 95% confidence interval of that predicted dose. Dose levels 3, 2 and 1 represented successive reductions of dose level 4 by a factor of $10^{1/2}$. Dose level 0 indicated the absence of a test agent. Litter examinations were performed on GD 22, regardless of the stage of parturition. For the identification of possible interactions between the three agents, four continuous endpoints were analysed by linear regression and five discrete endpoints were analysed using a logistic regression model. Effects seen in the three-agent experiment were generally consistent with the known effects for the individual components. There were no effects on maternal death. However, DEHP and heptachlor were found to be synergistic. All three agents showed effects on

maternal weight gain on GD 6-8 and there was synergism between TCE and DEHP and antagonism between DEHP and heptachlor. Maternal weight gain (adjusted for litter weight) showed effects due to TCE and heptachlor, but there were no interactions between these chemicals. Effects were evident for all three chemicals regarding full litter resorptions and prenatal loss, although the effect for heptachlor was not expected. For full-litter loss, data analysis suggested antagonistic interactions between TCE and heptachlor and DEHP and heptachlor, although this may have reflected a ceiling effect of the high-dose response. For prenatal loss, there was apparent synergism between TCE and DEHP. Postnatal loss showed effects due to DEHP and heptachlor but there was no evidence for any interactions. Pup weight data from postnatal day 1 suggested effects (reduction in pup weights) due to TCE and DEHP and an antagonistic interaction between the DEHP and heptachlor: on postnatal day 6 there were effects due to DEHP and heptachlor, an apparent synergism between TCE and DEHP and antagonism between DEHP and heptachlor. Both TCE and DEHP showed effects on microphthalmia and anophthalmia but there was no indication of any interaction.

8.79 Calciu *et al*[66] studied the teratogenic effects of camphechlor, two of its congeners T2 and T12, and mixtures of T2 and T12 in cultured rat embryos. Employing a 4 x 4 factorial experimental design, explanted embryos (0-2 somites, obtained from 40 pregnant Sprague Dawley females on gestation day 10) were exposed to 100, 1000 and 5000 ng/ml of camphechlor, T2, T12 or 50:50 mixtures of T2 and T12, for 48 hours. Total morphological score, crown-rump length and head-length were significantly decreased by all treatments, individually and in combination. Effects were concentration-dependent. Significant adverse effects were also observed on somite number and CNS scores. T2 and T12 congeners differed in the spectrum of abnormalities they caused (T2 caused limb and flexion malformations not observed with T12). Furthermore, the combination showed an apparent synergistic effect on decreasing crown-rump and head lengths, but inhibited the strong adverse effects of the individual congeners on otic development, relative to the actions of individual components alone.

8.80 You *et al*[67] investigated the effect of genistein, a phytoestrogen, on the developmental toxicity of methoxychlor in rats. Genistein was given at two dose levels (300 and 800 ppm) and methoxychlor at one (800 ppm) in the diet. Diets containing mixtures of methoxychlor (800ppm) with genistein at either 300 or 800 ppm and control diets were also administered. The diets were given to groups of 8 mated rats post-natal day 1 until parturition; offspring were weaned at post-natal day 21, and put on the diet until post-natal day 100. The adverse outcomes measured were accelerated vaginal opening and delayed preputial separation in the female and male offspring respectively. The former was seen with methoxychlor and both doses of genistein. When the two compounds were given together, the effect was greater, but there was insufficient dose/response information to describe the nature of the combined action. Delayed preputial separation was seen with methoxychlor and not with either dose of genistein, but genistein at 800 ppm, with methoxychlor, enhanced this effect. This was described as potentiation but could simply be an additive effect, the effect of genistein alone not having been clinically detectable. These authors also performed *in vitro* studies using estrogen and androgen receptor-based transcriptional activational assays, but these do not aid the interpretation of the results.

8.81 You *et al*[68] carried out a study in pregnant rats, in which genistein was given at two concentrations in the diet (300 and 800 ppm) and methoxychlor was given at one concentration (800 ppm) in the diet. No significant effects were seen in the female pups, but both compounds increased mammary development in the males, methoxychlor more so. The two compounds in combination caused more pronounced effects. This study did not provide information on the type of combined effect.

Endocrine Disruption

Compounds with estrogenic activity and the estrogenic potency/activity of mixtures

8.82 Several chemicals, that include industrial chemicals, waste chemicals, pesticides and natural plant products, can mimic the actions of estrogen. Generally speaking, the estrogenic potency of each of these compounds is several orders of magnitude lower than that of the naturally occurring hormone, 17β-estradiol. However, there is some concern that mixtures of these substances act in concert to modulate the endocrine systems in humans and wildlife, even at very low levels of environmental exposure.

Organochlorine pesticides

8.83 One study in particular reported in 1996 by Arnold *et al*[69] temporarily caused a great deal of concern in the mid-nineties. Despite the fact that the authors later retracted their findings and there is now evidence that data were falsified,[70] the study raised a possibility in the minds of some which continues to cause them concern. Arnold *et al* reported that the organochlorine pesticides dieldrin, endosulfan or camphechlor alone weakly increased human ER (hER)-dependent reporter gene (β-gal) activity in a recombinant yeast system. EC_{50} values were found to be › 33 μM. However, equimolar combinations of any two of the compounds produced a synergistic increase (160 – 1600-fold greater activity than was observed by any one chemical alone) in reporter gene expression. Chlordane, which alone had no appreciable activity within the yeast system, significantly enhanced the potency of the other chemicals. Competitive estrogen binding assays employing hER showed that dieldrin, endosulfan or camphechlor only weakly inhibited the binding of labelled 17ß-estradiol. To inhibit binding to the same extent, concentrations of the combined chemicals were at most 1/200th that required for either chemical alone. Chlordane alone did not inhibit 17ß-estradiol binding, but it did enhance the competitive binding activity of the other pesticides.

8.84 The implications of these data were far reaching and demanded verification. Consequently, the Arnold study was the stimulus for several other studies from other groups. However, other workers investigating a series of endpoints, failed to reproduce the original findings or find any other corroborating evidence (see below).[71,72] Even more significantly, the report of Arnold *et al*[69] was subsequently retracted by the authors when attempts to replicate the work within the same laboratory were also without success.[73]

[a] A minor component of the insecticide DDT.

8.85 Soto *et al*[74] tested the estrogenicity of endosulfan β, endosulfan α, camphechlor, dieldrin, DDT, o,p'DDT[a], 2,2',3,3',6,6'-hexachlorobiphenyl, 1,1'-(2,2-=dichloroethenylidene)-bis(4-chlorobenzene (p,p'-DDE), 1,1'-(2,2-=dichloroethylidene)-bis(4-chlorobenzene (p,p'-DDD)[b], methoxychlor in an E-screen cell proliferation assay employing estrogen responsive human breast tumour MCF-7 cells. All compounds were found to be weakly estrogenic at a concentration of 10 μM with relative proliferative potencies (RPP) compared to estrogen of ~0.001% and relative proliferative efficiencies (RPE) ranging from ~50-85%. Above concentrations of 25 μM, endosulfan β and endosulfan α were found to be cytotoxic.

8.86 Individually, none of the chemicals caused significant cell proliferation when tested alone at 1 μM. However, significant proliferation was observed when the compounds were tested as an equimolar mixture with each chemical present at a concentration of 1 μM, demonstrating an additive affect.

8.87 In response to reports of reproductive abnormalities in the alligators of Lake Apopka, Florida, following a spill of DDT and other pesticides suspected to have hormone-like activity, Vonier *et al*[75] examined the ability of chemicals to bind to estrogen receptors (aER) in protein extracts prepared from the alligator oviduct. The aER binding of 23 pesticides/pesticide metabolites (including all those mentioned below) found in the lake was tested in competition with [^{3}H]17β-estradiol. Individual IC_{50} values ranged from approximately 2 to >50 μM (IC_{50} for 17β-estradiol being less than 0.01 μM). Some pesticides (camphechlor, 2,4-D, endosulphan β, dieldrin, chlordane) failed to show significant binding. Four DDT components/metabolites were tested, individually, or as binary, ternary and quaternary mixtures, each component at a concentration of 1 μM. Individually, dicofol, p,p'-DDD or p,p'-DDE did not significantly reduce the binding of 17β-estradiol. However, o,p'-DDT inhibited binding by 18% and a mixture of all four chemicals resulted in a 40% loss of 17β-estradiol binding. The authors concluded that effects of the quaternary mixture on 17β-estradiol binding were additive. Seven chemicals identified in alligator eggs from Lake Apopka were tested individually, and as mixtures, at approximately the concentrations in which they were found in egg samples (p,p'-DDE 18 μM; p,p'-DDD 2.6 μM; dieldrin 0.63 μM; arochlor 1242 0.53 μM; trans-nonachlor 0.25 μM; chlordane 0.22 μM; camphechlor 0.2 μM; cis-nonachlor 0.16 μM). Of these chemicals, only p,p'-DDD was found to cause a significant decrease in 17β-estradiol binding (20%) when tested alone. However, a mixture of all seven components reduced 17β-estradiol binding by 60%. This led the authors to suggest that, unlike the mixture of DDT metabolites, the displacement effects of the mixture were greater than additive. However, no supporting data were provided to allow any independent substantiation of this claim. Furthermore, since receptor binding is not necessarily an indication of functional activity, any extrapolation of these data to imply increased estrogenic activity would be invalid.

8.88 Ramamoorthy *et al*[71] investigated estrogen receptor (ER) binding and estrogenic activity of dieldrin, camphechlor, and equimolar mixtures of both compounds, using both *in vivo* and *in vitro* methodology. In a uterotrophic assay employing immature (21-day old B6C3F1) female mice, treatment with dieldrin, camphechlor or equimolar mixtures of the two compounds (2.5, 15 and 60 (mol/kg/day x 3 days, i.p.) failed to induce any significant or dose-dependent estrogenic response. In contrast, treatment with 17β-estradiol (0.0053 kg/day x 3 days, i.p.) resulted in approximately 3, 5 and 8-fold

[a] A minor component of the insecticide DDT.

[b] p,p'-DDD and p,p'-DDE are metabolites of DDT.

increases in uterine wet weight, peroxidase activity and progesterone receptor binding, respectively. Neither the individual pesticides nor an equimolar mixture of the two were found to bind appreciably to estrogen receptors derived from mouse uterus in an *in vitro* competitive receptor binding assay [where 10^{-9} M [^{3}H]17β-estradiol was incubated in the presence or absence of 2×10^{-7} M unlabeled 17β-estradiol, dieldrin (10^{-5} M), camphechlor (10^{-5} M) or an equimolar (10–5 M) dieldrin/camphechlor mixture].

8.89 Neither the individual pesticides nor equimolar mixtures of the two compounds, over a concentration range of 10^{-8} – 10^{-5} M, stimulated the proliferation of estrogen-responsive MCF-7 human breast cancer cells. Neither did they induce chloramphenicol acetyl transferase reporter activity in MCF-7 cells transiently infected with plasmid constructs containing estrogen-responsive 5'-promoter regions from rat creatine kinase B and human cathepsin D genes. In both assays, 17β-estradiol (10^{-9} M) was used as a positive control. Treatment with 10^{-6} – 10^{-4} M chlordane, dieldrin, camphechlor, or an equimolar mixture of dieldrin and camphechlor, failed to induce β-gal activity in yeast transformed with hER and a double estrogen responsive element (ERE) upstream of the β-gal reporter. In contrast, endosulfan (10^{-4} M) and 17β-estradiol (10^{-8} M) caused 2000 and 5000-fold increases in activity, respectively. In yeast transformed with mouse ER (mER) and a single estrogen responsive element upstream of the β-gal reporter gene, diethylstilbestrol (10^{-8} M) caused an approximately 20-fold increase in reporter gene activity. In comparison, dieldrin, chlordane, camphechlor and endosulfan induced relatively small (1.5 – 4-fold) increases in activity at a concentration of 2.5×10^{-5} M. Synergistic transactivations were not observed for any equimolar binary combination at concentrations of 2.5×10^{-5} M or 2.5×10^{-4} M.

8.90 Stelzer and Chan[76] assessed the relative estrogenic activity of technical camphechlor and two of its congeners (T2 [2-exo,3-endo,5-exo,6-endo, 8,8,10, 10-octachlorobornane] and T12 [2-exo,3-endo,5-exo,6-endo,8,8,9,10,10-nonachlorocamphene]) over a range of concentrations (0.001 or 0.01 up to 1000 (M, increments of x10) in an estrogen responsive (MCF7-3E) cell proliferation assay. The minimal effective concentration for camphechlor, T2 and T12 individually was in each case 10 μM. In comparison, 17β-estradiol exhibited a minimal effective concentration of 30 pM. The proliferative effect of congeners T2 and T12 was, respectively, 16 and 30% lower than that of camphechlor. The activities of binary combinations of camphechlor, T2, T12, PCB-136 and p,p'-DDE were assessed with camphechlor or individual constituents, each present at a concentration of 5 (M (individually a sub-effective dose). No differences were observed between the treatment of 10 μM camphechlor alone and combinations of camphechlor with T2 or T12. The mixture of T2 and T12 showed a similar proliferative effect to that seen with 10 μM T2 alone. Consequently, the authors described the effects of camphechlor, T2 and T12 as additive. A decrease in proliferation was observed in binary mixtures of PCB-136 and camphechlor, T2 or T12 compared to 10 μM PCB-136 alone. Similarly, a decrease in proliferative effect was observed with all binary combinations of p,p'-DDE and camphechlor as compared to 10 μM p,p'-DDE alone. However, the authors suggested that the decreases could be accounted for by difference in strength/potency of the individual compounds regarding proliferative effects.

8.91 Graumann *et al*[77] tested the ability of endosulfan and dieldrin, alone or in equimolar combination, over a concentration range of 10^{-10} – 10^{-4} M, to induce ER-dependent gene expression in a recombinant

yeast assay system. The β-gal (reporter gene) response to endosulfan and dieldrin, either singly or as a mixture, was significant above concentrations of 10^{-6} M. However, neither ligand efficiency nor ligand potency was changed significantly when the two compounds were combined. The herbicide atrazine and its metabolites desethylatrazine and desisopropylatrazine were tested alone, in combination with each other, and combined with increasing concentrations of 17β-estradiol. None of the individual compounds elicited a response over a concentration range of 10^{-9} and 10^{-4} M. There was no evidence that equimolar mixtures of all three compounds acted synergistically. Similarly, combinations of the different compounds at various concentrations with increasing concentrations of 17β-estradiol failed to influence the 17β-estradiol-mediated response.

8.92 Tully *et al*[78] evaluated the ability of six organochlorine pesticides or metabolites thereof (p,p'-DDT[a], p,p'-DDD[b], p,p'-DDE[b], aldrin, dieldrin and endrin) to modulate transcriptional activation of an estrogen-responsive reporter gene in transfected HeLa cells. Cells were exposed to these pesticides individually and in defined combinations. While 17β-estradiol (0.01-1 nM) consistently elicited a 10-23-fold dose-dependent induction of reporter gene activity, the organochlorine compounds (0.001-10 μM) showed no detectable dose-related response either individually or as equimolar binary mixtures.

8.93 Rajapakse *et al*[79] tested bisphenol A, o,p'-DDT[c] and 17β estradiol and mixtures thereof, using a yeast reporter gene (*Saccharomyces cerevisiae* hERα) assay. They reported that at molar ratios proportional to those normally found in the human body (1:500 17β estradiol: bisphenol A or17β estradiol:DDT) the two xenoestrogens had too weak an effect to have an impact on the activity of 17β estradiol. With mixtures of 17β estradiol, with either bisphenol A or DDT, at higher concentration ratios of the xenoestrogens (1:20,000 to 1:100,000), bisphenol A and DDT exerted estrogenic effects and the effects of the mixtures suggested additivity (simple similar action).

8.94 Payne *et al*[80] studied mixtures of four OCs on cell proliferation in MCF-7 human breast cancer cells. The four OCs used were o,p'-DDT[c], p,p'-DDE[b], β-hexachlorocyclohexane and p,p'-DDT[a]. Concentration-response analyses were carried out. When the mixtures were tested the effects were additive.

(Other) Polychlorinated biphenyls (PCBs)

8.95 Arnold *et al*[69] reported a mixture of two (unnamed) hydroxylated PCBs synergistically activated hER-dependent reporter gene activity in transfected Ishikawa cells. *In vitro* binding experiments confirmed that that the PCBs interacted with hER and when mixed, showed a synergistic reduction in the inhibition of labelled 17β-estradiol binding. IC_{50} values for each of the hydroxy-PCBs and an equimolar mixture were found to be 55 nM, 120 nM and 5 nM respectively. However, as with the studies on organochlorine pesticides (see above), attempts within the same laboratory and by others have failed to reproduce the findings of Arnold *et al*[69] and the report was subsequently retracted.[73] It appears that data were intentionally falsified.[70] (see also section 8.83)

[a] The major component of the insecticide DDT.

[b] Metabolite of DDT.

[c] Minor component of DDT.

8.96 Ramamoorthy *et al*[81] investigated ER binding and estrogenic activity of 2',4',6'-trichloro-4-biphenylol (OH-PCB3), 2',3',4',5'-tetrachloro-4-biphenylol (OH-PCB4), and equimolar mixtures of both compounds, using both *in vivo* and *in vitro* methodology. In a uterotrophic assay employing immature (21-day old B6C3F1) female mice, treatment with 17β-estradiol (0.02 μg/kg/day x 3 days, i.p.) caused 2-3, 4-6 and 6-7-fold increases in uterine wet weight, peroxidase activity and progesterone receptor binding, respectively. Treatment with OH-PCB3, OH-PCB4 or equimolar mixtures of both compounds (18, 73, 183 or 366 μmol/kg/day x 3 days, i.p.) resulted in dose-dependent increases in uterine wet weight, peroxidase activity and progesterone receptor binding. However, the estrogenic activity of either PCB at any dose tested was sub-maximal and described as weak. The activity of the PCB mixture was comparable to that of each individual PCB.

8.97 As part of the same study, the binding of 17β-estradiol, HO-PCB3, HO-PCB4 and equimolar mixtures of the two PCBs was tested in an *in vitro* competitive receptor binding assay using estrogen receptors derived from mouse uterus and [^{3}H]17β-estradiol as the radioligand. IC_{50} values obtained for 17β-estradiol, HO-PCB3, HO-PCB4 and the PCB mixture were 1.1 x 10^{-8}, 3.4 x 10^{-6}, 9.9 x 10^{-7} and 4.25 x 10^{-6}M, respectively.

8.98 HO-PCB3 and HO-PCB4 stimulated both the proliferation of MCF-7 cells and the expression of estrogen-responsive reporter genes in MCF-7 cells transfected with two different hER reporter constructs (hER-CKB-CAT or hER-C3-LUC) in a dose-dependent manner. Maximal stimulation occurred at concentrations in the region of 10^{-6} M. The potency of the PCBs was 1000-10,000 times lower than that of 17β-estradiol. The estrogenic activity of the HO-PCB3/HO-PCB4 mixture was comparable to that of each individual PCB. Similar results were obtained using yeast transformed with hER and a double ERE upstream of the β-gal reporter gene.

8.99 It had been suggested[73] that synergistic action of estrogenic components within a mixture may be determined by the level of ER expression within an assay system. However, Ramamoorthy *et al*[81] failed to demonstrate any such influence. Investigations in HepG2 cells co-transfected with C3-LUC and variable levels of ER expression plasmid showed that, as ER levels decreased, the magnitude of reporter gene induction by 17β-estradiol, the individual PCBs or the PCB mixture also decreased. No synergistic activity was observed with the PCB mixture at either high or low levels of ER expression. Similar results were obtained in a system that employed MDA-MB-231 cells co-transfected with C3-LUC and variable amounts of ER expression plasmid.

8.100 The red-eared turtle (*Trachemys scripta*) displays temperature-sensitive sex determination, in which the incubation temperature of the egg determines the sex of the individual. In temperature-sensitive sex determination species, exogenous estrogens applied to the eggshell during the period of sexual differentiation can counteract the effects of male-producing temperatures and induce ovarian development. Bergeron *et al*[82] applied eleven PCBs, individually, at two dose levels, to turtle eggs (15 eggs/dose) at 4 weeks (stage 17), incubated at 27.8 °C (male producing temperature). 17β-Estradiol was used as a positive control. Two of the hydroxybiphenyl compounds, 2',4',6'-trichloro-4-biphenylol and 2',3',4',5'-tetrachloro-4-biphenylol, caused significant sex reversal ($p < 0.001$, with 2',4',6'-trichloro-4-

biphenylol causing 100% sex reversal) at a male producing temperature, at dose levels of 100 μg (9ppm), but not at 10 μg. Egg incubations were then carried out with low (10 μg), medium (100 μg) or high (145-190 μg) doses of PCB compounds in ethanol. Some eggs received a cocktail of all PCBs, except for the two (2',4',6'-trichloro-4-biphenylol and 2',3',4',5'-tetrachloro-4-biphenylol) found to cause sex reversal. Others were exposed to a combination of hydroxybiphenyls, again in the absence of F and 2',3',4',5'-tetrachloro-4-biphenylol. Further groups of eggs were exposed to non-hydroxylated PCB. In all conditions, there was no evidence of sex reversal. Combined exposure to 2',4',6'-trichloro-4-biphenylol and 2',3',4',5'-tetrachloro-4-biphenylol resulted in apparent synergism, with a significant ($p < 0.01$) increase in ovarian development at a dose of 10 μg (> 1 ppm) of each chemical. 2',4',6'-Trichloro-4-biphenylol alone and 2',3',4',5'-tetrachloro-4-biphenylol alone required at least a 10-fold higher dose to show sex reversal. 17β-Estradiol produced a similar effect at a dose of 0.5 μg (or 0.04 ppm). In the absence of clearer definition of the shapes of the individual dose response curves, the occurrence of potentiation would need confirmation by further work in this test system.

8.101 Arcaro *et al*[83] examined the estrogenicity of binary mixtures of the hydroxylated polychlorinated biphenyls 2,4,6-trichloro-4'-biphenylol and 2,3,4,5-tetrachloro-4'-biphenylol and the pesticides endosulfan and dieldrin in the MCF-7 cell focus assay and a competitive hER binding assay. Although individual hydroxylated polychlorinated biphenyls were found to be estrogenic (EC50 values 0.22 and 0.72 μM for 2,4,6-trichloro-4'-biphenylol and 2,3,4,5-tetrachloro-4'-biphenylol, respectively), no synergistic action was observed when they were combined at various concentrations as equimolar mixtures (EC50 0.18 μM). Of the pesticides, endosulfan was found to be weakly estrogenic in the MCF-7 cell assay at concentrations $\geq$ 10 μM. Again no synergy was observed with equimolar mixtures of the two pesticides. Additionally, no synergy was observed in MCF-7 cells between 2,4,6-tetrachloro-4'-biphenylol and physiologically relevant concentrations of 17β-estradiol.

Other mixtures

8.102 Using a yeast reporter gene assay with the human estrogen receptor α (hERα), Rajapakse *et al*[84] assessed the ability of 11 compounds with estrogenic activity to affect the actions of 17β-estradiol. The compounds were genistein, resorcinol monobenzoate, phenyl salicylate, benzyl-4-hydroxyparabene 2,4-dihydroxybenzophenone, bisphenol A, 4'-chlorobiphenyl-4-ol, 2,3,4-trichlorobiphenyl, 2',5'-dichloro-biphenyl-4-ol, 2,3,4,5-tetrachlorobiphenyl and 2',3',4',5'-tetrachlorobiphenyl-4-ol. Concentration/effect relationships for 17β-estradiol and the other 11 compounds were established. The observed combined effects of mixtures of the 12 compounds were explicable assuming additive combination effects, and the effects of 17β-estradiol were enhanced, even when each other component of the mixture was present at below its individual no observable effect concentration (NOEC).

8.103 Silva *et al*,[85] using the yeast hERα screen as above, studied eight estrogenic compounds, none being pesticides. These were 2',3',4',5-tetrachloro-4-biphenylol, 2',5-dichloro-4-biphenylol, 4'-chloro-4-biphenylol, genistein, 2,4-dihydroxybenzophenone, benzyl-4-hydroxyparabene, bisphenol A and resorcinol monobenzoate. They found that concentration addition and the toxic equivalency approach described the effects observed and that effects could be produced by the mixture, when the constituents were present at below their individual NOECs. This suggests simple similar action at the hERα.

Effects of chemical mixtures on other hormones

Thyroxine and somatotropin

8.104 Porter *et al*[86] used a full factorial study design to investigate the interactive effects of low concentrations of the carbamates aldicarb and methomyl and the triazine compound metribuzin, at two dose levels with centre replicates, on thyroxine and somatotrophin levels in male and female Sprague Dawley rats. Animals (6 per group per sex) were treated with each chemical individually (10 ppb aldicarb, 10,000 ppb metribuzin or 1000 ppb methomyl) or in combination as binary mixtures (10 ppb aldicarb + 10,000 ppb metribuzin, 10 ppb aldicarb + 1000 ppb methomyl, 10,000 ppb metribuzin + 1000 ppb methomyl and as ternary mixtures (10 ppb aldicarb + 10,000 ppb methomyl + 1000 ppb methomyl or 1 ppb aldicarb + 1000 ppb methomyl + 100 ppb metribuzin), in the drinking water, for up to 6 weeks (females) or 16 weeks (males). The authors stated that this study design allowed the investigation of all main effects and interactions for all three chemicals. However, interpretation of the data must be limited by the absence of any characterisation of the dose-responses for the individual compounds. The concentrations used were chosen as they represented levels found in contaminated groundwater. Response surface analysis showed changes in free thyroid index as a main effect for metribuzin and a three-way interaction between aldicarb + methomyl + metribuzin significantly increased thyroxine levels in females after 2 weeks exposure. After 6 weeks, in females metribuzin caused an even bigger increase in plasma free thyroxine but the three-way interaction at this time was less. In male rats, the outcome after 7 or more weeks of treatment was similar to that seen in females after 6 weeks, with metribuzin alone causing a significant and dose-dependent increase in thyroxine levels. Somatotropin levels in females after 2 and 6 weeks exposure were not significantly altered in any of the treatment groups. However, after 7 weeks, data suggested aldicarb treatment caused a reduction in somatotropin, although the dose-response was not linear. After 12 weeks treatment, there was "moderate evidence" for a three-way interaction.

Summary

8.105 There has been much interest recently in so-called endocrine modulators, substances present in the environment or ingested in food and water, which affect the activities of the physiologically essential oestrogen and androgen systems in the body, either directly or by a change in genomic imprinting.[87-89]

8.106 The study of endocrine disruption by environmental chemicals, using either *in vitro* or *in vivo* methodology, is fraught with difficulties of reproducibility whether employing similar or different methods or procedures. Ashby and Elliott[90] have pointed out that inter-laboratory variation regarding study outcome is notorious. Positive data are not always reproducible and therefore confirmation of endocrine disruption is difficult. Consequently, the assessment of mixtures of compounds containing weakly estrogenic chemicals at concentrations relevant to low levels of environmental exposure is also difficult.

8.107 There have been very few studies that have investigated the combined functional effects of mixtures of estrogenic compounds. There is no evidence that any of these forms of toxicity failed to follow normal dose-response relationships, despite earlier claims to the contrary, which have now been totally withdrawn on the grounds that the findings were irreproducible and appear to have been fraudulent. Generally, the effects of mixtures of weakly estrogenic compounds, which have included organochlorine pesticides, PCBs and certain weakly estrogenic plant-derived compounds have been shown to be additive or competitively antagonistic. Where synergistic interaction has been claimed, the study design was not adequate to justify such conclusions.[82]

8.108 In terms of assessing the risks of mixtures of endocrine modulating substances, it continues to be appropriate to consider biological data on the activities of the individual compounds, especially by studies of reproductive function and development, and to consider the concentrations likely to occur in subjects potentially liable to be affected because they are at an appropriate stage of development. Assessment of any risk can then be based on standard procedures.

Immunotoxicity

8.109 Immunotoxicology defines the adverse health effects that may result from the interactions of chemicals with the immune system. Such effects may be classified broadly into two main types, immunotoxicity and allergy. In the first, immunotoxicity, exposure results in functional impairment of the immune system. The concern here is that the compromised immune function will translate into reduced host resistance and increased susceptibility to infectious disease and malignancy. The second is allergy, which is associated with stimulation of a specific immune response by a xenobiotic. This results in sensitization. If the now sensitized individual is exposed again to the inducing chemical, then an accelerated and more aggressive secondary immune response may be provoked, resulting in an allergic reaction or, in some instances, autoimmunity.

8.110 Both major classes of immunotoxic action display basic dose-response relationships as are found with other forms of target organ toxicity. However, it must be recognised that chemical allergy, in common with other forms of allergic disease, develops in two phases; induction and elicitation. The levels of exposure required for the effective acquisition of sensitisation (induction) and for the subsequent provocation of an allergic reaction in a previously sensitised subject (elicitation), may be very different. The view is that, in most cases, the amount of chemical required for sensitisation is greater than that required for the elicitation of an allergic reaction in sensitised individuals.

8.111 It is likely that dose-additivity and effect additivity may occur in immunotoxic actions although these processes do not appear to have been investigated directly. The small number of studies which have attempted to evaluate the effects of various mixtures on immune functions, both *in vivo* in animal studies, and *in vitro* using human or animal cells (described above), have shown potential immunomodulatory effects of the mixtures tested. However, studies have generally not assessed the effects of mixture components individually and, thus, conclusions regarding potential interactive effects cannot be made.

8.112 Two additional aspects of allergic sensitisation are relevant when considering the relevance of exposure to mixtures. The first of these is immunologic cross-reactivity when allergic reactions in sensitised subjects can be elicited following exposure to chemicals that antigenically cross-reactive with the inducing allergen.

8.113 The other issue is that of adjuvant-like effects wherein exposure of an antigen together with another substance (that is itself not necessarily immunogenic – an adjuvant) may augment the vigour or modify the quality of induced immune responses. It is well known that the acquisition of skin sensitisation to a chemical allergen is influenced by the vehicle or formulation in which the active chemical is encountered at the skin surface, and in certain circumstances this might be regarded as being an adjuvant-like effect.[91] It is possible also that certain exposure conditions (certain environmental pollutants, for instance) can enhance and/or modulate respiratory immune responses.[92]

8.114 In considering risk assessment of aggregate exposure in relation to effects on the immune system, it appears extremely unlikely that an adjuvant effect would ever be important unless there were very considerable exposure to a limited range of materials. Apart from that still hypothetical exception, it appears appropriate to apply conventional considerations of dose and exposure in assessing the possible immunotoxicity of aggregate exposures, whilst being aware of the potential for a greatly heightened response that may occur in a previously sensitised individual.

8.115 Germolec *et al*[93] carried out studies with mice to evaluate the immunological effects of a complex mixture containing 19 organic and 6 inorganic chemicals representing 25 common contaminants identified by the USEPA as frequently found in contaminated groundwater. Concentrations of each chemical present in the maximum concentration mixture are shown in Table 8.2.

8.116 Female C57BL/6 X C3H mice (B6C3F1) were dosed, via drinking water, with 0.2, 2 and 20% (14 day study) or 1, 5 and 10% (90 day study) solutions of the maximum concentration stock solution, whilst control animals were given deionised water only. A paired-water study was also conducted to parallel the water intake of animals in the high (20%) dose, 14 day group. Following exposure, mice were killed and pathologic and haematologic analyses were performed. A range of immunological tests was also carried out. Treatments had no effects on body, thymus or liver weights, nor were any significant histological changes observed. Slight increases in kidney weights were noted in both the 14- and 90-day groups, but this effect was also seen in the paired-water group. Mice exposed to the high dose for 90 days showed a 15% decrease in spleen weight. Mice treated for 14 days showed no differences from controls in haematological values. However, animals in the 90-day study showed significant, dose-related decreases in haematocrit, mean corpuscular volume, haemoglobin, and mean corpuscular haemoglobulin, consistent with mild microcytic anaemia. Bone marrow analysis showed significant decrease in the number of granulocyte-macrophage progenitor cells in the 20% (14 day) and the 5 and 10% (90 day) treatment groups. Animals in the highest dose (14- and 90-day) groups also showed suppressed antibody response (to sheep red blood cells), but no decrease in spleen cellularity B or T lymphocyte numbers. None of the groups showed effects of exposure to the mixture on parameters of tumour immunity. Host susceptibility tests to *Listeria monocytogenes*, PYB6 syngeneic tumour cells or *Plasmodium yoelii* were carried out. Mice challenged with the parasite, *P. yoelii* showed increased numbers of parasitised red blood cells as compared with control groups. Resistance to challenge with *L. monocytogenes* or PYB6 tumour cells was not affected by mixture treatment. The authors noted that resistance to the latter agents is primarily mediated by macrophages and activated T cells, whilst humoral immunity plays a major role in resistance to malarial parasites. They concluded that the results of this study suggested that long-term exposure to contaminated groundwater may represent a risk to the immune system in humans. As no single-compound studies were carried out, this study did not provide any information on potential interactions of compounds within the mixture tested.

Table 8.2 EPA survey concentrations of groundwater contaminants and composition of a complex chemical mixture representing a contaminated groundwater sample (reprinted from Fundamental and Applied Toxicology, Vol 13, Germolec *et al*, 1989, Toxicology studies of a chemical mixture of 25 groundwater contaminants II. Immunosuppression in B6C3F$_1$ mice, page 379, 1989, by permission of the publisher Academic Press/Elsevier Science).[93]

	Average EPA survey concentrations (ppm)	Maximum EPA survey concentrations (ppm)	Maximum concentrations used in the study (ppm)[a]
Acetone	6.90	250.0	106.00
Aroclor 1260	0.21	2.9	0.02
Arsenic	30.60	3680.0	18.00
Benzene	5.00	1200.0	25.00
Cadmium	0.85	225.0	102.00
Carbon tetrachloride	0.54	20.0	0.80
Chlorobenzene	0.10	13.0	0.20
Chloroform	1.46	220.0	14.00
Chromium	0.69	188.0	72.00
Diethylhexyl phthalate	0.13	5.8	0.03
1,1-Dichloroethane	0.31	56.1	2.80
1,2-Dichloroethane	6.33	440.0	80.00
1,1-Dichloroethylene	0.24	38.0	1.00
1,2-trans-Dichloroethylene	0.73	75.2	5.00
Ethylbenzene	0.65	25.0	0.60
Lead	37.00	31000.0	140.00
Mercury	0.34	50.0	1.00
Methylene chloride	11.20	7800.0	75.00
Nickel	0.50	95.2	13.60
Phenol	34.00	7713.0	58.00
Tetrachloroethylene	9.68	21570.0	6.80
Toluene	5.18	1100.0	14.00
1,1,1-Trichloroethane	1.25	618.0	4.00
Trichloroethylene	3.82	790.0	13.00
Xylenes	4.07	150.0	3.20
Total concentration of all chemicals (ppm)	131.05		756.05

Note

a. The highest dose level of the mixture used in the study was a 1:5 dilution (i.e., 20%) of the technically achievable stock mixture which is not shown.

8.117 Omara *et al*[94] carried out *in vitro* studies of the immunological effects of mixtures containing low levels of methylmercury, polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and Aroclor polychlorinated biphenyls (PCBs). Chemicals were selected by their presence in human food sources and their known ability to cause various biological adverse effects on isolated rat leucocytes. Concentrations tested were based on the following data from an environmental survey of concentrations found in the flesh of fish from the St. Lawrence river, Quebec; methylmercury; 0.1 – 2 μg/g: Arochlor PCB mixtures, composed of Aroclor 1242, 125 and 1260 at a proportion of 3:4:3 by weight; 0.01-0.5 μg/g: PCDD/PCDF mixtures, composed of TCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin, 2,3,7,8-tetrachlorodibenzofuran, and 1,2,3,7,8-pentachlorodibenzofuran at a proportion of 20:5:2:5:80:15 by weight; 1-15 pg/g. Final cell culture concentrations of chemicals and composition of mixtures are shown in Table 8.3.

Table 8.3 Composition and final culture concentrations of chemical mixtures used in studies on rat leukocytes in vitro in the study of Omara *et al*,[94] reprinted with permission from Omara *et al*,[95] Environmental Toxicology and Chemistry 1997: 16: 576-581. Copyright, SETAC, Pensacola, Florida, USA.

Treatment[a]	MeHg[b] (μg/ml)	Aroclor PCB mixtures[c] (μg/ml)	PCDD/PCDF mixtures[d] (pg/ml)
A	–	–	15
B	–	–	1
C	–	0.5	–
D	–	0.01	–
E	0.1	–	–
F	0.1	0.5	15
G	0.1	0.5	1
H	0.1	0.01	15
I	0.1	0.01	1
J	2	–	–
K	2	0.5	15
L	2	0.5	1
M	2	0.01	15
N	2	0.1	1

Notes

a. Media and dimethylsultoxide (DMSO) controls were included

b. MeHg = methylmercuric chloride

c. PCB = polychlorinated biphenyls

d. PCDD = polychlorinated dibenzo-p-dioxins; PCDF = polychlorinated dibenzofurans.

8.118 Mixtures were tested on single-cell suspensions of splenocytes, peritoneal leukocytes and peripheral blood lymphocytes isolated from male Long-Evans rats. Assays were performed for cytolethality and viability, mixed leukocyte response (an assessment of T-lymphocyte-mediated immune function), macrophage phagocytic activity, and natural killer cell cytolethal activity. Significant cytolethality was observed in splenocytes, peritoneal leucocytes and peripheral blood lymphocytes treated with 2 μg/ml MeHg. A similar decrease in viability was observed in cells treated with mixtures containing 2 μg/ml methylmercury, suggesting that the effect was due to methylmercury alone, with no interactive cytolethal effects. Because of this effect, other tests were carried out using a non-cytolethal concentration of methylmercury (0.1 μg/ml). All other tests showed no significant immunotoxic effects of either methylmercury or the various mixtures tested as compared to controls. The authors concluded that the results of this study demonstrated a cytolethal effect of 2 μg/ml methylmercury in rat splenocytes, peritoneal leukocytes and peripheral blood lymphocytes. Previous studies by the same authors had shown significant inhibition of T- and B-cell mitogenic responses with 0.1 μg/ml methylmercury, but no suppression with Aroclor PCB or PCDD/PCDF mixtures, and furthermore no enhancement of the effect of methylmercury by the Aroclor PCB/PCDD/PCDF mixtures, indicating a lack of additive toxicity.[95] However, the authors noted that, as the experiments did not include evaluation of the toxicity of individual chemicals, possible antagonistic interactions (particularly among Aroclor PCB/PCDD/PCDF mixtures) could not be excluded by the results.

8.119 Institorís *et al*[96] examined the immunotoxic effects in male Wistar rats of oral exposure to various concentrations of the type II synthetic pyrethroid pesticide, cypermethrin, alone or in combination with cadmium or lead. Preliminary experiments determined an acute oral LD_{50} of 554 mg/kg bw in 5 week old rats. For subsequent studies, groups of rats were treated by gavage, 5 days per week, for a 28 day period as follows:

Experiment 1: determination of immunotoxic (IT) and non-effective (NE) doses of cypermethrin. Animals were treated with high (55.4 mg/kg bw/day [LD_{50}/10]), middle (22.2 mg/kg bw/day [LD_{50}/25]) or low (11.1 mg/kg bw/day [LD_{50}/50]) doses of cypermethrin. The high and low doses were determined as significantly immunotoxic (IT) and non-effective (NE), respectively. Data from a previous study had determined IT and NE doses of cadmium chloride ($CdCl_2$, 6.43 and 1.61. mg/kg bw) and lead acetate (80.0 and 20.0 mg/kg bw).

Experiment 2: cypermethrin (IT) + $CdCl_2$ (NE); cypermethrin (IT); $CdCl_2$ (IT) + cypermethrin (NE); $CdCl_2$ (IT); vehicle control.

Experiment 3: cypermethrin (IT) + lead acetate (NE); cypermethrin (IT); lead acetate (IT) + cypermethrin (NE); lead acetate (IT); vehicle control.

Subsequent analyses were carried out for general toxicological and haematological parameters. Immunological parameters assessed were IgM-plaque forming cell (PFC) assay and delayed type hypersensitivity, which was examined by footpad swelling assay. IT doses of cypermethrin, cadmium and lead all caused significantly reduced relative liver weights. Combinations of cypermethrin (NE)

with either cadmium (IT) or lead (IT) caused a further significant increase as compared to cadmium (IT) or lead (IT) alone. In immune function assays, the authors reported that no evaluable changes of the PFC count were observed (results were not shown). In the delayed type hypersensitivity (DTH) test, all three doses of cypermethrin significantly decreased the maximum DTH reaction in experiment 1. This immunomodulatory effect of cypermethrin was altered inconsistently by concurrent exposure to cadmium or lead, but there was no evidence of potentiation.

8.120 Gauthier *et al*[97] studied the effects of camphechlor on isolated human neutrophils. Tests with camphechlor alone (the complete complex mixture over a dose range from 0.1-50 μg/ml) showed that the mixture induced a dose-dependent increase in neutrophil superoxide production, an increase in neutrophil phagocytosis and a dose-dependent increase in the numbers of apoptotic neutrophils after 24 hours. Studies were also carried out with T_2 and T_{12}, two environmentally prevalent congeners of the mixture. Both congeners, when tested singly, induced a significant increase in neutrophil superoxide production (which was substantially lower than that induced by camphechlor itself). A mixture of T_2 and T_{12} also induced neutrophil superoxide production, although the effects were similar, or slightly lower than those obtained with either congener alone, showing no additive or synergistic effects.

8.121 In summary, the small number of studies that have attempted to evaluate the effects of various mixtures on immune functions, both *in vivo* and *in vitro* using human or animal cells, have shown potential immunodilatory effects of the mixture tested. However, studies have generally not assessed the effects of mixture components individually and thus conclusions regarding potential interactive effects cannot be made.

Cytotoxicity in vitro

Isolated hepatocytes

8.122 The cytotoxicity of cadmium chloride and chloroform, alone and in combination, was investigated in isolated rat hepatocytes.[98] One hour incubations were carried out in the presence of 25, 50 or 100 μM cadmium chloride or 15, 30 or 60 mM chloroform, individually, or in the presence of both chemicals simultaneously, in all possible combinations of these concentrations. Exposure of hepatocytes to the two chemicals simultaneously resulted in greater cytotoxicity than that observed with each chemical alone (as assessed by loss of intracellular potassium and aspartate aminotransferase). Furthermore, cytotoxic effects of the combination were observed at concentrations where the individual chemicals alone had failed to elicit a response, although the lactate/pyruvate ratio (an index of disrupted cellular metabolism) was less consistently affected. The nature of the combination effect could not be determined from this data because the study was undertaken at the high end of the dose response curve.

8.123 Many studies have investigated the toxic effects of combinations of chlorinated solvents *in vitro*. For example, the cytotoxicity of carbon tetrachloride and chloroform was investigated, alone and in combination, in isolated rat hepatocytes.[91] Concentration-response curves were determined for both compounds individually. For interaction studies, concentrations of carbon tetrachloride (1.0, 2.5 and 5.0

mM) and chloroform (5.0, 10.0 and 25 mM) were chosen that, respectively, gave a moderate response (50-60% lactate dehydrogenase (LDH) leakage, 0-20% initial potassium ion concentration), a minimal response (20-30% LDH leakage, 60-80% initial potassium ion concentration) and no effect (same as control). All possible combinations were evaluated. Data were analysed using response surface methodology. It was stated that analysis suggested that, in combination, the cytotoxicity of carbon tetrachloride and chloroform was greater than additive.

HeLa Cells

8.124 Malich *et al*[100] compared the cytotoxicity of binary and ternary chemical mixtures of four structurally different chemicals (cupric sulfate, mercuric chloride, phenol and xylene) in HeLa cells, using the colorimetric MTS assay. Binary and ternary mixtures were composed in any combination of the four test chemicals. In addition, each combination was prepared at different ratios of concentration so as to obtain information on the effective potency of each chemical within the mixture. The study was performed blind. IC_{50} values were determined for each mixture. Experimental data were compared and set against data predicted by a mathematical algorithm that did not account for synergism or antagonism. The data indicated mainly additive effects of the individual components in the mixture. However, some less than additive (antagonistic) and some more than additive effects were also identified, the latter even at low doses.

Ciliate protozoon *(Colipidium campylum)*

8.125 Bonnemain and Dive[101] studied the toxicity of seven dithiocarbamates and reported the occurrence of apparently complex interactive effects with copper on the growth of the ciliate protozoon (*Colipidium campylum*, Stokes). Cultures were exposed to propineb, mancozeb, nabam, thiram, metiram, ferbam or ziram at four concentrations (0.01, 0.1, 1 and 10 mg/l) in the presence, or in the absence, of copper sulfate pentahydrate (0.089 or 0.178 mg/l). Propineb and ziram were toxic at a concentration of 10 mg/l but not below. Metiram, nabam and ferbam were lethal at 10 mg/l but were non-toxic below this concentration. Thiram and mancozeb were lethal at 1 mg/l but no effect was observed at 0.1 mg/l. Experiments to study interactions with copper were performed at sub-lethal pesticide concentrations. Copper itself appeared to have a small but reproducible adverse effect on growth at the higher concentration. The authors reported no or only slight interactions between copper and propineb or mancozeb. At non-toxic levels of ziram or ferbam (0.1 mg/l) there was, respectively, 70% and 90% inhibition of growth in the presence of the lower (non-toxic) concentration of copper. However, 100% lethality was observed in the presence of the higher copper concentration. At pesticide concentrations of 1 mg/ml, the enhancing effect of the copper was reduced and no lethal effect was observed even at the higher copper concentration. With nabam and metiram at concentrations of 0.1 mg/l and thiram at 0.01 mg/l, the effects of copper and pesticide were described as additive. With higher pesticide concentrations, there were apparent synergistic effects between thiram and low dose copper and nabam or thiram and high dose copper.

Genotoxicity

8.126 A reasonable amount of data are available from genotoxicity studies of mixtures of pesticides and/or herbicides. Some studies have suggested that concentrations of such compounds at or above those representative of the levels which may occur within human foodstuffs, or as groundwater contaminants, may produce effects (such as clastogenic effects) when tested as combined mixtures. Many studies have not, however, tested individual compounds alone and so do not provide useful information on which chemicals within the mixtures tested may be of particular concern, or on any interactions that may occur between mixture components. Studies reported by Dolara *et al*[102] and Piatti *et al*[103] suggest that benomyl may have been the component responsible for the genotoxic effects produced by the mixtures assessed in these studies, although the (limited) data available did not suggest any potentiating effects of benomyl with any of the other chemicals tested.

8.127 Studies involving triazine herbicides suggested that some of these compounds may produce genotoxic effects both alone and in combination with other compounds, but no additive or potentiating effects were reported.[104,105]

8.128 A small number of genotoxicity studies using bacterial mutagenicity tests have been carried out with mixtures of model compounds, chosen for their well-established direct or indirect genotoxic properties. Studies with the direct-acting mutagens, N-methyl-N'-nitro-N-nitrosoguanidine + ethylmethane sulfonate, demonstrated clear, linear dose-responses for single compounds, whilst mixtures of the two compounds showed a linear dose-response with effects very closely approximating those predicted by additivity.[106] Studies with mixtures of pentachlorophenol and 2,4,6-trinitrotoluene (TNT), suggested inhibition of benz(a)pyrene mutagenicity by TNT. The authors speculated that this phenomenon might be caused by limitation by TNT of the capacity for benz(a)pyrene to access DNA.[108]

Cytogenetic aberrations

8.129 Kligerman *et al*[109] analysed two simulated groundwater contamination mixtures for cytogenetic toxicity in rodents. The mixtures tested were as follows:

California chemical mixture (CCM): aldicarb 9 ppm, atrazine 0.5 ppm, dibromochloropropane 0.01 ppm, 1,2-dichloropropane 4.5 ppm, ethylene dibromide 0.9 ppm, ammonium nitrate 10,000 ppm, simazine 0.3 ppm.

Iowa chemical mixture (ICM): alachlor 0.9 ppm, atrazine 0.5 ppm, cyanazine 0.4 ppm, metribuzin 0.6 ppm, metolachlor 0.4 ppm, ammonium nitrate 10,000 ppm.

8.130 Concentrations of chemicals in the mixtures were based upon median survey values in groundwater under normal agricultural use in the USA (except for nitrate, which was calculated to reflect expected exposure levels). Dosing solutions 10- and 100-fold above the stated mixture concentrations were also tested. Animals (male Fischer 344 rats, female C57B1/6 X C3H (B6C3F1) mice) were dosed with test

solutions, given in drinking water, for 71 days (rats) or 91 days (mice), following which animals were killed. Cultured lymphocytes were analysed for sister chromatid exchange (SCE), chromosomal aberration (CA) and micronucleus (MN) frequencies. The CCM caused a statistically-significant, dose-related increase in SCE frequency of splenocytes at all mixture concentrations in rats, with only the 100-fold mixture positive in mice. There were no consistent, significant effects on CAs or on MN frequency in rats or mice. The ICM was negative in mice, whilst studies with rats were not carried out. In their conclusions the authors noted that mixtures containing pesticides at ppb levels could produce cytogenetic effects, but that there was no consistent evidence for clastogenicity. The increase in SCE frequency suggested potential genotoxicity of components of the CCM. A literature review suggested that 1,2-dichloropropane, 1,2-dibromo-3-chloropropane and ethylene dibromide were likely candidates in producing these results. However, as dose-response curves for individual chemicals were not established, it is not possible to draw any conclusions regarding potential interactions of mixture components from these data.

8.131 Atrazine and linuron are herbicides found in USA groundwater samples. Some studies have suggested that the triazine herbicide, atrazine, may be mutagenic and carcinogenic. Studies of the phenylurea herbicide, linuron (a group C or possible human carcinogen according to the USEPA) showed no effect in a two year rat-feeding experiment at levels up to 125 ppm. Doses of 625 ppm caused growth depression and increased erythropoiesis associated with red blood cell haemolysis, although there was no evidence of carcinogenicity. One study showed that pretreatment with linuron caused a decrease in AChE activity in Wistar rats subsequently treated with parathion-methyl, suggesting that linuron may increase the toxicity of other agents (Roloff *et al*[109] and references therein). Roloff *et al*[109] carried out *in vitro* and *in vivo* chromosome aberration studies of low concentrations of linuron, alone or in combination with non-clastogenic concentrations of atrazine. In studies *in vivo*, female ICR mice were treated for 90 days with water containing either 20 μg/ml atrazine, 10 μg/ml linuron, or 10 μg/ml atrazine + 5 μg/ml linuron. Cyclophosphamide was used as a positive control. After the treatment period, animals were killed and harvested bone marrow cells were analysed for chromosomal aberrations (CAs). Dividing bone marrow cells (including the positive control) did not show any significant increase in CAs, as compared with negative controls. However, the authors reported that cultured spleen cells from all treated cultures showed increased chromosome breakage for all treatments compared to controls, with all but linuron producing damage that was statistically significant ($P < 0.05$). The cyclophosphamide and linuron groups also showed significantly increased spleen weights and splenic indices, whilst linuron-treated mice showed significantly increased liver weights. *In vitro* studies were carried out on isolated human peripheral blood lymphocytes. Cultured cells were treated with either 0.001 μg/ml atrazine, 1 μg/ml linuron, or 0.0005 μg/ml atrazine + 0.5 μg/ml linuron and then scored for CAs. The authors stated that "synergism was determined following the method in the National Research Council's committee on methods for toxicity testing (1988) where the expected damage from individual treatments is equal to the observed damage minus the background damage. The expected combination (observed damage of the combination minus background) is compared to the sum of the individual expected frequencies of damage." Neither linuron nor atrazine alone caused a significant increase in CA frequency. The combination of the two compounds resulted in a significant increase in chromosome breakage ($P < 0.01$), suggesting an additive model of joint action.

8.132 Dolara *et al*[110] evaluated the effects of dimethoate and omethoate (two OP insecticides), deltamethrin (a synthetic pyrethroid insecticide) and benomyl (a systemic fungicide) on SCE frequency in human peripheral lymphocytes *in vitro*. When tested individually, dimethoate (approximate test range 25-75 μg/ml) and omethoate (approximate test range 20-110 μg/ml) produced significant, dose-related increases in SCE frequency, whilst weak, but non-significant increases were seen with deltamethrin (approximate test range 5-55 μg/ml) and benomyl (approximate test range 0.5-5 μg/ml). Mixtures of the four compounds, containing concentrations of each individual chemical selected as those inducing SCE frequency in the range of control means (total mixture concentrations 41.5 and 83 μg/ml, in each case 43% dimethoate, 43 % omethoate, 12% deltamethrin, 1.2% benomyl) produced significant, dose-dependent increases in SCE frequency. The authors noted that these experiments demonstrated that sub-threshold doses of pesticides may increase SCEs when present in a mixture. They commented that the mechanism of the observed increase in SCEs is not known, but speculated that one possibility may be that interference of the orderly assembly of genetic material during mitosis by benomyl may render cells more susceptible to small doses of other genotoxic compounds. From these data, It is not possible to determine if the combined effect represented additivity or synergy.

8.133 Dolara *et al*[111] evaluated the genotoxic effects of a mixture of 15 pesticides, based on analyses of residues found in common foods in central Italy (those which were found to occur at a calculated dose/day of $\geq$ 1 μg). Pesticide mixtures contained each of the 15 compounds in the relative proportions as calculated for the daily intakes (Table 8.4).

8.134 Bacterial mutagenicity studies showed no mutagenic activity of the mixture at concentrations of 500 μg/plate in different strains of *S. typhimurium*, with or without metabolic activation. *In vitro* tests were also carried out to assess the effects of the mixture on SCE in cultured human lymphocytes. At 1 μg/ml pesticide mixture, a small, statistically significant increase in SCE frequency was observed, compared with controls. This effect was not, however, noted using higher concentrations of the mixture (up to 20 μg/ml). *In vivo* studies were carried out to assess the effects of the mixture on MN frequency in rat bone marrow. None of the pesticide mixture doses tested (100-fold, 1000-fold and 10,000-fold higher than the estimated exposure in humans) caused a significant increase in MN frequency. The authors concluded that the pesticide mixture tested (representing that in the Italian diet) does not have a clear-cut genotoxic effect, although they noted that the overall effect of the mixture was consistent with an inhibitory action of some compounds on the mutagenic activity of some others (by comparison with previously-reported data regarding single-compound effects). However, as this study did not include analysis of the genotoxic effects of single compounds, it is not possible to draw any direct conclusions regarding potential interactions between the compounds tested.

8.135 Dolara and colleagues[102] reported further cytogenetic studies using a mixture of 15 pesticides commonly found in foods of central Italy, with percentages of each pesticide in the mixture proportional to the average concentrations observed in foods (see above paragraph; Table 8.4). Concentrations of 1-20 (g/ml pesticide mixture did not induce significant variations in ploidy in human lymphocytes *in vitro*, or in the number of chromosome aberrations. However, a significant, dose-dependent increase was observed in the number of non-synchronous centromeric separations. This

effect was not observed when benomyl was excluded from the mixture (although apparently only one mixture concentration, approximately 15 μg/ml, was tested without benomyl). The authors noted that removal of other pesticides from the mixture did not affect the effects of the mixture as a whole (data were not reported). Analysis of the effects of benomyl alone, at concentrations up to 8 μg/ml, also showed an increased frequency of nonsynchronous centromeric separations (concentrations of benomyl present in the 1-20 μg/ml test mixture were approximately 0.2-3.9 μg/ml). The authors considered that the elimination of benomyl from use on food products may reduce the potential of common pesticide residues to cause genotoxic effects. (It may be worth noting that benomyl shows a threshold effect).

Table 8.4 Pesticide residues analysed in foods of central Italy; generic names are used; the minimum detected levels are given in ppb (from Dolara *et al*, 1993, table 1, Cell Biology and Toxicology, Vol 9, 1993, pp 333-343, by kind permission of Kluwer Academic Publishers, Dordrecht, the Netherlands, and the authors).[111] The spelling and identity of some of the pesticides is clearly incorrect in some cases; unless totally clear the names have been left as in the cited paper.

Compound	Concentration	Compound	Concentration
2,4-DDD	10	Carbofenothion	5
2,4-DDT	10	Chlorfenvinphos	10
2,4-DMA	200	Chlorpyrifos	10
4,4-DDD	10	Chlorpropham	200
4,4-DDE	10	Chlorpyriphos-methyl	5
4,4-DDT	10	Chlorothalonyl	10
Acephate	10	Chlorthion	5
Aldrin	10	Cyanophos	5
Anilazine	100	Deisopropyl	20
Atrazine	20	Desmethrin	50
Azinphos	20	Diazinon	5
Azinphos-ethyl	20	Dichloflunid	200
Benalaxyl	200	Dichlorvos	5
Benfluralin	10	Dieldrin	10
Benomyl	100	Dimethoate	10
Binapacryl	500	Diphenylamine	50
Bitertanol	200	Disulfoton	10
Bromophos-ethyl	5	Ditalimfos	5

Compound	Concentration	Compound	Concentration
Dithiocarbamates	400	Naled	10
Endosulphan α	10	Omethoate	10
Endosulphan β	10	Paraoxon	10
α-Esachlorcyclohexane	10	Paraoxon-methyl	10
Ethion	5	Parathion	5
Fenarimol	200	Parathion-methyl	5
Fenchlorphos	5	Pendimethalin	10
Fenthoate	10	Phenitrothion (presumably fenitrothion)	5
Fonofos	5	Phenmedipham	500
Formothion	10	Phention	5
Fosfamidone	10	Phorate	5
Heptachlor	10	Phosalone	10
Heptachlor epoxide	10	Pirimicarb	50
Heptenophos	5	Pirimiphos-ethyl	5
Imazalil	100	Pirimiphos-methyl	10
Iprodione	250	Procymidone	200
Lenacyl	200	Promecarb	50
Lindane	10	Promethon	50
Linuron	200	Promethrin	20
Malaoxon	10	Propazine	20
Malathion	5	Propham	200
Methylbenzimidazoyl carbamate	100	Prophenofos	10
Metalaxil	200	Propiconazole	100
Methamidophos	5	Propoxur	200
Methidathion	5	Pyrazophos	10
Methomyl	200	Quinalphos	5
Methoprotryn	50	Simazine	20
Mevinphos	5	Thiabendazole	100
Monocrotophos	10	TEPP	10

Compound	Concentration	Compound	Concentration
Terbumethon	20	Trichlorfon	20
Terbuthrin	20	Trifluralin	10
Tetrachlorvinfos	10	Vamidothion	50
Triadimefon	100	Vinclozolin	10

8.136 The genotoxicities, separately and in combination, of 5 pesticides (benomyl and the organophosphate insecticides, azinphos-methyl, diazinon, dimethoate and pirimiphos-methyl) were evaluated using a micronucleus (MN) test with cultured human peripheral lymphocytes.[112] Concentrations of compounds tested were calculated as representative of the estimated daily intake (EDI), calculated from the concentrations of the compounds in foodstuffs. The concentrations used were benomyl 1 μg/ml, azinphos-methyl 0.06 μg/ml, diazinon 0.04 μg/ml, dimethoate 0.1 μg/ml, pirimiphos-methyl 0.15 μg/ml. Concentrations 10 and 100 times higher were also tested. The following mixtures (and equivalent 10- and 100-fold concentrations of each mixture) were also tested;

Mixture 1 — 1 μg/ml benomyl + 0.15 μg/ml pirimiphos-methyl

Mixture 2 — 0.06 μg/ml azinphos-methyl + 0.04 μg/ml diazinon + 0.1 μg/ml dimethoate

Mixture 3 — 0.06 μg/ml azinphos-methyl + 1 μg/ml benomyl + 0.04 μg/ml diazinon + 0.1 μg/ml dimethoate + 0.15 μg/ml pirimiphos-methyl

8.137 When tested singly, all compounds except pirimiphos-methyl induced a low, significant elevation of MN frequency compared to controls, but these were not clearly dose-related. Mixtures similarly showed significant responses, which were not dose-related in the case of mixture 2 and were not clearly so with the other two mixtures. The authors concluded that the data indicated that none of the compounds tested, either alone or in the mixture, showed a dose-related response and that the various mixtures did not give additive or synergistic effects. They also suggested that the low increase in MN frequency induced by the pesticides, both alone and in mixtures, may be due to the absence of metabolic activation.

8.138 Piatti *et al*[103] assessed the pesticides benomyl and pirimiphos-methyl, separately and in combination, for genotoxic effects in a micronucleus (MN) assay, using primary cultured hepatocytes isolated from Sprague-Dawley, male albino rats. Dose ranges were selected on the basis of low cytotoxicity in preliminary assays. In single-compound tests benomyl (0.5 to 25 μg/ml) induced a significant, dose-related increase in MN frequency, whilst pirimiphos-methyl (0.8 to 50 μg/ml) was negative at all doses tested. Mixtures of benomyl:pirimiphos-methyl (at a ratio of 6:1, cited as the ratio frequently observed in foodstuffs) (dose ranges from 0.5 μg/ml + 0.08 μg/ml to 25 μg/ml + 4.2 μg/ml) showed progressive enhancement of MN frequency similar to that of benomyl alone, suggesting that there was no interaction between the two compounds at this ratio.

8.139 Guigas *et al*[104] examined the effects of the plant flavonol, quercetin, and the triazine herbicides atrazine, cyanazine, and "Gesamprim"[a], individually and in combination, using SCE and gene mutation (HPRT) assays with Chinese hamster ovary (CHO) cells. There was no evidence of an increased SCE rate. The test substances caused a slightly increased mutation rate in the HPRT assay after metabolic activation. However, the authors reported that combination studies with 2 or 3 of the test substances did not result in higher mutation rates than those observed for the individual compounds tested singly.

8.140 Taets *et al*[105] reported a study of the single and combined effects of three triazine herbicides, atrazine, simazine and cyanazine (described as the most frequent herbicide contaminants identified in a study of USA groundwater samples), on levels of whole cell and chromosomal damage in cultured Chinese hamster ovary (CHO) cells. Each compound was tested at 2 dose levels;

- maximum contamination levels (MCLs) set by the USEPA (3 ppb atrazine, 3ppb cyanazine [this arbitrary level was chosen as there is no established MCL], 1 ppb simazine)

- maximum levels observed in a study of USA groundwater samples (18 ppb atrazine, 12 ppb cyanazine, 4 ppb simazine).

8.141 Compounds were tested singly or in binary and ternary combinations of the lower or higher doses. As compared with controls, in single compound studies atrazine and simazine showed significant increases in whole cell clastogenicity (both doses), whilst only atrazine was positive for chromosomal damage. No significant increases in either effect were seen with either doses of cyanazine. In binary studies, combinations (at both lower and higher doses) of atrazine with simazine or cyanazine were positive for whole cell clastogenicity and chromosomal damage. Simazine/cyanazine mixtures did not show significant genotoxicity, apart from the high dose mixture, which was positive for whole cell clastogenicity. The high (but not low) dose ternary mixture produced a significant increase in whole cell clastogenicity, but this mixture showed no effects at either dose on chromosome aberration levels. In cases where mixtures showed a significant effect, as compared with controls, the magnitude of none of these effects was significantly different from those of either of the single compounds tested at the same dose, suggesting lack of additive effects or potentiation.

[a] This is stated to be a commercial preparation of atrazine, but this trade name is not listed in the Pesticide Manual. Gesaprim is, as a mixture with terbutryn (Novartis).

Oxidative DNA damage

8.142 Lodovici *et al*[113] evaluated the quantitative effects of pesticide mixtures on oxidative damage in the rat liver. Animals (male Wistar rats) were fed for 10 days with doses of 1 mg/kg bw/day pesticide mixture (a proportionate mixture of the 15 pesticides commonly found in Italian foods, as reported by Dolara *et al*, 1993; see Table 8.4). Oxidative damage was assessed by measuring the levels of 8-hydroxy-2-deoxyguanosine relative to 2-deoxyguanosine in DNA. Subgroups of pesticides were also tested, as follows:

Mix 1: dithiocarbamate 0.207 mg/kg bw/day, benomyl 0.196 mg/kg bw/day.

Mix 2: procymidone 0.08 mg/kg bw/day, methidathion 0.023 mg/kg bw/day, chlorpyrifos 0.02 mg/kg bw/day, parathion-methyl 0.01 mg/kg bw/day, chlorpropham 0.007 mg/kg bw/day, parathion 0.007 mg/kg bw/day, vinclozolin 0.003 mg/kg bw/day, chlorfenvinphos 0.003 mg/kg bw/day, pirimiphos-ethyl 0.001 mg/kg bw/day.

Mix 3: thiabendazole 0.149 mg/kg bw/day, fenarimol 0.02 mg/kg bw/day, diphenylamine 0.140 mg/kg bw/day, chlorothalonil 0.130 mg/kg bw/day.

8.143 Liver DNA levels of 8-hydroxy-2-deoxyguanosine were significantly increased after treatment with mix 3, but not mix 1 or mix 2. Components of mix 3 were also tested individually, at the same dose as in the mixture. Diphenylamine and chlorothalonil, but not thiabendazole or fenarimol, significantly increased 8-hydroxy-2-deoxyguanosine levels. Further analysis of dose-response curves for diphenylamine and chlorothalonil showed that the effects were dose-related in both cases. The minimum doses at which oxidative DNA damage was observed were 0.13 and 0.09 mg/kg bw/day, for diphenylamine and chlorothalonil, respectively, considered by the authors to be approximately 1/100 the calculated human exposure through food in Italy. The authors concluded that diphenylamine and chlorothalonil may generate reactive oxygen species capable of inducing cell genetic damage, and that elimination of these compounds from use on crops destined for human consumption would reduce the risk of inducing DNA oxidative damage through ingestion of residues in foods. The study did not directly address the possibility of interactions between pesticides within mixtures. However, the data reported suggested that the magnitude of oxidative DNA effects observed with mix 3 (containing 0.14 mg/kg bw/day diphenylamine and 0.13 mg/kg bw/day chlorothalonil) was not greater than that observed with either of the two compounds tested separately at these doses, indicating no obvious additivity.

Plant clastogenicity assays

8.144 Gill and Sandhu[114] compared the genotoxic activities of 3 chemicals commonly found as pollutants at industrial waste sites (arsenic trioxide, dieldrin and lead tetraacetate), and also their 9 binary and 1 ternary mixtures using the *Tradescantia* micronucleus (Trad-MN) assay. Single chemicals were applied either by addition to soil (at 4 mg/kg) or directly to plant cuttings in aqueous solution (at 4 μg/ml). Binary mixtures contained ratios of 1:1, 2:1 or 1:2 of the 2 chemicals tested, whilst ratios in the ternary

mixture were 1:1:1. The total concentration of all chemicals in binary and ternary mixtures was 4 mg/kg in soil and 4 μg/ml in aqueous solution. Solvent (negative) and 10 ppm maleic hydrazide (positive) were used as controls. Statistical analysis was carried out using Dunnett's *t* test. Arsenic trioxide and dieldrin induced significant increases in MN frequency when tested in both aqueous and soil media, whilst lead tetraacetate induced a significant increase only when tested in soil. Results with mixtures were as follows:

- arsenic trioxide:lead tetraacetate: 1:1 and 1:2 mixtures induced significant MN increase when tested in soil.
- dieldrin:lead tetraacetate: 2:1 mixture induced significant MN increase when tested in aqueous and soil media.
- arsenic trioxide:dieldrin: 2:1 mixture induced significant MN increase when tested in aqueous medium.
- arsenic trioxide:dieldrin:lead tetraacetate: equimolar mixture in aqueous solution induced a significant increase in MN frequency.

8.145 The variable results suggested possible potentiating and/or antagonistic effects of the compounds tested, dependent on the medium in which the compounds were applied and also the relative mixing concentrations. However, as dose-response curves were not available for individual compounds it is not possible to draw any firm conclusions from these data.

DNA adducts

8.146 Howard and Beland[115] investigated the effect of pyrene on the metabolism and the DNA binding of 1-nitropyrene, and the effect of pyrene and 1-nitropyrene on the metabolism and DNA adduct formation of 1,6-dinitropyrene in male B6C3F1 mice.

8.147 Metabolism studies showed that pyrene was a mixed-type metabolic inhibitor *in vitro* but was found not to affect the excretion of 1-nitropyrene (single i.p. dose of 10 nmol) *in vivo* when co-administered to mice at either a 20-fold or 250-fold molar excess. However, at the high dose, the urinary excretion of 1-nitropyrene metabolites was decreased by 20% and faecal excretion increased by the same extent. Treatment-related DNA adducts could not be detected by ^{32}P-postlabelling analyses of liver DNA when 1-nitropyrene was administered either by itself or with a 20- or 250-fold molar excess of pyrene. Co-administration of pyrene or 1-nitropyrene had no effect on the total excretion of 1,6-dinitropyrene metabolites. A single major adduct that co-eluted with N-(dexoyguanosin-8-yl)-1-amino-6-nitropyrene was detected in hepatic DNA from mice treated with 1,6-dinitropyrene, the concentration of which was significantly decreased by co-administration of a 25-fold molar excess of pyrene and significantly increased by simultaneous treatment with a 25-fold molar excess of 1-nitropyrene.

Bacterial mutagenicity assays

8.148 Salamone *et al*[106] carried out Ames genotoxicity tests with single and binary combinations of model compounds, benzo[a]pyrene + benzo(rst)pentaphene (promutagens), and N-methyl-N'-nitro-N-nitrosoguanidine + ethylmethane sulfonate (directly-acting mutagens). Testing of the promutagen binary mix (1 μg benz(a)pyrene, 2 μg benzo[rst]pentaphene per plate) showed no additive effects in comparison to those calculated from dose-response curves for the individual compounds. The two direct-acting mutagenic compounds, when tested individually (dose ranges, 0.1-0.5 μg/plate N-methyl-N'-nitro-N-nitrosoguanidine, 0.2-1.0 μg/plate ethyl methane sulfonate, *Salmonella typhimurium*, strain TA100, no metabolic activation), both showed linear dose responses. Testing of a binary combination of the two compounds within the same dose ranges as those tested singly also showed a linear dose-response curve which very closely approximated the theoretical additivity curve, indicating that the mutagenicity of the mixture was equal to the sum of the activities of each component.

8.149 Donnelly *et al*[107] assessed the mutagenic response of 3 model compounds, benzo(a)pyrene, pentachlorophenol and 2,4,6-trinitrotoluene (TNT), singly and in binary and ternary mixtures, using 3 bacterial strains in the *Salmonella typhimurium* microsome assay. *Salmonella typhimurium* tester strains TA98, TA97a and TA100 were used, with and without metabolic activation. Single chemicals were tested at 5 dose levels; (benz(a)pyrene and pentachlorophenol at 0.5, 1, 2.5, 5 and 10 μg/plate; TNT at 2, 20, 50, 120 and 240 μg/plate), whilst mixtures contained combinations of chemicals at doses within the above ranges. Benz(a)pyrene induced a dose-dependent positive mutagenic response in all strains, only in the presence of metabolic activation. TNT induced a dose-dependent mutagenic response in all strains without metabolic activation, whilst this effect was reduced in the presence of metabolic activation. Pentachlorophenol was negative, both with and without metabolic activation. Results with mixtures of pentachlorophenol/benz(a)pyrene and pentachlorophenol/TNT were comparable to those obtained with benz(a)pyrene and TNT alone, respectively. However, benz(a)pyrene/TNT mixtures did not show the predicted additive response; in the presence of metabolic activation this mixture induced lower maximal response than benz(a)pyrene alone at the equivalent dose. Almost complete inhibition of the mutagenic response of benz(a)pyrene by TNT was observed in strain TA98 (in the presence of metabolic activation). Titration of the response of mixtures of benz(a)pyrene/TNT, with increasing doses of TNT showed increasing inhibition of benz(a)pyrene mutagenicity. Ternary mixtures of pentachlorophenol/benz(a)pyrene/TNT also showed responses that were less than additive with respect to individual responses of each of the components of the mixture. In the absence of metabolic activation the ternary mixture induced a response that was lower than that of TNT alone, whilst the response with activation was less than that observed with benz(a)pyrene alone.

The authors of this report concluded that:

- The bacterial mutagenicity of benz(a)pyrene was reduced or eliminated in the presence of TNT.
- In a ternary mixture with all 3 chemicals, inhibition was also observed, although the effect was not as great as was observed for the binary mixtures.

- The addition of increasing concentrations of TNT produced increasing inhibition of benz(a)pyrene mutagenicity.

- The exact mechanism of inhibition of benz(a)pyrene mutagenicity by TNT is unknown at the present time. The data suggest that an interaction in the presence of TNT limits the concentration of benz(a)pyrene which is capable of reaching or binding with bacterial DNA.

Multiple Endpoints

8.150 Jonker *et al*[116] investigated the individual and combined effects of sodium metabisulfite, mirex, loperamide, metaldehyde, di-*n*-octyltin dichloride, stannous chloride, lysinoalanine and potassium nitrite, in male and female Wistar rats (n = 10/group for each sex) for 4 weeks. Potassium nitrate was administered in the drinking water and the other compounds in the feed. Combination treatment was at the predetermined LOAEL, NOAEL, 1/3 NOAEL and 1/10 NOAEL for each individual chemical. Treatment with individual chemicals was at the predetermined LOAEL. Control animals received standard diet and normal drinking water. Individual treatment-related effects included growth retardation, reduced water and food intake, reduced food/body weight conversion efficiency, decreased haemoglobin and albumin, increased relative testes and thyroid weights, increased liver weights, swollen and vacuolated hepatocytes, hyperplasia and hyperkeratosis of the forestomach and reduced weight and lymphoid depletion of the thymus. More severe effects (including growth retardation, reduced food intake and liver damage) and less severe effects (including changes in weight and morphology of the thymus less than with di-*n*-octyltin dichloride) were observed with the combined treatment at the LOAEL compared to those observed following treatment with the compounds individually at the same dose. Furthermore, some adverse effects found with the combined treatment at the LOAEL (decreased prothrombin time, increased plasma alanine aminotransferase activity and aspartate aminotransferase activity, increased kidney weight, reduced number of corpus lutea, increased multinucleated giant cells in the epididymides [many effects related to severe growth retardation]) had not been observed with the individual treatments at the LOAEL or in previous dose ranging studies (details not provided) at levels higher than the LOAEL, indicating that component interactions had occurred at this exposure level. In the NOAEL combined treatment group, some minor adverse effects were observed (slight decreases in haemoglobin levels and slight increases in relative kidney weight in male animals). No treatment-related adverse effects were seen in the lower dose groups.

8.151 Groten *et al*[117] investigated the effects of a combination of nine chemicals with different target organ toxicity and/or different modes of action. Male Wistar rats (8 animals/group) were simultaneously exposed to dichloromethane, formaldehyde, aspirin, DEHP, cadmium chloride, stannous chloride, BHA, loperamide and spermine, at concentrations predetermined in the same laboratory to be equal to each chemical's individual LOAEL, NOAEL or NOAEL/3, for four weeks. Exposure to the dichloromethane and formaldehyde was by inhalation (6 hours/day, 5 days/week). The remaining compounds were included in the diet. Control animals received standard diet and breathed fresh air. Sixteen satellite groups (5 animals/group) were exposed to various combinations of five chemicals, all at the LOAEL, in

a 2 level factorial design with 9 factors (i.e.1/32 x 2^9 groups).[a] The combinations of chemicals were chosen such that the results would allow analysis of interactions between the 9 chemicals and also allow optimal analysis between main effects of the individual compounds. Standard toxicology endpoints were assessed including clinical biochemistry, haematology, biochemistry and pathology. Statistical analysis of all parameters, except those related to pathology, was first by one way analysis of (co)variance followed by Dunnett's multiple comparison. Data from satellite groups were analysed for possible interactions by performing factorial analysis (forward selection/stepwise regression analysis).

8.152 For the complete combination, several effects on haematology, and clinical chemistry parameters were encountered at the LOAEL, along with hyperplasia of the transitional epithelium and/or squamous metaplasia of the respiratory epithelium in the nose. A few minor changes were found at the NOAEL (decreased plasma alkaline phosphatase activity and plasma triglyceride levels, increased relative kidney weight, histopathological changes in the liver and nasal cavity) and at the NOAEL/3 (decreased bilirubin, increased relative kidney weight). In the satellite experiment, factorial analysis revealed main effects of the individual compounds and interaction (non-additivity) between compounds for some effects (namely: interaction between BHA and DEHP resulting in decreased palmitoyl CoA activity; interactions between cadmium chloride and loperamide and between stannous chloride and cadmium chloride caused more than and less than expected increase in plasma aspartate aminotransferase activity, respectively).

8.153 The authors concluded that simultaneous exposure to these chemicals did not constitute any evidently increased hazard compared to exposure to each of the chemicals separately, provided the exposure level of each chemical in the mixture was at most similar or less than its own NOAEL.

8.154 The herbicides alachlor, atrazine or picloram, were administered to male ICR mice (n = 40 per group) in the drinking water, either individually, as one of three binary combinations or as a ternary combination, over a period of up to 90 days.[118] The concentration of each constituent was 10 ppm. Drinking water treatments also contained 0.13% v/v ethanol and control animals received drinking water containing the same amount of ethanol. Groups of mice were also treated with the individual herbicides or their mixtures by oral gavage (in corn oil, 10 ml/kg) daily for 21 days at doses of 100 mg/kg/day for each constituent. Standard toxicological/biochemical endpoints and pentobarbitone sleeping times were assessed. None of the mixtures, including the ternary mixture, caused toxic effects that were significantly greater than those observed with the most active component individually. Moreover, certain effects with some of the mixtures were significantly less than the effects of the components separately. The design of this study did not permit identification of the nature of any interaction between the mixture components.

[a] In the 16 groups of this satellite study the rats were simultaneously exposed to various combinations of chemicals at the LOAEL. The 16 groups jointly comprise a two-level study (i.e. 1/32 x 2^9 groups). Each compound was absent in 8 of the experimental groups and present in the other 8. For any pair of compounds, 4 of the 16 groups contained both compounds, 4 groups contained neither, 4 groups contained only the first one of the pair, and 4 groups contained only the second compound of the pair. The combinations of chemicals were chosen such that the results would allow analysis of the interactions between the nine chemicals (two-factor interactions), but would also allow for optimal analysis between the main effects of the individual compounds.

8.155 van Birgelen *et al*[119] investigated the possible interactive effects of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Female Sprague Dawley rats were fed diets containing PCB 126 (0, 7, 50 or 180 μg/kg diet), TCDD (0, 0.4 or 5 μg/kg diet) or combinations of both compounds (at all the dose levels given individually), for 13 weeks. PCB 126 alone caused a dose-related decrease in food consumption associated with a dose-related deficit in body weight gain. A significant and dose-related reduction in terminal body weight was observed in the middle (daily intake, 3.18 μg/kg/day) and the highest (daily intake,10.1 μg/kg/day) dose groups, along with enlargement of the liver and kidneys and decreases in plasma thyroid hormone. Significant thymic atrophy, loss of hepatic retinoids and marked induction of CYP1A1 and CYP1A2 were observed at intakes $\geq$ 0.47 μg/kg/day (the lowest dietary concentration) with maximal levels of induction being achieved at sub-maximal doses. Feeding TCDD alone indicated a toxic equivalency factor (TEF) for PCB 126 of 0.01 – 0.1 for these effects. Co-administration of PCB 126 and TCDD resulted in a further decrease in body weight gain, thymic atrophy and increase in relative liver weight. However, the authors noted that these effects were less than predicted if additivity was assumed. These findings were explained by the fact that nearly maximum effects had been achieved for the various parameters with the single compounds, illustrating the importance of defining the dose response curve of the individual constituents. A similar explanation was also given for a less than predicted CYP1A2 activity, with maximal induction having been obtained with each compound individually. However, an antagonistic (a less than additive response) effect was suggested to occur between TCDD and PCB 126 regarding changes in CYP1A2 activity and hepatic retinol levels.

8.156 Porter *et al*[120] studied the interactive effects of mixtures of aldicarb, atrazine and nitrate on endocrine, immune and nervous system function when given to male wild deer mice and Swiss Webster ND4 mice in the drinking water. Using a full factorial study design, the ternary and all possible binary combinations of the three chemicals were tested at a low concentrations (the authors stated that this was usually zero but no analytical data were provided for drinking water) and high concentrations (aldicarb 10 ppb, atrazine 10 ppb, nitrate 28 ppm; the same order of magnitude as current United States maximum contaminant levels [MCLs]). In addition, each chemical was tested alone at an intermediate level as a centre replicate. Cyclophosphamide was used as a positive control. The number of animals per group was 6. These experiments were repeated several times (6 times with Swiss Webster mice, 3 times with wild deer mice) over 5 years at different times of the year. The length of dosing was 22-103 days. Not all parameters were assessed in each experiment. Thyroid hormone levels, antibody making ability (plaque forming cell assay) and behaviour (aggression and exploratory holepoke tests) were assessed. The results were poorly reported. Generally, at concentrations that were representative of current ground water concentrations, the pesticides individually showed little or no biological effect, although aldicarb alone (at the intermediate, but unspecified concentration) caused a significant change in free thyroxine index in one of 4 experiments and in plaque forming cells in one out of three experiments and atrazine caused a significant change in plaque forming cells in one out of three experiments. Significant effects were also seen when the chemicals were given as combinations, although again these were not consistent between experiments. The authors suggested that inconsistencies were due to species difference, seasonal influences and differences in duration of exposure. The significance of these findings is uncertain.

Other effects *in vivo*

Intestinal transport of amino acids

8.157 Sędrowicz *et al*[121] studied the effect of chlorfenvinphos, cypermethrin and their mixture on the intestinal transport of the amino acids leucine and methionine in male Wistar rats. Rats were administered chlorfenvinphos, cypermethrin or their mixture p.o. at doses equivalent to 5% of their respective LD_{50} dose levels, daily for two weeks. Exposure to the mixture caused less pronounced changes in parameters describing active transport of amino acids than either of the two pesticides administered alone. However, the authors noted that this phenomenon could not be explained on the basis of the data presented.

Effect on antioxidant enzymes

8.158 Panemangalore *et al*[122] studied alterations in antioxidant enzymes in erythrocytes and liver of young adult rats following dermal exposure to low doses of commonly used pesticides, either individually or in combination. Rats (12 – 16 animals per group) were exposed dermally (area 25 mm^2/200g) to acephate, methamidophos or nicotine, individually, at doses equivalent to 0.04, 0.03 and 0.43% of the respective oral LD_{50}, or as combinations containing 1 x, 2 x and 3 x those concentrations, daily for 4 weeks. Vehicle control animals were treated with ethyl alcohol (50%, 0.25 ml). Half the animals of each treatment group were killed at the end of the exposure period and the remainder 4 weeks later. Individual treatments resulted in a 17% decrease ($p < 0.05$) in erythrocyte superoxide dismutase activity. Four weeks after the cessation of treatment, erythrocyte superoxide dismutase was restored in acephate and methamidophos but not in nicotine-treated rats. Erythrocyte catalase, erythrocyte glutathione peroxidase, liver superoxide dismutase, liver catalase and liver glutathione peroxidase were not modified by individual treatments. In contrast, exposure to the acephate + methamidophos + nicotine mixture resulted in significant dose-related increases in erythrocyte superoxide dismutase (maximal 22% after 4 weeks [$p < 0.05$], rising to 200% 4 weeks post exposure [$p < 0.05$], although SD values for test groups were relatively large compared to controls), erythrocyte catalase (maximal 13 %, $p < 0.05$) and liver glutathione peroxidase (maximal 34%, rising to 65% post exposure, $p < 0.05$) activities that persisted to the end of the post-exposure period. Liver catalase activity was inhibited by about 12% in all groups. Neither erythrocyte glutathione peroxidase nor liver superoxide dismutase nor liver superoxide dismutase were affected in any group. Kidney enzymes were not modified by exposure to either individual or mixtures of pesticides. Data were not analysed to determine whether the nature of the combined effects were additive or interactive.

Study on *Daphnia*

8.159 Deneer *et al*[123] repoted a study on *Daphnia*. Surface waters are frequently contaminated by mixtures of non-reactive non-ionized chemicals, whose toxicity towards aquatic organisms is a function of their hydrophobicity, and is usually referred to as narcosis. The combined effect of 50 of these chemicals towards *Daphnia magna* was described as approximating to concentration addition.

Other effects *in vitro*

Inhibition of gap junctional intercellular communication

8.160 Mills *et al*[124] investigated the interaction of binary chemical mixtures in the process of gap-junctional transfer of metabolites, using a V79 cell metabolic co-operation assay. Dose-response curves of one constituent were determined in the presence or absence of a fixed concentration of the second constituent. The effects of phorbol-12-myristate-13-acetate and phorbol-12-13-dibutyrate, inhibitors of metabolic co-operation thought to operate through the same receptor-mediated process (probably through binding to and activation of protein kinase C), were additive until a maximum was reached with saturation of protein kinase binding sites. The effects of a mixture of two other inhibitors, the pesticide aldrin and cyclohexylamine, were slightly less than predicted by summation, indicating that these chemicals act through similar mechanisms. However, when phorbol-12-myristate-13-acetate was combined with either aldrin or cyclohexylamine, a synergistic interaction was noted. It was suggested that phorbol-12-myristate-13-acetate and aldrin act by stimulating different sub-species of protein kinase C. At the high concentration at which cyclohexylamine was used in this study, inhibition of metabolic co-operation was thought due to non-specific binding. Thus, phorbol-12-myristate-13-acetate and cyclohexylamine were thought to have acted through different mechanistic pathways. Interactions also occurred between phorbol-12-myristate-13-acetate, aldrin or cyclohexylamine and 2,4-diaminotoluene. 2,4-Diaminotoluene appeared to enhance metabolic co-operation and its inclusion in a mixture reversed the effects of all inhibiting compounds to a lesser or greater extent, although the pattern of response was different for each combination. This indicated that the mechanism of interaction in each case was unlikely to be the same. The concentrations used for each chemical permitted $\geq$ 70% viability of cells (0-0.1 ng/ml for phorbol-12-myristate-13-acetate; 0.5 ng/ml for phorbol-12,13-dibutyrate; 0-7.5 μg/ml for aldrin; 0-0.3 mg/ml for cyclohexylamine; 30 or 50 μg/ml for 2,4-diaminotoluene).

8.161 Kang *et al*[125] investigated various halogenated hydrocarbons for their ability to inhibit gap junctional intercellular communication in normal human breast epithelial cells, when given alone or in combination. Dieldrin, DDT and camphechlor inhibited gap junctional intercellular communication in a thresholded dose-dependent manner that was reversible. Binary mixtures of these chemicals were tested at the maximal no-effect concentration for each individual chemical. Significant inhibition of gap junctional intercellular communication was observed in cells treated with mixtures of DDT and 2,4,5-hexachlorobiphenyl, dieldrin and 2,4,5-hexachlorobiphenyl or dieldrin and 2,4,5-hexabromobiphenyl, It was not clear from the data provided as to whether this effect was more than additive.

Inhibition of protein synthesis

8.162 The effects of the OP pesticides, azinphos-methyl, dimethoate, diazinon and pirimiphos-methyl and a benzimidazole fungicide, benomyl, on protein synthesis, were investigated, both singly and as binary mixtures, in human leukaemia HL-60 cells.[126] Azinphos-methyl and diazinon individually inhibited protein synthesis at 24 hours at doses of 60 and 40 μg/ml, respectively. Inhibition in each case was

dose-related. Dimethoate and pirimiphos-methyl were not inhibitory at concentrations up to 100 μg/ml. Benomyl strongly inhibited protein synthesis at 50 μg/ml and the polymerisation of F-actin cytoskeletal microfilaments at 30 μg/ml. When present at equimolar (benomyl:pirimiphos-methyl 30:30 μg/ml) concentrations, pirimiphos-methyl caused apparent antagonism of the inhibitory effect of benomyl on protein synthesis at 4 hours but not at 24 hours. At lower concentrations of pirimiphos-methyl (benomyl:pirimiphos-methyl 30:14 and 30:7.5 μg/ml), there was no antagonism of benomyl activity. The inhibitory effect ($P < 0.01$) of a mixture of dimethoate (100 μg/ml) and diazinon and azinphos-methyl at their individually minimal effective concentrations was greater than the effects of each of the chemicals alone. The same mixture was not found to be significantly inhibitory at 1/10th this concentration but, surprisingly, was found to be inhibitory at 1/100th the concentration ($P < 0.05$).

Inhibition of acetylcholine esterase (AChE)

8.163 The same workers[127] went on to study the toxicity of a similar list of compounds, either singly or as combinations (dimethoate + diazinon + azinphos-methyl; benomyl + pirimiphos-methyl; a mixture of all 5 chemicals together) at concentrations from 0.4 μg/ml up to 100 μg/ml, in human neuroblastoma (SH-SY5Y) cells. Diazinon, azinphos-methyl and pirimiphos-methyl, but not dimethoate or benomyl, were found to inhibit AChE activity. All compounds were found to inhibit protein synthesis (benomyl > azinphos-methyl > diazinon > pirimiphos-methyl = dimethoate). The inhibition of AChE by the various mixtures was equal to that of the most effective constituent present. In contrast, the inhibitory effect of the mixtures on protein synthesis was greater than the single compounds alone and, in certain cases, the authors reported that potentiation of effects had occurred. However, the concentrations of the components of the mixtures were the same as those used when the compounds were tested individually. Consequently, potentiation cannot be inferred from these data.

Studies of metabolic interactions within cell-free systems

8.164 Hu and Kulkarni[128] studied the effects of efficient substrates for lipoxygenase to produce shuttle oxidants and stimulate the formation of reactive species from other chemicals. In the presence of soybean lipoxygenase and hydrogen peroxide, the generation of chlorpromazine cation radicals was shown to trigger a 42-fold stimulation of the oxidation of benzidine, another lipoxygenase substrate, to form benzidine diimine. Other phenothiazines were also found to stimulate benzidine oxidation, albeit to a lesser degree. Chlorpromazine radical generation also stimulated the oxidation of several other xenobiotic substrates, including a 94-fold increase in the oxidation of tetramethyl phenylenediamine to the Wursters blue radical. Preliminary data showed that human placental lipoxygenase displayed a similar stimulatory response in the benzidine oxidation in the presence of chlorpromazine. The significance of this finding *in vivo* remains to be established.

Inhibition of cerebral synaptosome ATPase

8.165 ATPase (Mg^{2+}-activated and total) in cerebral synaptosomes prepared from rat brain was used as a biomarker for membrane effects of pyrethrin and piperonyl butoxide, both individually and in combination.[129] Exposure to 0.1-10 μM pyrethrin inhibited total ATPase activity by ~15% and exposure to 100-1000 μM caused 40% inhibition. Pyrethrin failed to inhibit Mg^{2+}-activated ATPase over the concentration range tested, although a marked increase in activity was observed at a pyrethrin concentration of 1000μM. Exposure to piperonyl butoxide alone had no effect on total ATPase activity over the concentration range of 0.4-4 μM. However, exposure to 40 – 4000 μM piperonyl butoxide resulted in 10-60% dose-related inhibition. Any inhibitory effect on Mg^{2+} ATPase was not significant. The authors noted that 1:4 mixtures of pyrethrin and piperonyl butoxide were "the most efficient", although the meaning and basis for this statement was unclear. Combinations of pyrethrin (0.1- 10 μM) and piperonyl butoxide (0.4 – 40 μM) in a ratio of 1:4 were found to inhibit total ATPase activity by 15-60%. Higher concentrations of the chemicals combined in the same ratio resulted in a maximal 85% inhibition of ATPase activity. Mg^{2+} dependent activity was significantly inhibited by 1:4 mixtures containing pyrethrin $\geq$ 10 μM with maximal inhibition (40-50%) obtained with pyrethrin:piperonyl butoxide concentrations of 100:400 μM. The authors suggested that their results demonstrated piperonyl butoxide to be a potent synergistic agent for the effects of pyrethrin and that this synergism was not only based upon disturbance of biotransformation as previously described but also at the level of the target site on the neural membrane. The term pyrethrin generally implies a complex mixture and the sources of the pyrethrin and the pyrethrin/piperonyl butoxide mixture were two commercial preparations. There is no assurance that the pyrethrin in the two was comparable, which complicates interpretation of the above study.

Toxic interactions in humans following exposure to mixtures of pesticides, drugs, solvents or gaseous environmental pollutants

Symptomatic exposure to mixtures of pesticides and similar compounds in humans

8.166 There are almost insuperable ethical difficulties in studying the effects of mixtures of pesticides in humans experimentally, at symptom-producing doses. Although a number of cases of exposure to more than one pesticide, in humans at doses sufficient to cause symptoms, have been described in the literature, these cases rarely give much information on any toxicological interactions that may be occurring. The reason for this is that the dose of each component of a mixture is rarely known and the toxicity of each component alone in man is rarely known. Thus, in cases of exposure to more than one OP, it is usually not possible to say how much each component contributed to the combined toxicity of the mixture.[130,131] In the case of exposure to phenoxy acid herbicides and dicamba, such as that described by Fraser *et al*,[132] Proudfoot[133] states that the effects of phenoxy acid herbicides predominate: this could be said to support the two compounds acting independently, but confirmation in animal studies would be desirable before such a conclusion could be acted upon. In the case of a combined poisoning of a pregnant woman with two herbicides, metobromuron (a substituted urea) and metolachlor (an acetanilide), methaemoglobinaemia developed severe enough to warrant treatment

with methylene blue. The authors ascribed the methaemoglobinaemia to the metobromuron ingested, although of the acetanilides, propanil has been reported to produce methaemoglobinaemia and it is possible that the methaemoglobinaemia was a combined effect of the two xenobiotics.[134] Diquat and paraquat have rather different systemic toxicities in that only the latter affects the lungs. Both however produce local effects on the oropharynx and less commonly the skin. Ronnen *et al*[135] described severe skin burns from leaking sprayers in two men produced by a proprietary mixture of these two bipyridilium herbicides; while it is likely that this was caused by both compounds acting together it is unclear how much was contributed by each. In some cases, usually suicide attempts, exposure has been to a complex mixture of pesticides as well as solvents. Hancock *et al*[136] described an attempted suicide with pentachlorophenol, benzene hexachloride, dieldrin, metaldehyde, phenoxyacetates, diquat, paraquat and solvents. It is difficult to be clear which signs and symptoms were caused by which component of this mixture. Likewise, in the case described by Callander *et al*,[137] in which neurological sequelae of acute poisoning were ascribed to "phosphorothioate", it is by no means clear that the delayed effects were in fact caused by the "phosphorothioate", as the signs observed were not typical of the delayed effects of OPs, were partly unilateral and other potential neurotoxins were present in the mixture including pyrethrin and petroleum distillates.

Drug-drug interactions

8.167 Adverse drug-drug interactions result from an undesirable modification of the action of one or more concurrently administered agents. A comprehensive treatise of all documented and potential drug-drug interactions is beyond the scope of this report. However, Johnson and colleagues[138] have recently reviewed some clinically significant interactions and listed many more potential interactions. The possible outcomes of an interaction are treatment failure, increased pharmacological effect or toxicity. Most drug-drug interactions occur at the pharmacodynamic and/or pharmacokinetic (ADME) level. Generally speaking, these types of interaction follow a specific time course and are therefore predictable (some examples are given in Table 8.5). Adverse interactions do not occur in all patients who are given the same drug combination and more than one variable can influence a response. Variables affecting susceptibility to drug-drug interactions include health/nutritional status, dietary and other environmental influences, age, genetics (sex, metabolic polymorphisms, ethnicity). Crucially, the dose given is an important factor in determining the occurrence of an adverse drug-drug interaction. Generally, adverse interactions are observed only at relatively high pharmacologic/therapeutic doses and the potential for an interaction is likely to be greatest with those drugs that have a particularly steep dose-response curve (e.g. phenytoin, aminoglycosides, vancomycin) or have a low therapeutic ratio (e.g. theophylline, digoxin).

Table 8.5 Categories of drug interactions, with examples of drugs potentially involved in each

Category	Subcategory	Examples
Pharmacodynamic:- Alteration caused by competition for same receptor site or similar or antagonistic drug actions		Theophylline + albuterol Enflurane + atracurium Morphine + diazepam
Pharmacokinetic:- Alteration in drug concentration caused by change in one or more of the following actions:	Absorption	Cimetidine + ketoconazole Erythromycin + digoxin Sucralfate + ciprofloxacin Iron + tetracycline
	Distribution	Aspirin + warfarin Desipramine + guanethidine Phenylbutazone + phenytoin
	Metabolism	Erythromycin + prednisone Phenytoin + theophylline Rifampin + chlorpropamide Fluoxetine+ desipramine
	Excretion	Probenecid + penicillin Cimetidine + procainamide Hydrochlorothiazide + lithium

Toxic interactions between solvents

8.168 Mutual metabolic inhibition interactions between trichloroethylene and 1,1,1-trichloroethane, benzene and toluene, ethylbenzene and m-xylene, xylene and toluene, trichloroethylene and tetrachloroethylene, m-xylene and methylethyl ketone and m-xylene and isobutanol, characterised by a reduced/delayed production and excretion of metabolites and/or an increased concentration of parent chemical in blood and expired air, have been confirmed in human volunteer or occupational exposure studies.[139-142] However, the human health significance of these metabolic interactions has yet to be confirmed in workers occupationally exposed to binary solvent mixtures.[143,144]

8.169 Supra-additive effects between carbon tetrachloride and propanol were suspected following two instances when environmental concentrations of carbon tetrachloride exceeded allowable exposure limits.[145,146] Enzyme induction by acetone (major product of propanol metabolism) was thought to have potentiated the effects of the carbon tetrachloride. It is likely that this type of interaction would not be significant at lower levels of carbon tetrachloride or of propanol exposure.

Toxic interactions among gaseous pollutants

8.170 It has been suggested that supra-additive toxicity from combined exposures to sulfur dioxide and ammoniacal compounds and to sulfur dioxide and ozone may have been responsible, respectively, for the London fog disaster of 1952 and the high mortality of Japanese children in the 1970s.[147,148] A study reported by Hazucha and Bates[149] suggested human volunteers exposed to realistic amounts of ozone and sulfur dioxide (SO_2) experienced respiratory symptoms that were not experienced when they were exposed to the same level of sulfur dioxide alone and more severe than those experienced with ozone alone. Bell *et al*[150] failed to reproduce these findings although it was suggested that differences in response activities might have been attributable to adaptation by some volunteers to their respective ambient environmental conditions and/or to the extent of formation of respirable (sulfur-containing) aerosols during exposure. Several other volunteer studies involving exposures to combinations of commonly occurring gaseous pollutants (mixed oxides of nitrogen $[NO]_x$, mixed oxides of sulfur $[SO_x]$, ozone, aerosols) have not reported enhanced effects of mixtures.[151-153]

8.171 More recently, Mustajbegovic *et al*[154] compared people exposed to low concentrations (below the maximum allowable concentrations [MACs]) of organic and inorganic air pollutants at work with matched workers who were not exposed and found that respiratory symptoms in the exposed group were primarily associated with smoking, although the authors suggested that environmental effects, possibly due to an interaction of pollutants could not be discounted.

Increased asthma risk and bronchial hyper-responsiveness following exposure to environmental pollutants, solvents or smoking

8.172 There is evidence to suggest that allergic respiratory diseases such as rhinosinusitis and bronchial asthma are becoming more common. It has been suggested that increased atmospheric concentrations of pollutants such as ozone, NO_x, respirable particles (PM10) and volatile organic chemicals may be responsible. Various studies (reviewed by D'Amato *et al*[155]) have suggested that inhalation of air pollutants either individually or in combination, can enhance the airways response to inhaled allergens in atopic subjects, thus inducing asthma exacerbations. Some data suggest that air pollutants can interact with aeroallergens in the atmosphere and/or human airways, potentiating their effects. It has been suggested that increased airway inflammation may increase epithelial permeability allowing some pollutants to overcome the mucosal barrier and induce an allergen-induced response. However, this area remains hotly debated. For example, von Mutius[156] is of the opinion that while passive smoking has been shown convincingly to increase the risk for asthma and bronchial hyper-sensitiveness among exposed children, the evidence that outdoor pollutants (such as sulfur dioxide, particulate matter, diesel exhaust and ozone) is causally related to the inception of allergic disease is poor and that lifestyle, childhood disease, exposure to allergens and socio-economic and other factors may prove to be of greater relevance.

8.173 Some studies have suggested that exposure to water-based paints, volatile organic solvents and some insecticide aerosols may result in bronchial hyper-responsiveness, as determined by the methacholine provocation test.[157-159]

Evidence on possible toxicokinetic interactions between pesticides

8.174 There is a paucity of data on toxicokinetic interactions in humans exposed to pesticides, and the majority of studies involve the administration of high doses to animals. In consequence, while the data may be of relevance to high-dose human exposure, the observations are not necessarily relevant to the exposure of humans to pesticide residues in food. The route of administration in animal studies is often different from that experienced by humans. The route of administration can have a major effect on the concentrations of a chemical that reach the systemic circulation and nervous system. For some compounds the two main routes of exposure for humans can represent important barriers to entry into the general circulation: the skin barrier usually results in only very slow and incomplete absorption, whereas the gastrointestinal tract usually allows rapid absorption, but the extent of absorption may be limited by extensive first-pass metabolism. A number of the animal studies that have reported interactions between pesticides have involved intraperitoneal injection or other routes that allow rapid and complete delivery of the dose into the general circulation. Route of administration differences can give rise to problems of interpretation with respect to the likelihood of interactions in exposed humans. Some relevant studies have already been discussed above.

8.175 Lauwerys and Murphy[160] reported that the pretreatment of rats with tri-*O*-tolyl phosphate (which is not a pesticide) decreased the binding of paraoxon to non-vital tissue in rat liver and plasma, and increased the acute toxicity of paraoxon in rats. Although this probably represents a toxicokinetic interaction, the time course, reversibility and pharmacokinetic basis of this interaction were not defined, and the response of the combination was not compared with adequate paraoxon plasma concentration-response data.

8.176 There is evidence for a protective effect of aldrin and chlordane against the acute toxicity of the carbamate carbanolate, which, at least in part, is due to the induction of CYP-mediated carbanolate detoxification.[1] Piperonyl butoxide, which inhibits CYP activities, abolished the protection afforded by these organochlorine pesticides, and this supports an underlying metabolic mechanism.

8.177 Gaughan *et al*[2] (see also section 8.4) reported the effects of OP pesticides (profenofos, sulprofos, EPN and S,S,S-tributylphosphorotrithioate given by intraperitoneal injection) on liver pyrethroid esterase and on the LD_{50} of fenvalerate, malathion, *trans*-permethrin and *cis*-cypermethrin (given by intraperitoneal injection). The OPs inhibited the esterase, and at the same doses enhanced the toxicity of fenvalerate, malathion and cis-cypermethrin, but not of *trans*-permethrin. The authors concluded that the studies demonstrated a toxicokinetic interaction in which inhibition of metabolism caused increased toxicity. The conclusion was not supported by any *in vivo* toxicokinetic evidence.

8.178 The effects of four OP insecticides on the toxicity of fenobucarb were studied by Takahashi *et al*[3] (see also section 8.5). The study design was based on analysis of the ratios of the oral LD_{50} of fenobucarb alone to its LD50 when co-administered with 4-5% of the LD_{50} of cyanophos, dichlorvos, fenitrothion or malathion. The toxicity of fenobucarb was increased with the phosphorothioates (cyanophos, fenitrothion and malathion), and these also caused an increase in the plasma concentrations of

fenobucarb. Inhibition of fenobucarb metabolism with SKF525A, which is a P450 inhibitor, enhanced plasma concentrations of fenobucarb, and enhanced its inhibition of brain acetylcholine esterase, but did not produce an equivalent shift in the LD_{50}. These findings suggest that both toxicokinetic and toxicodynamic interactions may have been occurring at these acutely toxic doses. The relevance of interactions at such high doses to human exposures is questionable.

8.179 Johnston[5] reviewed the increased sensitivity to the neurotoxicity of malathion following treatment with prochloraz (a fungicide), and concluded that the mechanistic basis was the induction of the enzymatic oxidation of malathion to its active metabolite.

8.180 The paper by Abou-Donia *et al*[27] has received considerable attention in the media in relation to chemical exposures and their possible consequences during the Gulf War (see also section 8.37). The paper reports greater toxicity from a combination of pyridostigmine (a reversible acetylcholine esterase inhibitor given orally), DEET (an insect repellent given by sub-cutaneous injection) and permethrin (a synthetic pyrethroid insecticide given by subcutaneous injection). Fixed doses were studied alone and in combinations, and their effects on toxicity in hens, and inhibition of plasma acetylcholine esterase were reported. The data cannot be interpreted because of the study design, and there appeared to be a poor relationship between the potency to inhibit plasma acetylcholine esterase and toxicity outcomes. Despite this the authors hypothesised that "competition for liver and plasma esterases by these compounds leads to their decreased breakdown and increased transport of the parent compound to nervous tissues." The authors provided no toxicokinetic data to support this hypothesis.

Overview

8.181 There are many papers that describe the toxic effects of mixtures. However, relatively few studies have adequately investigated the nature of interactions that may be occurring between the constituents within a mixture and deviations from additivity.

8.182 In general, studies of the toxicity of mixtures have fallen into distinct types of investigation. Studies of toxicity of a complete mixture as a single entity have often involved complex mixtures that represent those found in the environment (e.g. water contaminants). With some exceptions, these have not been included in this review as they generally provide little information regarding the nature of any interactions that may occur between the individual constituents as they have not concurrently investigated the effects of those constituents alone. However, some studies have assessed the toxicity of mixtures at very low doses in which the concentrations of the individual constituents are believed to be below independently reported ADIs (and therefore likely to be well below the individual NOAELs). While these studies cannot provide any information regarding the nature of interactions that may occur to enhance toxicity, where there is a failure to demonstrate toxic effects, a certain amount of reassurance may be gained in the fact that, for that particular mixture, any interactions that may occur do not enhance toxicity at these levels of exposure.

8.183 Many studies involving less complex mixtures have studied the effects of chemicals individually and in various combinations. In some cases, the data obtained have shown effects that could not be predicted by simple summation of the effects of the single constituents. The authors have then claimed to show deviations from additivity and therefore evidence for interaction. However, for various reasons, the design of many of these experiments has not been adequate to substantiate their claims. Primarily, this is because the investigators have studied chemicals only at single doses, individually and in combination. In the absence of knowledge of the shape of the dose-response, the nature of any interaction cannot be elucidated. For example, for simple similar action (simple joint action or concentration/dose addition), the effects of a mixture may not appear to be additive either at doses close or just below the NOAEL or at high doses where the response is near saturation. A conclusion that effects are summative is valid only if the dose-response is linear. Furthermore, extrapolation of high dose data to low dose exposures is not necessarily valid.

8.184 Other workers have given more consideration to their study design for the specific purpose of investigating interactions of the constituents of mixtures, taking into consideration dose-response and the potential of effects of mixtures at low doses.

8.185 Some studies have predetermined the NOAEL and/or LOAEL for the individual constituents and then tested the mixture at NOAEL and/or the LOAEL and at sub-NOAEL concentrations. These types of experiments have generally looked at mixtures containing up to 10 constituents. The series of studies involving mixtures of nephrotoxins by Jonker *et al*[38-40] strongly suggested that single or repeated exposure to a mixture of chemicals that have the same target organ, but different modes of action, did not constitute an evidently increased hazard, provided that the level of exposure to each individual chemical was at a non-toxic level. For a mixture of chemicals that have the same target organ and the same mode of action, the effect induced by simultaneous administration of levels of chemicals that individually are non-toxic corresponds to an effect expected on a basis of simple additivity. However, some adverse effects, albeit minor, may occur when the mixture components are present at a level equivalent to their respective NOAELs, regardless of the mechanism by which the components exert their toxic effect. Furthermore, a range of interactive effects is possible when co-exposure occurs to mixture components present at a level equivalent to their individual LOAELs. Similarly designed studies that investigated mixtures of toxins (mycotoxins, aldehydes and others) with multiple target organs suggested the same (e.g. Groten *et al*).[117]

8.186 Some studies have used various levels of factorial dose-group design for investigation of the interactive and non-interactive effects of mixtures. Such study designs and sophisticated mathematical modelling should allow the identification of all main effects and interactions of the chemicals under investigation. Data from these types of study have demonstrated that combination effects may simultaneously include both simple addition and both less than and more than additive effects, depending on the endpoint studied. However, the models used to analyse data are limited by the assumptions they make e.g. the monotonic nature of dose-response curves. Interpretation may be limited if dose-response characteristics for individual chemicals are not known.

8.187 Other studies have firstly characterised dose responses then determined interaction by isobolic analysis using mixtures at concentrations predicted to be isoeffective. Where chemicals were thought to act by similar/common mechanisms, observed effects are predicted according to a linear dose-addition model. The interpretation of data obtained using isobolic methods is limited by the precision with which the equi-effective doses of each of the constituents is known.

8.188 It is clear that study of the toxicology of mixtures is a challenging area. Sophisticated study designs are required for determination of the nature of interactions that may occur in simple mixtures. The situation is vastly more complicated when dealing with more complex mixtures. The usefulness of data derived from these studies is determined by how well the dose response curves have been defined for the individual compounds. The extrapolation of effects and interactions at high doses for risk assessment of low-level exposures may not always be valid. Studies of acute effects are not thought to be helpful because the critical target for acute toxic effects may not be the same as those at lower levels of exposure, particularly over more prolonged periods.

8.189 Where interactions occur, the precise mechanisms of the interactions between the constituents of a mixture will be, in most cases, unknown. It is quite feasible that the nature of an interaction of one component with a second could be different from one that may occur between the first and a third component. Some interactions may be countered by other interactions and therefore their existence may be masked. Based on our current understanding, the likelihood of higher-order interactions is, however, small. Where the absorption, distribution, metabolism and excretion (ADME) and pharmacological and toxicological actions of each individual component are well understood at a quantitative level, it may be possible to predict the occurrence and extent of an interaction within a simple mixture purely on a mechanistic basis and using for example pharmacologically-based pharmacokinetic (PBPK) modelling. In the absence of this knowledge, appropriate experimentation is required. However, data from studies involving binary or simple mixtures provide limited information when considering more complex mixtures or cocktails.

Implications for Assessing Potential Health Risks for Humans Exposed to Pesticide Mixtures

8.190 Toxicity studies with defined chemical mixtures have shown that the type of combined action or interaction found at clearly-toxic-effect levels does not predict what will happen at non-toxic-effect levels including levels only slightly lower than the LOAEL (see Cassee *et al*[41] and Groten *et al*[16] for reviews). Even if one of the chemicals occurs at a slightly-toxic-effect level, the type of combined action of the mixture may be different from that occurring at clearly-toxic-effect levels. However, precisely what happens at non-toxic-effect levels (including exposure levels only slightly lower than the LOAEL) is paramount to the assessment of the potential health risk for humans exposed to mixtures of these chemicals. Generally, when exposure levels of the chemicals within a mixture are in the range of the NOAELs, no additivity and no potentiating interaction are found, indicating the applicability of the basic concept of independent joint action.

8.191 On the other hand, *in vivo* studies with chemicals that exhibit the same target organ and the same mode of action (e.g. nephrotoxicants, sensory irritants, mycotoxins, and compounds with estrogenic activity) have revealed that the toxicity of a mixture of similarly acting toxicants, even at levels slightly below the LOAEL of the individual compounds, correspond to the effect expected on the basis of the additivity assumption.[7,40,75,76,162] In these cases the dose addition model represents the basic concept to be used for hazard assessment. This model is applicable over the whole range of exposure levels from low non-toxic levels to LOAELs.

8.192 Some studies (acute toxicity, sub-chronic toxicity, genetic toxicity, carcinogenicity) have addressed the combined effect of mixtures of pesticides and in a few studies clear cases of potentiation were observed in animals exposed to effect levels of individual compounds. However, extrapolation of these findings to much lower dose levels is invalid and the probability of increased health hazard due to additivity or potentiating interaction of mixtures of pesticides at (low) non-toxic doses of the individual chemicals is likely to be small, since the dose of pesticides to which humans are exposed is generally much lower than the NOAEL. Exceptions to these rules may be mixtures of pesticides with similarities in their mode of action or with clear evidence of physico-chemical and/or toxicokinetic interactions, and to mixtures of pesticides with no or very small margins of safety.

High risk subgroups within the population

8.193 It has already been mentioned that young children are often the critical group in exposure assessment because of their high intake of food on a body weight basis. Furthermore, it has been hypothesized that fetuses, infants and children might be more sensitive to certain toxicological effects of pesticides, notably endocrine disruption and neurotoxicological effects (see National Research Council[163] and Aggett and Kuiper[164] for reviews). Data on such toxicological endpoints from animal studies cited in this chapter suggest that the conclusions drawn in sections 8.190-8.192, in respect of combined exposure would also hold true for these endpoints and subgroups within the population. The extent to which genetic differences between individuals affects susceptibility to mixtures of pesticides is not known. The margins of exposure are such that interactions are not anticipated, but this is a topic that will need to be monitored carefully over the coming years, to ensure that any findings of significance for the risk assessment of mixtures are taken into account adequately.

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9. Probabilistic methods for risk assessment

Introduction

9.1 Characterisation of chemical risk involves comparing assessments of exposure and toxicity. Current methods used by United Kingdon (UK) regulators and industry are based on deterministic derivations of both quantities. For example, dietary exposure is based on a high-level consumer (97.5 percentile) for pesticides, or a standard food basket for veterinary medicines (see Chapter 4). An estimate of chronic pesticide exposure is then obtained by multiplying this daily consumption value by the median residue level obtained from treated crop trials; for acute exposure, the multiplier is usually the highest residue level detected in treated crop trials adjusted to take account of item-to-item[a] variability (see 9.14).

9.2 An exposure level is considered to be acceptable for risk evaluation if it does not exceed a prescribed reference level, the acceptable daily intake (ADI) for chronic exposure, or the acute reference dose (ARfD) for acute exposure. These reference levels are usually derived from an estimate of the no adverse effect level (NOAEL) in animal studies.

9.3 Any health risk assessment should take account of variability and uncertainty in the characterisation process. Variability refers to the natural variation between individuals in sensitivity or between residue levels in dietary commodities; uncertainty to the lack of knowledge in risk specification. In a deterministic risk assessment, uncertainty and variability are usually allowed for by the use of safety factors. Thus, an ADI may be derived from the estimated NOAEL for an animal species (expressed relative to body weight) using a divisor to take account of the uncertainty from between-species differences and a further divisor to take account of within-species variability. Typically a divisor of 10 is used in both cases. Rubery *et al*[1] and Renwick[2] identified issues which influence the size of the assessment factor.

9.4 For acute dietary exposure, where residues levels are derived from bulked samples, a multiplier (often 7, but occasionally up to 10) may be used to reflect variation among individual items consumed (e.g. individual fruits).

9.5 Current deterministic methods for characterising risk attempt to deal with variability (and uncertainty) by adopting a conservative approach. For acute dietary exposure, this allows for the fact that those individuals with highest consumption levels may consume food items with the highest residue levels; or, for chronic exposure, that those individuals identified from short-term surveys as having high consumption may persist as high-level consumers for long periods of time. Furthermore, these high input levels are compared with ARfD or ADI reference levels, which are set to include those individuals most sensitive to toxic challenge. However, a major shortcoming of the deterministic approach is that it provides no quantification of the joint probability that these safety thresholds are exceeded, i.e. of the proportion of the population that is still at risk.

[a] Item here means a fruit or vegetable that may be consumed whole eg an apple or a carrot.

9.6 The inbuilt conservatism of a deterministic approach to risk assessment is currently considered appropriate for UK regulatory purposes. However, while the approach may work for risk assessment for a single chemical with a single source and route of exposure, it is found to be untenable when dealing with several chemicals or many exposure pathways.[3] In particular, an estimate of aggregate exposure to a chemical from all possible pathways, based on a conservative estimate of exposure for each pathway, will usually exceed any realistic reference safety level. This property of the deterministic approach is referred to as compounded conservatism[4,5] since, while one individual might hypothetically receive high-level intakes from all pathways, the likelihood of this happening is exceptionally small. Probabilistic methods provide a mechanism for assessing the degree of deterministic conservatism and estimating the level of aggregate or cumulative risk.

9.7 A probabilistic approach to risk assessment takes account of the inherent variability of all sample components by replacing point estimates with distributions. This approach can also provide a formal framework for incorporating parameter uncertainty and expert judgement into the risk assessment process. Outputs are expressed in the form of probabilities of particular outcomes. Hence, in contrast to the deterministic approach, it is possible to estimate the proportion of the population at risk. Recent work has mainly been directed to applying probabilistic methods to estimate aggregate exposure.

Assessment of dietary exposure

9.8 Aggregate exposure to a single chemical combines exposures from all possible sources, including food, air, water and soil. We here describe the probabilistic approach only in relation to dietary sources of exposure. An individual's dietary exposure to a chemical on a single day is calculated from the equation

$$\text{Exposure} = \Sigma \text{ consumption} \times \text{residue level}$$

where the summation is over all food commodities in the diet which may contain the chemical residue. Consumption is standardised by dividing by body weight to give a residue exposure in mg per kg body weight per day, which can be compared with reference levels such as the ADI or ARfD.

9.9 In practice, information on consumption and residue levels for each food product come in the form of sample distributions from independent surveys and trials, as discussed earlier. These can be combined, probabilistically, to give the distribution of exposure across the population. Different calculations and data are required for chronic and acute exposure.

Data sources and variability

Residue levels

9.10 Within UK, the main sources of data on chemical residues in food are the surveillance programmes overseen by the Pesticide Residues Committee and Veterinary Residues Committee. These surveys contain some elements that are targeted to address specific issues of concern and provide sample distributions of residues for up to 100 pesticides and 160 veterinary substances for a wide range of food products. The number of samples, collected each year, for each product varies from as low as 24, for minor commodities, to 200 for dietary staples (bread, milk and potatoes). Samples are collected from twelve centres, two from each of six UK regions.

9.11 If residue distributions are to be used to assess population exposure, it is essential that samples are representative of all available sources of each food commodity. Targeted surveys are unlikely to meet this requirement.

Chronic exposure

9.12 To estimate chronic exposure to a chemical, it is assumed that the concentrations of residues met by an individual over time represent a random sample from the residue distribution obtained for each food commodity in the diet. This assumption is reasonable only if the effect of local sourcing by consumers is small. It follows that, for any single commodity, the expected total intake over a period is equal to the total consumption multiplied by the mean residue level in that commodity. This equivalence holds good, provided there is no daily correlation between the consumption of a dietary commodity and the residue level in that commodity. Hence, summing over all commodities, and expressing exposure and consumption on a daily basis

Average exposure for an individual = Σ usual consumption x mean residue level

This may be compared with the ADI for the chemical.

9.13 The information on residue levels which is required to estimate chronic dietary exposure is thus limited to the mean of the distribution for each food commodity. The number of samples required to estimate a mean with given level of precision will depend on the skewness of the residue distribution. This distribution is usually very long tailed with more than half the samples having residue levels below the reporting limit (RL), so that the estimated mean may be sensitive to the values of a small number of positive observations. Research is currently being directed to study the usefulness of parametric distributions for improving the robustness of the statistics estimated from residue data.

Acute exposure

9.14 Acute dietary exposure is based on an assessment of the amount of chemical residue that an individual might ingest in a single day. In contrast to chronic exposure, this requires information on the variability of residues between daily food portions or items for each commodity.[6] Obtaining a true measure of this variability is demanding. Variability assessment from field trials may be misleading since field trials are usually carried out under controlled conditions, resulting in residue levels that may be more uniform than those following commercial chemical application. Variability in field trials may be different from variability in retail samples as the latter might include non-treated samples resulting in a lower mean level and a higher variability for the same peak individual level. Information from surveillance data may also be unrepresentative, unless the surveys are properly designed. Furthermore, when residue levels are measured in composite samples consisting of several dietary items (as was formerly invariably the case), variability will be underestimated since any item-to-item variability within samples will be ignored.[7] Within the UK, residue levels in retail samples of individual items of fruit and vegetables have been found to exceed 10 times the value for the composite sample on occasion.[8]

Consumption

9.15 The most extensive UK data on consumption of individual food commodities are the food purchase data collected by large retail chains. This, however, provides poor information on individual variability in diet. Surveys of individual diets are usually based on diet diaries or recall questionnaires.[9] In addition to providing statistics on the distribution of consumption across a population for individual food commodities, such surveys should also provide inter-commodity correlations in consumption.

Chronic exposure

9.16 Assessment of chronic exposure requires information on the long-term dietary history of individuals. Surveys of usual daily intake of individuals are less common and provide less accurate data than daily records. However, daily records will overestimate the variability in long-term consumption because of within-individual variation which is often larger than that between individuals. Nusser *et al*[10] show how the distribution of usual daily intake may be estimated from records of daily dietary intakes taken over several days.

Acute exposure

9.17 Assessment of acute exposure is based on the distribution of consumption of food commodities for a random individual on a random day. This is provided by daily records or 24-hour recall questionnaires. Surveys should sample consumption over the whole year to include differences between the days of the week as well as seasonal variation. Intakes between different food commodities are often correlated and correlations are higher when the unit of observation is the meal or the day, than when it is the mean or usual daily intake.

Temporal patterns

9.18 In addition to seasonal changes in diet, residue levels on particular food commodities may also change during the year due to changes in agricultural management, storage or sourcing. When pronounced, this may require separate exposure assessments to be made at different times of the year.

9.19 Changes in diet with age also demands separate acute exposure assessments for different population age groups, particularly, infants, children and the elderly. Assessment of chronic, lifetime exposure may also be improved by fitting an age-related model to consumption data.[11]

Monte Carlo methods

9.20 Monte Carlo is a general probabilistic method which allows the distribution of the total exposure of a population to residues in food to be constructed by repeated sampling from the distributions for residue level and consumption for each commodity. The method is currently being promoted for estimating acute exposure, but can also be used for chronic exposure.

Chronic exposure

9.21 The distribution of chronic exposure for a single dietary commodity (see 9.12) is the distribution of long-term consumption with values multiplied by the mean residue level for that commodity. The total exposure for a random individual from the population is obtained by addition of single sample values drawn from the exposure distribution for each dietary commodity. This is repeated several thousand times to form a distribution of exposures for the population from which the higher order percentiles can be extracted.

9.22 If the consumption of different dietary commodities is assumed to be uncorrelated for the study population, sampling from the exposure distributions can be carried out independently. However, when the consumption of two commodities is correlated, sampling should be carried out on their joint exposure distribution.[12] Including all possible correlations among dietary commodities that may contain residues is rarely feasible, but ignoring large positive correlations will lead to underestimation of the variability of total exposure.

Acute exposure

9.23 To construct the distribution of acute exposure requires Monte Carlo sampling from both the acute consumption and acute residue distributions. The product of the two samples values for a single dietary commodity then gives the acute exposure for a random individual on a random day. The total dietary exposure is given by summing these exposure values for each dietary commodity. Once again, correlations in consumption should be taken into account where possible. The distribution of total exposure is then obtained from repeated sampling for several thousand random individuals.

9.24 Fig 9.1 illustrates the outcome of this process for a single chemical and dietary commodity, the acute exposure to chlormequat from consumption of pears. The derived exposure distribution is based on 5000 Monte Carlo samples.

Parametric approaches

9.25 For acute exposure, Monte Carlo sampling is usually applied to the empirical distributions for consumption and residue levels. However, data for particular residue and food commodity combinations are often scarce and the form of the exposure distribution can then be very sensitive to a small number of unrepresentative sample values. This is not always apparent since a large number of Monte Carlo samples will usually produce a smooth output distribution, obscuring any outliers in one of the parent distributions.

Fig 9.1 Acute Exposure to chlormoquat in pears

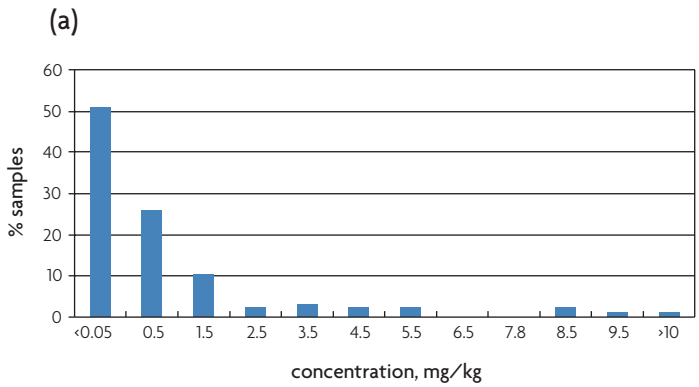
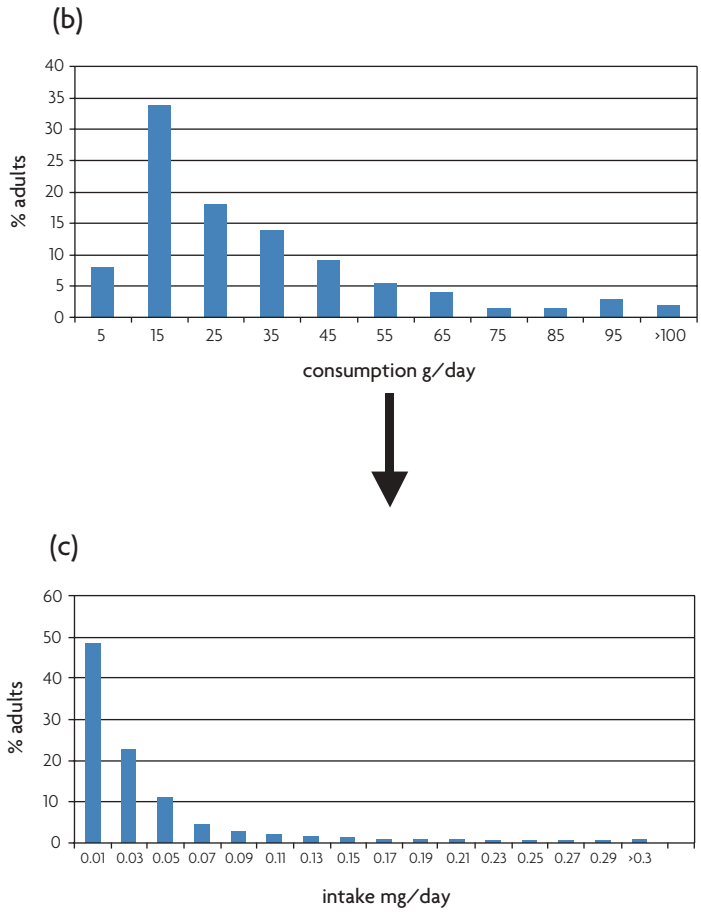


Fig 9.1 Acute Exposure to chlormoquat in pears *(continued)*



Notes Illustrative example of derivation of the distribution of acute exposure to chlormequat on pears by Monte Carlo sampling from the empirical distributions for residues and consumption (redrawn with amendments from presentation by Tennant).[24] Labels for horizontal axis indicate mid-points for each grouping interval.

a) distribution of pesticide residues (mg/kg) for 97 retail samples of pears.[25]
b) distribution of reported average daily pear consumption (g/day) among UK adults.[9]
c) distribution of acute adult exposure (mg/day) derived from the products of 5000 random samples of residue and consumption distributions.

9.26 One solution to this problem, currently being explored in a number of research programmes, is to describe these distributions by parametric models. These models provide a smoothing of the data prior to Monte Carlo sampling, and yield more robust estimates of high percentiles of the resultant exposure distribution. A parametric modelling approach also allows prior information and expert judgement to be incorporated into the assessment process. However, use of parametric approaches has been restricted because of uncertainty over the chosen form for the distribution.

9.27 Empirical residue distributions typically have a large proportion of values below the reporting limit of the residue assay, as well as a long tail of higher values. Distributions are often found to be well modelled by assuming a proportion of samples with zero residue level and a lognormal distribution of positive residues.[13] When the fit is good, the parametric approach provides an estimate of the proportion of samples with positive residues below the RL, and an adjustment to the mean residue level for estimating chronic exposure.

9.28 Consumption distributions also tend to be long tailed and have most commonly been fitted by the lognormal distribution.[14,15] Survey estimates of between-individual and within-individual (day-to-day) variances in consumption can then be used immediately to derive the parametric distributions for acute and chronic exposure assessment. Correlations in the consumption of different food commodities may be taken into account by deriving their joint distribution.

9.29 Estimation of aggregate exposure can proceed as before with Monte Carlo sampling now based on the fitted parametric distributions. When the size of the residue and consumer surveys are both large, the distributions will usually be sufficiently well specified for empirical and parametric approaches to produce similar results, except for very high percentiles. However, when the number of residue levels sampled is less than 100, van der Voet and colleagues[13] recommend that a parametric approach is adopted and that a theoretical distribution (e.g. lognormal) is first fitted to the residue distribution (the number of consumers surveyed is usually much larger than 100). An exception is made when the number of samples with residue levels above the RL is less than 10, since the variability in the residue distribution is poorly estimated and a parametric approach cannot then be recommended.

Assessment of cumulative dietary exposure

9.30 Cumulative risk assessment relates to multiple chemicals that have a common mechanism of toxicity. This introduces a further layer of complexity into exposure assessment, since it is necessary to allow for multiple chemical residues in a single food commodity. These residue levels may be correlated, for example two chemicals may be alternative crop treatments and therefore unlikely to occur together. This is an important consideration when assessing acute exposure, since it implies that Monte Carlo sampling should be performed on the joint residue distribution of the group of chemicals for each food commodity. In practice, the available data are unlikely to support an informative empirical distribution, so approximations will need to be explored.

9.31 Extending the Monte Carlo techniques described earlier for total dietary exposure will provide levels of exposure to each chemical for all food commodities, for a sample individual. If doses are assumed to act additively, the cumulative dietary exposure may be obtained by summing these exposures, after scaling for the chemicals' toxic equivalence. Repeated Monte Carlo sampling then provides a distribution for cumulative exposure.

Toxicity assessment

9.32 For a fully probabilistic assessment of population risk, it is necessary to take account of the variability, not only in exposure levels to the chemicals, but also in human thresholds for their toxic effects.[16] This requires consideration of the variability in the estimate of the threshold of toxicity or benchmark dose in animals[17,18] and replacement of assessment factors used to allow for uncertainty in extrapolation to humans by assessment distributions.[19] Uncertainty due to possible non-additivity in action of multiple chemicals could also be included at this stage. However, probabilistic methods in this area are still relatively unexplored, compared with developments in exposure assessment.

Adoption by regulatory agencies

9.33 The United States of America (USA) Office of Pesticide Programs (OPP) has recently advocated a probabilistic approach to the assessment and regulation of food uses of pesticides (see http://www.epa.gov/oppfead1/trac/science). Most progress has been made in assessing acute dietary exposure, where Monte Carlo techniques are used to derive the distribution of exposure to a chemical from all food pathways. A policy paper[20] suggests that safety concerns should be raised if the 99.9 percentile of the exposure distribution is greater than the ARfD of the chemical. Before taking action, however, a sensitivity analysis is recommended to determine whether the high percentile value results from unusually high food consumption or residue values. As noted earlier, modelling the residue and consumption levels by parametric distributions may produce a more robust analysis, but this practice is not currently promoted. In December 2001, the OPP released its preliminary assessment of the cumulative risks of OP pesticides. (http://www.epa.gov/pesticides/cumulative/pra-op/)

9.34 Within the UK, regulators are also exploring the use of probabilistic methods for acute risk assessment.[3] FSA has recently considered the requirements for aggregate exposure assessment and has initiated an exercise to identify information held by Government Departments on different sources of exposure. The Interdepartmental Group on Health Risks from Chemicals and the MRC Institute for Environment and Health have promoted probabilistic methods for risk assessment through a number of fora, particularly in relation to estimation of toxicity thresholds.[21,22]

9.35 It is likely that uniform regulation procedures for risk assessment will eventually be adopted across the European Union (EU). The Fifth Framework programme is currently funding the Monte Carlo Project (http://www.tchpc.tcd.ie/projects/montecarlo) which has as its aim to develop, validate and apply probabilistic modelling of human exposure to food chemicals and nutrients. The Project will report its findings in February 2003.

9.36 Realistic exposure modelling requires support of sophisticated databases to describe possible scenarios for individual members of the population. Some progress has been made in the USA where a number of modelling programs have been developed which were the topic of an International Life Sciences Workshop in 2000 (see http:hesi.ilsi.org/file/agaworkshopreport.pdf). A number of groups in the USA are developing software in response to the Food Quality Protection Act of 1996.[23] These include Calendex (Novigen Sciences), Lifeline (Hampshire Research Institute) and CARES (Crop Life America). The systems cover a range of lifestyle exposure opportunities from individual diet, workplace, home and leisure environments. Although the models and databases are not directly transferable to a UK environment, lessons learnt in development may prove valuable when methods for managing multiple residues and multiple routes of exposure are being considered.

9.37 The adoption of a probabilistic approach for risk assessment brings transparency to the debate over what is an acceptable level of population risk. This is illustrated by the public response to the OPP's choice of the 99.9 percentile as a threshold level for daily exposure to a pesticide residue above the ARfD.[16] Comments ranged from an insistence that "a policy is flawed if it protects less than 100 percent of the population" to suggestions that a level as high as the 99.9 percentile is "very sensitive to the shape of the curve and the extent and quality of data" and that "protecting a binge eater from the health effects of minute doses of a pesticide is not reasonable". Faced with media which regularly demand regulations which guarantee absolute safety, establishing UK protocols which openly accept non-zero levels of risk may still be difficult.

Conclusions

9.38 The development of probabilistic modelling techniques for risk assessment within a regulatory framework is still in its infancy. However, as the requirements for risk regulation become more demanding, the limitations of deterministic methods are increasingly evident. In particular, probabilistic methods are now widely seen as essential for aggregate and cumulative risk assessment, although major shortcomings over data and methodology need to be overcome before these methods can be routinely implemented. The greater transparency brought to the risk assessment process will require greater engagement with the public in setting acceptable risk thresholds.

9.39 Some of the advantages and the likely difficulties of implementation are summarised below:

Advantages

- Based on a formal model which can be extended to incorporate additional risk factors as they are identified
- Specifies variability in risk factors, e.g. individual exposure and susceptibility, as explicit distributions which are comprehensible and verifiable
- Avoids the problem of compounding conservatism, which severely limits the use of deterministic methods for aggregate and cumulative risk assessment
- Provides an estimate of the proportion of the population at risk and allows outcomes of different risk management strategies to be investigated

Difficulties of implementation

- No regulatory guidelines currently exist
- Requires extensive databases or expert knowledge and sophisticated software
- No track record that the technique is robust for routine implementation

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10. Conclusions

General issues

10.1 There is concern that premarketing authorisation systems for pesticides and veterinary products found in foods do not address the combined toxic effects of different substances (see Chapter 3). For this reason the Food Standards Agency requested the Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment to establish a working party to draft a report on these matters. Another stimulus to the reconsideration of the risk assessment of pesticides and similar materials has been the Food Quality Protection Act (FQPA)[1] in the United States of America (USA).

10.2 There are a number of ways in which exposure to pesticide and other chemical residues might theoretically result in unexpected toxicity and these have been considered in detail in this report. Evaluation of the literature together with information supplied by stakeholders has shown that there is limited exposure of humans to multiple residues and that such exposure occurs at low levels, at least through food. There are no substantiated accounts of adverse reactions to such exposures except under laboratory conditions. Nevertheless the nature and extent of combined exposure, together with the likelihood of any adverse effects which might result, should be evaluated when carrying out risk assessment.

Nomenclature

10.3 The working group decided to use the terminology for combined actions of mixtures, as described in Chapter 7.[2] This is summarised in Table 10.1. It was considered to be the most practicable way of describing the various ways in which combined actions may occur.

Table 10.1 Nomenclature used in this report for combined actions of components of mixtures

Concept	Term used in report	Synonym(s)	Effects observed
Non-interaction	Simple similar action	Simple joint action	Concentration/dose addition
	Simple dissimilar action	Simple independent action, independent joint action	Effect/response addition
Interaction	Potentiation	Synergy, supra-additivity	Greater than additive effect
	Antagonism	Sub-additivity	Less than additive effect

10.4 Simple similar action is a non-interactive process, in which the individual dose of each component of a mixture contributes in proportion to the dose of the chemical which, alone, would be required to obtain the given effect. In simple dissimilar action, the components of a mixture do not affect each other's toxic response. Interaction is used to mean the situation which exists, where the response is greater than would be expected assuming additivity (potentiation) or less than would be expected assuming additivity (antagonism).

10.5 The United States Food Quality Protection Act,[1] introduced the terms aggregate and cumulative risk assessment. The former term means exposure to a single chemical from multiple sources, whilst the term cumulative risk assessment means taking account of exposure to multiple chemicals with a common mechanism of toxicity. The usage of the term cumulative risk assessment, which can be confused with pharmacokinetic accumulation has unfortunately become so widespread that it is unlikely to be dropped, and will be used here.

Stakeholder concerns

10.6 Concern has been expressed about the "cocktail" effect: that is the possibility that adverse effects that may arise from exposure to residues of many different pesticides and similar substances, not necessarily possessing toxicological similarity. Evidence of the occurrence and importance of such combined actions in humans remains uncertain.

10.7 Groups of substances, which are of specific concern, include the anticholinesterases (organophosphates [OPs] and carbamates) and the dicarboximide, dithiocarbamate and benzimidazole fungicides. Concern has also been expressed about the possible impacts of mixtures of endocrine disruptors. It has been suggested that certain groups in the population, notably pregnant women and young children may be at higher risk from these effects than adults; the developing brain and endocrine systems of the fetus and of children are particularly of concern. Moreover, young children have a high intake of food compared to adults on a body weight basis.

10.8 Public interest groups have also pointed to the multiplicity of sources of exposure to pesticides: food, drinking water, and home and garden use as well as occupational exposure. Pesticides used in public hygiene and, in some countries, for vector control are other possible sources of exposure. Consumer goods, especially textiles and carpets, may be treated with insecticides, while paint may contain fungicides. Timber treatment is another potential source of exposure to fungicides or insecticides. The active ingredients of some veterinary medicines are the same as the active ingredients of pesticides. In relation to food, it should be noted that animal products may contain veterinary residues in the same way as plant products can contain pesticide residues. Furthermore, there may be direct exposure of humans to veterinary medicines during and after use, for example to pet flea treatments. Products containing the same active ingredients as those used in pesticides may be used in human medicine, for example as head louse treatments.

Regulation of pesticides, veterinary medicines, human pharmaceuticals and other compounds

10.9 Current regulation of pesticides and veterinary medicines in the UK is based upon the evaluation of the toxicity of individual pesticides or veterinary medicines and comparing the toxicity with calculated or measured intakes. The impact of combined exposure to multiple pesticides of either toxicologically different or similar groups is rarely addressed. Moreover, the impact of multiple sources of exposure is not often considered.

10.10 Many of the procedures for the regulation of pesticides and veterinary medicines, particularly the latter, are being harmonised at European Union (EU) and International level.

10.11. The Committee was made aware that, with certain groups of compounds, measures are in place to deal with mixed exposures in occupational settings.

Evidence of exposure

10.12 Residue surveillance for pesticides in food crops and for veterinary residues in animal products is carried out in the UK. The surveillance is not random but is targeted on products where previous experience or other information suggests that there are likely to be problems. Therefore, it is extremely difficult to assess the frequency with which residues, below or above legally enforcible maximum residue limits (MRLs) occur. It is even more difficult to assess the frequency of multiple residues occuring in the same product. There are further difficulties with limits of detection and reporting limits for assays, and the MRLs for pesticides in crops exist primarily to assess good agricultural practice (GAP); moreover exceedences of MRLs for pesticides have *per se* no human health implications.

10.13 A representative program of surveillance would be necessary to assess the frequency of residues, including multiple residues. Decisions about which products and which pesticides or veterinary medicines are to be analysed are made by expert groups at intervals based on knowledge of products believed to be in use at the time in question. It is recognised that sources of information about the usage of pesticides, veterinary medicines and growth promoters outside the UK are limited.

10.14 Data on exposure from sources other than food and water seem to be extremely poor or non-existent. With a few exceptions, biomarkers and markers of effect, which would help enable the estimation of exposure are not available; nor are adequate intake data available.

10.15 Both biological monitoring and biological effect monitoring can be useful in validating exposure models and identifying internal and effective doses. Metabolites common to groups of compounds, such as the urinary excretion of alkylphosphates as biomarkers of exposure to OPs can only be used with caution, since the toxicity of the parent compounds may vary markedly, whilst producing the same pattern of alkylphosphate excretion. Data show that organochlorines (OCs) are present in human breast milk, albeit at declining levels.

Modelling exposure

10.16 Current deterministic methods of risk assessment are sometimes considered to be highly conservative. Furthermore these methods do not make use of all the available information on multiple sources of exposure, normal variation in dietary and other routes of exposure and exposure to more than one compound. The alternative is to utilize probabilistic methods, which use all the information available on the distribution of intake from all sources. These can be used for aggregate risk assessment, for

both acute and chronic exposure, and for cumulative risk assessment where an assumption of additivity is made.

10.17 The mathematical and statistical techniques required have been seldom employed in toxicological risk assessment until recently and the procedures, their underlying assumptions and the limitations of the computer programs need to be better understood.

Toxicology of mixtures

10.18 Because of the complexity and variability of chemical mixtures that may occur in the environment, risk assessment of their potential toxic effects is an extremely difficult task. Almost all information on effects of combinations of pesticides comes from studies in experimental animals or in *in vitro* systems. Although there are reports of mixed exposures in humans, insufficient information is available to draw any conclusions about the presence or nature of any interactions. Furthermore, most attention has been directed at toxic effects due to combined actions on biological targets at levels of exposure, that are high compared to those likely to be encountered as residues in food.

10.19 Direct chemical reactions can occur between the components of a mixture: there are relatively few studies of mixtures that have investigated such reactions.

10.20 Several studies claim to have identified toxicological potentiation in some mixtures. However, for the most part, these studies have been inadequately designed and based on an incomplete understanding of the concepts involved, thus highlighting a need for a well-defined and universally accepted terminology and methodology for this field. A few well-designed studies have demonstrated the occurrence of both synergistic and antagonistic interactions, as well as additive effects in mixtures, usually at high concentrations or high exposure levels, which are probably unrepresentative of doses likely to be ingested with food.

10.21 The underlying mechanisms for most interactions will not be known. Where there is sound knowledge of pharmacokinetics, pharmacology, metabolism or toxicology of individual components of a mixture, some interactions may be predictable.

10.22 The type of combined action or interaction found at clearly toxic effect levels does not necessarily predict what will happen at non-toxic effect levels, including levels only slightly lower than the lowest observed adverse effect levels (LOAELs).

10.23 Some interactions may not be readily predictable. Investigation of the potential for interaction at the transcriptional or transductional level of the genome level may, in the future, require focussed molecular screening studies using modern technologies, for example genomics, proteomics and/or metabonomics. Such studies will need to address dose-response relationships in order to interpret the data.

10.24 In relation to most examples of possible human exposure to multiple residues, it will be important to evaluate critically whether any effects are likely to occur at low levels of exposure.

Implications for assessing potential health risks for humans exposed to mixtures of pesticides and similar substances

10.25 Studies with chemicals that exhibit the same target organ and the same mode of action (nephrotoxicants, sensory irritants, mycotoxins, and compounds with estrogenic activity) have revealed that the toxicity of a mixture of similarly acting toxicants, even at levels slightly below the LOAEL of the individual compounds, correspond to the effect expected on the basis of the dose additivity assumption. In these cases the dose addition model represents the basic concept to be used for hazard assessment (simple similar action).

10.26 Animal toxicity studies with defined chemical mixtures have shown that the type of combined action or interaction found at clearly-toxic-effect levels does not necessarily predict what will happen at non-toxic-effect levels including levels only slightly lower than the LOAEL. Even if one of the chemicals occurs at a slightly-toxic-effect level, the type of combined action of the mixture may be different from that occurring at clearly-toxic-effect levels. However, precisely what happens at non-toxic-effect levels (including exposure levels only slightly lower than the LOAEL) is paramount to the assessment of the potential health risk for humans exposed to mixtures of pesticides and similar substances. Generally, when exposure levels of the chemicals within a mixture are in the range of the no observed adverse effect levels (NOAELs), no dose additivity and no potentiating interactions are found, indicating the applicability of the basic concept of "simple dissimilar action". Thus, where exposure is to multiple pesticides or other chemicals at doses less than the NOAEL, adverse reactions to such exposure is unlikely.

10.27 Some studies (acute and subacute toxicity, genetic toxicity, carcinogenicity) have addressed the combined effect of mixtures of pesticides and in a few studies clear cases of potentiation were observed in animals exposed to effect levels of individual compounds. However, direct extrapolation of these findings to much lower dose levels may be invalid and the probability of any health hazard due to additivity or potentiating interaction of mixtures of pesticides at (low) non-toxic doses of the individual chemicals is likely to be small, since the dose of pesticides to which humans are exposed is generally much lower than the NOAEL.

10.28 Some endpoints that have been studies in animals or in *in vitro* systems are relevant to groups in the population believed to be at higher risk than the general population. Such endpoints include developmental toxicity studies, endocrine and neurotoxic effects and genotoxicity studies. On the basis of limited information and despite their possible greater sensitivity, it seems likely that the default assumptions in relation to mixtures in children and pregnant and nursing mothers, would be the same as for the rest of the population.

10.29 The evidence base for the default assumptions on simple similar action and are sufficiently robust for cumulative risk assessments to be carried out on well-defined groups with similar toxic actions, for example anticholinesterases. However, in other cases further work may need to be carried out to define common mechanism groups (CMGs).

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11. Recommendations

Regulatory

11.1 We *recommend* that the approval of pesticides used on crops, and authorization of similar compounds used in veterinary medicine should consider all sources of exposure.

11.2 We *recommend* that a scientific and systematic framework should be established to decide when it is appropriate to carry out combined risk assessments of exposures to more than one pesticide and/or veterinary medicine.

11.3 In the event that it is considered appropriate to carry out risk assessment of combined exposure, the default assumptions should be that chemicals with different toxic actions will act independently (simple dissimilar action), and those with the same toxic action will act additively (simple similar action). In the latter circumstances a toxic equivalency approach might be considered. In specific instances the possibility of interaction, particularly potentiation, may have to be considered. In such circumstances adequate dose-response data will be essential in the interpretation of findings in relation to dietary intakes and other human exposures.

11.4 We *recommend* that the approval of pesticides and authorization of compounds used in veterinary medicine, should include more formal analysis, and possibly experimental investigation, of the potential for combined toxic action or interaction due to the addition of other substances to the formulations employed. This consideration should also include tank mixes of pesticides.

11.5 Analysis of all sources of exposure to pesticides and of concurrent exposure to more than one pesticide will require changes in the methods used for risk assessment, including, in some cases, the use of probabilistic exposure assessment. This will be contingent on changes in residue surveillance.

Surveillance

11.6 Dietary and food consumption surveys in the UK should continue to cover all social, age, and ethnic groups within the population. Consideration should be given as to whether additional groups need to be covered.

11.7 Aggregate exposure assessment will require acquisition of robust data on all pathways of exposure to pesticides and veterinary medicines and on sources of variation in such exposure.

11.8 We *recommend* that residue surveillance programmes should be modified in the light of the need for representative data for probabilistic exposure assessment. The effect of food processing and preparation on the bioavailability and chemical nature of residues should be further investigated.

Research

11.9 We *recommend* that methods be developed to provide valid and cost-effective biomarkers or other robust indicators of population exposure and systemic (body) burdens of mixtures of pesticides and relevant veterinary residues.

11.10 We *recommend* that valid markers be developed to enable the early and reliable detection of systemic responses and health effects arising from such exposures (biomarkers of effect).

11.11 This work should be extended to the characterisation of the possible variability in human responses to mixtures of pesticides and veterinary medicines.

11.12 We *recommend* that further work be undertaken, in suitable experimental systems, to characterise both the nature of, and dose-response relationships for, combined actions of pesticides, veterinary medicines and similar substances. Such studies should be performed at doses that include those potentially ingested by humans in the diet. Groups of pesticides having common targets of toxicological action should be identified. Such work might include the identification of sites of action at a molecular level, to identify those groups of compounds that would be expected to show simple similar action. Studies of protein and/or RNA expression, using modern array technology, in relevant systems may be appropriate in some cases. These may be followed up by more detailed mechanistic studies of gene expression and/or enzyme or hormonal activity as necessary. Array technology (RNA and protein) may be appropriate in some cases, or enzyme or hormonal activity in others.

Public information

11.13 A central and accessible repository of information about all forms of human exposure to pesticides and similar substances should be established.

11.14 The extent and adequacy of the information available to the domestic user of pesticides and veterinary medicines requires review of its extent and ease of comprehension.

Appendix 1

Glossary and Abbreviations

AαC: 2-Amino-9H-pyrido[2,3b]indole, a heterocylclic amine

Acaricide: Substance that kills mites

Acceptable daily intake: Dose of a compound which, on the basis of present knowledge, can be ingested every day over a lifetime

ACE: Acetaldehyde

AChE: Acetylcholinesterase; enzyme in cholinergic synapses and the neuromuscular junction, which hydrolyses acetylcholine

Acephate: An organophosphate insecticide

Active ingredient: In a pesticide or veterinary medicine, the chemical substance in the formulation which is responsible for the pesticidal or therapeutic activity

ACP: Advisory Committee on Pesticides; a committee of independent experts that advises Ministers on matters relating to pesticides

ACR: Acrolein

Acute reference dose: Dose of a compound which, on the basis of present knowledge can be ingested over a day or at a single meal

Acypetacs-zinc: Mixture of aliphatic and branched-chain carboxylic acids (C_8-C_{10}) with zinc. Used as a fungicidal wood preserver

Additivity: In dose additivity, each of the chemicals in a mixture, contributes to the toxicity of the mixture in proportion to its dose, expressed as a percentage of the dose of that chemical alone which would elicit the given effect of the chemical. Response additivity is a situation which exists where each individual in a population has a certain tolerance to the individual components of a mixture and will only exhibit a response where the dose exceeds the tolerable dose. Response additivity can be determined by summing the quantal responses of the animals to each toxicant in a mixture

ADI: Acceptable daily intake: dose of a compound which, on the basis of present knowledge, can be ingested every day over a lifetime

ADME studies: Studies of absorption, distribution, metabolism and excretion of chemicals; these are routinely required of pesticide approval holders and veterinary product marketing authorization holders

aER: Alligator estrogen receptor

Aggregate risk assessment: Term introduced by the FQPA (qv) to describe risk assessment taking all sources of intake of a given pesticide into account

Aldicarb: A carbamate insecticide

Aldrin: An organochlorine insecticide of the cyclodiene group

ALT: Alanine aminotransferase

Ames test: Mutagenicity test using various strains of histidine-dependent *Salmonella typhimurium*

Aminoglycosides: A group of antibiotics

Aniline herbicide: Herbicides derived from aniline, such as trifluralin

Anticholinesterases: Substances that inhibit cholinesterases; they include many organophosphate and carbamate (qv) insecticides

AOEL: Acceptable operator exposure level

ARfD: Acute reference dose: dose of a compound which, on the basis of present knowledge, can be ingested over a day or at a single meal

AST: Aspartate aminotransferase

Azinphos-methyl: An organophosphate insecticide

Azole fungicides: A group of fungicides

Azoxystrobin: A strobilurin fungicide

Benomyl: A benzimidazole fungicide

Benzimidazole fungicides: A group of fungicides: it includes carbendazim, benomyl and thiophanate-methyl

BHA: Butylated hydroxyanisole

Biomarker of exposure: The substance to which a human or an animal is exposed, or a metabolite of such a substance, which can be used to assess exposure to that substance qualitatively or quantitatively

Bipyridilium herbicides: Group of herbicides comprising paraquat and diquat

BuChE: Butyryl cholinesterase; cholinesterase enzyme that is present in the plasma and whose preferred substrate is butyryl choline. Also known as pseudocholinesterase

Butyric acid: A short chain fatty acid

CA: Chromosomal abberration

Camphechlor: A mixture of chlorinated camphenes used as an insecticide. Also known as toxaphene, it is composed of more than 800 congeners

Carbamates: Insecticides and fungicides. The insecticides have anticholinesterase properties and are mostly derivatives of N-methyl carbamic acid. Carbamate fungicides (methyl dithiocarbamates, dimethyl dithiocarbamates and ethylene*bis*dithiocarbamates) are not anticholinesterases. There are also carbamate herbicides

Captan: A fungicide belonging to the chloroalkylthio group

Carbanolate: Carbamate insecticide, also known as Banol

Carbaryl: A carbamate insecticide

Carbendazim: A benzimidazole fungicide

CCM: California chemical mixture

Chlordimeform: An acaricide

Chlordane: An organochlorine insecticide

Chlorfenvinphos: An organophosphate insecticide

Chlormequat: A quaternary ammonium plant growth regulator

Chlorpromazine: A pharmaceutical of the phenothiazine group used in human medicine. It has antipsychotic and sedative properties

Chlorpyrifos: An organophosphate insecticide

Chlorothalonil: A nitrile fungicide

Chlorpropham: A carbamate herbicide

Chlortetracycline: An antibiotic

CMG: Common mechanism group. Group of compounds sharing a common mechanism of action

Complex mixture: For the purposes of this report a complex mixture is one that consists of many chemicals and where the composition is not fully characterised qualitatively and/or quantitatively

COPR: Control of Pesticide Regulations (1986). UK Statutory Instrument 1986 No 1510

COSHH: Control of Substances Hazardous to Health (1994). UK Statutory Instrument 1994 No 3246

Cox's dessert apple: Cox's orange pippin, variety of *Malus domestica*

Cumulative risk assessment: Term introduced by the United States FQPA (qv) to describe risk assessment taking intake of more than one pesticide into account

CVMP: Committee on Veterinary Medicinal Products. The Committee which prepares the opinion of the European Medicines Evaluation Agency on any matter to do with the evaluation of veterinary medicinal products

Cyanazine: A triazine herbicide

Cyanophos: An organophosphate insecticide

Cycad: Family (cycadaceae) of plants, resembling palms, but which are gymnosperms. In some countries, flour is made from them (especially *Cycas rumphii*)

Cyclophosphamide: A cytotoxic drug used in human medicine. An organic phosphorus compound

Cypermethrin: A synthetic pyrethroid insecticide

Cyproconazole: An azole fungicide

DARDNI: Department of Agriculture and Rural Development in Northern Ireland

DBF: Decreased breathing frequency

p,p'-DDD: Metabolite of DDT (1,1'-(2,2-dichloroethylidene)-bis(4-chlorobenzene)

p,p'-DDE: Metabolite of DDT (1,1'-(2,2-dichloroethenylidene)-bis(4-chlorobenzene)

DDT: A largely obsolete organochlorine insecticide (it is still used in malaria control). It is a mixture of isomers, p,p'-DDT being the main one. Other isomers include o,p'-DDT

DEDTC: Diethyldithiocarbamate

DEFRA: Department for the Environment, Food and Rural Affairs. British Government Department

DEET: N,N-diethyl-*m*-toluamide, an insect repellent.

DEHP: Di(2-ethylhexyl) phthalate

Deltamethrin: A synthetic pyrethroid insecticide

DEN: Diethylnitrosamine, a hepatocarcinogen

Demeton-S-methyl: An organophosphate insecticide

DH: Department of Health. British Government Department

Diazinon: An organophosphate insecticide, also used in veterinary medicine

Dicamba: An organic acid herbicide

Dicarboximide: A group of fungicides: includes vinclozolin, iprodione and procymidone

Dichlorvos: An organophosphate insecticide

Dieldrin: An organochlorine insecticide of the cyclodiene group

Diflufenican: A herbicide

Dimetridazole: A nitroimidazole bateriocidal drug, used in veterinary medicine

Diphenylamine: A fungicide

Diquat: A bipyridilium herbicide

DMDTC: Sodium dimethyldithiocarbamate

DMDTP: Dimethyldithiophosphate, a metabolite of some organophosphates

Dimethoate: An organophosphate insecticide

DMP: Dimethylphosphate, a metabolite of some organophosphates

DMSO: Dimethylsulfoxide

DMTP: Dimethylthiophosphate, a metabolite of some organophosphates

DNA: Deoxyribonucleic acid

Disulfiram: International non-proprietary name for the drug also known as Antabuse, used to combat alcoholism. It is the teraethyl homologue of thiram, an agricultural fungicide

Dithionon: A fungicide

Dithiocarbamates: Group of fungicides: in residues in food and water, they cannot generally be measured separately

Dose additivity: In dose additivity, each of the chemicals in a mixture, contributes to the toxicity of the mixture in proportion to its dose, expressed as a percentage of the dose of that chemical alone which would elicit the given effect of the chemical

DPN: Dipentylnitrosamine

EBDC: Ethylene*bis*dithiocarbamate (group of fungicides) (qv)

EC: European Community

EDC: Endocrine disruptor; substance capable of disrupting endocrine systems, especially those concerned with reproduction

EDI: Estimated daily intake

EEC: European Economic Communities

EFSA: European Food Safety Authority

EFTA: European Free Trade Area

EMEA: European Medicines Evaluation Agency

Endosulfan: An organochlorine insecticide; it is a mixture of 2 stereoisomers known as α-endosulfan and β-endosulfan

Endrin: An organochlorine insecticide of the cyclodiene group

Enrofloxacine: Fluoroquinoline antibacterial agent used in veterinary medicine

EPN: An organophosphate insecticide

Epoxyconazole: An azole fungicide

ER: Estrogen receptor

ERE: Estrogen-responsive element

17β-estradiol: An estrogenic sex hormone, sometimes spelt 17β-oestradiol

Ethylene*bis*dithiocarbamate fungicides: Group of fungicides, including maneb, zineb and mancozeb

ETU: Ethylene thiourea

EU: European Union

Excipient: Substances in a medicinal formulation, other than the active ingredient

FAO: Food and Agricultural Organization of the United Nations

Fenarimol: A fungicide

Fenbucarb: A carbamate insecticide

Fenitrothion: An organophosphate insecticide

Fenpropimorph: A morpholine fungicide

Fenvalerate: A synthetic pyrethroid herbicide

FEPA: Food and Environment Protection Act (1985). Act of the UK parliament, relating to Great Britain

Ferbam: An iron-containing dimethyldithiocarbamate fungicide

FETAX test: Test undertaken in a species of South American frog (*Xenopus laevis*). The name is an acronym for Frog Embryo Teratogenesis Assay – *Xenopus*

FIFRA: Federal Insecticide, Fungicide and Rodenticide Act. Act of the US Congress

Fluazifop-P-butyl: An organic acid herbicide

Fluroxypyr: An organic acid herbicide

Flusilazole: An azole fungicide

Formulation: Mixture or solution in which an active ingredient of a pesticide or drug is present in a product

FQPA: Food Quality Protection Act 1996. An Act of the US Congress

FRM: Formaldehyde

FSA: Food Standards Agency. UK Government agency concerned with food safety

Fungicide: Substance that kills fungi

GAP: Good agricultural practice

GD: Gestation day

Genistein: Phytoestrogen found in soybeans

Genomics: The study of genes and their functions

Gesaprim: Trade name for atrazine

GLP: Good Laboratory Practice

GST: Glutathione S-transferase

Glu-P-1: 2-Amino-pyrido[1,2-a:3',2'-d]-imidazole, a heterocyclic amine

Glu-P-2: 2-Amino-6-methylpyridol[1,2-a:3',2'-d]-imidazole, a heterocyclic amine

HCB: Hexachloro-1,3-butadiene

HCH: Hexachlorocyclohexane, an organochlorine insecticide. As usually synthesized it is a mixture of isomers. The γ isomer is lindane

HeLa cells: A line of human cells in culture

Heptenophos: An organophosphate insecticide

hER: Human estrogen receptor

hERα: Human estrogen receptor α

Herbicide: Substance that kills plants

Hexachlorobenzene: An organochlorine fungicide

HI: Hazard index

HSE: Health & Safety Executive. A British Government Agency

Internal dose: The amount of a toxicologically significant metabolite to which an organism is exposed

Iprodione: A dicarboximide fungicide

IQ: 2-Amino-3-methylimidazo[4,5-f]quinoline, a heterocyclic amine

ISO: International Organization for Standardization

Isomalathion: Impurity present in some preparations of malathion

Isoniazid: A drug used to treat tuberculosis

Isoproturon: A urea herbicide

JECFA: Joint Expert Committee on Food Additives. Committee convened jointly by the Food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO) to consider reference doses for food additives and veterinary residues

JMPR: Joint Meeting on Pesticide Residues. Meeting convened jointly by the Food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO). It advises the Codex Alimentarius Commission Committee on Pesticide Residues in food

LDH: Lactate dehydrogenase, an enzyme present in many tissues, including the blood

LGC: Laboratory of the Government Chemist

Lindane: γ Isomer of hexachlorocyclohexane, an organochlorine insecticide

Linuron: A urea herbicide

LOAEL: Lowest observed adverse effect level

LOAEC: Lowest observed adverse effect concentration

LOEL: Lowest observed effect level

LONEL: Lowest observed nephrotoxic effect level

Loperamide: A drug used to treat diarrhea; one proprietary brand is Imodium

MAC: Maximum allowable concentration

MAFF: Ministry of Agriculture, Fisheries and Food; former British Government Ministry

Malathion: An organophosphate insecticide

Mancozeb: An ethylene*bis*dithiocarbamate fungicide

Maneb: An ethylene*bis*dithiocarbamate fungicide

MCL: Maximum contaminant level

MCPA: A phenoxy herbicide

MeAαC: 2-Amino-3-methyl-9H-pyrido[2,3]indole, a heterocyclic amine

MeIQ: 2-Amino-3,8-dimethylimidazo[4,5-f]quinoline, a heterocyclic amine

MeIQx: 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline, a heterocyclic amine

MEL: Maximum exposure limit

Metaldehyde: A molluscicide

Methamidophos: An organophosphate insecticide

Methidathion: An organophosphate insecticide

Methomyl: A carbamate fungicide

Methoxychlor: An organochlorine insecticide

Methylmercury: Organic compound of mercury

Metiram: An ethylene*bis*dithiocarbamate fungicide

Metribuzin: A triazinone herbicide

Metolachlor: An acetanilide herbicide

Metobromuron: A urea herbicide

Metsulfuron-methyl: A sulfonylurea herbicide

Michaelis-Menten kinetics: Kinetics typically exhibited by saturable biological processes

Mirex: An obsolete organochlorine insecticide

MN: Micronucleus

MNNG: N-methyl-N'-nitrosoguanidine

MOEC: Minimal observed effect concentration

Monocrotophos: An organophosphate insecticide

Molluscicide: A substance used to kill molluscs eg slugs

Morpholine fungicides: A group of fungicides containing a morpholine group

4-MP: 4-Methoxyphenol

MRL: 1) Maximum residue level (for pesticides). Legally enforceable limit on the maximum concentration of a pesticide or allowed in food. In the case of pesticides it is calculated from trials data and is not a safety limit *per se*.

2) Maximum residue limit for veterinary products. Legally enforceable limit on the maximum concentration of a veterinary drug allowed in food. The MRL for veterinary drugs is a safety limit.

Nabam: An ethylene*bis*dithiocarbamate fungicide

NBBN: N-butanol-butyl nitrosamine

NDNS: National diet and nutrition survey

NEDI: National estimate of dietary intake

NESTI: National estimated short term intake

Nicotine: An alkaloid from tobacco, formerly used as an insecticide, and widely enjoyed in cigarettes &tc

Nitrile fungicides: A group of fungicides, including chlorothalonil

NOAEC: No observed adverse effect concentration

NOAEL: No observed adverse effect level

NOEL: No observed effect level

NONEL: No observed nephrotoxic effect level

NO_X: Mixed oxides of nitrogen

NTA: Nitrilotriacetic acid

OES: Occupational exposure standard

o.g.: Oral gavage (method of administration of test substances to animals using a stomach tube)

OH-PCB3: 2'4'6'-Trichloro-4-biphenylol

OH-PCB4: 2'3'4'5'-Tetrachloro-4-biphenylol

Omethoate: An organophosphate insecticide; also a metabolite of dimethoate (qv)

OP: Organophosphate ester

OPP: Office of pesticide programs – office within the United States Environmental Protection Agency, which deals with pesticide registration

Organochlorine insecticides: A group of insecticides containing chlorine and acting on sodium channels in the insect nervous system

Organophosphate insecticides: A group of insecticides containing phosphorus, usually esters of phosphoric and related acids, which inhibit esterases *inter alia* acetylcholinesterase

Oxytetracycline: An antibiotic

Paclobutrazole: A plant growth regulator

PAH: Polyaromatic hydrocarbon

PAN UK: Pesticide Action Network UK

Paraquat: A bipyridylium herbicide; chemical name N,N'-dimethyl-4,4'-bipyridylium

Parathion-methyl: An organophosphate insecticide; also known as methyl parathion

PBPK modelling: Physiologically-based pharmacokinetic modelling

PBTK modelling: Physiologically-based toxicokinetic modelling

PCB: Polychlorinated biphenyl

PCB 126: 3,3',4,4',5-Pentachlorobiphenyl

PCB 153: 2,2,4,4',5,5'-Hexachlorobiphenyl

PCB 156: 2, 3,3',4,4',5-Hexachlorobiphenyl

PCDD: Polychlorinated dibenzo-*p*-dioxin

PCDF: Polychlorinated dibenzofurans

Pendimethalin: An aniline herbicide

Penicillamine: Drug used *inter alia* to treat Wilson's disease, a condition in which inappropriate amounts of copper are stored in the body

Permethrin: A synthetic pyrethroid insecticide

Pesticide: Substance intended to kill unwanted living organisms

Pheasant: Family of large gallinaceous birds. *Phasianus colchicus mongolicus et torquatus* is the common or ring-necked pheasant

Phenothrin: A synthetic pyrethroid insecticide

Phenoxy herbicide: A group of selective herbicides; chemical analogs of plant auxins

Phenytoin: A drug used to treat epilepsy

Phosphorothioate: Type of organophsophate (qv), that is derived from phosphorothioic acid. There are two subtypes: i) those containing a P=S bond; these are widely used as pesticides and are inactive until the P=S is converted to P=O; these are sometimes called phosphorothionates. ii) those containing S-alkyl or S-aryl groups; these are sometimes called phosphorothiolates

Ph1p: 2-Amino-1-methyl-6-phenylimidazol[4,5-b]pyridine, a heterocyclic amine

PIAP: Pesticides Incidents Appraisal Panel

Picloram: A herbicide

Piperonyl butoxide: An insecticide synergist

Pirimicarb: A carbamate insecticide

Pirimiphos-methyl: An organophosphate insecticide

p.o.: Acronym for *per os*, Latin for by mouth

Potentiation: A toxicological interaction between two or more compounds where a greater than additive effect is seen

PPPR: Plant Protection Products Regulations (1995). UK Statutaory Instrument 1995 No 887. Regulations controlling the marketing, sale and storage of pesticides in Great Britain

PRC: Pesticides Residue Committee

Processing factor: The ration of the pesticide residue level in a food as it is customarily consumed, compared to the pesticide residue level in the raw state of that food

Prochloraz: A fungicide

Procymidone: A dicarboximide fungicide

Profenofos: An organophosphate insecticide

Propachlor: An aniline herbicide

Propineb: A propylene*bis*dithiocarbamate fungicide

Propyzamide: An amide fungicide

Proteomics: Study of protein properties on a large scale to obtain a global, integrated view of cellular processes including expression levels, post-translational modifications, interactions and locations

PSD: Pesticides Safety Directorate (an agency of DEFRA)

Pyrethrin: Term used in several senses. Pyrethrins comprise a group of natural insecticides that are present in *Chrysanthemum cinerariaefolium*. They are permitted for use in organic agriculture. As usually marketed, pyrethrin or "pyrethrins" are a mixture. Amongst the components are pyrethrin I and pyrethrin II, as well as numerous other components

Pyridostigmine bromide: Carbamate drug, used in the prophylaxis of soman poisoning and the treatment of myasthenia gravis

Quercetin: A plant flavonol

Red-eared turtle: *Trachemis scripta*

Red-legged partridge: *Alectoris rufa*. European bird of the phasanidae family

Reference dose: General term used in regulatory toxicology for doses of a compound that are considered safe. In the USA, the term is used to mean the dose of a compound which, on the basis of present knowledge, can be ingested every day over a lifetime and is sometimes referred to as the chronic reference dose. Thus, it is the same as the term acceptable daily intake (ADI) used in the UK. See also acute reference dose

Reporting limit: For pesticides, this is the lowest calibrated level employed during analysis to detect residues. The reporting limit may very from laboratory to laboratory for methodological reasons. For veterinary medicines, the reporting limit is 50% of the maximum residue limit

Response additivity: A situation which exists where each individual in a population has a certain tolerance to the individual components of a mixture and will only exhibit a reponse where the dose exceeds the tolerable dose. Response additivity can be determined by summing the quantal responses of the animals to each toxicant in a mixture

RL: Reporting limit (qv)

RNA: Ribonucleic acid

ROS: Reactive oxygen species

Salmonella typhimurium: An organism that causes food poisoning: strains of it are used to test chemicals for the ability to induce mutations in tests such as the Ames test

s.c.: Subcutaneous; used in this report to mean subcutaneous injection

SCE: Sister chromatid exchange

SCAN: Scientific committee on animal nutrition

Simazine: A triazine herbicide

Simple mixture: For the purposes of this report, a simple mixture is one that consists of a small number of chemicals, which is characterised both qualitatively and quantitatively

SLS: Sodium lauryl sulfate, a surfactant

$SnCl_2$: Stannous chloride, a salt of tin and a reducing agent

SOP: Standard operating procedure

SOx: Mixed oxides of sulfur

Streptomycin: An antibiotic

Sulfonamide: A group of bacteriocidal drugs

Sulfadimidine: A sulfonamide bacteriocidal drug, used in veterinary and human medicine

Sulphamethazine: A sulfonamide bacteriocidal drug

Sulprofos: An organophosphate insecticide

SVS: State Veterinary Service

Synergist: A substance that interacts with one or more other compounds producing synergy. A pesticidal synergist is defined as "any substance other than water, without significant pesticidal properties, which enhances or is intended to enhance the effectiveness of a pesticide, when added to that pesticide"

Synergy: A synonym for potentiation

Synthetic pyrethroids: A type of insecticide, similar in chemical structure to components of pyrethrum

TCDD: 2,3,7,8-Tetrachlorodibenzo-p-dioxin

TCE: Trichloroethylene

Tebuconazole: An azole fungicide

TEF: Toxic equivalency factor

TH: Tyrosine hydroxylase

Thiabendazole: A benzimidazole fungicide

Thiram: A dithiocarbamate fungicide

TNF: Tumor necrosis factor

Toxaphene: See camphechlor

Triazine herbicides: A group of herbicides, including simazine

S,S,S-Tributylphosphorotrithioate: An organophosphate cotton defoliant, also known as DEF

TDS: Total Diet Survey

Trp-P: 3-Amino-methyl-5H-pyrido[4,3-b]indole, a heterocyclic amine

Trp-P-1: 3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole, a heterocyclic amine

TNT: 2,4,6-Trinitrotoluene, an explosive

Trinexapac: Plant growth regulator

Trifluralin: An aniline herbicide

TWA: Time-weighted average; term used in occupational hygiene

UK: United Kingdom

Urea herbicides: A group of herbicides that are chemically substituted ureas

USA: United States of America

USDA: United States Department of Agriculture

USEPA: United States Environmental Protection Agency

Valproic acid: Drug used to treat epilepsy

Vancomycin: A glycopeptide antibiotic

VICH: International harmonisation of data requirements for registration of veterinary medicinal products

Vinclozolin: One of the dicarboximide group of fungicides (qv); vinclozolin is suspected of having anti-androgenic properties

VMD: Veterinary Medicines Directorate (agency of DEFRA)

VMP: Veterinary Medicinal Product

VPC: Veterinary Products Committee. Statutory Committee established under Section 4 of the Medicines Act (1968). It advises ministers on the marketing authorization of veterinary medicinal products and comprises independent experts

WHO: World Health Organization

WPPR: Working Party on Pesticide Residue. Former MAFF (qv) working party that oversaw the pesticides residue surveillance programme. Its function was taken over by the Pesticides Residues Committee

Xenopus laevis: A species of frog from South America, used in teratological tests – see also FETAX

XMC: A carbamate insecticide

Xylylcarb: A carbamate insecticide

Zebrafish: *Danio rerio, a small striped Indian fish*

Zineb: An ethylene*bis*dithiocarbamate fungicide

Ziram: A dimethyldithiocarbamate fungicide

Appendix 2

Examples of Treatment Histories

The mode of use of pesticides on a small number of food items is described below. It should be remembered that the use of pesticides in any one season will depend on many factors including particularly pests and the weather.

Lettuce

UK winter lettuce is one of the crops treated most frequently with insecticides – there were five applications on average[1] using seven products (which means products were sometimes mixed together) and seven active ingredients, and mainly against aphids and caterpillars. The three active ingredients used most by weight were pirimicarb, heptenophos and demeton-S-methyl.

Lettuce was also one of the crops most frequently treated with fungicides, with 2 applications using three products and four active ingredients, principally against botrytis and mildew. The three fungicides used most by weight were mancozeb, zineb and a mixture of metalaxyl and thiram. Mancozeb, maneb and zineb are dithiocarbamate fungicides, which degrade to ethylene thiourea (ETU), an animal carcinogen.[2]

There were on average two herbicide applications, mainly for general weed control and also for broad-leaved weeds. The herbicides used most by weight were propachlor followed by propyzamide and chlorpropham.

Winter Barley

Winter barley is the second largest crop by area grown in Great Britain and is used in beer and animal feed.[3] Crops received on average two fungicide sprays, for broad spectrum disease control and for seed treatment. Flusilazole, fenproprimorph and epoxyconazole were the fungicides most used by weight.

There were also two herbicide sprays for general weed control and against blackgrass and wild oats. The herbicides most used by weight were isoproturon, a mixture of diflufenican and isoproturon and a mixture of isoproturon and pendimethalin.

There was also a growth regulator spray and some limited use of insecticides. Chlormequat was the growth regulator most used by weight and cypermethrin the most common insecticide.

Strawberries

Strawberries receive on average twelve applications during the growing season.[4] There were six fungicide sprays to control botrytis, fruit rot and mildew. The fungicides most used by weight were copper compounds and iprodione.

There were on average three herbicide applications, to control broad leaved weeds and grass. The herbicides most used by weight were propachlor and napropamide.

On average there was one insecticide application against aphids and spider mite. The insecticide most used by weight was chlorpyrifos. There was one acaricide application – the acaricide most used was endosulfan. One molluscicide was used against slugs – the most common was metaldehyde.

A small percentage of the area grown was treated with methyl bromide and other soil sterilants.

Apples

The most recent survey of pesticide usage on apples was in 1996.[5] Cox's dessert apples received on average 16 spray applications. The number of active ingredients applied (including repeat applications of the same active ingredient) was about 36. Other dessert apples received on average one application less.

Most of the applications on Cox apples were the 13 fungicide sprays, for a broad spectrum of disease. The fungicides most used by weight were captan, dithianon and mancozeb.

Three growth regulators on average were applied. Those most used by weight were carbaryl, which has been considered by the Committee on Carcinogenicity as an animal carcinogen,[6] and paclobutrazol.

Two herbicide applications on average were applied. Those most used by weight were simazine and MCPA.

There was also on average one acaricide spray, and clofentazine was the most used by weight.

References

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Appendix 3

Detected Frequencies of Occurrence of Multiple Residues on Individual Samples of Food Commodities, 1997-2000 (see also Chapter 5). Table A3.1 ranks pesticides found in each commodity in order of frequency of occurrence, while table A3.2 ranks the frequency of pesticide occurrence in combination with at least one other pesticide.

Table A3.1 Worst-case occurrence of pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001

Apples	%	Apples (cont)	%	Aubergines	%	Baby vegetables	%	Bananas	%	Green beans	%	Blackberries	%
diphenylamine	43	Phosmet	1	Methamidophos	8	iprodione	6	imazalil	68	dithiocarbamates	6	dithiocarbamates	19
carbendazim	39	tecnazene	1	Procymidone	8	metalaxyl	6	thiabendazole	56	chlorothalonil	4	iprodione	14
chlorpyrifos	35	tolylfluanid	1	iprodione	4	carbendazim	4	aldicarb	4	dimethoate	4	pirimicarb	14
dithiocarbamates	19	diazinon	1			chlorfenvinphos	4	dithiocarbamates	4	omethoate	4	carbendazim	5
captan	17	omethoate	1			pendimethalin	2	chlorpyrifos	2	bromopropylate	2	vinclozolin	5
thiabendazole	17	Dicofol	11			triazophos	2			carbendazim	2		
dodine	14	Fenitrothion	1										
metalaxyl	14	Methomyl	1										
carbaryl	13	Tebufenpyrad	1										
phosalone	13												
pirimicarb	10												
propargite	10												
azinphos-methyl	9												
dimethoate	8												
bifenthrin	4												
bromopropylate	4												
iprodione	4												
myclobutanil	4												
dithianon	3												
bupirimate	3												
ethoxyquin	1												
paclobutrazole	1												

Pesticides are ranked by their worst-case frequency* of overall occurrence in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the pesticide. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.1 Worst-case occurrence of pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001 *(continued)*

Broccoli	%	Brussels sprouts	%	Cabbages	%	Chinese cabbage	%	Carrots	%	Cauliflowers	%	Celery	%
phosmet	1												
bifenthrin	4	iprodione	8	iprodione	6	no residues		iprodione	42	no residues		inorganic bromide	58
chlorpyrifos	4	triazophos	4	tebuconazole	6			pendimethalin	24			chlorothalonil	28
iprodione	4			carbendazim	3			chlorfenvinphos	9			carbendazim	12
methamidophos	4			cypermethrin	3			trifluralin	8			thiabendazole	12
				deltamethrin	1			quinalphos	6			chlorpyrifos	11
				dimethoate	1			triazophos	4			dithiocarbamates	8
								dithiocarbamates	3			prometryn	8
								phorate	2			phorate	6
												procymidone	4
												disulfoton	3
												chlorpyrifos-methyl	3
												endosulfan	3
												pirimicarb	3
												propyzamide	3
												dichlofluanid	1
												heptenophos	1
												methamidophos	1
												propoxur	1
												vinclozolin	1

Pesticides are ranked by their worst-case frequency* of overall occurrence in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the pesticide. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.1 Worst-case occurrence of pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001 *(continued)*

Cherries	%	Cucumbers	%	Currants	%	Coconuts	%	Grapes	%	Grapes continued	%	Lettuce	%
Myclobutanil	17	Procymidone	17	chlorothalonil	20	no residues		captan	22	propargite	4	dithiocarbamates	44
Pirimicarb	13	Oxadixyl	14	fenpropathrin	17			iprodione	22	carbaryl	3	propamocarb	44
Iprodione	8	Dithiocarbamates	13	pirimicarb	12			myclobutanil	17	chlorpyrifos-methyl	3	tolclofos-methyl	41
Carbaryl	4	Chlorothalonil	6	bupirimate	10			dithiocarbamates	14	deltamethrin	3	Inorganic bromide	32
Dithiocarbamates	4	Carbendazim	4	dichlofluanid	10			carbendazim	11	dicofol	3	iprodione	32
		Bupirimate	3	iprodione	10			chlorpyrifos	11	endosulfan	3	cypermethrin	25
		Buprofezin	3	tolylfluanid	10			procymidone	11	ethion	3	propyzamide	18
		Metalaxyl	3	pyrimethanil	7			parathion	8	fenbutatin oxide	3	quintozene	16
		Pyrimethanil	3	chlorpyrifos	2			pyrimethanil	8	fenitrothion	3	procymidone	14
				cypermethrin	2			bifenthrin	6	fenpropathrin	3	folpet	9
				dithiocarbamates	2			cypermethrin	6	fenvalerate	3	oxadixyl	8
								dimethoate	6	flusilazole	3	pirimicarb	8
								lambda-cyhalothrin	6	metalaxyl	3	metalaxyl	6
								parathion-methyl	6	methomyl	3	gamma-HCH	5
								pyrazophos	6	omethoate	3	acephate	4
								bromopropylate	4	penconazole	3	methamidophos	4
								methamidophos	4	phosmet	3	dichlofluanid	4

Pesticides are ranked by their worst-case frequency* of overall occurrence in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the pesticide. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.1 Worst-case occurrence of pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001 *(continued)*

Cherries	%	Cucumbers	%	Currants	%	Coconuts	%	Grapes	%	Grapes continued	%	Lettuce	%
								propiconazole	3			furalaxyl	4
								tetradifon	3			malathion	4
								cyhalothrin	1			ofurace	4
								diphenylamine	1			dimethoate	3
								methiocarb	1			carbendazim	1
								phosalone	1			omethoate	1
								tebuconazole	1			tebuconazole	1

Pesticides are ranked by their worst-case frequency* of overall occurrence in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the pesticide. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.1 Worst-case occurrence of pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001 *(continued)*

Melons	%	Mixed leaf salad	%	Mushrooms	%	Olives	%	Parsnips	%	Passion fruit	%	Peas fresh/frozen	%
endosulfan	13	oxadixyl	21	prochloraz	15	no residues		trifluralin	11	dithiocarbamates	19	gamma-HCH	11
methamidophos	11	procymidone	17	carbendazim	13					bromopropylate	10	fluazifop	4
thiabendazole	10	iprodione	13	gamma-HCH	2					carbendazim	10	bifenthrin	2
dithiocarbamates	7	inorganic bromide	8							methamidophos	5		
carbendazim	3	acephate	4							omethoate	5		
diazinon	3	propyzamide	4							triazophos	5		
fenpropathrin	3												
propamocarb	3												
bupirimate	1												
buprofezin	1												
captan	1												
oxadixyl	1												
permethrin	1												
pirimicarb	1												

Pesticides are ranked by their worst-case frequency* of overall occurrence in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the pesticide. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.1 Worst-case occurrence of pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001 *(continued)*

Peas edible pods	%	Peaches/nectarines	%	Pears	%	Pears oriental	%	Peppers sweet	%	Pineapples	%	Plums	%
Endosulfan	15	iprodione	25	chlormequat	63	dithiocarbamates	31	methamidophos	42	no residues		phosalone	11
Methamidophos	10	methamidophos	21	dithiocarbamates	60	diphenylamine	15	endosulfan	33			chlorpyrifos	5
Pyrazophos	6	dithiocarbamates	15	carbendazim	50	azinphos-methyl	8	procymidone	8			isazophos	5
Carbendazim	4	acephate	15	diphenylamine	26	bromopropylate	8	acephate	4			carbendazim	2
Bifenthrin	2	carbendazim	11	iprodione	22	captan	8					dimethoate	2
Cypermethrin	2	phosalone	10	captan	21	chlormequat	8					parathion	2
Dimethoate	2	chlorfenvinphos	5	tolylfluanid	19	ethoxyquin	8						
Iprodione	2	methomyl	5	phosalone	16	monocrotophos	8						
Lambda-cyhalothrin	2	carbaryl	4	carbaryl	12								
Procymidone	2	chlorpyrifos	4	diethofencarb	12								
Tetradifon	2	fenitrothion	3	phosmet	12								
Triazophos	2	pirimicarb	3	bromopropylate	10								
		dimethoate	1	dimethoate	8								
		ethion	1	omethoate	8								
		vinclozolin	1	ethoxyquin	7								
		chlorpyrifos-methyl	1	imazalil	7								

Pesticides are ranked by their worst-case frequency* of overall occurrence in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the pesticide. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.1 Worst-case occurrence of pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001 *(continued)*

Peas edible pods		Peaches/nectarines		Pears		Pears oriental		Peppers sweet		Pineapples		Plums	
	%		%		%		%		%		%		%
		parathion-methyl	1	azinphos-methyl	5								
		quinalphos	1	dodine	4								
				procymidone	4								
				thiabendazole	4								
				chlorpyrifos	4					malathion	4		
				fenitrothion	2								
				metalaxyl	2								
				pirimicarb	2								
				chlorpyrifos-methyl	1								

Pesticides are ranked by their worst-case frequency* of overall occurrence in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the pesticide. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.1 Worst-case occurrence of pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001 *(continued)*

Potatoes	%	Potatoes processed	%	Radishes	%	Raspberries	%	Spinach	%	Strawberries	%	Sweet corn	%
chlorpropham	26	chlorpropham	13	chlorfenvinphos	4	oxadixyl	25	lambda-cyhalothrin	9	cyprodinil	45	no residues	
thiabendazole	22	maleic hydrazide	10			chlorothalonil	21	permethrin	9	pyrimethanil	45		
oxadixyl	21	oxadixyl	8			dichlofluanid	21	deltamethrin	6	bupirimate	36		
maleic hydrazide	21					iprodione	13	iprodione	5	penconazole	36		
tecnazene	21					dithiocarbamates	8	chlorpyrifos	2	iprodione	31		
imazalil	8					pirimicarb	8	metalaxyl	2	tolylfluanid	27		
propamocarb	3					bupirimate	4			captan	18		
						captan	4			vinclozolin	18		
						cypermethrin	4			chlorothalonil	10		
						fenpropimorph	4			myclobutanil	10		
						myclobutanil	4			kresoxim-methyl	9		
										dichlofluanid	8		
										malathion	8		
										dithiocarbamates	7		
										procymidone	7		
										fenpropimorph	6		
										endosulfan	4		
										carbaryl	2		

Pesticides are ranked by their worst-case frequency* of overall occurrence in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the pesticide. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.1 Worst-case occurrence of pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001 *(continued)*

Potatoes	Potatoes processed	Radishes	Raspberries	Spinach	Strawberries		Sweet corn
%	%	%	%	%		%	%
					carbendazim	2	
					tetradifon	2	
					fenarimol	2	
					fenpropathrin	2	
					fenpropidin	2	
					metalaxyl	2	
					methomyl	2	
					pirimicarb	2	
					propargite	2	

Pesticides are ranked by their worst-case frequency* of overall occurrence in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the pesticide. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.1 Worst-case occurrence of pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001 *(continued)*

Tomatoes	%	Turnips/swedes	%	Yams	%	Fruit juices	%	Wine	%	Infant food	%	Bread	%
Procymidone	8	Chlorfenvinphos	5	carbendazim	75	2-phenylphenol	13	ETU	10	propargite	35	pirimiphos-methyl	51
Bupirimate	6			imazalil	10	carbaryl	9	oxadixyl	3	carbendazim	25	chlormequat	41
Dithiocarbamates	6					carbendazim	7	procymidone	3	phosalone	25	malathion	9
Endosulfan	6					metalaxyl	4			diphenylamine	23	glyphosate	7
Fenbutatin oxide	6					imazalil	3			thiabendazole	23	chlorpyrifos-methyl	2
Iprodione	4					thiabendazole	3			chlormequat	6	alpha-HCH	1
Lambda-cyhalothrin	4					biphenyl	1			carbaryl	4	gamma-HCH	1
Oxamyl	4									ethoxyquin	4	permethrin	1
Carbendazim	2									iprodione	3		
Propargite	2									oxadixyl	3		
Oxadixyl	2									bromopropylate	2		
Vinclozolin	2									penconazole	2		
										methiocarb	1		
										deltamethrin	1		
										pirimiphos-methyl	1		

Pesticides are ranked by their worst-case frequency* of overall occurrence in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the pesticide. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.1 Worst-case occurrence of pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001 *(continued)*

Bread (fruit)	%	Beer	%	Biscuits	%	Breakfast cereals	%	Cakes	%	Crackers	%	Nuts	%
Carbendazim	16	Chlormequat	35	pirimiphos-methyl	8	etrimfos	1	carbaryl	4	pirimiphos-methyl	2	inorganic bromide	23
Cypermethrin	16							iprodione	4			endosulfan	4
Iprodione	16											dieldrin	2
Pirimiphos-methyl	12												
Endosulfan	4												

Nut butters	%	Pasta	%	Rice	%	Milk, cows'	%	Milk, human	%	Infant formulae	%	Bacon	%
Inorganic bromide	17	No residues		phosphine	54	gamma-HCH	14	DDT	98	endosulfan	15	no residues	
DDT	8			inorganic bromide	19	DDT	1	beta-HCH	36	gamma-HCH	10		
				pirimiphos-methyl	3			HCB	23	HCB	5		
								dieldrin	15				
								gamma-HCH	2				

Beef	%	Burgers	%	Butter	%	Cheese, cows'	%	Chicken	%	Chocolate	%	Cooking fats	%
No residues		No residues		DDT	19	gamma-HCH	12	no residues		inorganic bromide	98	DDT	3
										gamma-HCH	75	HCB	3
										fenvalerate	2		

Pesticides are ranked by their worst-case frequency* of overall occurrence in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the pesticide. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.1 Worst-case occurrence of pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001 *(continued)*

Meat cooked	%	Cream	%	Ducks	%	Eggs	%	Ice cream	%	Cheese goat/ewe	%	Kidney	%
DDT	6	No residues		no residues		DDT	1	no residues		gamma-HCH	26	DDT	2
										DDT	9		
										alpha-HCH	4		
Lamb	**%**	**Liver**	**%**	**Pate**	**%**	**Rabbit**	**%**	**Pies, pasties**	**%**	**Pork**	**%**	**Pork Chinese can**	**%**
DDT	42	DDT	2	no residues		beta-HCH	53	no residues		no residues		DDT	100
Diazinon	3					DDT	41					beta-HCH	17
Gamma-HCH	1					alpha-HCH	6					alpha-HCH	8
Turkey	**%**	**Veal**	**%**	**Yoghurt**	**%**	**Fish oils**	**%**	**Fish oily**	**%**	**Fish sticks**	**%**	**Fish white/sea**	**%**
No residues		No residues		no residues		DDT	96	DDT	83	no residues		no residues	
						dieldrin	52	dieldrin	47				
						chlordane	9	gamma-HCH	3				
						gamma-HCH	9						
						HCB	9						
						alpha-HCH	4						

Pesticides are ranked by their worst-case frequency* of overall occurrence in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the pesticide. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.1 Worst-case occurrence of pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001 *(continued)*

Shellfish	Trout/salmon		Mayonnaise	Water, bottled	Sausages
%		%	%	%	
No residues	DDT	100	no residues	no residues	no residues
	Dieldrin	100			
	HCB	100			
	Gamma-HCH	54			
	Chlordane	17			

Pesticides are ranked by their worst-case frequency* of overall occurrence in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the pesticide. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.1 Worst-case occurrence of pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001 *(continued)*

Oranges	%	Soft citrus	%
imazalil	99	imazalil	96
thiabendazole	86	thiabendazole	82
2,4-D	58	2-phenylphenol	74
2-phenylphenol	39	chlorpyrifos	52
methidathion	30	2,4-D	42
chlorpyrifos	12	methidathion	32
metalaxyl	12	carbendazim	14
dicofol	6	malathion	12
carbaryl	3	pirimiphos-methyl	12
dimethoate	3	azinphos-methyl	10
tetradifon	3	tetradifon	10
biphenyl	2	chlorpyrifos-methyl	6
carbendazim	2	ethion	4
chlorpyrifos-methyl	2	dimethoate	2
malathion	2	fenthion	2
mecarbam	2		
diazinon	1		
ethion	1		

Pesticides are ranked by their worst-case frequency* of overall occurrence in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the pesticide. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.2 Worst-case occurrence of multiple pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001

Apples	%	Apples (cont)	%	Aubergines	%	Baby vegetables	%	Bananas	%	Green beans	%	Blackberries	%
Carbendazim	39	Bifenthrin	4	no multiple		iprodione	6	imazalil	38	dimethoate	4	dithiocarbamates	10
Diphenylamine	39	Bromopropylate	4	residues		metalaxyl	6	thiabendazole	34	omethoate	4	pirimicarb	10
Chlorpyrifos	25	Ethoxyquin	4			carbendazim	4	aldicarb	4			iprodione	5
Thiabendazole	17	Iprodione	4					dithiocarbamates	4			vinclozolin	5
Dithiocarbamates	14	Dithianon	3					chlorpyrifos	2				
Metalaxyl	14	Bupirimate	3										
Captan	10	Myclobutanil	3										
Phosalone	8	Diazinon	1										
Propargite	8	Paclobutrazole	1										
Carbaryl	8	Tolylfluanid	1										
Dimethoate	8	Omethoate	1										
Pirimicarb	8	Dicofol	1										
Dodine	6	Methomyl	1										
Azinphos-methyl	4	Tebufenpyrad	1										

Pesticides ranked by their worst-case frequency* of occurrence as multiple residues in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the given pesticide in combination with at least one other pesticide residue. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.2 Worst-case occurrence of multiple pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001

Broccoli	%	Brussels Sprouts	%	Cabbages	%	Chinese Cabbage	%	Carrots	%	Cauliflowers	%	Celery	%
No multiple		No multiple		carbendazim	3	no multiple		iprodione	15	no multiple		chlorothalonil	19
Residues		Residues		iprodione	3	residues		pendimethalin	13	residues		inorganic bromide	16
				cypermethrin	1			chlorfenvinphos	5			chlorpyrifos	11
				deltamethrin	1			trifluralin	4			dithiocarbamates	8
								triazophos	3			prometryn	8
								dithiocarbamates	2			phorate	6
								quinalphos	2			procymidone	4
												thiabendazole	4
												carbendazim	3
												chlorpyrifos-methyl	3
												endosulfan	3
												pirimicarb	3
												propyzamide	3
												dichlofluanid	1
												disulfoton	1
												methamidophos	1
												vinclozolin	1

Pesticides ranked by their worst-case frequency* of occurrence as multiple residues in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the given pesticide in combination with at least one other pesticide residue. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.2 Worst-case occurrence of multiple pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001

Cherries	%	Cucumbers	%	Currants	%	Coconuts	%	Grapes	%	Grapes continued	%	Lettuce	%
Carbaryl	4	Dithiocarbamates	9	chlorothalonil	17	no multiple		dithiocarbamates	14	chlorpyrifos-methyl	3	dithiocarbamates	44
Dithiocarbamates	4	Procymidone	9	fenpropathrin	17	residues		iprodione	11	deltamethrin	3	propamocarb	40
Myclobutanil	4	Oxadixyl	3	pirimicarb	10			myclobutanil	11	dicofol	3	iprodione	32
Pirimicarb	4	Chlorothalonil	1	bupirimate	7			captan	8	endosulfan	3	toloclofos-methyl	32
		Metalaxyl	1	dichlofluanid	7			carbendazim	8	ethion	3	inorganic bromide	24
		Pyrimethanil	1	iprodione	7			pyrimethanil	8	fenitrothion	3	cypermethrin	22
				tolylfluanid	7			bifenthrin	6	fenpropathrin	3	propyzamide	18
				pyrimethanil	5			dimethoate	6	fenvalerate	3	quintozene	16
				cypermethrin	2			parathion	6	lambda-cyhalothrin	3	procymidone	12
				dithiocarbamates	2			parathion-methyl	6	metalaxyl	3	pirimicarb	8
								pyrazophos	6	omethoate	3	metalaxyl	6
								cypermethrin	4	penconazole	3	oxadixyl	6
								methamidophos	4	phosmet	3	folpet	5
								bromopropylate	3	procymidone	3	gamma-HCH	5
								carbaryl	3	propargite	3	acephate	4
								chlorpyrifos	3	propiconazole	3	methamidophos	4

Pesticides ranked by their worst-case frequency* of occurrence as multiple residues in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the given pesticide in combination with at least one other pesticide residue. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.2 Worst-case occurrence of multiple pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001

Cherries	%	Cucumbers	%	Currants	%	Coconuts	%	Grapes	%	Grapes continued	%	Lettuce	%
								tetradifon	3			dichlofluanid	4
								cyhalothrin	1			furalaxyl	4
								fenbutatin oxide	1			malathion	4
								methomyl	1			ofurace	4
								phosalone	1			dimethoate	3
								tebuconazole	1			carbendazim	1
								dichlofluanid	4			omethoate	1
												tebuconazole	1

Pesticides ranked by their worst-case frequency* of occurrence as multiple residues in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the given pesticide in combination with at least one other pesticide residue. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.2 Worst-case occurrence of multiple pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001

Melons	%	Mixed leaf salad	%	Mushrooms	%	Olives	%	Parsnips	%	Passion Fruit	%	Peas fresh/frozen	%
Methamidophos	10	Oxadixyl	17	carbendazim	2	no multiple		no multiple		bromopropylate	5	no multiple	
Endosulfan	8	Procymidone	13	prochloraz	2	residues		residues		dithiocarbamates	5	residues	
Thiabendazole	6	Acephate	4							methamidophos	5		
Dithiocarbamates	4	Iprodione	4							triazophos	5		
Bupirimate	1												
Buprofezin	1												
Diazinon	1												
Fenpropathrin	1												
Oxadixyl	1												
Permethrin	1												
Pirimicarb	1												

Pesticides ranked by their worst-case frequency* of occurrence as multiple residues in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the given pesticide in combination with at least one other pesticide residue. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.2 Worst-case occurrence of multiple pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001

Peas Edible Pods	%	Peaches/Nectarines	%	Pears	%	Pears Oriental	%	Peppers Sweet	%	Pineapples	%	Plums	%
Endosulfan	8	Dithiocarbamates	8	chlormequat	54	dithiocarbamates	31	methamidophos	8	no multiple		carbendazim	2
Methamidophos	4	Iprodione	8	dithiocarbamates	46	diphenylamine	15	procymidone	6	residues		chlorpyrifos	2
Carbendazim	2	Methamidophos	8	carbendazim	44	bromopropylate	8	acephate	4			isazophos	2
Lambda-cyhalothrin	2	Carbendazim	7	iprodione	22	captan	8	endosulfan	4			phosalone	2
Pyrazophos	2	Acephate	5	captan	21	monocrotophos	8						
Triazophos	2	Carbaryl	3	diphenylamine	20								
		Phosalone	3	tolylfluanid	19								
		Chlorpyrifos	3	diethofencarb	13								
		Fenitrothion	3	phosalone	12								
		Pirimicarb	3	bromopropylate	10								
		Dimethoate	1	dimethoate	8								
		Vinclozolin	1	phosmet	8								
		Methomyl	1	azinphos-methyl	8								
		Parathion-methyl	1	ethoxyquin	7								
		Quinalphos	1	imazalil	7								

Pesticides ranked by their worst-case frequency* of occurrence as multiple residues in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the given pesticide in combination with at least one other pesticide residue. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.2 Worst-case occurrence of multiple pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001

Peas Edible Pods	Peaches/Nectarines	Pears		Pears Oriental	Peppers Sweet	Pineapples	Plums
%	%		%	%	%	%	%
		procymidone	4				
		omethoate	4				
		carbaryl	4				
		dodine	2				
		fenitrothion	2				
		malathion	2				
		metalaxyl	2				
		thiabendazole	2				
		chlorpyrifos	2				
		chlorpyrifos-methyl	2				
		pirimicarb	2				

Pesticides ranked by their worst-case frequency* of occurrence as multiple residues in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the given pesticide in combination with at least one other pesticide residue. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.2 Worst-case occurrence of multiple pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001

Potatoes	%	Potatoes processed	%	Radishes	%	Raspberries	%	Spinach	%	Strawberries	%	Sweet corn	%
Thiabendazole	18	Maleic hydrazide	6	no multiple		chlorothalonil	17	lambda-cyhalothrin	5	cyprodinil	45	no multiple	
Chloropham	17	Oxadixyl	4	residues		dichlofluanid	17	deltamethrin	3	pyrimethanil	45	residues	
Maleic hydrazide	12	Chloropham	2			iprodione	13	iprodione	3	bupirimate	36		
Tecnazene	9					oxadixyl	13	permethrin	3	penconazole	36		
Imazalil	7					pirimicarb	13			iprodione	27		
						bupirimate	4			captan	18		
						dithiocarbamates	4			tolylfluanid	18		
						fenpropimorph	4			vinclozolin	18		
						myclobutanil	4			chlorothalonil	10		
										myclobutanil	10		
										kresoxim-methyl	9		
										pirimicarb	9		
										dichlofluanid	8		
										malathion	8		
										dithiocarbamates	7		
										fenpropimorph	6		
										procymidone	4		

Pesticides ranked by their worst-case frequency* of occurrence as multiple residues in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the given pesticide in combination with at least one other pesticide residue. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.2 Worst-case occurrence of multiple pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001

Potatoes	Potatoes processed	Radishes	Raspberries	Spinach	Strawberries		Sweet corn
%	%	%	%	%		%	%
					carbaryl	2	
					carbendazim	2	
					endosulfan	2	
					fenarimol	2	
					fenpropathrin	2	
					fenpropidin	2	
					methomyl	2	
					propargite	2	

Pesticides ranked by their worst-case frequency* of occurrence as multiple residues in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the given pesticide in combination with at least one other pesticide residue. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.2 Worst-case occurrence of multiple pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001

Tomatoes	%	Turnips/swedes	%	Yams	%	Fruit juices	%	Wine	%	Infant food	%	Bread	%
Dithiocarbamates	6	No multiple		carbendazim	6	2-phenylphenol	6	oxadixyl	1	propargite	35	chlormequat	7
Endosulfan	6	Residues		imazalil	6	imazalil	3	procymidone	1	carbendazim	25	glyphosate	5
Procymidone	6					metalaxyl	3			phosalone	25	pirimiphos-methyl	2
Carbendazim	2					thiabendazole	3			diphenylamine	23	alpha-HCH	1
Lambda-cyhalothrin	2					biphenyl	1			thiabendazole	23	malathion	1
Oxadixyl	2									ethoxyquin	4		
Oxamyl	2									bromopropylate	2		
Propargite	2									carbaryl	2		
Vinclozolin	2									iprodione	1		

Pesticides ranked by their worst-case frequency* of occurrence as multiple residues in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the given pesticide in combination with at least one other pesticide residue. The worst-case means the year with the highest frequency of the given pesticide.

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Table A3.2 Worst-case occurrence of multiple pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001

Bread (fruit)	%	Beer	%	Biscuits	%	Breakfast cereals	%	Cakes	%	Crackers	%	Nuts	%
Carbendazim	16	No multiple		no multiple		no multiple		no multiple		no multiple		endosulfan	4
Cypermethrin	16	Residues		residues		residues		residues		residues		inorganic bromide	4
Iprodione	12												
Pirimiphos-methyl	8												
Endosulfan	4												
Nut butters	**%**	**Pasta**	**%**	**Rice**	**%**	**Milk, cows'**	**%**	**Milk, human**	**%**	**Infant formulae**	**%**	**Bacon**	**%**
DDT	4	No multiple		inorganic bromide	13	no multiple		DDT	45	endosulfan	5	no multiple	
Inorganic bromide	4	Residues		phosphine	9	residues		beta-HCH	33	HCB	5	residues	
				pirimiphos-methyl	3			HCB	21				
								dieldrin	15				
								gamma-HCH	2				
Beef	**%**	**Burgers**	**%**	**Butter**	**%**	**Cheese, cows'**	**%**	**Chicken**	**%**	**Chocolate**	**%**	**Cooking fats**	**%**
No multiple		No multiple		no multiple		no multiple		no multiple		no multiple		DDT	3
Residues		Residues		residues		residues		residues		residues		HCB	3

Pesticides ranked by their worst-case frequency* of occurrence as multiple residues in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the given pesticide in combination with at least one other pesticide residue. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.2 Worst-case occurrence of multiple pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001

Meat cooked	%	Cream	%	Ducks	%	Eggs	%	Ice cream	%	Cheese goat/ewe	%	Kidney	%
No multiple		No multiple		no multiple		no multiple		no multiple		alpha-HCH	4	no multiple	
Residues		Residues		residues		residues		residues		gamma-HCH	4	residues	
Lamb	**%**	**Liver**	**%**	**Pate**	**%**	**Rabbit**	**%**	**Pies, pasties**	**%**	**Pork**	**%**	**Pork Chinese can**	**%**
DDT	1	No multiple		no multiple		beta-HCH	29	No multiple		no multiple		DDT	25
gamma-HCH	1	Residues		residues		DDT	24	Residues		residues		beta-HCH	17
						alpha-HCH	6					alpha-HCH	8
Turkey	**%**	**Veal**	**%**	**Yoghurt**	**%**	**Fish oils**	**%**	**Fish oily**	**%**	**Fish sticks**	**%**	**Fish white/sea**	**%**
no multiple		No multiple		no multiple		DDT	57	DDT	47	no multiple		no multiple	
residues		Residues		residues		dieldrin	52	Dieldrin	47	residues		residues	
						chlordane	9						
						gamma-HCH	9						
						HCB	9						
						alpha-HCH	4						

Pesticides ranked by their worst-case frequency* of occurrence as multiple residues in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the given pesticide in combination with at least one other pesticide residue. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.2 Worst-case occurrence of multiple pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001

Shellfish	Trout/salmon		Mayonnaise	Water, bottled	Sausages
%		%	%	%	%
no multiple	DDT	100	no multiple	no multiple	no multiple
residues	Dieldrin	100	residues	residues	residues
	HCB	100			
	Gamma-HCH	54			
	Chlordane	17			

Pesticides ranked by their worst-case frequency* of occurrence as multiple residues in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the given pesticide in combination with at least one other pesticide residue. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.2 Worst-case occurrence of multiple pesticide residues in different food commmodities using data from the surveillance programmes 1997-2001

Oranges	%	Soft citrus	%
imazalil	92	Imazalil	94
thiabendazole	85	Thiabendazole	82
2,4-D	58	2-phenylphenol	74
2-phenylphenol	39	Chlorpyrifos	46
Methidathion	30	2,4-D	44
Chlorpyrifos	12	Methidathion	32
Metalaxyl	12	carbendazim	14
Dicofol	6	malathion	12
Carbaryl	3	azinphos-methyl	10
Carbendazim	3	tetradifon	10
Dimethoate	3	pirimiphos-methyl	8
Tetradifon	3	chlorpyrifos-methyl	6
Biphenyl	2	ethion	4
Malathion	2	dimethoate	2
Mecarbam	2	fenthion	2
Diazinon	1		
Ethion	1		

Pesticides ranked by their worst-case frequency* of occurrence as multiple residues in each commodity surveyed from 1997-2001. Frequency is measured as the percentage (%) of samples measured containing the given pesticide in combination with at least one other pesticide residue. The worst-case means the year with the highest frequency of the given pesticide.

* Worst-case frequencies were adopted to circumvent problems associated with different pesticides being sought, and different reporting limits being adopted, in different surveys of the same commodity during 1997 to 2001. They were also adopted because apparent changes in the residues profile of a particular commodity from year to year may or may not have been real. The surveys are targeted towards foods which are widely consumed and where residues are most likely to be present, or when information is made available to suggest that misuse may have occurred. Hence the surveys are not designed to be representative.

Table A3.3A to A.3.3J

Detected frequencies of occurrence of multiple residues in individual samples of food commodities, 1997-2001

The table shows the occurrence of multiple residues of pesticides in individual samples of different food commodities in the years surveyed since 1997.

No data are shown for samples containing single pesticide residues or for samples where no pesticides were found.

Each row of the table represents a sample or samples in a given year that have a particular combination of residues. A cross indicates the occurrence of a pesticide in the sample or samples. Reading across the table from left to right, the first column is the year of the survey. The columns that follow are headed by individual pesticides and the crosses in each row indicate the combination of particular pesticide residues detected. Towards the right hand side of the table, the column headed pesticides shows the number of pesticide residues found in the sample, which tallies with the number of crosses in the row. The occurrences column shows the number of samples that contained that particular combination of pesticides and the samples column indicates the total number of samples analysed in that year. As stated above the samples that contained either only one or no detectable pesticide are not represented in the rows, but the total number of samples includes these samples.

Highlighted columns in the table represent organophosphorus and carbamate compounds. Some sections of the table indicated by (OP/C) beneath the year are surveys that were restricted to organophosphorus and carbamate compounds.

It should be noted that it may be necessary to read from more than one row of the table to find out how many times a given combination of say, 2 pesticides occurs. This is because a combination of 2, 3 or 4 or more pesticides is each listed as a separate row. A particular combination of 2 pesticides can be present in a sample with 3 or more.

Table A3.3A Detected frequencies of occurrence of multiple residues in Apples, 1997-2001

year	azm	bif	bpp	bup	cap	Cby	cbz	cpf	dic	dim	diz	dod	dpa	dtc	dtn	etq	ipr	met	mtx	myc	ome	pac	pgt	phs	pir	tbz	teb	tol	pesticides	occurrences	samples
1997													X													X			2	5	72
					X		X																						2	3	
							X						X																2	2	
							X												X										2	2	
							X																	X					2	2	
													X	X															2	2	
						X	X																						2	1	
								X					X																2	1	
											X		X																2	1	
										X			X																2	1	
		X											X																2	1	
		X			X			X		X																			2	1	
								X												X									2	1	
								X						X															2	1	
					X																			X					2	1	
					X		X												X										3	2	
				X			X												X										3	2	
							X						X						X										3	1	
						X	X												X										3	1	
							X												X			X							3	1	
							X						X											X					3	1	
			X				X																					X	3	1	
													X	X												X			3	1	
													X											X		X			3	1	
		X											X													X			3	1	
													X	X			X												3	1	
					X	X								X															3	1	
							X						X				X						X						4	1	
							X						X										X			X			4	1	
							X						X											X		X			4	1	
							X						X	X		X													4	1	
					X		X						X											X					4	1	
					X		X							X									X						4	1	
										X			X										X			X			4	1	
					X												X		X	X					X				5	1	
			X				X						X			X							X						5	1	
							X						X	X		X										X			5	1	
			X				X						X	X									X						5	1	

azinphos-methyl (azm), bifenthrin (bif), bromopropylate (bpp), bupirimate (bup), captan (cap), carbaryl (cby), carbendazim (cbz), chlorpyrifos (cpf), dicofol (dic), dimethoate (dim), diazinon (diz), dodine (dod), diphenylamine (dpa), dithiocarbamates (dtc), dithianon (dtn), ethoxyquin (etq), iprodione (ipr), methomyl (met), metalaxyl (mtx), myclobutanil (myc), omethoate (ome), paclobutrazol (pac), propargite (pgt), phosalone (phs), pirimicarb (pir), thiabendazole (tbz), tebufenpyrad (teb), tolylfluanid (tol)

Table A3.3A Detected frequencies of occurrence of multiple residues in Apples, 1997-2001

year	azm	bif	bpp	bup	cap	Cby	cbz	cpf	dic	dim	diz	dod	dpa	dtc	dtn	etq	ipr	met	mtx	myc	ome	pac	pgt	phs	pir	tbz	teb	tol	pesticides	occurrences	samples
1997						X		X																					2	3	78
(OP/C)								X																	X				2	1	
						X				X																			2	1	
								X		X																			2	1	
								X			X																		2	1	
	X					X																							2	1	
	X							X																					2	1	
						X		X		X																			3	1	
	X							X		X																			3	1	
								X		X														X					3	1	
								X		X											X								3	1	
1998							X						X																2	5	96
													X	X															2	4	
							X																	X					2	2	
							X												X										2	1	
						X	X																						2	1	
			X				X																						2	1	
														X										X					2	1	
					X																			X					2	1	
										X				X															2	1	
								X						X															2	1	
									X				X																2	1	
					X								X																2	1	
				X				X																					2	2	
							X						X											X					3	2	
							X						X	X															3	1	
			X				X						X																3	1	
					X		X						X																3	1	
						X		X						X															3	1	

azinphos-methyl (azm), bifenthrin (bif), bromopropylate (bpp), bupirimate (bup), captan (cap), carbaryl (cby), carbendazim (cbz), chlorpyrifos (cpf), dicofol (dic), dimethoate (dim), diazinon (diz), dodine (dod), diphenylamine (dpa), dithiocarbamates (dtc), dithianon (dtn), ethoxyquin (etq), iprodione (ipr), methomyl (met), metalaxyl (mtx), myclobutanil (myc), omethoate (ome), paclobutrazol (pac), propargite (pgt), phosalone (phs), pirimicarb (pir), thiabendazole (tbz), tebufenpyrad (teb), tolylfluanid (tol)

Table A3.3A Detected frequencies of occurrence of multiple residues in Apples, 1997-2001

year	azm	bif	bpp	bup	cap	Cby	cbz	cpf	dic	dim	diz	dod	dpa	dtc	dtn	etq	ipr	met	mtx	myc	ome	pac	pgt	phs	pir	tbz	teb	tol	pesticides	occurrences	samples
1999	X							X																					2	4	144
								X																	X				2	2	
								X		X														X	X				2	1	
	X					X																							2	1	
						X		X		X																			3	1	
	X					X		X																					3	1	
2000							X	X																					2	10	144
								X																	X				2	5	
								X						X															2	5	
								X				X																	2	4	
						X		X																					2	2	
						X	X																						2	2	
								X																X					2	1	
							X																		X				2	1	
							X					X																	2	1	
							X																	X					2	1	
																								X	X				2	1	
															X									X					2	1	
	X													X															2	1	
	X									X																			2	1	
								X		X														X					3	2	
						X		X				X																	3	2	
							X	X						X															3	1	
								X							X										X				3	1	
								X		X														X					3	1	
								X			X	X																	3	1	
	X							X						X															3	1	
							X								X										X				3	1	
							X																		X		X		3	1	
							X								X									X					3	1	
							X								X			X						X					3	1	
												X			X										X				3	1	

azinphos-methyl (azm), bifenthrin (bif), bromopropylate (bpp), bupirimate (bup), captan (cap), carbaryl (cby), carbendazim (cbz), chlorpyrifos (cpf), dicofol (dic), dimethoate (dim), diazinon (diz), dodine (dod), diphenylamine (dpa), dithiocarbamates (dtc), dithianon (dtn), ethoxyquin (etq), iprodione (ipr), methomyl (met), metalaxyl (mtx), myclobutanil (myc), omethoate (ome), paclobutrazol (pac), propargite (pgt), phosalone (phs), pirimicarb (pir), thiabendazole (tbz), tebufenpyrad (teb), tolylfluanid (tol)

Table A3.3B Detected frequencies of occurrence of multiple residues in lettuce, 1997-2001

year	dtc	fpt	gHCH	ipr	pcb	ppz	tcm	ace	cbz	dcf	dim	inb	mdp	mtx	ome	oxl	Pcm	qui	tbc	cyp	pir	pym	flx	mal	ofu	pesticides	occurrences	samples
1997					X		X																			2	1	22
				X	X																					2	1	
	X						X																			2	1	
				X			X																			2	1	
					X	X																				2	1	
	X				X		X																			3	1	
			X			X	X																			3	1	
		X		X		X																				3	1	
	X			X	X		X																			4	1	
	X			X		X	X																			4	1	
1998					X												X									2	1	25
				X																			X			2	1	
							X														X					2	1	
					X															X						2	1	
	X			X																						2	1	
										X															X	2	1	
	X				X							X														3	1	
					X		X											X								3	1	
	X											X								X						3	1	
	X			X								X														3	1	
	X				X		X										X									4	2	
	X			X	X													X								4	1	
	X			X	X		X																			4	1	
	X			X	X							X						X								5	1	
				X	X													X		X	X					5	1	
	X						X					X				X				X						5	1	
	X			X		X	X					X								X				X		7	1	

dithiocarbamates (dtc), folpet (fpt), gamma-HCH (gHCH), iprodione (ipr), propamocarb (pcb), propyzamide (ppz), tolclofos-methyl (tcm), acephate (ace), carbendazim (cbz), dichlofluanid (dcf), dimethoate (dim), inorganic bromide (inb), methamidophos (mdp), metalaxyl (mtx), omethoate (ome), oxadixyl (oxl), procymidone (pcm), quintozene (qui), tebuconazole (tbc), cypermethrin (cyp), pirimicarb (pir), pyrimethanil (pym), fluralaxyl (flx), malathion (mal), ofurace (ofu)

Table A3.3B Detected frequencies of occurrence of multiple residues in lettuce, 1997-2001

year	dtc	fpt	gHCH	ipr	pcb	ppz	tcm	ace	cbz	dcf	dim	inb	mdp	mtx	ome	oxl	Pcm	qui	tbc	cyp	pir	pym	flx	mal	ofu	pesticides	occurrences	samples
1999					X													X								2	2	72
	X			X																						2	2	
	X																	X								2	2	
	X						X																			2	1	
							X					X														2	1	
										X				X			X									2	1	
	X													X												2	1	
								X					X													2	1	
				X	X																					2	1	
	X			X								X														3	1	
					X							X						X								3	1	
	X				X		X					X														4	2	
				X			X				X				X											4	1	
		X		X						X									X							4	1	
		X						X					X			X										4	1	
	X			X	X		X					X														5	1	
	X			X			X		X			X														5	1	
	X			X			X					X		X												5	1	
		X		X				X					X			X										5	1	
	X			X			X					X		X		X										6	1	

dithiocarbamates (dtc), folpet (fpt), gamma-HCH (gHCH), iprodione (ipr), propamocarb (pcb), propyzamide (ppz), tolclofos-methyl (tcm), acephate (ace), carbendazim (cbz), dichlofluanid (dcf), dimethoate (dim), inorganic bromide (inb), methamidophos (mdp), metalaxyl (mtx), omethoate (ome), oxadixyl (oxl), procymidone (pcm), quintozene (qui), tebuconazole (tbc), cypermethrin (cyp), pirimicarb (pir), pyrimethanil (pym), fluralaxyl (flx), malathion (mal), ofurace (ofu)

Table A3.3B Detected frequencies of occurrence of multiple residues in lettuce, 1997-2001

year	dtc	fpt	gHCH	ipr	pcb	ppz	tcm	ace	cbz	dcf	dim	inb	mdp	mtx	ome	oxl	Pcm	qui	tbc	cyp	pir	pym	flx	mal	ofu	pesticides	occurrences	samples
2000												X								X						2	2	71
																	X			X						2	1	
																X	X									2	1	
	X																X									2	1	
				X		X																				2	1	
	X																	X								2	1	
								X					X													2	1	
												X						X		X						3	1	
				X	X													X								3	1	
				X								X								X						3	1	
				X			X													X						3	1	
								X					X			X										3	1	
				X		X												X								3	1	
					X							X						X								3	1	
	X													X			X									3	1	
	X			X			X					X														4	2	
	X				X		X					X														4	1	
					X	X												X			X					4	1	
	X			X			X							X												4	1	
				X			X					X						X								4	1	
	X			X			X													X						4	1	
				X	X													X		X						4	1	
	X			X		X	X													X						5	1	
				X	X						X							X		X						5	1	
	X			X	X		X											X		X						6	1	
	X			X			X					X				X				X		X				7	1	
	X			X	X		X				X	X		X						X						8	1	

Dithiocarbamates (dtc), folpet (fpt), gamma-HCH (gHCH), iprodione (ipr), propamocarb (pcb), propyzamide (ppz), tolclofos-methyl (tcm), acephate (ace), carbendazim (cbz), dichlofluanid (dcf), dimethoate (dim), inorganic bromide (inb), methamidophos (mdp), metalaxyl (mtx), omethoate (ome), oxadixyl (oxl), procymidone (pcm), quintozene (qui), tebuconazole (tbc), cypermethrin (cyp), pirimicarb (pir), pyrimethanil (pym), fluralaxyl (flx), malathion (mal), ofurace (ofu)

Table A3.3C Detected frequencies of occurrence of multiple residues in pears and oriental pears,, 1997-2001

year	cap	cby	cbz	clq	dim	dpa	dtc	etq	imz	ipr	pcm	phs	pir	pmt	tol	ome	mon	azm	bpp	fnt	mtx	tbz	cpf	cpm	dfc	dod	mal	dim	pesticides	occurrences	samples
1997			X							X																			2	3	54
			X	X																									2	3	
		X				X																							2	1	
				X			X																						2	1	
			X	X		X																							3	2	
			X	X											X														3	2	
			X			X				X																			3	1	
							X			X			X																3	1	
							X					X		X															3	1	
	X		X	X																									3	1	
			X	X			X								X														4	4	
			X	X		X				X																			4	1	
			X			X	X			X																			4	1	
			X	X			X			X																			4	1	
				X		X	X			X																			4	1	
				X			X	X	X																				4	1	
				X		X			X	X																			4	1	
	X		X	X											X														4	1	
			X				X	X		X	X																		5	1	
			X			X		X	X			X																	5	1	
			X		X	X			X	X				X															6	1	
	X		X		X	X	X	X						X															7	1	
1997					X														X										2	1	25
(OP/C)												X		X															2	1	
												X													X				2	1	
					X							X		X		X													4	1	

captan (cap), carbaryl (cby), carbendazim (cbz), chlormequat (clq), dimethoate (dim), diphenylamine (dpa), dithiocarbamates (dtc), ethoxyquin (etq), imazalil (imz), iprodione (ipr), procymidone (pcm), phosalone (phs), pirimicarb (pir), phosmet(pmt), tolylfluanid (tol), omethoate (ome), monocrotophos (mon), azinphos-methyl (azm), bromopropylate (bpp), fenitrothion (fnt), metalaxyl (mtx), thiabendazole (tbz), monocrotophos (mon), azinphos-methyl (azm), bromopropylate (bpp), fenitrothion (fnt), metalaxyl (mtx), thiabendazole (tbz), chlorpyrifos (cpf), chlorpyrifos-methyl (cpm), diethofencarb (def), dodine (dod), malathion (mal), dimethoate (dim)

Table A3.3C Detected frequencies of occurrence of multiple residues in pears and oriental pears,, 1997-2001

year	cap	cby	cbz	clq	dim	dpa	dtc	etq	imz	ipr	pcm	phs	pir	pmt	tol	ome	mon	azm	bpp	fnt	mtx	tbz	cpf	cpm	dfc	dod	mal	dim	pesticides	occurrences	samples
1998	X						X																						2	1	72
				X						X																			2	1	
			X			X																							2	1	
				X			X																						2	3	
				X																X									2	1	
	X			X																									2	1	
			X																		X								2	1	
	X			X			X																						3	1	
				X			X												X										3	2	
				X			X				X																		3	2	
	X		X	X																									3	1	
			X	X			X																						3	2	
				X			X								X														3	2	
			X	X			X			X																			4	2	
	X		X	X											X														4	2	
			X	X			X								X														4	2	
	X		X			X								X				X											5	1	
						X	X		X			X		X															5	1	
			X	X				X	X			X																	5	1	
			X		X	X	X					X																	5	1	
	X														X			X	X			X							5	1	
			X	X			X								X				X										5	1	
	X			X	X	X	X		X																				6	1	
	X		X	X			X								X				X										6	1	

captan (cap), carbaryl (cby), carbendazim (cbz), chlormequat (clq), dimethoate (dim), diphenylamine (dpa), dithiocarbamates (dtc), ethoxyquin (etq), imazalil (imz), iprodione (ipr), procymidone (pcm), phosalone (phs), pirimicarb (pir), phosmet(pmt), tolylfluanid (tol), omethoate (ome), monocrotophos (mon), azinphos-methyl (azm), bromopropylate (bpp), fenitrothion (fnt), metalaxyl (mtx), thiabendazole (tbz), monocrotophos (mon), azinphos-methyl (azm), bromopropylate (bpp), fenitrothion (fnt), metalaxyl (mtx), thiabendazole (tbz), chlorpyrifos (cpf), chlorpyrifos-methyl (cpm), diethofencarb (def), dodine (dod), malathion (mal), dimethoate (dim)

Table A3.3C Detected frequencies of occurrence of multiple residues in pears and oriental pears,, 1997-2001

year	cap	cby	cbz	clq	dim	dpa	dtc	etq	imz	ipr	pcm	phs	pir	pmt	tol	ome	mon	azm	bpp	fnt	mtx	tbz	cpf	cpm	dfc	dod	mal	dim	pesticides	occurrences	samples
2000			X	X																									2	16	84
							X											X											2	3	
				X			X																						2	2	
				X														X											2	1	
		X																X											2	1	
																							X			X			2	1	
				X																				X					2	1	
				X								X																	2	1	
			X				X																						2	1	
		X		X																									2	1	
							X					X																	2	1	
							X																			X			2	1	
												X		X															2	1	
			X	X																					X				3	7	
			X	X			X																						3	2	
		X	X	X																									3	1	
			X	X								X																	3	1	
			X	X																			X						3	1	
							X					X						X											3	1	
		X		X								X																	3	1	
							X					X		X															3	1	
				X			X							X															3	1	
				X			X																				X		3	1	
			X				X							X														X	4	1	
			X	X			X																		X				4	4	
			X	X																					X				4	1	
			X	X																					X	X			4	1	
				X			X					X		X													X		5	1	

Oriental pears

year	cap	cby	cbz	clq	dim	dpa	dtc	etq	imz	ipr	pcm	phs	pir	pmt	tol	ome	mon	azm	bpp	fnt	mtx	tbz	cpf	cpm	dfc	dod	mal	dim	pesticides	occurrences	samples
1997						X	X																						2	2	13
							X										X												2	1	
	X						X												X										3	1	

captan (cap), carbaryl (cby), carbendazim (cbz), chlormequat (clq), dimethoate (dim), diphenylamine (dpa), dithiocarbamates (dtc), ethoxyquin (etq), imazalil (imz), iprodione (ipr), procymidone (pcm), phosalone (phs), pirimicarb (pir), phosmet(pmt), tolylfluanid (tol), omethoate (ome), monocrotophos (mon), azinphos-methyl (azm), bromopropylate (bpp), fenitrothion (fnt), metalaxyl (mtx), thiabendazole (tbz), monocrotophos (mon), azinphos-methyl (azm), bromopropylate (bpp), fenitrothion (fnt), metalaxyl (mtx), thiabendazole (tbz), chlorpyrifos (cpf), chlorpyrifos-methyl (cpm), diethofencarb (def), dodine (dod), malathion (mal), dimethoate (dim)

Table A3.3D Detected frequencies of occurrence of multiple residues in peaches and nectarines, 1997-2001

year	ace	cby	cpf	fen	mdp	met	pam	phs	pir	qin	cbz	dim	dtc	ipr	vin	pesticides	occurrences	samples
1997	X				X											2	3	100
			X							X						2	1	
					X		X									2	1	
					X	X										2	1	
					X			X								2	1	
				X					X							2	1	
		X	X	X					X							4	1	
1998					X								X			2	3	72
	X										X					2	1	
													X	X		2	1	
		X									X					2	1	
												X		X		2	1	
											X		X			2	2	
	X													X		2	1	
					X										X	2	1	
					X			X						X		3	1	
		X						X						X		3	1	
					X						X			X		3	1	

acephate (ace), carbaryl (cby), chlorpyrifos (cpf), fenitrothion (fen), methamidophos (mdp), methomyl (met), parathion-methyl (pam), phosalone (phs), pirimicarb (pir), quinalphos (qin), carbendazim (cbz), dimethoate (dim), dithiocarbamates (dtc), iprodione (ipr), vinclozolin (vin)

Table A3.3E Detected frequencies of occurrence of multiple residues in mandarins and clementines, 1997-2001

year	24D	2pp	azm	cbz	cpf	cpm	dim	fnt	imz	mal	mdt	pim	tbz	tet	etn	pesticides	occurrences	samples
1997	X								X							2	2	34
		X							X				X			3	4	
	X								X				X			3	1	
	X	X											X			3	1	
		X			X				X				X			4	3	
	X	X				X			X							4	3	
	X								X		X		X			4	3	
	X	X							X				X			4	1	
					X				X		X		X			4	1	
	X	X		X					X				X			5	2	
	X	X			X				X				X			5	3	
		X		X	X				X				X			5	1	
					X		X		X		X		X			5	1	
					X				X		X		X	X		5	1	
	X	X			X				X		X					5	1	
		X							X		X	X	X	X		6	1	
	X	X			X				X		X		X			6	1	
		X	X		X				X			X	X			6	1	
		X	X		X				X				X	X		6	1	
	X	X		X	X				X				X			6	1	
		X			X			X	X	X		X	X			7	1	

2,4-D, 2-phenylphenol (24D), azinphos-methyl (azm), carbendazim (cbz), chlorpyrifos (cpf), chlorpyrifos-methyl (cpm), dimethoate (dim), fenitrothion (fnt), imazalil (imz), malathion (mal), methidathion (mdt), pirimiphos-methyl (pim), thiabendazole (tbz), tetradifon (tet), ethion (etn)

Table A3.3E Detected frequencies of occurrence of multiple residues in mandarins and clementines, 1997-2001

year	24D	2pp	azm	cbz	cpf	cpm	dim	fnt	imz	mal	mdt	pim	tbz	tet	etn	pesticides	occurrences	samples
1997										X			X			2	1	16
			X										X			2	1	
		X			X				X							3	1	
		X							X		X		X			4	1	
		X			X				X				X			4	1	
				X	X				X				X			4	1	
		X	X						X				X		X	4	1	
		X			X				X	X			X			5	1	
	X	X		X					X		X					5	1	
		X							X	X	X		X			5	1	
		X		X	X				X				X			5	1	
		X							X	X	X		X			5	1	
		X	X						X		X		X		X	5	1	
	X				X				X	X			X	X		6	1	
	X	X			X				X		X	X		X		7	1	
		X	X						X		X		X		X	6	1	

2,4-D, 2-phenylphenol (24D), azinphos-methyl (azm), carbendazim (cbz), chlorpyrifos (cpf), chlorpyrifos-methyl (cpm), dimethoate (dim), fenitrothion (fnt), imazalil (imz), malathion (mal), methidathion (mdt), pirimiphos-methyl (pim), thiabendazole (tbz), tetradifon (tet), ethion (etn)

Table A3.3F Detected frequencies of occurrence of multiple residues in strawberries, 1997-2001

year	bup	cap	cby	cln	cbz	dcf	ens	fdn	fnm	fpm	fpp	ipr	mal	met	myc	pcm	pgt	pir	tol	vin	pen	pym	dtc	krm	cyd				Pesticides	Occurrences	Samples
1997	X					X																							2	1	48
										X		X																	2	1	
	X																		X										2	1	
				X								X																	2	1	
	X			X																									2	1	
								X												X									2	1	
					X											X													2	2	
												X							X										2	1	
				X		X									X														3	1	
	X									X		X																	3	1	
	X			X											X														3	1	
						X	X			X																			3	1	
		X										X	X																3	1	
											X			X			X												3	1	
			X										X		X		.												3	1	
						X							X		X														3	1	
	X			X								X						X											4	1	
									X			X	X		X														4	1	

bupirimate (bup), captan (cap), carbaryl (cby), chlorothalonil (cin), carbendazim (cbz), dichlofluanid (dcf), endosulfan (ens), fenpropidin (fdn), fenarimol (fnm), fenpropimorph (fpm), fenpropathrin (fpp), iprodione (ipr), malathion (mal), methomyl (met), myclobutanil (myc), procymidone (pcm), propargite (pgt), pirimicarb (pir), tolylfluanid (tol), vinclozolin (vin), penconazole (pen), pyrimethanil (pym), dithiocarbamates (dtc)

Table A3.3F Detected frequencies of occurrence of multiple residues in strawberries, 1997-2001

year	bup	cap	cby	cln	cbz	dcf	ens	fdn	fnm	fpm	fpp	ipr	mal	met	myc	pcm	pgt	pir	tol	vin	pen	pym	dtc	krm	cyd				Pesticides	Occurrences	Samples
1999												X										X						2	1	45
	X											X																2	1	
																X						X						2	1	
																X			X									2	1	
																			X			X						2	1	
												X									X							2	1	
	X					X																X						3	1	
												X						X					X					3	1	
	X			X								X																3	1	
	X														X							X						3	1	
	X																	X				X						3	1	
	X			X																		X						3	1	
	X											X										X						3	1	
	X											X											X					3	1	
	X	X																				X						3	1	
						X												X				X	X					4	1	
	X	X													X							X						4	1	
	X	X										X										X						4	1	
	X									X		X						X				X						5	1	
2000																			X						X				2	1	11
		X																							X				2	1	
	X																					X							2	1	
												X										X							2	1	
																					X				X				2	1	
	X											X																	2	1	
	X																		X			X							3	1	
	X																				X	X							3	1	
												X									X	X							3	1	
																				X	X			X					3	1	
		X																		X	X								3	1	

bupirimate (bup), captan (cap), carbaryl (cby), chlorothalonil (cin), carbendazim (cbz), dichlofluanid (dcf), endosulfan (ens), fenpropidin (fdn), fenarimol (fnm), fenpropimorph (fpm), fenpropathrin (fpp), iprodione (ipr), malathion (mal), methomyl (met), myclobutanil (myc), procymidone (pcm), propargite (pgt), pirimicarb (pir), tolylfluanid (tol), vinclozolin (vin), penconazole (pen), pyrimethanil (pym), dithiocarbamates (dtc), kresoxim-methyl (krm), cyprodinil (cyd)

Table A3.3F Detected frequencies of occurrence of multiple residues in grapes, 1997-2001

year	bif	bpp	cap	cby	cbz	cpf	cpm	cyh	cyp	del	dic	dim	dtc	ens	etn	fbt	fnp	fnt	fnv	ipr	lcy	mdp	met	mtx	myc	ome	pam	pan	pcm	pmt	pcz	pgt	phs	pnz	pym	pyz	tbc	tet	pest's	occ's	samp's
1997						X																			X															2	1	36
		X			X																																		2	1	
				X																					X														2	1	
	X									X																											X			3	1	
					X								X								X																			3	1	
	X												X													X													3	1	
												X	X							X																			3	1	
			X									X													X															3	1	
															X				X					X	X			X									X				4	1	
													X				X										X										X			4	1	
					X								X												X			X												4	1	
			X								X				X			X	X									X	X										8	1	
1999			X																X																				2	1	72
			X																	X																			2	5	
		X																																		X			2	1	
																								X				X											2	1	
																													X						X				2	1	
																				X															X				2	1	
									X					X																										2	1	
						X																											X						2	1	
		X						X															X																3	1	
					X				X											X																				3	1	
		X																		X		X																		3	1	
																									X							X				X			3	1	
											X						X																				X			3	1	
									X					X														X									X			4	1	
																								X					X			X				X			4	1	
							X									X						X										X				X				5	1	
							X															X										X				X				5	1	

bifenthrin (bif), bromopropylate (bpp), captan (cap), carbaryl (cby), carbendazim (cbz), chlorpyrifos (cpf), chlorpyrifos-methyl (cpm), cyhalothrin (cyh), cypermethrin (cyp), deltamethrin (del), dicofol (dic), dimethoate (dim),dithiocarbamates (dtc), endosulfan (ens), ethion (etn), fenbutatin oxide (fbt), fenpropathrin (fnp), fenitrothion (fnt), fenvalerate (fnv), iprodione (ipr), lambda-cyhalothrin (lcy), methamidophos (mdp), methomyl (met),metalaxyl (mtx), myclobutanil (myc), omethoate (ome), parathion-methyl (pam), parathion (pan), procymidone (pcm), phosmet (pmt), propiconazole (pcz), propargite (pgt), phosalone (phs), penconazole (pnz), pyrimethanil (pym), pyrazophos (pyz), tebuconazole (tbc), tetradifon (tet)

Table A3.3H Detected frequencies of occurrence of multiple residues in oranges, 1997-2001

year	24d	2pp	bpy	cby	cbz	cpf	cpm	dic	dim	diz	etn	imz	mal	mdt	mec	mtx	tbz	tet	Pesticides	occurrences	samples
1998												X		X					2	7	66
												X					X		2	4	
	X											X							2	4	
		X										X							2	1	
						X											X		2	1	
	X											X					X		3	12	
												X		X			X		3	1	
						X						X					X		3	1	
												X		X	X				3	1	
						X						X	X						3	1	
	X	X										X					X		4	6	
		X				X						X					X		4	2	
		X										X		X			X		4	2	
	X				X							X					X		4	1	
		X	X									X					X		4	1	
	X											X				X	X		4	1	
	X											X		X			X		4	1	
	X	X										X		X			X		5	4	
	X	X										X				X	X		5	4	
				X		X						X		X			X		5	1	
		X				X	X					X					X		5	1	
	X	X				X						X					X		5	1	
	X	X		X								X					X		5	1	
	X	X										X		X		X	X		6	3	

2,4-D (24d), 2-phenylphenol (2pp), biphenyl (bpy), carbaryl (cby), carbendazim (cbz), chlorpyrifos (cpf), chlorpyrifos-methyl (cpm), dicofol (dic), dimethoate (dim), diazinon (diz), ethion (etn), imazalil (imz), malathion (mal), methidathion (mdt), mecarbam (mec), metalaxyl (mtx), thiabendazole (tbz), tetradifon (tet)

Table A3.3H Detected frequencies of occurrence of multiple residues in oranges, 1997-2001

year	24d	2pp	bpy	cby	cbz	cpf	cpm	dic	dim	diz	etn	imz	mal	mdt	mec	mtx	tbz	tet	Pesticides	occurrences	samples
1999												X					X		2	7	72
		X										X							2	2	
	X											X							2	2	
														X			X		2	1	
								X						X					2	1	
	X											X					X		3	21	
						X						X					X		3	3	
												X					X	X	3	1	
		X										X					X		3	1	
		X						X				X							3	1	
												X		X			X		3	1	
											X	X					X		3	1	
	X	X										X					X		4	7	
	X											X		X			X		4	4	
		X										X		X			X		4	2	
	X								X			X					X		4	1	
						X						X		X			X		4	1	
	X											X					X	X	4	1	
								X				X		X			X		4	1	
		X				X						X					X		4	1	
	X	X										X		X			X		5	2	
								X		X		X		X			X		5	1	
						X			X			X		X			X		5	1	
	X			X								X		X		X	X		6	1	
	X					X						X		X		X	X		6	1	
	X	X		X								X		X		X	X		7	1	

2,4-D (24d), 2-phenylphenol (2pp), biphenyl (bpy), carbaryl (cby), carbendazim (cbz), chlorpyrifos (cpf), chlorpyrifos-methyl (cpm), dicofol (dic), dimethoate (dim), diazinon (diz), ethion (etn), imazalil (imz), malathion (mal), methidathion (mdt), mecarbam (mec), metalaxyl (mtx), thiabendazole (tbz), tetradifon (tet)

Table A3.31 Detected frequencies of occurrence of multiple residues in celery, 1997-2001

year	cbz	cln	cpf	cpm	dcf	dsf	dtc	ens	inb	mdp	pcm	pho	pir	ppz	pyn	tbz	vin	pesticides	occurrences	samples
1997		X													X			2	2	36
												X			X			2	1	
		X										X						2	1	
	X	X																2	1	
			X						X									2	1	
									X				X					2	1	
			X	X														2	1	
		X					X											2	1	
		X					X		X									3	1	
		X	X													X		3	1	
					X		X	X						X				4	1	
1999		X							X									2	9	68
	X															X		2	2	
		X				X												2	1	
										X							X	2	1	
							X				X							2	1	
									X		X							2	1	
											X					X		2	1	
					X		X											2	1	
						X			X									2	1	

carbendazim (cbz), chlorothalonil (cln), chlorpyrifos (cpf), chlorpyrifos-methyl (cpm), dicofol (dic), disulfoton (dsf), dithiocarbamates (dtc), endosulfan (ens), inorganic bromide (inb), methamidophos (mdp), procymidone (pcm), phorate (pho), pirimicarb (pir), propyzamide (ppz), prometryn (pyn), thiabendazole (tbz), vinclozolin (vin), prometryn (pyn), thiabendazole (tbz), vinclozolin (vin)

Table A3.3J Detected frequencies of occurrence of multiple residues in other crops, 1997-2001

Cooking fat	DDT	HCB										pesticides	occurrences	samples
1997	X	X										2	1	33

DDT, HCB

Lamb	DDT	gHCH										pesticides	occurrences	samples
1997	X	X										2	1	71

DDT, gamma-HCH (gHCH)

Rabbit	bHCH	DDT	aHCH									pesticides	occurrences	samples
1997	X	X										2	6	36
1999	X	X										2	6	17
	X		X									2	1	

beta-HCH (bHCH), DDT, gamma-HCH (gHCH)

Green beans	dim	ome										pesticides	occurrences	samples
1997	X	X										2	2	54

dimethoate (dim), omethoate (ome)

Infant formula	ens	HCB										pesticides	occurrences	samples
1997	X	X										2	1	20

endosulfan (ens), HCB

Mixed leaf salads	ace	ipr	oxl	pcm								pesticides	occurrences	samples
1998			X	X								2	3	24
	X	X	X									3	1	

acephate (ace), iprodione (ipr), oxadixyl (oxl), procymidone (pcm)

Mushrooms	cbz	prz										pesticides	occurrences	samples
1998	X	X										2	1	47

carbendazim (cbz), prochloraz (prz)

Tomatoes	cbz	dtc	ens	lcy	oxl	oxm	pcm	pgt	vin			pesticides	occurrences	samples
1998		X					X					2	2	48
	X								X			2	1	
		X	X									2	1	
			X	X								2	1	
					X	X						2	1	
			X				X	X				3	1	

carbendazim (cbz), dithiocarbamates (dtc), endosulfan (ens), lambda-cyhalothrin (lcy), oxadixyl (oxl), oxamyl (oxm), procymidone (pcm), propargite (pgt), vinclozolin (vin)

Table A3.3J Detected frequencies of occurrence of multiple residues in other crops, 1997-2001

Wine	oxl	pcm										pesticides	occurrences	samples
1998	X	X										2	1	72

oxadixyl (oxl), procymidone (pcm)

Yams	cbz	imz										pesticides	occurrences	samples
1998	X	X										2	1	16
2000	X	X										2	2	42

carbendazim (cbz), imazalil (imz)

Spinach	cyh	del	ipr	per								pesticides	occurrences	samples
1998	X			X								2	1	66
		X		X								2	1	
	X		X									2	1	
	X	X										2	1	
			X	X								2	1	

cyhalothrin (cyh), deltamethrin (del), iprodione (ipr), permethrin (per)

Blackberries	dtc	ipr	pir	vin								pesticides	occurrences	samples
1999	X		X									2	2	21
		X		X								2	1	

dithiocarbamates (dtc), iprodione (ipr), pirimicarb (pir), vinclozolin (vin)

Carrots	cfv	ipr	pnd	tfn	tri	dtc	qui					pesticides	occurrences	samples
1998		X	X									2	4	66
	X	X										2	3	
		X				X	X					3	1	
		X		X	X							3	1	
1999		X	X									2	5	72
	X	X										2	2	
2000		X	X									2	7	71
		X			X							2	2	
			X	X								2	2	
		X		X								2	1	
	X	X										2	1	

chlorfenvinphos (cfv), iprodione (ipr), pendimethalin (pnd), trifluralin (tfn), triazophos (tri), dithiocarbamates (dtc), quinalphos (qui)

Passion fruit	bpp	dtc	mdp	tri								pesticides	occurrences	samples
1999	X			X								2	1	21
	X		X									2	1	

bromopropylate (bpp), dithiocarbamates (dtc), methamidophos (mdp), triazophos (tri)

Table A3.3J Detected frequencies of occurrence of multiple residues in other crops, 1997-2001

Goat cheese	aHCH	gHCH										pesticides	occurrences	samples
1999	X	X										2	1	23

alpha-HCH (aHCH), gamma-HCH (gHCH)

Fish oils	aHCH	cld	DDT	die	gHCH	HCB						pesticides	occurrences	samples
1999			X	X								2	9	23
			X		X							2	1	
			X	X	X							3	1	
		X	X	X		X						4	1	
	X	X	X	X		X						5	1	

alpha-HCH (aHCH), chlordane (cld), DDT, dieldrin (die), gamma-HCH (gHCH), HCB

Sweet peppers	ens	mdp	pcm	ace								pesticides	occurrences	samples
1999		X	X									2	3	71
	X	X										2	1	
	X	X	X									3	1	
1999	X	X										2	5	48
(special surveys)		X		X								2	1	
	X	X		X								3	1	

endosulfan (ens), methamidophos (mdp), procymidone (pcm), acephate (ace)

Baby vegetables	cbz	ipr	mtx									pesticides	occurrences	samples
1997		X	X									2	1	49
	X	X	X									3	2	

carbendazim (cbz), iprodione (ipr), metalaxyl (mtx)

Cherries	cby	dtc	myc	pir								pesticides	occurrences	samples
1997		X		X								2	1	24
	X		X									2	1	

carbaryl (cby), dithiocarbamates (dtc), myclobutanil (myc), pirimicarb (pir)

Orange juice	2pp	bpy	imz	mtx	tbz							pesticides	occurrences	samples
1997	X			X								2	2	72
	X		X		X							3	1	
	X	X	X		X							4	1	

2-phenylphenol (2pp), biphenyl (bpy), imazalil (imz), metalaxyl (mtx), thiabendazole (tbz)

Table A3.3J Detected frequencies of occurrence of multiple residues in other crops, 1997-2001

Raspberries	bup	cln	dcf	dtc	fnp	ipr	myc	oxl	pir			pesticides	occurrences	samples
1997		X						X				2	1	24
			X			X						2	1	
		X	X									2	1	
		X							X			2	1	
			X						X			2	1	
				X		X		X				3	1	
			X		X	X						3	1	
	X	X					X	X				4	1	

bupirimate (bup), chlorothalonil (cln), dichlofluanid (dcf), dithiocarbamates (dtc), fenpropimorph (fnp), iprodione (ipr), myclobutanil (myc), oxadixyl (oxl), pirimicarb (pir)

Processed potatoes	cpp	mh	oxl	pph	tec							pesticides	occurrences	samples
1998	X	X										2	12	97
	X				X							2	5	
		X			X							2	2	
	X			X								2	2	
	X	X			X							3	8	
2001		X	X									2	2	48
	X	X										2	1	

chlorpropham (cpp), maleic hydrazide (mh), oxadixyl (oxl), propham (pph), tecnazene (tec)

Rice	hp	inb	pim									pesticides	occurrences	samples
2000	X	X										2	9	96
		X	X									2	3	

phosphine (hp), inorganic bromide (inb), pirimiphos-methyl (pim)

Infant food	cbz	pgt	dpa	ipr	phs	tbz						pesticides	occurrences	samples
fruit & veg-based	X			X								2	2	143
1998		X	X									2	2	
	X					X						2	2	
			X		X							2	1	
	X	X			X	X						4	1	
	X	X	X		X	X						5	2	
fruit-based, 2000	X	X										2	2	140

carbendazim (cbz), propargite (pgt), diphenylamine (dpa), iprodione (ipr), phosalone (phs), thiabendazole (tbz)

Nut butter	DDT	inb										pesticides	occurrences	samples
2000	X	X										2	1	24

DDT, inorganic bromide (inb)

Table A3.3J Detected frequencies of occurrence of multiple residues in other crops, 1997-2001

Cucumbers	dtc	pcm	cln	mtx	oxl	pym						pesticides	occurrences	samples
2000	X	X										2	2	59
		X			X							2	1	
		X	X									2	1	
				X	X							2	1	
		X			X	X						3	1	

dithiocarbamates (dtc), procymidone (pcm), chlorothalonil (cln), metalaxyl (mtx), oxadixyl (oxl), pyrimethanil (pym)

Plums	cbz	cpf	izp	phs								pesticides	occurrences	samples
2000	X	X										2	1	44
			X	X								2	1	

carbendazim (cbz), chlorpyrifos (cpf), isazophos (izp), phosalone (phs)

Nuts	ens	inb										pesticides	occurrences	samples
2000	X	X										2	2	47

endosulfan (ens), inorganic bromide (inb)

Cabbages	cbz	cyp	del	ipr								pesticides	occurrences	samples
2000	X			X								2	2	72
		X	X									2	1	

carbendazim (cbz), cypermethrin (cyp), deltamethrin (del), iprodione (ipr)

Bread	clq	gly	mal	pim	aHCH	bHCH	cbz	cyp	ens	ipr		pesticides	occurrences	samples
speciality, 1997				X	X							2	1	215
			X	X								2	1	
speciality, 1997							X	X				2	2	25
(fruit-containing)							X			X		2	1	
				X						X		2	1	
				X				X	X			3	1	
							X	X		X		3	1	
ordinary, 1999			X	X								2	1	144
speciality, 1999					X	X						2	1	68
ordinary, 2000	X	X										2	9	216
	X			X								2	5	
	X	X	X									3	1	

chlormequat (clq), glyphosate (gly), malathion (mal), pirimiphos-methyl (pim), alpha-HCH (aHCH), beta-HCH (bHCH), carbendazim (cbz), cypermethrin (cyp), endosulfan (ens), iprodione (ipr)

Table A3.3J Detected frequencies of occurrence of multiple residues in other crops, 1997-2001

melons	buf	bup	diz	dtc	ens	fnp	mdp	oxl	per	pir	tbz	pesticides	occurrences	samples
1999					X		X					2	2	72
				X			X					2	2	
							X				X	2	1	
				X							X	2	1	
						X			X			2	1	
			X		X							2	1	
		X						X				2	1	
					X		X				X	3	2	
	X				X					X		3	1	

buprofezin (buf), bupirimiate (bup), diazinon (diz), dithiocarbamates (dtc), endosulfan (ens), fenpropathrin (fnp), methamidophos (mdp), oxadixyl (oxl), permthrin (per), pirimicarb (pir), thiabendazole (tbz)

bananas	ald	cpf	dtc	imz	tbz							pesticides	occurrences	samples
1997				X	X							2	14	50
			X	X								2	2	
	X			X	X							3	2	
		X		X	X							3	1	

aldicarb (ald), chlorpyrifos (cpf), dithiocarbamates (dtc), imazalil (imz), thiabendazole (tbz)

human milk	bHCH	DDT	die	gHCH	HCB							pesticides	occurrences	samples
1997	X	X										2	27	168
		X			X							2	11	
		X	X									2	4	
		X		X								2	1	
	X	X			X							3	11	
	X	X	X									3	7	
		X	X		X							3	2	
		X	X	X								3	1	
	X	X	X		X							4	10	
	X	X	X	X	X							5	1	

beta-HCH (bHCH), DDT, dieldrin (die), gamma-HCH (gHCH), HCB

Salmon & trout	cld	DDT	die	gHCH	HCB							pesticides	occurrences	samples
1997		X	X		X							3	7	24
		X	X	X	X							4	10	
	X	X	X		X							4	4	
		X	X	X	X							4	3	

chlordane (cld), DDT, dieldrin (die), gamma-HCH (gHCH), HCB

Table A3.3J Detected frequencies of occurrence of multiple residues in other crops, 1997-2001

Potatoes	cpp	mh	tbz	tec	imz							pesticides	occurrences	samples
1997	X		X									2	9	96
			X	X								2	3	
		X		X								2	2	
	X			X								2	1	
		X	X									2	1	
	X		X	X								3	2	
	X	X	X									3	2	
	X	X		X								3	1	
1999	X		X									2	8	142
	X				X							2	3	
	X	X										2	3	
	X				X							2	1	
	X			X								2	1	
		X			X							2	1	
	X	X			X							3	2	
	X	X	X									3	1	
	X	X		X								3	1	
2000	X	X										2	10	144
	X			X								2	4	
	X				X							2	3	
		X		X								2	2	
	X		X									2	2	
		X	X									2	1	
		X			X							2	1	
				X	X							2	1	
	X	X			X							3	4	
		X	X		X							3	1	
	X	X		X								3	1	

chlorpropham (cpp), maleic hydrazide (mh), thiabendazole (tbz), tecnazene (tec), imazalil (imz)

Currants	bup	cln	cyp	dcf	dtc	fnp	ipr	pir	pym	tol		pesticides	occurrences	samples
1999		X				X						2	4	41
							X			X		2	3	
				X					X			2	2	
				X				X				2	1	
			X		X							2	1	
	X	X				X		X				4	3	

bupirimate (bup), chlorothalonil (cln), cypermethrin (cyp), dichlofluanid (dcf), dithiocarbamates (dtc), fenpropathrin (fnp), iprodione (ipr), pirimicarb (pir), pyrimethanil (pym), tolylfluanid (tol)

Table A3.3J Detected frequencies of occurrence of multiple residues in other crops, 1997-2001

Oily fish	DDT	die										pesticides	occurrences	samples
2000	X	X										2	17	36

DDT, dieldrin (die)

Edible-podded peas	cbz	lcy	ens	mdp	pyz	tri						pesticides	occurrences	samples
1998			X	X								2	2	48
			X		X							2	1	
		X				X						2	1	
	X		X									2	1	

carbendazim (cbz), lambda-cyhalothrin (lcy), endosulfan (ens), methamidophos (mdp), pyrazophos (pyz), triazophos (tri)

Chinese canned pork	aHCH	bHCH	DDT									pesticides	occurrences	samples
1999		X	X									2	4	24
	X		X									2	2	

alpha-HCH (aHCH), beta-HCH (bHCH), DDT

Appendix 4

Estimation of population based exposure to organophosphate pesticides from food and drinking water using UK data

In the United States of America (USA), guidance is in place for performing risk assessments based on aggregate exposure.[1] The USA model for aggregate exposure relies on probabilistic modelling methods. (Chapter 4 provides a discussion of the current regulation of pesticides in the USA). The United States Environmental Protection Agency (USEPA) has also recently published its first assessment of cumulative toxicity (for organophosphates [OPs]).[2] This assessment is a preliminary view of the results of a new way of analysing data about potential exposure to pesticides. It considers the aggregate and cumulative assessment of risk posed by exposure to multiple OPs by multiple pathways, that is food, drinking water and residential/non-occupational exposure to pesticides in air, or on soil, grass or indoor surfaces.

This appendix attempts to estimate cumulative exposure to OPs in the United Kingdom (UK), incorporating exposure from multiple pathways. A simple deterministic approach is used. The pathways considered are food and drinking water only, residential/non-occupational exposure is not accounted for as relevant data are not available. The estimate is based on OP data from the pesticides Total Diet Study (TDS) 1996-97 for food exposure and data provided by the Drinking Water Inspectorate on OP pesticides detected in drinking water in 2001 for drinking water exposure. Although it is likely that OP toxicity is additive, no attempt is made to scale for the toxicity of each OP.

The approach has a number of deficiencies including the following:

- Food and drinking water data cover different years, different OPs are sought and the full range of OPs is not sought.
- Consumption estimates (i.e. of food and water) are calculated using different methods.
- The approach is population based and is not considered to be a conservative estimate (population exposure estimates tend to result in underestimates of real consumption).
- The simple deterministic approach takes no account of the fact that the OPs found may not occur concurrently.
- No suitable data on evidence of exposure to residential/non-occupational exposure could be identified. USA data suggest that such exposure might be substantial.

Estimation of exposure through food

Data on the occurrence of multiple pesticide residues from Total Diet Studies is provided in Chapter 5 of this report. Intakes of OP pesticides from the 1996-97 TDS are given below. Total OP exposure through food is calculated as the sum of the exposures to the different OP pesticides detected.

Table A4.1 Estimation of OP exposure through food

OP Pesticide	Intake[a] (µg/person/day)	Acceptable Daily Intake (ADI)[b] For 60 kg Adult	
		µg/person/day	µg/kg bw/day
Chlorpyrifos	0.5	600	10
Chlorpyrifos-methyl	0.2	600	10
Dimethoate	0.2	120	2
Etrimfos	<0.1	180	3
Parathion	<0.1	240	4
Phosalone	0.7	1200	20
Phosphamidon total	<0.1	30	0.5
Pirimiphos-methyl	3.3	1800	30
Propetamphos	<0.1	No ADI	
Triazophos	<0.1	60	1
Malathion	Not found above RL[c]	18000	300
Total OP exposure (food)	**5.0**		

a OP Intake is calculated from 1996-97 TDS data and is taken from Table 5.2

b Joint Meeting on Pesticide Residues (JMPR) ADI (as of 2001)

c RL = reporting limit

Estimation of exposure through drinking water

Information on exposure to pesticides from drinking water is also provided in Chapter 5 of this report. An analysis of results from monitoring undertaken in 2001 in England and Wales is given below (the information is publicly available). Results from monitoring undertaken on OPs by individual water companies have been combined to give average mean residue levels for England and Wales for the range of OPs in drinking water. No samples breached regulatory standards. Total OP exposure through drinking water is estimated as the sum of the exposures to the different OP pesticides detected in the monitoring.

Table A4.2 Estimation of OP levels in drinking water and average consumption of drinking water

OP Pesticide	No. of samples[a]	Maximum residue level (μg/l)	Average mean residue level (μg/l)	Estimated average Consumpton[c] (l/person/day)
Chlorfenvinphos	5296	0.05	0.005	0.938
Chlorpyrifos	2898	0.03	0.006	0.938
Diazinon	2631	0.02	0.006	0.938
Dichlorvos	1369	0.09	0.01	0.938
Dimethoate	1369	0.02	0.01	0.938
Fenitrothion	1368	0.09	0.008	0.938
Malathion	1368	0.02	0.007	0.938
Phosalone	970	0.09	0.01	0.938
Fonophos	399	0.008	0.008	0.938

a Number of samples analysed for each OP is calculated by adding together the number of samples analysed by each water company for that pesticide. Five water companies undertook analysis for at least one OP in 2001.

b Mean residue levels provided by water companies for each OP are the mean of all samples analysed by the company. Where no residues were detected, the residue used in the calculation was the reporting limit. The value given in the table for each OP is the average of the means provided by the water companies. The data have not been weighted to account for the number of samples analysed by each water company. Given the use of reporting limits when no residues were detected, values are likely to be overestimates of drinking water concentrations.

c Estimate is derived using the Food Standards Agency's Food Consumption Database (Adult survey) and is population based.[3] This estimate is for a person weighing 70.1 kg.

Table A4.3 Estimation of OP exposure through drinking water

OP Pesticide	Intake[a] (µg/person/day)	Acceptable Daily Intake (ADI)[b] For 60 kg Adult	
		µg/person/day	µg/kg bw/day
Chlorfenvinphos	0.005	30	0.5
Chlorpyrifos	0.006	600	10
Diazinon	0.005	120	2
Dichlorvos	0.01	240	4
Dimethoate	0.01	120	2
Fenitrothion	0.007	300	5
Malathion	0.007	18000	300
Phosalone	0.009	1200	20
Fonophos	0.008	No ADI	
Total OP exposure (drinking water)	**0.067**		

ND no data

a Intake is calculated by multiplying the average mean residue level (Table A4.2) by estimated average consumption (Table A4.2).

b Joint Meeting on Pesticide Residues (JMPR) ADI (as of 2001).

Estimation of combined exposure

An estimation of combined population exposure to OP pesticides is calculated as the sum of food and drinking water exposure derived above.

Table A4.4 Estimation of combined OP exposure. (Figures are taken from A4.1 and A4.3)

Source	Intake (µg/person/day)
Food	5.0
Drinking water	0.067
Combined exposure	**5.1**

The data indicate that exposure to pesticide residues through food greatly outweighs exposure through drinking water. To carry out rigorous cumulative assessments of exposure it will be necessary to take an approach that involves several Government Departments and agencies.

Conclusion

Pesticide intakes for food and water in the UK suggest that cumulative exposure to OPs from food and drinking water are unlikely to erode traditional safety factors. However, the deficiencies pointed out above mean that this conclusion can only be tentative. Furthermore, the contribution from residential and public hygiene use of pesticides, as well as exposure to similar substances being used as veterinary medicines on pets, is virtually unknown.

References

1. Guidance for performing aggregate exposure and risk assessments. Washington: United States Environmental Protection Agency, 1999.

2. Organophosphate pesticides: Preliminary OP Cumulative Risk Assessment. Washington: United States Environmental Protection Agency, December 3rd, 2001. http://www.epa.gov/pesticides/cumulative/pra-op/

3. Gregory J, Foster K, Tyler H, Wiseman M. *Dietary and Nutritional Survey of British Adults* (mainly tables). London: HMSO, 2000.

Appendix 5

Substances which are currently used in the UK both as pesticides and veterinary medicines

For veterinary medicine uses, the Annex listing refers to their status under Regulation 2377/90. The information below on pesticide uses is based on an in-house database but is available in the UK pesticide guide, 2001 (CAB International)

Abamectin	Pesticides	Not to be used on food crops other than glass house cucumbers, tomatoes and lettuces (with restrictions)
	Veterinary medicines	*Annex I* (bovine meat)/*Annex III* (ovine meat). ADI currently under re-consideration by the EU Committee on Veterinary Medicinal Products
Alpha-cypermethrin	Pesticides	Brocolli, calabrese, cauliflower, oil seed rape, brussel sprouts, cabbages, kale, wheat, barley, peas & beans (various types), poultry houses
	Veterinary medicines	*Annex III* (bovine meat/milk, ovine meat/milk,chicken meat/eggs)
Amitraz	Pesticides	Apples & pears
	Veterinary medicines	*Annex I* (porcine meat, bovine meat/milk, ovine meat/milk, bees' honey)
Bromide, potassium salt	Pesticides	Not specifically approved – residues may arise from use of methyl bromide as a fumigant or other bromine containing pesticides, but are difficult to differentiate from natural levels
	Veterinary medicines	*Annex II* (all food producing species)
Bromide, sodium salt	Pesticides	As for potassium salt
	Veterinary medicines	*Annex II* (all food producing mammals, for topical use only)
Chrysanthemum cinerareaefolium flos (see also pyrethrins)	Pesticides	Not approved
	Veterinary medicines	*Annex II* (all food producing species, for topical use only)
Cyfluthrin	Pesticides	Extensive range of crops
	Veterinary medicines	*Annex III* (bovine meat/milk)
Cyhalothrin	Pesticides	Lambda isomer only:- extensive range of crops

	Veterinary medicines	*Annex I* (bovine meat/milk)
Cypermethrin	Pesticides	Extensive range of crops
	Veterinary medicines	*Annex III* (bovine meat/milk, ovine meat/milk, caprine meat/milk, porcine meat, chicken meat/eggs, *salmonidae* muscle/skin)
Cyromazine	Pesticides	Control of insects on animal manure
	Veterinary medicines	*Annex III* (ovine meat)
Deltamethrin	Pesticides	Extensive range of crops
	Veterinary medicines	*Annex III* (bovine meat/milk, ovine meat, chicken meat/eggs, fin fish muscle/skin)
Imazalil/enilconazole	Pesticides	barley, potatoes, courgettes, gherkins, cucumbers
	Veterinary medicines	*Annex II* (bovine, *Equidae*, for topical use only)
Permethrin	Pesticides	cucumbers, tomatoes, aubergines, chillies, mushrooms, celery, lettuce, peppers
	Veterinary medicines	*Annex III* (bovine meat/milk, caprine meat/milk, porcine meat, chicken meat/eggs)
Piperonyl butoxide	Pesticides	Not approved as an active substance. Present as a synergist in a wide range of products permitted for use on a very wide range of crops.
	Veterinary medicines	*Annex II* (bovine, ovine, caprine, *Equidae*, for topical use only)
Pyrethrum extract (pyrethrins)	Pesticides Veterinary medicines	All crops *Annex II* (All food producing species, for topical use only)
Tau fluvalinate	Pesticides	barley, wheat, rape
	Veterinary medicines	*Annex II* (bees' honey)
Teflubenzuron	Pesticides	Not approved on edible crops (ornamental and forestry only)
	Veterinary medicines	*Annex I* (*Salmonidae* muscle/skin)

Appendix 6

List of those individuals, organisations, and groups who have made written submissions or oral presentations to the Working Group

Date	From	Contents
24/01/01	Miss Margaret Reichlin	Letter enclosing a submission on synergies relating to domestic exposure.
05/02/01	Miss Margaret Reichlin	Further letter with submission (part 2) concerning the use of 'holiday insecticides'. Article on ' Holiday ills' : research into the effects of holiday chemicals – The Ecologist (2001).
03/03/01	Miss Margaret Reichlin	Further letter with submission (part 3) on chemical synergies containing extracts from scientific papers, newpaper articles and other sources of information.
17/04/01	Ms Sandra Bell	Presentation to stakeholder meeting 17 April 2001: A precautionary approach to pesticides.
17/04/01	Dr David Tennant	Presentation to stakeholder meeting 17 April 2001: Modelling exposure to multiple chemicals.
17/04/01	Dr Andreas Kortenkamp	Presentation to stakeholder meeting 17 April 2001: Concepts for the prediction and assessment of mixture effects of pesticide/veterinary medicines.
17/04/01	Mr Kim Travis and Ms Trish Malarky	Presentation to stakeholder meeting 17 April 2001: Health risks of residues of crop protection products in food.
17/04/01	Dr Vyvyan Howard	Presentation to stakeholder meeting 17 April 2001: The toxicity of mixtures.
17/04/01	Dr Philip Harvey	Presentation to stakeholder meeting 17 April 2001: Pesticides, endocrine disruption and potential human health effects.
17/04/01	Mrs Maureen Dennis	Presentation to stakeholder meeting 17 April 2001: Working with pesticides.

Date	From	Contents
30/04/01	Miss Joanna Wheatley	Letter and submission discussing a number of points: • substances other than the active ingredients of pesticides and also tank mixes • the reliance on animal rather than human data and the problems that may arise from this • the power of supermarkets and the desire for uniformly perfect food that may be responsible much use of pesticides.
May 2001	Dr David Tennant and Dr Christine Chaisson	Submission on modelling exposure to multiple chemicals setting out the problems of estimating cumulative exposure and describing a probabilistic modelling system (Lifeline) developed in the USA.
02/05/01 and 21/05/01	Mr Richard Bruce	The submission discusses: • the problems that arise from attempting to estimate the effects of cumulative exposure • the concept of synergy • the terms of reference of the Working Group • a paper by El-Demerdash et al (2001) on glysophate.
16/07/01	Dr Richard Bilington and Mr Kim Travis	Presentation from the Crop Protection Association (CPA) – CPA response to the Working Group's terms of reference.
16/07/01	Mr Howard Mason – Health and Safety Laboratory	Presentation to the Working Group : An occupational perspective on pesticide mixtures: the use of biological monitoring, human volunteer studies and biokinetic modelling.
22/08/01	Dr Carol Courage and Dr Len Levy – Interdepartmental Group on the Health Risk of Chemicals (IGHRC)	Presentation to the Working Group : The role, aims and current activities of IGHRC.

Date	From	Contents
1/10/2001	Iain Watts – Crop Protection Association (CPA)	Submission giving a detailed response giving the CPA's views; in summary: • the potential for multiple residues of pesticides to modify individual toxicity in humans • the assumptions that can be made about the toxicity of pesticides in combination • the potential impact of exposure to pesticides and veterinary medicines by different routes • the formulation of advice on the standard risk assessment procedures to the safety evaluation of individual pesticides and veterinary medicines.
9/10/01	Ms Rachel Benstead – Environment Agency	Presentation to the Working Group: The current use of Direct Toxicity Assessment within the National Centre for Ecotoxicology and Hazardous Substances, Environment Agency.
9/10/01	Ms Helen Ferrier – IGHRC	Presentation to the Working Group: Current knowledge and recent developments in pesticide exposure assessment – a UK perspective.
9/10/01	Miss Margaret Reichlin	Further letter enclosing article concerning chemically impregnated domestic products.
29/11/01	Mr Richard Bruce	Further letter drawing attention to the potential adverse effects of glysophate.
30/11/01	Mrs Shirley Bray	Letter regarding the treatment of fruit and vegetables with mixtures of pesticides.
30/11/01	Dr Edward Hamlyn	Letter drawing attention to homeopathy and its value alongside 'conventional' medicine.
3/12/01	Mrs Shirley Bray	Further letter regarding the amount of information available to victims of pesticide spraying from GPs and hospitals. With particular reference to OPs, phenols and disinfectants in combination. Newspaper articles covering Multiple Chemical Sensitivity (MCS), chemical cocktails, residues in food. Also included is a special report by the National Housewives Association on 'Pesticide and Chemical Food Problems' (1991); a report on fluoridation of water supplies (1987) and a report on adulteration of foods (1988)

Date	From	Contents
10/12/01	Ms Georgina Downs	Letter and supporting papers relating to the effects of spraying of mixtures of pesticides close to her property. Paper on the synergistic effects of chemical mixtures – can we rely on traditional toxicology? by Dr Vyvyan Howard – The Ecologist 1997. Paper on organophosphate pesticides – neurological and respiratory toxicity by Jeanette D Sherman – Toxicology and Industrial Health 1995. Submission to the Policy Commission on the Future of Farming and Food. Various papers regarding pesticide exposure and health effects including documents from the Pesticide Action Network and newspaper articles. Video films showing: • Crop spraying and spray drift in the vicinity of her home • Countryfile 2 December 2001 – cocktails and pesticides in food • Countryfile 9 December 2001 – pesticide related ill health following pesticide exposure
10/12/01	Dr Helen Fullerton	Letter and submission discussing Multiple Chemical Sensitivity (MCS) with regard to cross-sensitisation post chronic OP exsposure Case histories are also presented
Undated	Mrs V M Bryant	Letter concerning the adverse health effects of OPs

Appendix 7

List of those who commented on the draft report following the consultation exercise

The following individuals and organisations have submitted comments on the draft report in response to the consultation exercise initiated on 15 February. All the comments received have been submitted to the Working Group and where appropriate the draft report has been amended to reflect the comments made.

Date	Name of individual/organisation
22 Feb 2002	Mr Martin Grantley-Smith – Meat and Livestock Commission
23 Feb 2002	Mrs Shirley Bray – Public
26 Feb 2002	Miss Margaret J.Reichlin – Public
28 Feb 2002	Mr Gareth Digges La Touche – M J Carter Associates
28 Feb 2002	Professor J A Milne – The Macaulay Institute
11 March 2002	Ms Sylvia Owen – The National Council of Women of Great Britain
11 March 2002	Mr Johnathan R Hall – Scottish Landowners' Federation
12 March 2002	Ms Helen George – Food Standards Agency Wales
12 March 2002	Mr Martyn Evans – Scottish Consumer Council
20 March 2002	Dr Campbell Gemmell – Scottish Environment Protection Agency
20 March 2002	Ms Helen Ferrier – Imperial College of Science, Technology and Medicine
22 March 2002	Mr Richard A R Bruce – Public
25 March 2002	Dr Andreas Kortenkamp – School of Pharmacy
25 March 2002	Mrs K T Percival – Nestle UK Ltd
25 March 2002	Dr D Tennant – Food Chemical Risk Analysis
26 March 2002	Professor I D Aitken OBE – Chairman, Veterinary Products Committee
26 March 2002	Mrs E M Chapman – Public
26 March 2002	Mr D Gregory – Marks & Spencer
26 March 2002	Christine and Tanya Harrison – BRAME
27 March 2002	Miss Georgina Downs – Public
27 March 2002	Mrs Judy Brander BSc – Public
27 March 2002	Mr David Buffin – Pesticide Action Network UK
27 March 2002	Ms Emily Diamand – Friends of the Earth
27 March 2002	Mrs C A Harris – Novigen Sciences, Inc.

Date	Name of individual/organisation
28 March 2002	Mr Iain Watt – Crop Protection Assocation
28 March 2002	Ms Doris M Jones MSc – Independent Researcher + Writer
28 March 2002	Mr Sam Miskelly – Northern Ireland General Consumer Council
28 March 2002	Mr R G Aitken – The Scottish Agricultural College
28 March 2002	Mr Masood Khawaja – Halal Food Authority
28 March 2002	Miss Margaret J. Reichlin – Public
30 March 2002	Mr Oliver Dowding – Public
05 April 2002	Dr Carol Courage – Interdepartmental Group on the Health Risk Assessment of Chemicals
06 April 2002	Miss Margaret Anderson – Public
16 April 2002	Mr Steve Killen – Environment Agency

Appendix 8

Membership of the Working Group on the Risk Assessment of Mixtures of Pesticides and Veterinary Medicines

Chairman

Professor H F Woods *CBE* BSc BM BCh DPhil FFPM FIFST HonFFOM FRCP F Med Sci
(Formerly Chair of the COT until 31 March 2002)

Members

Professor P Aggett *OBE* MB ChB FRCP MSc DCH FRCPCH
Professor A Boobis PhD CBiol FIBiol
Professor A Dayan MD FRCP FRCPath FFOM FFPM FIBiol Hon MBIRA
Dr J Groten BSc PhD
Mr R A Kempton MA, BPhil CStat, FRSE
Professor A G Renwick *OBE* BSc PhD DSc
Professor J Timbrell BSc PhD DSc MRCPath FRFC FIBiol

Consumer Representatives

Mr P Beaumont BA
Ms S Payne

Observers

Dr J Fisher BSc PhD FRES

Secretariat

Dr T Marrs MD DSc MRCP FRCPath DipRCPath	Scientific Secretary
Mr K Butler	Administrative Secretary
Dr J Norman BSc PhD	
Dr D Benford BSc PhD	
Mr B Groves BSc MSc	
Ms Z Corbyn BSc	
Dr S Bowen BSc PhD	

Assessors

Dr I Dewhurst BSc PhD	DEFRA
Mr A Browning IDT MIBiol C Biol	DEFRA
Mr J Battershill BSc MSc	DH
Ms C McNicholas BSc	HSE

Appendix 9

Declaration of WiGRAMP Members' Interests

	Personal Interests		Non Personal Interests	
Member	Company	Interest	Company	Interest
Professor H F Woods (Chairman)	HBOS Bank (Halifax Bank)	Shares	University of Sheffield, Faculty of Medicine	University of Sheffield, Faculty of Medicine
	HSBC Bank	Shares		
	Abbey National Bank	Shares	Wide range of national and international food and chemical companies	Has extensive activity in teaching and research in nutrition and toxicology and in topics related to and supported by many companies in the food and chemical industry. Trustee of the Harry Bottom Charitable Trust.
	Royal & Sun Alliance	Shares		
	Scottish Power	Shares		
	Shell Transport and Trading	Shares		
	United Utilities	Shares		
	William Hill	Shares		
Mr P Beaumont	None	None	None	None
Professor A Boobis	OSI Pharmaceuticals	Ad hoc consultancy	Servier	Research support
			Food Standards Agency	Research support
			Department of Health	Research support
Professor A Dayan	ML plc	Non-executive Director and Shareholder	None	None
	Alkernes	All those below: Consultancies in (Human Medicine)		

	Personal Interests		Non Personal Interests	
Member	Company	Interest	Company	Interest
Professor A Dayan	Amgen Ares Serono Cantab Pharmmacia Smithkline Beecham Wyeth Ayerst Astra-Zeneca BP Amoco Glaxo Wellcome			
Dr J Groten	None	None	Wide range of food, chemical and pharmaceutical companies in a.o USA, UK, Germany, Netherlands, France, Switzerland & Japan.	Contract Research
			Governmental agencies in Belgium, Netherlands, UK, Germany and USA.	Ad Hoc Consultancy and research on risk assessment of chemical mixtures.
			Centre for Food Toxicology -TNO-WUR	TNO Zeist – Wageningen University Research Centre involved in research and training of graduate students in area of food toxicology and supported through national (private) grants.
Mr R A Kempton	None	None	None	None
Ms S Payne	None	None	None	None

	Personal Interests		Non Personal Interests	
Member	Company	Interest	Company	Interest
Professor A Renwick	International Sweeteners Association	Consultant	Hoffman-La Roche	Research Support
	Novartis	Occasional Fee	Unilever	Research Support*
			Smithkline Beecham	Research Support
	Targacept	Occasional Fee	Pfizer	Research Support
			Flavor and Extract Manufacturers Asociation (FEMA)	
			American Chemistry Council	Research Support
Professor J A Timbrell	Shook, Hardy & Bacon (Law Firm)	Occasional Fee	Glaxo Wellcome	
	Sorex	Occasional Fee	Taisho Pharmaceutical Co.	Research Support

Appendix 10

Membership of the Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment

Chairman

Professor I Hughes MA MD FRCP FRCP(C) FRCPH F Med Sci

Members

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Dr A Piersma MSc PhD
Dr L Rushton BA MSc PhD CStat
Professor I R Rowland BSc PhD
Ms J Salfield BSc MSc MIFST CERTED RPHN
Dr A G Smith BSc PhD CChem FRSC
Dr L Stanley BA PhD
Professor S Strobel MD PhD FRCP FRCPCH
Professor J A Timbrell BSc PhD DSc MRCPath FRFC FIBiol
Dr M Tucker BSc PhD FRCPath

Secretariat

Dr D J Benford BSc PhD	**Scientific Secretary**
Mr J M Battershill BSc MSc	**Scientific Secretary, DH**
Mr K V Butler	**Administrative Secretary**
Dr C Tahourdin BSc PhD	
Dr D Gott BSc PhD	
Ms C A Mulholland BSc	
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Dr J Shavila BSc MSc PhD	
Mr N Ball BSc MSc	
Mr B Maycock BSc MSc	

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