COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

COT STATEMENT ON FSA-FUNDED RESEARCH AND OTHER PROGRESS ON MIXTURES OF PESTICIDES AND SIMILAR SUBSTANCES

Introduction

1. A COT report on Risk Assessment of Mixtures of Pesticides and Similar Substances was published in September 2002. That report made a number of recommendations under the headings of "Regulatory", "Surveillance", "Research" and "Public Information".

2. The Food Standards Agency (FSA) subsequently funded seventeen research projects to address the report's recommendations. The final reports of these projects are now available and were provided to the Committee. The Committee was also briefed on progress in addressing the other recommendations of the 2002 report.

3. The Committee was asked for advice on the conclusions that could be drawn from the research reports, and the extent to which the research and other recommendations in the report had been addressed.

Background

4. In 2000, the Committee was asked by the FSA to establish a Working Group to review what was known about the toxicity of mixtures of chemicals and to consider the implications for the risk assessment process. In particular, the Working Group was to consider whether there was any scientific basis for consumer concerns that the occurrence of multiple residues of pesticides and veterinary medicines in food might lead to a "cocktail" effect. The rationale for including veterinary medicines was that some pesticides and veterinary medicines contained the same, or toxicologically similar, active ingredients.

5. The Working Group was asked also to consider exposure by routes other than consumption of foods containing residues of pesticides and veterinary medicines. These included consumption of drinking water containing traces of pesticides;

exposure by inhalation or skin contact to pesticides used in public hygiene and applied to gardens, parks or agricultural crops; exposure to veterinary products used to treat pets; and exposure to human medicines containing the same active ingredients as pesticides or veterinary medicines.

6. The following Terms of Reference were agreed for the COT Working Group:

- 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.

7. The report that was produced, which was agreed by the full Committee, is available at http://cot.food.gov.uk/cotreports/cotwgreports/cocktailreport.

8. The FSA subsequently funded a programme of research to address the report's recommendations. The recommendations addressed by the research programme are listed in Table 1. The other recommendations made in the report, and the progress that has been achieved in addressing them, are listed in Table 2.

The research projects

9. The FSA initially funded seventeen research projects within the programme. Subsequently, a further project was added to the programme in response to a recommendation of the COT's sister Committee on Mutagenicity (COM). This project, which is assessing the aneugenic effects of mixtures of benzimidazole fungicides and anthelmintics in vitro, is not due to be completed until 2012, and will then be reviewed by COM. It is not considered further in this report.

10. The seventeen research projects reviewed by COT are listed in Table 1, grouped according to the recommendations that they addressed. The table also summarises the main findings of each project. The Committee's assessment of the project reports is set out in the paragraphs that follow.

Table 1: Research projects funded by the FSA

Research requirement	Contractor	Project number	Project title
The development of methods to provide cost effective biomarkers or other robust indicators of population exposure and body burdens of mixtures of pesticides and relevant veterinary residues.	Health and Safety Laboratory	T10003	Cost effective biomarkers of exposure to mixtures of pesticides - method development Report available at: http://www.foodbase.org.uk/results.php?f_report_id=141 This project worked to develop biomarkers of exposure for pesticides, using methods based on the analysis of urinary metabolites by gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and immunoassay. Details of the biomarkers developed are in Annex A.
	ADAS Consulting	T10009	Development of diagnostic immunoassays for biomarkers of pesticide exposure Report available at: http://www.foodbase.org.uk/results.php?f_report_id=385 This project worked to develop biomarkers of exposure for various acetylcholinesterase-inhibiting pesticides based on immunoassays of urinary metabolites. Details of the biomarkers developed are in Annex A.
	Health and Safety Laboratory	T10013	Development of cost-effective biomarkers for herbicides and fungicides Report available at: http://www.foodbase.org.uk/results.php?f_report_id=407 This project worked to develop biomarkers of exposure to various herbicides and fungicides based on analysis of urinary metabolites using LC-MS. Details of the biomarkers developed are in Annex A.
	ADAS Consulting	T10021	Immunoassay detection of urinary biomarkers of pesticide exposure Report available at: http://www.foodbase.org.uk/results.php?f_report_id=502 This project worked to develop biomarkers of exposure for various pesticides based on immunoassays of urinary metabolites. Details of the biomarkers developed are in Annex A.

The development of markers to enable early and reliable detection of systemic responses and health effects arising from such exposures (biomarkers of effect).	Health and Safety Laboratory	T10002	Biomarkers of effect from exposure to mixtures of organophosphate pesticides Report available at: http://www.foodbase.org.uk/results.php?f_category_id=&f_report_id=742 This project explored three possible approaches to developing biomarkers of effect for organophosphate exposure, focusing on the phosphorylation of cholinesterases by organophosphates. No useful working assay was produced, but one of the approaches might be amenable to further development.
	University of Newcastle	T10010	Investigation of direct measurement of phosphorylation of the active site of esterases as sensitive biomarkers of organophosphate exposure Report available at: http://www.foodbase.org.uk/results.php?f_report_id=615 This project worked to develop a biomarker of effect for organophosphate exposure based on quantification of the relative amounts of phosphorylated and unphosphorylated acetylcholinesterase or butyrylcholinesterase in blood. However, the isolation and concentration of the enzymes from blood samples was not effective or reliable, and so a working assay was not produced.
	Centre for Ecology and Hydrology and University of Cambridge	T10014	A study to identify small metabolite biomarkers of effect following exposure to single or mixtures of pesticides Report available at: http://www.foodbase.org.uk/results.php?f_report_id=741 Metabolomics was applied to the urine of Fischer 344 rats treated individually with the benzimidazoles carbendazim and thiabendazole and the quaternary amines chlormequat and mepiquat at various dose levels in both acute and 29-day studies. Mixture studies were then performed using binary combinations and a quaternary mixture. However, there was a lack of shared metabolite responses between the single pesticide exposures and the mixture studies, and therefore mixture modelling of the metabolomic responses was not possible. A reduction in the relative weight of testes was observed in the quaternary mixture study, which was not predicted by either dose addition or independent action. See the COT consideration at paragraphs 19-20 of this Statement.

The characterisation of possible variability in human responses to mixtures of residues	CXR Biosciences	T10011	Interindividuality in cytochrome P450 and paraoxonase mediated metabolism of mixtures of pesticides Report available at: http://www.foodbase.org.uk/results.php?f_category_id=&f_report_id=737 This in vitro project tested a range of pesticides that were substrates and inhibitors of various CYP450s. With the exception of CYP2C19, highly polymorphic enzymes did not demonstrate high affinity to the tested compounds. No sex- or age-related differences in pesticide metabolism were found. Most oxidation reactions were strongly inhibited in a mixture experiment using all the tested pesticides, suggesting a potential for metabolic interactions between co-administered pesticides and other substances, e.g. pharmaceuticals.
	University of Newcastle	T10015	Functional impact of polymorphisms of transport proteins upon pesticide delivery to the CNS Report available at: http://www.foodbase.org.uk/results.php?f_report_id=495 This in vitro project indicated that dieldrin, lindane and heptachlor are substrates for the organic cation transporter OCT2, and that dichlorvos, chlorpyrifos, diazinon, deltamethrin and dieldrin are substrates for MDR1. Functional studies of two genetic variants of MDR1 revealed that they functioned identically to the 'normal' MDR1 protein. However, when inhibitors of MDR1 were used to model potential effects of polymorphisms, increased levels of pesticides in cells and increased toxicity to cells were observed.
Experimental research to characterise the nature and dose-response relationships for combined actions of chemicals when administered together.	Imperial College London	T10004	Use of protein profiles to characterise the concentration-effect curve of mixtures of estrogenic compounds Report available at: http://www.foodbase.org.uk/results.php?f_report_id=614 The four tested oestrogenic chemicals increased the proliferation of MCF-7 cells and produced similar changes in the protein profiles of MCF-7 cells. Mixture studies indicated that effects on 12 candidate protein biomarkers of effect were consistent with concentration addition; no synergism or antagonism was produced.

	Health and Safety Laboratory	T10008	Dose-response and mixture response of pesticides in vitro and in vivo Report available at: http://www.foodbase.org.uk/results.php?f_report_id=496 An in vitro part of this project indicated that the inhibition of acetylcholinesterase and butyrylcholinestase by binary mixtures of acetylcholinesterase-inhibiting pesticides was consistent with concentration addition. Human volunteer studies showed that consumption of combinations of chlorpyrifos-methyl with deltamethrin, and of chlorpyrifos-methyl with pirimicarb, each at ADI levels, produced no differences in the elimination kinetics of the pesticides from those seen when they were tested individually at ADI levels, thus indicating no toxicokinetic interactions.
	Central Science Laboratory (now the Food and Environment Research Agency)	T10016	Characterisation of the nature of combined actions of chemical mixtures and estimation of dose response relationships for pesticide mixtures Report available at: http://www.foodbase.org.uk/results.php?f_report_id=726 This in vitro project indicated that in all binary combinations of five organophosphates and N-methylcarbamates that were tested, the effects on acetylcholinesterase inhibition were consistent with concentration addition.
	Health and Safety Laboratory	T10017	A pilot study to assess the effects of co-exposure to organophosphates, carbamates and pyrethroids on the rate of metabolic detoxification via hydrolysis. Report available at: http://www.foodbase.org.uk/results.php?f_report_id=142 This in vitro project indicated that N-methyl carbamates and oxon forms of organophosphates have the potential to reduce the rate of detoxification of pyrethroids. It was unclear whether the in vivo concentrations of these pesticides from currently allowable dietary intakes would be sufficient to produce this effect.

The obtaining of robust data on all pathways of exposure to pesticides and veterinary medicines and on sources of variation in exposure. The assessment of the impact of implementation of the recommendations within the EU alone taking account of the exposure to products that will be imported from outside the EU.	Institute of Occupational Medicine and Central Science Laboratory (now the Food and Environment Research Agency)	T10005	Estimation of human uptake of pesticides and veterinary medicines from all potential exposure pathways and assessment of the impact within the EU of implementing COT regulatory recommendations on the risk assessment of mixtures of pesticides and similar substances. Report available at: http://www.foodbase.org.uk/results.php?f_report_id=281 This project assessed cumulative and aggregate exposure to groups of acetylcholinesterase-inhibiting pesticides and putative oestrogenic pesticides for various groups of the population (infants, adults and vegetarians in the general population; farmers; contractors and bystanders), using a single compartment pharmacokinetic model and Monte Carlo simulation. Cumulative exposures through food were below the equivalents of the combined ADIs. Using conservative assumptions, the possibility that some bystanders and workers might have excessive cumulative, aggregate exposure to acetylcholinesterase-inhibiting pesticides could not be excluded. See the COT consideration at paragraphs 28-29 of this Statement.
The effects of the nature of processing and preparation on the bioavailability and chemical nature of residues.	Queen's University Belfast, Agri-Food and Biosciences Institute	T10019	The effects of storage time, preparation and cooking method on residual pesticide levels in apples and potatoes treated with a suite of commonly used permitted pesticides. Report available at: http://www.foodbase.org.uk/results.php?f_category_id=&f_report_id=738 This project indicated that some residues decreased with storage (e.g. diphenylamine in apples) and some remained relatively stable (e.g. carbendazim in apples). Washing had no effect on residues of diphenylamine, carbendazim, chlorpyrifos or captan in apples, or maleic hydrazide in potatoes, but there was some decrease in imazalil and chlorpropham in potatoes. Peeling reduced the residues of all pesticides except maleic hydrazide (a systemic pesticide) in potatoes. Cooking had variable effects.

	Health and Safety Laboratory	T10018	Pilot study into the chemical nature of organophosphate pesticide residues and the implications for urinary metabolite analysis Report available at: http://www.foodbase.org.uk/results.php?f_report_id=408 One hundred apples were analysed for organophosphate residues and their dialkylphosphate metabolites. Dialkylphosphate levels ranged from 20 to 50,000 times the level of organophosphate residues in the same apple. The researchers concluded that the levels of dialkylphosphates detected in urine are therefore likely to reflect not only exposure to intact organophosphate pesticides, but also ingestion of dialkylphosphates in food.
Study of common mechanism groups to identify when it is appropriate to carry out risk assessment of combined exposure	Imperial College London	T10020	Application of protein profiles to identify common mechanism groups of pyrethrins and pyrethroids Report available at: http://www.foodbase.org.uk/results.php?f_report_id=615 This project assessed changes in protein profiles following treatment of SH-SY5Y cells in vitro with pyrethroids at concentrations that affected noradrenaline release. Two possible common mechanism groups were identified from the results, but these groupings were not evident in SK-N-SH cells, consistent with a lack of effect on noradrenaline release, or in a control non-neuronal cell line. Analysis to determine if type I and type II pyrethroids could be separated based on protein profiles was not successful with any cell line. Further work with two pyrethroids from each of the two previously identified groupings indicated four main patterns of response, suggesting that the pyrethroids comprise more than one common mechanism group.

The development of methods to provide cost effective biomarkers or other robust indicators of population exposure and body burdens of mixtures of pesticides and relevant veterinary residues

11. Details of the biomarkers of exposure developed are listed in the table in Annex A.

12. In some cases biomarkers were developed for the same chemical, using both liquid chromatography-mass spectrometry (LC-MS) and immunoassays. Immunoassay-based biomarkers had, in general, been less successful, due to matrix effects and cross-reactivity. Moreover, combined methods had been developed for the LC-MS analyses which meant that several pesticides could be analysed in the same assay. The choice between immunoassay and LC-MS may depend on the purposes for which a biomarker is to be used, and when screening large numbers of people for a wide range of chemicals, immunoassays followed by confirmatory LC-MS analyses might be the best approach, taking into account ease of use and cost. However, given the greater success in developing LC-MS methods, the Committee considered that these should be the priority for further development and validation, except where immunoassays are much less expensive.

13. The projects had aimed for limits of detection at low parts-per-billion levels. However, in the absence of good data on chronic dietary exposure to the pesticides concerned, the Committee found it difficult to determine whether the individual biomarkers were sufficiently sensitive to reflect dietary exposures. However, even if a biomarker were not sufficiently sensitive to quantify low level dietary exposures, it might still be useful in the assessment of exposures of bystanders, residents or workers.

14. The Committee considered that some of the immunoassay biomarkers – for several pyrethroids together, carbaryl, phosmet, imazalil, penconazole, carbendazim and thiabendazole – should be of low priority for further work, either because it was clear that they would not be sufficiently sensitive, or because more successful LC-MS biomarkers had been developed. In addition, an LC-MS biomarker for paraquat was of low priority since paraquat was no longer used in the EU and was not detected as a residue in food. On the other hand, diquat, although not detected as a residue in food, was still used in the UK, and a biomarker might therefore be useful in the assessment of non-dietary exposures.

15. The other biomarkers were considered worthy of further development, subject to further consideration of exposure levels. For two pesticides, penconazole and imazalil, further work would be required to confirm that the metabolite for which the biomarker had been developed was a urinary metabolite of the pesticide in humans. If six or seven pesticides were a particular priority because exposures might be at

levels of concern, then the focus should be on further developing and/or validating the biomarkers for those pesticides. However, the Committee recognised that the primary interest was the risk assessment of mixtures, which would require biomarkers to be available for a wide range of pesticides.

16. The biomarkers developed were all for urinary metabolites. Some of these urinary metabolites may themselves occur in treated crops, as shown for the dialkylphosphate metabolites of organophosphates (see para 31), which would complicate the interpretation of biomarker levels.

The development of markers to enable early and reliable detection of systemic responses and health effects arising from such exposures (biomarkers of effect)

17. Projects T10002 and T10010 were ambitious undertakings, which despite the best efforts of the research teams, had not delivered robust analytical methods or biomarkers. They had, however, been worth pursuing at the time they were commissioned.

18. Biomarkers developed in project T10014 were repeatable but had not been fully validated, and inconsistencies in mixture responses indicated that they may not be of practical use. The mixture experiments had not been successful due to a lack of shared metabolite responses between single pesticide exposures and the mixed exposures.

19. There was some evidence in the report for project T10014 that certain combinations of pesticides, notably a quaternary mixture of carbendazim, thiabendazole, chlormequat and mepiquat, produced a synergistic effect on testis weight when administered at doses some way below their individual no observed effect levels (NOELs) for this effect. Only carbendazim had an observable effect on its own, with a lowest observed effect level (LOEL) of 75 mg/kg bw and a NOEL of 50 mg/kg bw. The dose used in the quaternary mix was 20 mg/kg bw. However, the authors stated that the doses of carbendazim and thiabendazole used in the quaternary mix were in the region of, or slightly higher than NOELs identified in the literature for other toxicological effects. Hence, it was possible that effects of the quaternary mix on testis weight were confounded by above-threshold effects of one or more individual components on some other endpoint. For example, in some studies, the NOEL for effects of carbendazim on liver weight was similar to the dose used in the quaternary mix, which had produced a significant increase in liver weight.

20. The Committee considered that because of the uncertainties regarding possible effects of individual component compounds on other endpoints, further

confirmation would be necessary before a synergistic effect on testis weight from low dose exposure could be regarded as established.

The characterisation of possible variability in human responses to mixtures of residues

21. The report for project T10011 indicated a role for CYP2C19 in the detoxification of various pesticides. Therefore, the Committee considered the possible impact of polymorphism in CYP2C19. Approximately 3% of Caucasians are poor CYP2C19 metabolisers, but the prevalence in South-east Asians is higher (19%). The contribution of a polymorphic pathway to interindividual variation in the elimination of a pesticide will depend on the contribution of that pathway to the overall elimination of the compound. Thus, it was important to consider the relative contribution of CYP2C19. Few pesticides are metabolised uniquely by a single form of P450. The researchers had referred to two pesticides specifically, bupirimate and iprodione. These were metabolised also by CYP1A2, and bupirimate also by CYP3A4. The Committee concluded that polymorphism in CYP2C19, which is of low abundance in the liver, would contribute to inter-individual variability in detoxification, but only to a minor extent. Therefore, further investigation of the role of genetic variability in CYP2C19 as a determinant of interindividual differences in pesticide metabolism was not considered to be a high priority.

22. The T10011 researchers had used caffeine as a typical substrate of CYP1A2. They had noted that the Michaelis constant for the interaction of caffeine with CYP1A2 was higher than that for some of the pesticides in the project, suggesting that those pesticides could be more potent inhibitors of CYP1A2 than caffeine; and they had estimated that consuming certain pesticides at the levels of their ADIs would be equivalent in effect on CYP1A2 to consuming large numbers of cups of tea. The Committee concluded that while the approach followed was useful as a first step, it was important to take into account various pharmacokinetic considerations that were relevant to interpretation:

- Caffeine is almost completely absorbed following oral administration.[1,2] Hence, comparing pesticide intakes to an oral dose of caffeine would be a worst case, in that some of the pesticide may not be absorbed completely.

- The distribution of the compounds in the body will be determined in part by their lipid solubility. In general, the more lipid soluble a compound the larger the volume of its distribution, since it will tend to diffuse into lipid rich tissues. The log octanol:water partition coefficient of caffeine is only 0.01, whereas that of the pesticides tested ranged from 1.5-4.7 (except for propamocarb, but this compound was not inhibitory), indicating higher lipid solubility. For six of the compounds, the log octanol:water partition coefficient was ≥3.

- Consistent with this, caffeine has a very low volume of distribution – only around 0.6 l/kg.[3] Hence the plasma concentration from a given dose will be relatively high. In contrast, pesticides tend to have a much larger volume of distribution. This is certainly true for chlorpyrifos, which has been well studied in this respect.[4] Carbendazim has a volume of distribution of 10-20 l/kg in rodents.[5,6] Volumes of distribution tend to be relatively consistent across species.

- Plasma protein binding will influence the free concentration of a compound available to bind to the active site of an enzyme and so inhibit its activity. Caffeine is only modestly bound to plasma proteins (35%)[7] – i.e. approximately two thirds of the total plasma concentration is available to interact with enzymes. Protein binding of at least some of the pesticides tested is >95% – i.e. less than 1/20th of the total plasma concentration is available to inhibit hepatic enzymes.[8,9]

23. The Committee concluded that the pharmacokinetic differences between caffeine and the pesticides investigated in T10011 were such that any inhibition in vivo at the ADI was likely to be relatively modest if it occurred at all.

24. The Committee considered that the concentrations at which the T10011 report described interactions between pesticides were relatively high, and speculated on whether microsomal clearance values could be scaled up to obtain in vivo clearance estimates using an equation derived by Hallifax et al.[10] This equation was based on empirical data from a database of pharmaceuticals and there was no mechanistic explanation for it at present. The extent to which it would apply to pesticides was not yet clear. The use of Physiologically-Based Pharmacokinetic (PBPK) models might be less uncertain, but such models required extensive data.

25. Many of the pesticides studied in project T10015 are substrates for the P-glycoprotein efflux transporter (MDR1). Single nucleotide polymorphisms of MDR1 produced by the researchers were functionally indistinguishable from the reference MDR1, and the researchers had stated that, to date, there was no strong evidence that functionally distinct polymorphisms of MDR1 exist. However the Committee observed that evidence was available of variability in the function of P-glycoprotein. It would be useful to extend the research to include cellular permeability studies (which can now be performed with brain endothelial cells) with a view to incorporating the findings into PBPK models to estimate likely brain exposure in humans.

Experimental research to characterise the nature and dose-response relationships for combined actions of chemicals when administered together

26. The in vitro investigations of the dose-response relationship of mixtures of acetylcholinesterase-inhibiting organophosphates and N-methyl carbamates in vitro in projects T10008 and T10016 were consistent with dose-addition.

27. Project T10017, assessing the potential in vitro, for organophosphates, N-methylcarbamates and pyrethroids to interact toxicokinetically through inhibition of hydrolysis, was of interest since N-methylcarbamates and pyrethroids often co-occur in urine, indicating co-exposure. Low IC_{50} values (concentrations yielding 50% inhibition) for hydrolysis were reported, but the Committee questioned whether the pesticides would be confirmed as potent inhibitors in a study determining inhibition constants, K_i, which are relatively independent of study design, unlike IC_{50} values. Hydrolysis is not the only pathway of detoxification for pyrethroids, which are also oxidised, so this could offset any inhibition of hydrolysis. However, the Committee concluded that the research should be followed up to establish the mechanism of the inhibition, and to obtain parameters for use in risk assessment and possible incorporation into PBPK modelling of mixtures.

The obtaining of robust data on all pathways of exposure to pesticides and veterinary medicines and on sources of variation in exposure; and

The assessment of the impact of implementation of the recommendations within the EU alone taking account of the exposure to products that will be imported from outside the EU

28. One project (T10005) had been funded to address these recommendations. This assessed cumulative and aggregate exposure to various acetylcholinesterase-inhibiting and putative oestrogenic pesticides. Many assumptions had been necessary in order to derive the estimates of cumulative and aggregate exposure. Sensitivity analysis indicated that the assumptions were reasonable, but there were only limited exposure data for biocides.

29. The Hazard Index approach taken, which was based on ADIs, was conservative since not all ADIs were derived from the same toxic endpoint. From their analysis, the authors could not rule out the possibility that in some cases the aggregate exposures of workers or bystanders to acetylcholinesterase-inhibiting pesticides exceeded a level of concern, but because of various conservative assumptions in the assessments, it could not be concluded that over-exposure would, in fact, occur. The Committee sent a copy of this report to the Advisory Committee on Pesticides (ACP), which discussed it at a meeting on 5 July 2011.[11] The ACP identified data gaps and differences from current UK practice, and

concluded that "Taken together, the data gaps and differences from current UK practice mean that the research probably overstates the risks to the public."[12]

The effects of the nature of processing and preparation on the bioavailability and chemical nature of residues

30. Conservative assumptions are made in regulatory risk assessment for dietary exposure to pesticides – for example, that all of the peel of most fruits and vegetables is eaten. Project T10019 had been designed to enable more realistic estimates of exposure, which could be of particular value in cumulative risk assessments. The Committee was informed that the FSA was commissioning a further project to assess the effects of processing of different pesticides in a wider range of foods than were examined in T10019, focusing on those that are commonly present in the diet and which belong to large groups of pesticides with common modes of toxic action. Cumulative dietary risk assessments have recently been performed for some groups of pesticides (e.g. the organophosphates and triazoles) by the U.S. Environmental Protection Agency, the European Food Safety Authority (EFSA), the Danish Food and Veterinary Administration and the Dutch Institute of Food Safety (RIKILT), amongst others (see review by EFSA[13] and the subsequent EFSA Opinion on triazoles).[14] The Committee concluded that if the existing cumulative assessments did not indicate a risk, then the compounds in these groups should be a lower priority for further work on processing. Rather, the priority should be pesticides for which cumulative exposure could be of greater concern.

31. The results of project T10018 indicated that dialkylphosphates occurred in apples at higher levels than the parent organophosphates, which would complicate the use of urinary dialkylphosphates in assessing exposure to the parent organophosphates. If dialkylphosphates were to be used as biomarkers of exposure, data would be needed on their absorption, distribution, metabolism and elimination. The Committee concluded that the toxicity of dialkylphosphates would be adequately taken into account in risk assessments for the parent organophosphates, since: a) they were not expected to inhibit acetylcholinesterase; b) they were produced as metabolites of organophosphates in the laboratory species used in toxicity studies of organophosphates; and c) adverse effects were not observed in toxicological studies of organophosphates at dose levels which did not inhibit acetylcholinesterase. The Committee sent a copy of this report to the ACP for information.

Study of common mechanism groups to identify when it is appropriate to carry out risk assessment of combined exposure

32. The protein profiles produced by T10020 indicated that the pyrethroids and pyrethrins may comprise more than one common mechanism group. The results did

not accord with the prior hypothesis of the project that type I and type II pyrethrins/pyrethroids could be separated. To take this area of investigation forward, pathway analysis would be required. However, the Committee considered that this was a low priority, given the low levels of exposure to pyrethrins and pyrethroids, and their low toxicity.

Other recommendations of the 2002 report

33. The recommendations of the 2002 COT report not relating to research are listed in Table 2. A report by the Interdepartmental Group on Health Risks from Chemicals (IGHRC)[15] had gone some way to addressing the recommendation to provide a suitable framework for combined risk assessment of chemicals, as had opinions from the European Food Safety Authority (EFSA) Panel on Plant Protection Products and their Residues (PPR Panel).[13,14] In addition, the World Health Organization/International Programme on Chemical Safety (WHO/IPCS) had now developed a framework for assessing chemical combinations.[16] The Committee had recently commented[17] on a report commissioned by the European Commission Environment Directorate-General (DG Environment) on mixture toxicology,[18] and the non-food scientific advisory committees of DG Health had prepared an opinion on mixtures toxicology.[19]

34. Allocating pesticides to cumulative assessment groups was particularly challenging, and a forthcoming opinion of the EFSA PPR Panel may be useful in this respect.

35. It was confirmed to the Committee that product formulation is taken into account when assessing user safety and calculating withdrawal periods for veterinary medicines. Toxicokinetic interactions between the components of formulated products are considered.

36. The Committee discussed its previous recommendation that dietary surveys should continue to cover all social, age and ethnic groups. There were no plans to include pregnant and lactating women, or people in institutions, in the current rolling National Diet and Nutrition Surveys (NDNS) programme. The NDNS programme is primarily performed for nutritional reasons, and there were practical difficulties in including pregnant and lactating women. The Committee agreed that this was not specifically a matter of concern with regard to mixtures of pesticides.

37. The ACROPOLIS project, in which the UK's Health and Safety Executive Chemicals Regulation Directorate (HSE CRD) and Food and Environment Research Agency (Fera) are participants, would further investigate aggregate exposures. It

Table 2: Progress on the non-research COT recommendations

COT recommendation	Progress to date
STAGE 1	
11.2 Generate a framework to decide when to carry out combined risk assessments of exposures to more than one pesticide and/or veterinary medicine.	The Interdepartmental Group on Health Risks from Chemicals (IGHRC) has produced a framework for assessing risks to human health from chemical mixtures.[a] The European Food Safety Authority (EFSA) has considered methodologies to assess risks from exposure to multiple pesticides, and a PPR Panel Opinion was published in April 2008.[b] This included criteria for grouping pesticides into Cumulative Assessment Groups and recommendation of a tiered approach to both hazard characterisation and exposure assessment. The methodology was tested using the triazoles group in an Opinion published in June 2009.[c]
11.3 When it is appropriate to carry out risk assessment of combined exposure, certain toxicological approaches should be taken depending on the type of toxic action and/or interaction.	An inter-departmental Science Group concluded that both organophosphates and N-methylcarbamates should be included in a single common mechanism group.[d] An initial cumulative risk assessment for organophosphates and N-methylcarbamates was performed by the Pesticides Safety Directorate (now the Chemicals Regulation Directorate (CRD)) applying probabilistic modelling to Dutch consumption data and some UK residues data for 10 commodities. This work is described in the 2008 EFSA PPR Panel Opinion.[b] The Science Group also undertook work on benzimidazoles, which resulted in an opinion being sought from the COM.[e]

[a] See reference 15

[b] See reference 16

[c] See reference 17

[d] Available at http://www.food.gov.uk/multimedia/pdfs/papercmg.pdf.

	Reports by the Pesticide Residues Committee (PRC) now include deterministic acute cumulative risk assessments for multiple residues in single commodities, using the Hazard Index approach. To date this has been done for organophosphates/N-methylcarbamates (common mode of action based on acetylcholinesterase inhibition), captan/folpet (common metabolite), and thiophanate-methyl/carbendazim (common metabolic pathway via carbendazim). The EFSA PPR Panel is currently working on an Opinion identifying pesticides that can be grouped together for cumulative risk assessment (dose addition). In the preparatory phase of this Opinion, the PPR Panel outsourced the information collection, aimed at establishing a database with cumulative assessment groups. (This contract[f] was won by the National Food Institute and National Veterinary Institute of the Technical University of Denmark.) EFSA is currently procuring a contract to provide scientific information on different aspects of combined actions of chemicals in food acting through dissimilar modes of action, and to define criteria regarding the elaboration of cumulative assessment groups of pesticides which do not necessarily share a common mechanism or mode of action.[g] In order to obtain wider information, the review should be applied to chemicals present in food in general and not restricted to pesticides. The information will be used by the EFSA PPR Panel to further refine hazard assessment for dietary cumulative risk assessment.
11.4 Approval of pesticides and veterinary medicines should include more formal analysis and possibly experimental investigation of the potential for combined toxic action or interaction due to addition of other substances to the formulations employed.	CRD developed guidelines, approved by the ACP, on the assessment of mammalian toxicity to mixtures of chemicals in a pesticide product.[h] The Veterinary Medicines Directorate (VMD) currently takes product formulation into account when assessing user safety and calculating withdrawal periods. When setting veterinary medicine MRLs the Committee for Medicinal Products for Veterinary Use (CVMP) takes pesticide uses of the same active substance into account.

[e] The COM Statement is available at http://www.iacom.org.uk/statements/documents/COM07S3BenzimidazolesApril07.pdf.

[f] CFP/EFSA/PPR/2009/01 Identification of Common Assessment Groups of Pesticides http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902575207.htm

[g] See http://www.efsa.europa.eu/en/tendersevaluation/tender/cftefsappr201002.htm

[h] http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PSD/CombinedToxicity20050408.pdf

	The Biocidal Products Directive requires risk assessment for the whole product formulation. CRD funded a research project investigating the potential effects of tank mixing on dermal penetration.[i] The project did not identify major effects which had not been accounted for in previous predictions of dermal absorption for risk assessment.
11.5 To accommodate analysis of all sources of exposure and 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**.**	CRD has been supporting the EFSA Panel on Plant Protection Products and their Uses (PPR) Working Group in considering the principles of probabilistic exposure assessment. This resulted in a public consultation in March 2010 on the principles of modelling aggregate intakes for single substances,[j] as a prelude to consideration of multiple substances which will be worked on later.
11.6 Dietary and food consumption surveys in the UK should continue to cover all social, age and ethnic groups within the population.	A new National Diet and Nutrition Survey annual rolling programme started in April 2008 and involved a 4-day diary, with 1000 respondents across the UK from all age groups older than 18 months, living in private households. People living in institutions and pregnant and lactating women are excluded. For the new survey, foods are not weighed but consumption estimates are based on portion sizes. Year 1 results were published in February 2010 and year 1 and 2 results combined were published in July 2011. A survey for infants and children under 18 months is currently being undertaken, with the main-stage fieldwork completed in August 2011.

[i] The report for project PS2607 is downloadable from at http://randd.defra.gov.uk/.

[j] Public consultation on a Draft Guidance of the Scientific Panel on Plant Protection Products and their Residues (PPR) on the Use of Probabilistic Methodology for Modelling Dietary Exposure to Pesticide Residues (Part one: single active substances exposure assessment): http://www.efsa.europa.eu/en/consultationsclosed/call/ppr100301.htm

11.7 Aggregate exposure assessment will require robust data on all pathways of exposure and sources of variation in such exposure	See research project T10005. Veterinary medicine and biocide exposures were not included as initially envisaged due to data limitations. CRD is participating in a European Commission funded FP7 project, ACROPOLIS,[k] which will develop a scientifically sound framework for cumulative and aggregate risk assessment of pesticides. This work consists of: 1) studying the data needs, and the extent and organisation of data currently available (including uncertainties) for cumulative exposure and effect assessment in a probabilistic risk assessment framework; 2) integrating models describing various routes of exposure into an aggregate exposure model; 3) setting up new toxicological testing for identifying possible additive or synergistic effects and developing a strategy for refinement of cumulative assessment groups; 4) integrating cumulative and aggregate risk models including uncertainty analyses in a web-based tool, including accessible data for all stakeholders; 5) improving risk assessment strategies in Europe by analysing stakeholders attitudes, by training and by discussing the new methodology in several stakeholder conferences. The project commenced in June 2010 and will run for three years. Following a call for tenders in 2010, for the "collection and assessment of data relevant for non-dietary cumulative exposure to pesticides and proposal for conceptual approaches for non-dietary cumulative exposure assessment", EFSA had commissioned the UK Food and Environment Research Agency to undertake this work.[l]
11.8 Residue surveillance programmes should be modified in the light of the need for representative data for probabilistic exposure assessment. The nature of processing and preparation on the bioavailability and chemical nature of residues should be further investigated	The FSA provided papers on representative sampling for the Veterinary Residues Committee (VRC) and Pesticides Residue Committee (PRC). The scope for representative sampling is heavily constrained by current resources for the surveillance programmes. Both the PRC and VRC have acknowledged the need for collection of statistically representative data. See project T10019.

[k] Aggregate and Cumulative Risk Of Pesticides: an On-Line Integrated Strategy

[l] See http://www.efsa.europa.eu/en/tendersawarded/tender/cftefsappr201004.htm

STAGE 2	
11.1 Change to approval system such that pesticide and veterinary medicine authorisation considers mixtures from all sources of exposure.	Recent European regulations for pesticides, i.e. Regulation (EC) No. 396/2005 and Regulation (EC) No. 1107/2009, require account to be taken of known cumulative and synergistic effects when the methods to assess such effects are available.
11.13 Set up central and accessible repository of information about all forms of human exposure to pesticides and similar substances – on a web site or paper repository.	This recommendation will be addressed by the ACROPOLIS project (see 11.7 above).
11.14 Review extent and adequacy of information available to the domestic user of pesticides and veterinary medicines for its extent and ease of comprehension.	Information for domestic users is reviewed on an ongoing basis by the relevant regulatory agencies and their advisory committees.

would also address the recommendation to “Set up central and accessible repository of information about all forms of human exposure to pesticides and similar substances – on a web site or paper repository”. The Committee considered that the recommendation to “Review extent and adequacy of information available to the domestic users of pesticides and veterinary medicines for its extent and ease of comprehension” was a matter for the various committees overseeing pesticides and veterinary medicines.

Overall conclusions

38. The Committee concluded that the research programme established following its 2002 report had led to the development of a number of promising biomarkers for pesticides, and provided reassurance that combined risk assessment based on dose/concentration addition was adequately protective for compounds with similar modes of action. A few projects had not delivered their intended outcomes, but this did not indicate that they had been ill-chosen or poorly conducted. Overall, the recommendations for research in the 2002 report had been addressed well.

39. The non-research recommendations in the Committee’s 2002 report were largely in the process of being addressed. While UK Government departments had begun initial responses to the recommendations these had, in many cases, since been superseded by activity in the EU, in part as a result of regulatory changes relating to the approval of pesticides and the setting of Maximum Residue Levels (MRLs).

Recommendations

40. Following on from several projects in the programme, in vitro systems should be used to derive parameters for use in risk assessment and possible incorporation into PBPK modelling of mixtures.

41. While the development of biomarkers of effect would be the ideal, there will be a greater chance of success in developing biomarkers of exposure, and therefore these should be the priority for further work. The Committee has identified several biomarkers of exposure which should be prioritised for further development (see paragraphs 11-16).

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List of abbreviations

ACP	Advisory Committee on Pesticides
ADAS	No longer an abbreviation but the name of the company itself, ADAS was formerly known as the Agricultural Development Advisory Service
ADI	Acceptable Daily Intake
COM	Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment
CRD	Chemicals Regulation Directorate
CVMP	Committee for Medicinal Products for Veterinary Use
CYP1A2	Cytochrome P450 1A2
CYP2C19	Cytochrome P450 2C19
CYP3A4	Cytochrome P450 3A4
CYP450	Cytochrome P450 enzyme
EFSA	European Food Safety Authority
GC-MS	Gas chromatography-mass spectrometry
HSE	Health and Safety Executive
IC_{50}	The half-maximal inhibitory concentration
IGHRC	Interdepartmental Group on Health Risks from Chemicals
IPCS	International Programme on Chemical Safety
K_i	Inhibition constant
LC-MS	Liquid chromatography-mass spectrometry
MCF-7	A human breast adenocarcinoma cell line
MDR1	Multidrug Resistance Protein 1
MRL	Maximum Residue Level (for pesticides)
NDNS	National Diet and Nutrition Survey
NOAEL	No observed adverse effect level
NOEL	No observed effect level

OCT2	Organic cation transporter
PBPK model	Physiologically-based pharmacokinetic model
PRC	Pesticide Residues Committee
PPR Panel	EFSA Panel on Plant Protection Product and their Residues
RIKILT	Dutch Institute of Food Safety
SH-SY5Y	A human neuroblastoma cell line
SK-N-SH	A human neuroblastoma cell line
VMD	Veterinary Medicines Directorate
WHO	World Health Organization

Annex A: Table summarising the biomarkers of exposure. A list of abbreviations follows at the end.

Biomarker	Method	Project	Sensitivity	Specificity	Matrix effects?	Authorised for use on food crops in EU or otherwise occurs in food on sale in the UK?	Other notes
Pirimicarb (MDHP metabolite)	Immunoassay	T10003	LOD = 2 µg/l. Urine sample from a volunteer who had ingested pirimicarb indicated sufficient to identify exposure below the ADI	Some cross-reactivity with DDHP, another metabolite of pirimicarb (minor)	Some effect but overcome by matrix-matching standard	Authorised in the EU	
Pirimicarb (MDHP metabolite)	LC-MS	T10003	LOD = 2.5 µg/l. Capable of identifying exposures below the ADI, though not detected in the urine of 10 volunteers with no occupational exposure	Specific	No	Authorised in the EU	Combined method so can analyse for pirimicarb, benomyl, carbendazim, thiophanate-methyl and thiabendazole in a single assay

Pirimiphos-methyl (EMHP metabolite)	Immunoassay	T10003	LOD = 1 µg/l. Urine sample from a volunteer who had ingested pirimiphos-methyl indicated sufficient to identify exposure below the ADI.	Some cross-reactivity with a minor metabolite of pirimicarb (DDHP)	Some effect but overcome by matrix-matching standard	Authorised in the EU	
Carbendazim (hydroxyl carbendazim metabolite)	Immunoassay	T10021	LOD = 1.4 µg/l in buffer. Could quantify down to around 4 µg/l in urine but low recovery from urine affects accuracy	High cross-reactivity between the metabolites of carbendazim and thiabendazole	Yes – overcome to an extent, though recovery from urine is low and further optimisation required	Authorised in the EU	
Thiabendazole (hydroxyl thiabendazole metabolite)	Immunoassay	T10021	LOD = 1.8 µg/l in buffer. Could quantify in urine at around 8 µg/l and above, but inaccurate at lower levels.	High cross-reactivity between the metabolites of thiabendazole and carbendazim	Yes – overcome to an extent, though further optimisation required	Authorised in the EU	

Benomyl, carbendazim and thiophanate-methyl (5-OH-MBC common metabolite)	LC-MS	T10003	LOD = 5.0 µg/l. Sufficient to detect background exposure in two out of 10 volunteers with no occupational exposure.	A common metabolite, but specific to this group	No	Carbendazim and thiophanate-methyl are authorised in the EU	Combined method so can analyse for pirimicarb, benomyl, carbendazim, thiophanate-methyl and thiabendazole in a single assay
Thiabendazole (5-OH-TBZ metabolite)	LC-MS	T10003	LOD = 1.1 µg/l. Sufficient to detect background exposure in five out of 10 volunteers with no occupational exposure.	Specific	No	Authorised in the EU	Combined method so can analyse for pirimicarb, benomyl, carbendazim, thiophanate-methyl and thiabendazole in a single assay
Several pyrethroids (3-PBA common metabolite)	Immunoassay	T10003	LOD = 8 µg/l. Sufficient for occupational exposures but sensitivity questionable for non-occupational exposure	A common metabolite of most pyrethroids	Some effect but overcome by matrix-matching standard	Authorised in the EU	

Several pyrethroids (3-PBA metabolite)	Immunoassay	T10021	LOD = 1.5 µg/l in buffer, but matrix effects in urine	A common metabolite of most pyrethroids	Yes. Not developed to functioning assay in urine	Authorised in the EU	
Several pyrethroids (3-PBA metabolite)	LC-MS	T10013	1.0<LOD<2.1 µg/l (exact LOD not reported). Detected in unspiked urine, which was presumed to reflect background dietary exposure	A common metabolite of most pyrethroids	No	Authorised in the EU	Common extraction method for permethrin/cyper-methrin metabolite, deltamethrin metabolite and 3-PBA. Extraction could also be combined with the metabolites of iprodione and penconazole
Deltamethrin (DVBA metabolite)	GC-MS	T10003	LOD = 1 µg/l. Sufficient to detect background exposure in 29% of 80 volunteers with no occupational exposure.	Specific	No	Authorised in the EU	Assay accredited under the G-EQUAS quality assurance scheme (German External Quality Assessment Scheme for Analyses in Biological Materials). T10013 looked at using LC-MS to reduce costs

Deltamethrin (DBVA metabolite)	LC-MS	T10013	LOD = 2.1 µg/l	Specific	No	Authorised in the EU	Common extraction method for permethrin/cyper-methrin metabolite, deltamethrin metabolite and 3-PBA. Extraction could also be combined with the metabolites of iprodione and penconazole
Bifenthrin and cyhalothrin (CTFPA metabolite)	GC-MS	T10003	LOD = 1 µg/l. Sufficient to detect background exposure in 42% of 80 volunteers with no occupational exposure.	A common metabolite of bifenthrin and cyhalothrin	No	Bifenthrin: Provisional approvals in Members States. Resubmitted for inclusion in Annex I of Directive 91/414/EEC Lambda cyhalothrin authorised in the EU	T10013 looked at using LC-MS to reduce costs

Permethrin and cypermethrin (cis- and trans- DCVA metabolite)	LC-MS	T10013	LOD = 1 µg/l	A common metabolite of permethrin and cypermethrin	No	Permethrin: Not authorised in the EU but has been found in food sold in the UK Cypermethrin: Authorised in the EU	Common extraction method for permethrin/cyper-methrin metabolite, deltamethrin metabolite and 3-PBA. Extraction could also be combined with the metabolites of iprodione and penconazole
Ethylenebisdithio-carbamates (ETU common metabolite)	LC-MS	T10003	LOD = 250 µg/l. Sufficient to detect background exposure in 26% of 80 volunteers with no occupational exposure to ethylenebisdithiocar-bamates	Exposure to ETU itself in addition to the ethylenebisdi-thiocarbamates	No	Authorised in the EU	Possible to combine the assay with that for pirimicarb and the benzimidazoles, though there would be loss of sensitivity for ETU
Carbaryl (1-naphthol metabolite)	Immunoassay	T10009	LOD = 406 µg/l in buffer. Low ppb levels was the target. Unlikely the assay could be developed to be suitably sensitive	1-naphthol is also a metabolite of naphthalene	Likely appreciable effects (not developed as a urine assay due to low sensitivity)	No	

Chlorpyrifos (TCP metabolite)	Immunoassay	T10009	Developed into a semi-quantitative test that could detect 2.5 µg/l in spiked urine with specificity of 86% and sensitivity of 71%. Assay standardisation would be needed before application	TCP is a common metabolite of chlorpyrifos and chlorpyrifos-methyl	Appreciable – could be ameliorated but this resulted in the assay having to be developed into a semi-quantitative assay	Authorised in the EU	
Phosmet (phthalic acid metabolite)	Immunoassay	T10009	LOD = 13 µg/l in buffer. Assay unlikely to be improvable to the extent that low level background exposure could be detected in urine.	Phthalic acid is also a metabolite of folpet and phthalic acid esters	Likely to be appreciable effects (not developed as a urinary assay)	Authorised in the EU	
Paraquat	LC-MS	T10013	Lowest detected standards in spiked urine = 10 µg/l. No paraquat was detected in the urine of 10 volunteers with no occupational exposure.	Specific	No	No	Single extraction method feasible for paraquat, diquat and chlormequat though not a combined chromatographic method

Diquat	LC-MS	T10013	2 µg/l in spiked urine readily detected. No diquat was detected in the urine of 10 volunteers with no occupational exposure.	Specific	No	Authorised in the EU but not detected at or above reporting limits used in food	Single extraction method feasible for paraquat, diquat and chlormequat though not a combined chromatographic method
Chlormequat	LC-MS	T10013	LOD = 0.25 µg/l. Sufficient to detect background exposure in 1 out of 10 volunteers with no occupational exposure	Specific	No	Authorised in the EU	Single extraction method feasible for paraquat, diquat and chlormequat though not a combined chromatographic method
Penconazole (4(2,4-dichlorophenyl)-5-[1,2,4]triazol-1-yl-pentanoic acid metabolite)	Immunoassay	T10021	LOD = 0.4 µg/l in buffer. LOD in region of 0.5 – 2.5 µg/l in urine, and quantitative detection could be achieved at levels above 2.5 µg/l in urine.	Specific. Minimal cross-reactivity with other triazoles and imidazoles tested, but further work needed to confirm that the metabolite is produced in humans and validate the assay	Overcome	Authorised in the EU	

Penconazole (4-(2,4-dichloro-phenyl)-5-[1,2,4]triazol-1-yl-pentanoic acid metabolite)	LC-MS	T10013	LOD = 1.6 µg/l. Sufficient to detect background exposure in 5 out of 10 volunteers with no occupational exposure	Specific but further investigation is needed to confirm that the metabolite is produced in humans	No	Authorised in the EU	Could be chromatographed with imazalil metabolite (separate extraction needed) or with iprodione metabolite (common extraction). Extraction could also be combined with metabolites of permethrin/ cypermethrin and deltamethrin, and with 3-PBA.
Imazalil (1-(2,4-dichloro-phenyl)-2-imidazol-1-yl-ethanol metabolite)	Immunoassay	T10021	LOD = 9.3 µg/l in buffer, but lower in urine.	Cross reactivity with penconazole metabolite and penconazole. Further investigation is needed to confirm that the metabolite is produced in humans	Some – further optimisation of urine extraction required, but may still not be possible to have a sensitive, fully quantitative assay	Authorised in the EU	

Imazalil (alpha(2,4-dichlorophenyl)-1H-imidazole-1-ethanol metabolite)	LC-MS	T10013	LOD = 1 µg/l. Sufficient to detect background exposure in 3 out of 10 volunteers with no occupational exposure	Specific but the metabolite was identified from laboratory animals and needs confirmation as a metabolite in humans	No	Authorised in the EU	Could be extracted using same procedure as metabolites of pirimicarb, benomyl, carbendazim, thiophanate-methyl and thiabendazole developed in T10003.
Iprodione (3-(3,5-dichlorophenyl)-2,4-dioxoimidazolidine-1-carboxamide metabolite)	LC-MS	T10013	LOD = 2.9 µg/l. Sufficient to detect background exposure in 3 out of 10 volunteers with no occupational exposure	Specific	No	Authorised in the EU	Could be extracted using the same procedure as for metabolites of penconazole, permethrin/cyper-methrin and deltamethrin, and 3-PBA.

List of abbreviations used in the table

3-PBA	3-Phenoxybenzoic acid
5-OH-MBC	5-hydroxy-methyl-2-benzimidazolecarbamate
5-OH-TBZ	5-hydroxy-thiabendazole
ADAS	No longer an abbreviation but the name of the company itself, ADAS was formerly known as the Agricultural Development Advisory Service
ADI	Acceptable Daily Intake
CTFPA	2-Chloro-3,3,3-trifluoroprop-1-enyl-2,2-dimethylcyclopropane-1-carboxylic acid
DBVA	2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid
DCVA	3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid
DDHP	2-Dimethylamino-5,6-dimethyl-4-hydroxypyrimidine
EMHP	2-Ethylamino-6-methyl-4-hydroxypyrimidine
ETU	Ethylenethiourea
EU	European Union
GC-MS	Gas chromatography – mass spectrometry
HSL	Health and Safety Laboratory
LC-MS	Liquid chromatography – mass spectrometry
LOD	Limit of detection
MDHP	2-Methylamino-5,6-dimethyl-4-hydroxypyrimidine
TCP	3,5,6-Trichloro-2-pyridinol