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WORLD HEALTH ORGANISATION
COLLABORATING CENTRE
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Biological monitoring of pesticide exposures

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Levels of pesticide metabolites in the urine of people known to have been exposed to specific pesticides were compared with the level of these metabolites predicted from data obtained from the regulatory risk assessment process. Cypermethrin and mancozeb were selected as the pesticides to be studied. Subjects were recruited to one of four groups: pesticide applicators or sprayers; agricultural workers entering the fields after spraying (post-application workers); bystanders or neighbours and urban dwellers (consumers). Pesticide metabolites were detected in the urine of sprayers more frequently than in the urine of post-application workers, bystanders or consumers. Recruitment difficulties resulted in a smaller number of samples being obtained than had originally been intended.

A simple pharamacokinetic model was developed to predict urinary metabolite levels using dermal exposure level estimated through the regulatory risk assessment process or using data from the EUROPOEM model. Predicted median urinary metabolite levels were generally much higher than observed urinary metabolite values, for sprayers and post-application workers for both cypermethrin and mancozeb containing pesticides. This provides some confirmation that predicted values are conservative and provides reassurance that the current regulatory risk assessment is protective for sprayers and post-application workers. Where metabolites of cypermethrin or mancozeb were detected in the urine of bystanders, predicted median levels were lower than the observed values regardless of how dermal exposure was estimated. However, only a small number of bystanders were monitored and so it is not possible to conclude whether the regulatory system protects bystanders or not.

All measured values for bystanders were well below the acceptable level, i.e. the Allowable Operator Exposure Level (AOEL) or Acceptable Daily Intake (ADI). Since small numbers of subjects were involved, further fieldwork is recommended. We support the establishment of a biomonitoring database as suggested by the Royal Commission for Environmental Pollution and the Advisory Committee on Pesticides.

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SUMMARY

The purpose of this study was to compare the levels of pesticide metabolites in the urine of people known to have been exposed to specific pesticides with the level of these metabolites predicted from data obtained from the regulatory assessment process. In the first year of the study, cypermethrin was selected as the active ingredient to be studied because it was widely used in Great Britain and specific methods of analysis existed. However, once the study was underway it was apparent that cypermethrin was no longer as widely used and so in the second year of the study mancozeb was selected as the active ingredient to be studied since many sprayers and farmers reported using products containing it. Subjects were recruited to one of four groups: pesticide applicators or sprayers; agricultural workers entering the fields after spraying (post-application workers); bystanders or neighbours and urban dwellers (consumers). We had expected that sprayers and post-application workers were likely to have the highest exposures, bystanders less exposure and consumers should only have had exposure from the diet or the use of home and garden products containing the active ingredient. During the first year of the study a total 48 subjects participated: 11 sprayers; three post-application workers; six bystanders and 27 consumers. During the second year 16 subjects potentially exposed to mancozeb participated: eight sprayers; one post-application worker and seven bystanders. The number of samples was less than originally planned because of problems recruiting subjects into the study.

Cypermethrin metabolites were detected in the urine of sprayers more frequently than in the urine of post-application workers, bystanders or consumers. Ethylenethiourea (ETU), the metabolite of mancozeb was detected in the urine of sprayers more frequently than in the urine of bystanders. The levels in urine for both pesticide active ingredients for sprayers, post-application workers and bystanders were low and only one consumer had a detectable level of metabolite.

A simple pharmacokinetic model was developed to predict urinary metabolite levels using dermal exposures estimated using the regulatory risk assessment process and data from the EUROPOEM model (http://europoem.csl.gov.uk/). Predicted median urinary metabolite levels were generally much higher than the corresponding observed values, for sprayers and post-application workers for both cypermethrin and mancozeb containing pesticides. Such predicted values are therefore conservative and provide reassurance that the current regulatory risk assessment is protective for sprayers and post-application workers. Where metabolites of cypermethrin or mancozeb were detected in the urine of bystanders, predicted median levels were lower than the observed values regardless of how dermal exposure was estimated. Although this suggests that the regulatory systems may not be as protective for bystanders, only were a small number of bystanders studied. Further, the two bystanders with detectable levels of 3-phenoxybenzoic acid (3-PBA) in their urine following potential exposure to cypermethrin, had known previous occupational exposure. It would, however, be ill-advised to dismiss these results and it is recommended that consideration be given to how dermal exposure is estimated for bystanders in order to ensure that this group are protected with the same degree of overestimation of exposure as other exposed groups. It should also be noted that all measured urinary metabolite values for bystanders would have arisen from exposures that were well below the acceptable regulatory level, i.e. the Allowable Operator Exposure Level (AOEL) or Acceptable Daily Intake (ADI).

In summary, the number of people involved in this study were small and further field work is recommended to better understand potential exposure of bystanders. It is considered that studies should focus on active ingredients for which adequate toxicokinetic information is available, for example chlorpyrifos. Finally, the establishment of a biomonitoring database, as suggested by

the Royal Commission on Environmental Pollution (RCEP) and the Advisory Committee on Pesticides (ACP), would be a useful additional resource.

1 INTRODUCTION

In Great Britain the use of pesticides in agriculture, horticulture, forestry, food storage and the home or garden is regulated to protect human health and the environment. The regulatory system is administered for the Department of Environment Food, and Rural Affairs (DEFRA) by the Pesticide Safety Directorate (PSD). The scientific paradigm underpinning the approval of pesticides involves the comparison of estimated human exposure with some limit or limits, below which it is considered there are no adverse health effects. The system is generally considered to be conservative such that estimated exposures represent some multiple of the likely exposure. Similar regulatory systems exist for biocides (e.g. non-agricultural pesticides) and veterinary medicines, which may contain the same active substances as pesticides.

The exposures are typically estimated for those who apply the pesticide, workers who may be involved in post-application activities such as harvesting, and bystanders or neighbours. There are no mandated methods to estimate exposure and applicants for pesticide approval may use measurements made during application or other work with the product, other analogous measurement data or one of a number of exposure models. The exposure estimates of the applicant company are carefully reviewed by the PSD and, if appropriate, the basis for the assessments may be modified to ensure the estimated exposures are appropriate. Maximum Residue Levels (MRLs), derived from supervised field trials are set for each pesticide for a wide range of products including fruit, vegetables and cereals. MRLs are set to ensure that residues at the MRL do not represent a risk to human health.

Exposure to pesticides may arise by inhalation of aerosols or vapours, accidental ingestion or dermal contact, although the latter exposure route is generally considered to dominate amongst occupational groups. There has been very limited scientific investigation of the importance of accidental ingestion of chemicals and as a consequence little explicit consideration is generally given to this route of exposure in regulatory risk assessments.

The model most commonly used by PSD to estimate pesticide exposure during application is POEM (Pesticide Operator Exposure Model). POEM is based on a number of measurements of "external" exposure mostly made more than twenty to thirty years ago. These data comprise measurements of the mass of pesticide on workers clothing and/or skin using absorbent patches plus inhalation exposure data. The use of the model involves making a number of assumptions about the exposure situation, including the type of protective clothing worn. To estimate the fraction of this external exposure that may pass through the skin into the body or be absorbed in the lungs it is normal to use data from toxicological studies of uptake in experimental animal systems. POEM and other models used in these risk assessments are generally considered to be "screening" models, where the estimated exposures are expected to be much higher than the actual exposures that people may experience.

There have been a small number of research studies that have focused on comparison of regulatory exposure assessments with exposure estimated from biomarker studies. For example, Cochran (2002) describes an appraisal of the risks from chronic non-occupational exposure to chlorpyrifos in the USA. He compared the exposure estimates made using the US Environmental Protection Agency (US EPA) model incorporating standard default values with data from two biological monitoring studies (3,5,6 trichloro-2-pyridinol – (TCPy) in urine). The ratio of the average exposure (μg per kg body weight per day – μg/kg/day) to the average exposure estimated from the biological monitoring data ranged from 33 to 133. This analysis is somewhat reassuring, although it is important to recognise that the individuals monitored were selected in different ways. Those represented in the first study were a random population sample and in the second a sample of children from homes where chlorpyrifos containing products had

been used. In addition the comparison was made for average exposure and not some upper percentile of the exposed populations, which would be more reassuring in terms of protection of public health. If one were to arbitrarily assume a ten times safety factor to allow for interpersonal variation then the differences between the two assessment approaches would be less reassuring (i.e. 3 to 13).

Krieger et al (2001) also compare regulatory exposure assessments with biological monitoring data for non-occupational exposure. They had data for 13 people from three different residential situations: fogging, broadcast spraying and crack and crevice application. All of the situations involved exposure to chlorpyrifos and the biological monitoring was carried out by measuring TCPy in urine. Estimated exposure for an adult in the home where the broadcast spray was used using the US EPA model was judged to be 1mg chlorpyrifos/kg/day, whereas the measured exposure was a one thousand times less at 1µg/kg/day. Comparisons for other subjects were similar. Again these data are reassuring, but there are only a few measurements available from three exposure situations.

There have also been surveys undertaken to assess the exposure of the general population to pesticides, including chlorpyrifos. For example, the Centers for Disease Control and Prevention's (CDC) ongoing National Report on Human Exposure to Environmental Chemicals provides comprehensive biomonitoring information on a representative sample of the American population over two year periods. The second report was issued in 2003 and presented biomonitoring information on 116 environmental chemicals over the period 1999 to 2000 (CDC, 2003). The third report was issued in July 2005 (CDC, 2005) and presented data on 148 chemicals over the period 2001 to 2002 and also includes data from the second report. These surveys included measurement of urinary TCPy levels in samples of approximately 2000 adults and children in the years 1999-2000 and 2001-2002 (US DHHS, 2003). Ninety five percent of their data, which were not normalised for body mass, were less than 8.4µg/l and 9.2 µg/l urine in the years 1999-2000 and 2001-2002 respectively. Children also had higher excretion of TCPy, with the average level in those between 6 and 11 years almost twice that of adults aged 20 to 59 years. If these data were normalised to body mass then the difference would be even greater.

Interest in biomonitoring has increased in recent years and in 2004 an International Biomonitoring Workshop was held in the U.S. to discuss the information required for linking biomonitoring data with risk assessment and risk management (Albertini *et al.*, 2006). In Europe, a plan to develop a coordinated approach to human biomonitoring was started (EC, 2004) and the development of validated biomarkers useable for human biomonitoring. In California, The California Environmental Contaminants Biomonitoring Program (Senate Bill 1379, http://www.dhs.ca.gov/ehlb/BPP/PDF/SB1379.pdf) was passed in September 2006 to monitor the presence and concentration of designated chemicals in Californians.

2 AIMS

This study was based on the hypothesis that the levels of pesticide biomarkers in exposed people are less than the levels predicted from exposure information used in the regulatory risk assessment and the exposure assessments are protective.

The aim of this research was to compare the levels of a specific pesticide metabolite in the urine of people with documented exposure to that pesticide with the level of metabolite predicted from data obtained from the regulatory process.

To achieve this aim the following objectives were addressed:

1. Identify one active pesticide compound for the first year of the study with suitable biomarkers that may be found in the urine of exposed people, and identification of possible compounds for subsequent years;
2. Identify all approved pesticides, veterinary medicines and biocides that contain the selected compound;
3. Select two or three pesticide products containing this active compound for inclusion in the study;
4. Acquire the regulatory exposure estimates for each of the products from the PSD;
5. Develop a simple pharmacokinetic model to predict the level of the identified biomarker from the regulatory exposure estimates;
6. Identify and recruit people applying the pesticide products using suitable methods;
7. Recruit appropriate bystanders (e.g. neighbours) and workers who may enter treated areas after application, plus consumers;
8. Collect urine samples for biomarker analysis and diary information from all recruits, analyse urine samples;
9. Compare the measured biomarker levels with those predicted by the pharmacokinetic model from the regulatory exposure assessments and diary information.

3 METHODS

3.1 GENERAL SAMPLING STRATEGY

3.1.1 Cypermethrin

Cypermethrin was initially chosen as the active ingredient to be studied for a number of reasons. Firstly, it is widely used as an insecticide and it was fourth on the list of arable use in Great Britain (about 2 million hectares treated in 2004). It is mostly applied by boom spray during the autumn and so was thought to be particularly appropriate to investigate bystander exposure. There are some volunteer studies for cypermethrin and specific methods for analysis. Out of the top 10 it has the highest toxicity (lowest LD50) for mammals and might thus be of most concern for humans. The biological half-life is sufficiently long for there to be detectable amounts of the metabolites in the body 24hr after exposure.

There are three human volunteer studies using cypermethrin (Eadsforth and Balwin, 1983, Eadsforth *et al.*, 1988, Woollen *et al.*, 1992) and these would aid the development of the simple pharmacokinetic model needed for the study. Eadsforth *et al.* and Woollen *et al.* studied both oral and dermal exposures. Eadsforth *et al.* also studied different oral doses (1983 and 1988) and repeated oral doses (1988). These studies allow an estimation of dermal absorption and a determination of an appropriate sampling strategy. The majority of the oral doses were excreted within 24 hours whereas it was 36 hours for the dermal doses.

The primary metabolites are cis- and trans- 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (DCVA), 3-phenoxybenzoic acid (3-PBA) and 3-(4'-hydroxyphenoxy) benzoic acid (4OH3PBA). The metabolites, 3-PBA and 4OH3PBA are common to a number of synthetic pyrethroids whereas cis- and trans-DCVA are specific to permethrin and cypermethrin. The cis- and trans-DCVA metabolites have been widely studied in respect of cypermethrin exposure (noting any co-exposure to permethrin) and are the accepted biomarkers of choice. There are several analytical methods (Angerer and Ritter, 1997, Leng *et al.*, 1997, Angerer and Schaller, 1999) including a well validated method published by the Deutsche Forschungsgemeinschaft and there is an external quality assurance scheme (G-EQUAS) in operation.

Selection of cypermethrin containing pesticides

Information about the usage of cypermethrin containing products was obtained from Central Science laboratory (CSL). This was based on information from surveys across all crops in 2003. It was estimated that 49% of the total area treated with cypermethrin was attributable to unspecified generic cypermethrin use. Of the remaining area, 35% was treated with Toppel 10 and 35% with Permasect C. Cyperkill 10 accounted for 12%, Fernpath Banjo for 8% and Cleancrop Pyrimet for 5%. It was therefore initially decided to focus on Toppel 10, Permasect C and Cyperkill 10.

The original intention was to look at these products only, however, this soon proved too narrow and the study was expanded to include all cypermethrin containing products.

3.1.2 Mancozeb

In the second year of the study, mancozeb was chosen as the active ingredient to be studied. It is a widely used fungicide and approximately one million hectares were treated with it in 2004.

Mancozeb degrades to form ethylenethiourea (ETU). The biological half life is around 100 hours and so there will be detectable amounts of metabolite in the urine on the morning following exposure.

Based on experiences with cypermethrin, all mancozeb containing products were included in the study from the start.

3.2 ETHICAL APPROVAL

Submission for ethical approval was made to the Multi-Centre Research Ethics committee (MREC) for Scotland. After amendments to meet the committee's requirements, approval was given in October 2004.

3.3 RECRUITMENT

Subjects fell into one of four groups: pesticide applicators or sprayers; agricultural workers undertaking crop re-entry (post-application workers); bystanders or neighbours and urban dwellers (consumers).

Sprayers and post-application workers are likely to have the highest exposures. Bystanders will have considerably lower exposures. Children (bystanders) living or working on farms will have exposure dependent on their activity patterns. Amongst these "rural" groups, exposure is likely to be predominately from the dermal deposition or inhalation of pesticide spray or vapour. Ingestion exposure should be of less importance for applications on bystanders. "Urban" consumers are likely to have considerably lower exposures with the majority of their internalised dose coming from ingestion of foodstuffs with trace residues of pesticides.

3.3.1 Recruitment of subjects potentially exposed to cypermethrin

Four manufacturers of the cypermethrin containing products (Toppel 10, Permasect C, Cyperkill 10) were contacted to ask for their support for the project. Four suppliers of these products were identified and contacted to ask for their advice and assistance. One supplier no longer distributed the selected product. The remaining three suppliers were willing to help identify spraying contractors, farmers and other users of their products.

A list of crop sprayers was obtained from the National Association of Agricultural Contractors (NAAC) website (http://www.naac.co.uk/Sections/membersByWork.asp?WorkType=GroundCropSpraying). The NAAC represents agricultural and amenity contractors in the UK, including crop sprayers. Members on this list were initially contacted by telephone. When recruitment for this study started, there were 131 companies listed on this website. However, 10 companies were located in Scotland which was initially not included in the study. Further, not all companies were involved in crop spraying; some were seed merchants, potato dealers etc and were therefore not suitable for inclusion in the study.

A number of strategies were employed for recruiting farmers. Farmers may be directly exposed to pesticides as sprayers, post-application workers or bystanders. One approach was to ask spraying contractors for details of farms where they were spraying. The Crown Estates Office was approached and supplied a list of rural estate managing agents who were contacted by telephone to enlist their support in recruiting farm tenants. The Crown Estates Office supplied lists of farm tenants and tenants were then approached, initially by letter and subsequently by a telephone call. Finally, lists of farmers were downloaded from www.yell.com and farmers approached by telephone.

Initially, recruitment of sprayers and farmers, was restricted to England and Wales, however, as the project progressed, recruitment was extended to Scotland.

Potential consumers were originally selected from the Manchester and Sheffield areas. Page numbers from the respective telephone books were randomly selected and the first name on that page contacted by telephone. If this person was unsuitable i.e. for age or medical reasons or residence in a rural area, then the next number on the list was selected. The recruiting strategy was later modified, with individuals also being recruited from the offices of the IOM and HSL and also through personal contacts of IOM staff.

3.3.2 Recruitment of subjects potentially exposed to mancozeb

Initially, it was intended to recruit individuals potentially exposed when orchards and vineyards were sprayed. Lists of vineyard owners and orchard owners were obtained from the following sites on the internet.

The English wine site, http://www.english-wine.com/
English wine producers, www.englishwineproducers.com/vineyard.htm
UK cider – the real cider website, http://www.ukcider.co.uk/wiki/index.php/Orchards
Apple journal, www.applejournal.com
PickYourOwn.org, www.PickYourOwn.org
Welsh Perry and Cider Society Ltd, www.welshcider.co.uk

Other individuals, including sprayers, post application workers and bystanders associated with a local spraying company who had previously participated in the study agreed to participate in the second phase of the study.

3.3.3 Information sent to subjects

All subjects who were interested in participating in the study were sent further details, a consent form, an initial questionnaire, a diary and a sampling pack comprising a sampling container for collecting urine, insulated holder and packaging material. If, after reading the further information, they were still willing to participate, subjects completed the consent form and initial questionnaire and returned them in a pre-paid envelope. The diary was completed and the urine sample provided when convenient first thing in the morning for sprayers and first thing in the morning after spraying or exposure for sprayers, bystanders and post-application workers.

3.4 QUESTIONNAIRES AND DIARIES

In order to obtain information about the circumstances of exposure, questionnaires tailored to each of the four groups were designed. In addition to personal details, the questionnaires asked for details on the daily consumption of fruit, vegetables, salad, bread and drinks. The same questionnaire was completed by consumers, bystanders and post-application workers (Appendix 1). This questionnaire was partially based on one previously developed by HSL. The questionnaire for sprayers also collected general information on spraying, such as how many days a year on average they sprayed and how long they had been working with pesticides, clothing and personal protective equipment used and spraying equipment (Appendix 2). This questionnaire was based on sheets used by CSL in their Survey of Current Farm Sprayer Practices in the United Kingdom (Garthwaite, 2002). For the second year of the study the questionnaire for sprayers was simplified, with questions about clothing and personal protective equipment being omitted (this information was collected as part of the information collected associated with the spraying event).

3.5 COLLECTION OF DIARIES AND URINE SAMPLES

All participants were asked to keep a diary of what they ate and where they were for roughly seven days before providing a urine sample. Additionally, bystanders were asked questions designed to obtain information on their proximity to the field being sprayed and whether or not they entered the field. Post-application workers were asked when they had entered the sprayed field and for how long, the location of the field, products used on the field and clothing and equipment worn. Sprayers were asked questions related to spraying, including location, equipment used, environmental conditions, products used, clothing and equipment worn and accidental exposure to pesticide. Daily diary sheets for each group are shown in Appendices 3 to 6. During the second year of the study, all participants completed the same diary (Appendix 3). Additional specific sheets were completed by bystanders, post application workers and sprayers as necessary (Appendices 7 to 10).

For consumers the diary was completed and the urine sample provided at their convenience. Sprayers, bystanders and post-application workers were asked to provide a urine sample first thing in the morning after the last exposure of the week.

After the sample was collected and placed in the secure packaging, subjects were instructed to post it immediately back to the IOM. On arrival at the IOM samples were frozen at -20^{o}C.

3.6 ANALYSIS OF URINE SAMPLES

Samples from individuals exposed to cypermethrin were acid hydrolysed to release any conjugated metabolites (e.g. glucuronides etc). The analytes were then extracted by liquid:liquid extraction using chlorobutane, evaporated to dryness and derivatised using pentafluorobenzyl bromide. Samples were analysed by gas chromatography - mass spectrometry using selected ion monitoring with chemical ionisation (using methane). The method measures cis- and trans-DCVA (specific metabolites of permethrin and cypermethrin), a specific metabolite of deltamethrin and 3-PBA which is a generic metabolite of pyrethroids. The detection limit of the method was 5 nmol/l for 3-PBA, cis-DCVA and trans-DCVA. The method is valid over the range 1 to 100 μg/l. Internal quality control samples indicate that the day to day imprecision (r.s.d) of 3-PBA is 18% and cis- and trans-DCVA 23%. HSL has certification for DCVA from the German External quality assurance scheme.

Samples from individuals exposed to mancozeb were extracted using diamotaceous earth extraction columns. The analyte was eluted with DCM and evaporated to dryness under a stream of nitrogen and reconstituted in the mobile phase. Samples were analysed by liquid chromatography – mass spectrometry. The method measures ethylenethiourea (ETU). The method is valid over the range 0.25 to 25 μg/l. Internal quality control samples indicate that the day to day imprecision (r.s.d) of ETU is 15%.

3.7 ESTIMATION OF EXPOSURE AND DOSE

3.7.1 Estimation of exposure

Estimates of dermal exposure were provided from two sources. Firstly, estimates were based on data in the EUROPOEM database. This database contains information on the exposure of sprayers, bystanders and post application workers. For spraying, information on potential and actual exposure related to mixing/loading, application and mixing/loading/application related to different scenarios is recorded. For the purposes of this project, actual exposure data relating to hand held spraying and other (e.g. boom, airblast) spraying were used to estimate exposure for

sprayers. No distinction was made between mixing, loading and application, i.e. we used aggregate data for all phases of use. Information on actual exposure extracted from the EUROPOEM database for sprayers is shown in Table 1.

Table 1 Dermal exposure (mg/kg active substance) – summary statistics

	Hand held		Other	
	ADE	**AHE**	**ADE**	**AHE**
Minimum	0.06	0.01	0.0001	0.0002
Median	1.82	0.09	0.03	0.02
Maximum	1364.80	71.51	2.11	18.59

where ADE is actual dermal exposure (mg/kg active substance) and AHE is actual hand exposure (mg/kg active substance). These values were re-expressed as a proportion of the amount being sprayed (k_f) as shown in Table 2.

Table 2 Proportion of pesticide sprayed which is deposited on skin (k_f)

	Hand held	**Other**
Minimum	1.60×10^{-7}	3.00×10^{-10}
Median	1.91×10^{-6}	5.00×10^{-8}
Maximum	1.44×10^{-3}	2.07×10^{-5}

A triangular distribution using the minimum, median and maximum, was assumed to be appropriate to represent the potential uncertainties involved in the modelling.

Exposure was then calculated as follows.

$$E = M.k_f \qquad \text{Equation 1}$$

M – amount of pesticide used (μg)
k_f – fraction of amount of pesticide sprayed that gets onto the skin

Information on potential bystander exposure was collected from EUROPOEM and reported as a percentage of the applied dose (l or kg/ha as applied to 2 m^2 (assumed surface area of a subject)) per pass of the sprayer. A summary of exposures as a proportion for arable spraying is given in Table 3.

Table 3 Proportion of applied dose rate per pass of the sprayer which contaminates bystander

	Proportion
Minimum	0.0003
Median	0.002
Maximum	0.032

The exposure per pass of sprayer can then be calculated as follows.

$$E = 2.\frac{M}{A}.k_p \qquad \text{Equation 2}$$

where A is the area sprayed (m^2) and k_p is the proportion of contamination per pass. In order to calculate the total exposure per pass of the sprayer, an average of three passes per field were assumed (Twining, 2006). It should be stressed that only potential exposure for bystanders is available from EUROPOEM. Since no information is available about the protection afforded by ordinary clothing, the actual exposures were assumed to be equivalent to the potential exposures, which is the worst scenario whereby ordinary clothing is assumed to provide no protection.

Secondly, exposure assessments for the scenarios and pesticides reported in this study were provided by PSD (Paul Hamey, personal communication) and are shown below in Tables 4 to 9. It was assumed that 10% cypermethrin was absorbed through the skin for sprayers, bystanders and post-application workers. When mixing and loading it was assumed that 0.11% mancozeb was absorbed through the skin and that 0.24% mancozeb was absorbed through the skin when spraying. It was also assumed that 0.24% mancozeb was absorbed through the skin of bystanders and post-application workers.

Dermal exposure and the amount absorbed by this route are shown in Tables 4 and 5 for sprayers using cypermethrin and mancozeb products respectively. Two sprayers (SP62 and SP64) used two mancozeb containing products, Electis and Rhapsody, their exposure was assumed to be that of the lowest regulatory assessment, since that will provide the least protective estimate.

Table 4 Dermal exposure for sprayers using products containing cypermethrin

Pesticide	Active ingredient concentration (mg/ml)	Application method	Dermal exposure (mg)	Amount absorbed (mg)
Cyperguard 100 EC, Permasect C, Toppel 10 [1]	100	Boom	11	1.12
Toppel 10 [2]	100	Boom	20	2.04

1 – brussel sprouts, winter wheat, 2 – oilseed rape

Table 5 Dermal exposure for sprayers using products containing mancozeb

Pesticide	Active ingredient concentration (mg/kg)	Application method	Dermal exposure (mg)	Amount absorbed (mg)
Karamate Dry Flo Newtec [1]	750	Hand held	246	0.59
Karamate Dry Flo Newtec [1]	750	Air-assisted	293	0.70
Unikat 75 WG [1]	667	Air-assisted	146	0.35
Electis 75 WG [2]	667	Boom	41	0.10
Rhapsody [2]	680	Boom	46	0.11
Matilida [2]	680	Boom	49	0.11

1 – vines, 2 - potatoes

Dermal exposure and the amount absorbed by this route are shown in Tables 6 and 7 for bystanders exposed to cypermethrin and mancozeb products respectively. It was assumed that bystander exposure to Matilda was the same as that for bystander exposure to Rhapsody. The reported exposures refer to whole day exposure and for the purposes of pharmacokinetic modelling it was assumed that bystanders exposed to the same product had the same exposure.

Table 6 Dermal exposure for bystanders exposed to products containing cypermethrin

Pesticide	Active ingredient concentration (mg/ml)	Application method	Dermal exposure (μg)	Amount absorbed (μg)
Cyperguard 100 EC	100	Boom	12.5	1.25

Table 7 Dermal exposure for bystanders exposed to products containing mancozeb

Pesticide	Active ingredient concentration (mg/ml)	Application method	Dermal exposure (μg)	Amount absorbed (μg)
Electis 75 WG	667	Boom	600	1.44
Rhapsody	680	Boom	680	1.63

Dermal exposure and the amount absorbed by this route are shown in Tables 8 and 9 for post-application workers exposed to cypermethrin and mancozeb products respectively. It was assumed that exposure occurred over a two hour period.

Table 8 Dermal exposure for post-application workers exposed to products containing cypermethrin

Pesticide	Active ingredient concentration (mg/ml)	Application method	Dermal exposure (mg)	Amount absorbed (mg)
Cyperguard 100 EC	100	Boom	0.38	0.04

Table 9 Dermal exposure for post-application workers exposed to products containing mancozeb

Pesticide	Active ingredient concentration (mg/ml)	Application method	Dermal exposure (mg)	Amount absorbed (mg)
Electis 75 WG	667	Boom	18	0.04
Rhapsody	680	Boom	20	0.05

The above dermal exposures were then used in the pharmacokinetic model along with the proportion absorbed and information about spraying and exposure to predict urinary concentration. These were then compared with the actual values and those predicted using exposures estimated using data from EUROPOEM (section 3.8).

For the purposes of this study only the dermal route of exposure has been considered with exposure via the inhalation and ingestion routes being assumed to be minimal.

3.7.2 Estimation of dose

The dose rate (μg/kg.BW/hr) is calculated from uptake of the pesticide (U (μg)) and body weight (BW (kg)) of an individual as follows.

$$D = \frac{U}{BW} \Big/ h \qquad \text{Equation 3}$$

where h is the number of hours over which exposure occurs. Uptake can be calculated from exposure (E (μg)) and the fraction of the pesticide absorbed over the skin (F_a).

$$U = E.F_a \qquad \text{Equation 4}$$

3.8 PHARMACOKINETIC MODELLING

3.8.1 Methods

The use of biomarkers for human health risk assessment is attractive because they are an indicator of the dose that actually enters the body by all routes of exposure (inhalation, ingestion and dermal). However, the relationships between biomarker and environmental exposure are often unclear, because what is seen in a urine sample depends on the route of exposure plus the intensity and duration of the exposure. Thus, there is a need to clarify the underlying mechanisms behind the pathways between biomarkers and internal dose, which can be achieved using a pharmacokinetic model. Such models can vary from simple one-compartment models to complex multi-compartment models. Although generally more realistic, the more complex a model, the more information with respect to anatomical, physiological, biochemical and physiochemical that is required. Often, only limited information pertaining to humans is available. Or, only information from animal studies may be available and its relevance to humans is questionable. Alternatively, a simple model may not be fully representative of the processes occurring in the body. Therefore, when selecting a model, issues of complexity and simplicity must first be considered.

For the purposes of this study a simple pharmacokinetic model was developed in order to investigate the relationship between pesticide exposure, internal dose and biomarker level in the urine. This was considered most appropriate given the available toxicology and pharmacokinetic data available for the pesticides and metabolites of interest. The model describes the pathways from uptake to absorbed dose, to metabolite concentration in target tissues and, finally, to urinary excretion.

The approach adopted was based on that of Rigas *et al.* (2001) and assumes that pesticide is absorbed into a single body compartment and that the distribution of the pesticide or its metabolite in the body is approximated by a volume of distribution.

This model is represented by the following equations.

$$\frac{dC_a}{dt} = -k_a.C_a + \frac{S.R}{V_d.\frac{M_o}{M_m}}D \qquad \text{Equation 5}$$

$$\frac{dC_b}{dt} = k_a.C_a - k_e.C_b \qquad \text{Equation 6}$$

where C_a is the concentration of metabolite in the absorption reservoir, in this case the skin (µg/l) and C_b is the body burden concentration of metabolite or concentration available systemically (µg/l). It is assumed that absorption through the skin and elimination are governed by the first-order rate constants k_a (per hr) and k_e (per hr). S is the selectivity, which is the amount on a molar basis of the absorbed material that can be collected as the metabolite of interest and lies between 0 and 1. R is the stochiometric ratio of the pesticide to the metabolite of interest. V_d is the volume of distribution (l/kg). The molecular weights of the pesticide and metabolite are represented by M_o and M_m respectively. Finally, D is the background dose rate (µg/kg/hr).

The average urinary excretion rate ($\overline{UER}$, µg/hr) can be determined from a single urine collection by integrating the concentration of metabolite in the body from the time of last urination (t_l) and the time of current urine collection (t_c) as follows:

$$\overline{UER} = \frac{V_d.k_e.BW}{t_c - t_l}\int_{t_l}^{t_c} C_b(s)ds \qquad \text{Equation 7}$$

In practice, $\overline{UER}$ is the total mass of the metabolite in a complete urine void divided by the time over which it accumulated in the bladder.

$$\overline{UER} = \frac{C_u.V_u}{(t_c - t_l)} \qquad \text{Equation 8}$$

where C_u is the measured metabolite concentration in urine (µg/l), which can be estimated by rearranging the above equation as follows.

$$C_u = \frac{\overline{UER}.(t_c - t_l)}{V_u} \qquad \text{Equation 9}$$

The values of the parameters used to calculate the urinary concentration of metabolites are presented in Table 10. Values for some parameters are known, for example, the molecular weights of pesticides and metabolites and the stochiometric ratios. Values of other parameters were estimated, for example, the urine volume from the time of the last void to the time of sample collection, the time of the last collection and sample collection. Values for some parameters were derived from the literature (the fraction absorbed over the skin, the half life). The elimination rate was calculated by dividing log(2) by the half life.

The volume of distribution for the metabolites of cypermethrin have been estimated (Tornero-Velez *et al.*, 2006), but no information on this parameter was available for ETU. Further, the absorption rates and selectivity are not known for either cypermethrin or mancozeb. Estimates for these parameters were made based on knowledge of the pesticides used and the advice of colleagues.

Information about the exposure scenario was obtained from the completed diaries and spraying sheets, including start and finish time of spraying and the amount of active ingredient used.

3.8.2 Implementation of model

The pharmacokinetic model was implemented using the software package Matlab v.6 (see Appendix 11 for an example of the program used). The concentration of metabolite in the absorption reservoir (C_a) and the body burden concentration of metabolite available systemically (C_b) were modelled on a minute by minute basis.

Allowance for uncertainties in the model parameters was built in using Monte Carlo simulation techniques. The absorption rate constants, selectivity and volume of distribution (ETU only) were assigned uniform distributions while the fraction of pesticide on the skin was assigned a triangular distribution. The values assigned to these parameters are shown in Table 10. It should be noted that these distributions were selected based on the expert judgement of the main author, equally normal or lognormal distributions may be selected and values assigned accordingly.

It was also assumed that once spraying stopped the exposure ceased. However, unless the subject showered immediately this will not be the case. The effect of this is that predicted urinary concentrations are likely to be underestimated. Further, the effects of repeated exposures to pesticide over a number of days have not been accounted for. This is particularly relevant for mancozeb because it has a half life of about 100 hours. For cypermethrin (half life of 13 hours) it can be assumed that exposure that occurred some days prior to the collection of the urine sample would have had less impact on the estimated urine concentration than exposure events that occurred on the day prior to sampling.

Once parameters were set for all tasks, the model was run for 1000 iterations and the values saved to a file. This file was then exported to Microsoft Excel and summary statistics calculated.

3.8.3 Comparison with the regulatory exposure assessments

Urinary metabolite levels were predicted using the pharmacokinetic model for exposure levels derived from the EUROPOEM database and the regulatory risk assessments and compared with the observed urinary metabolite levels.

Table 10 Values for parameters used in pharmacokinetic modelling

Factor	Cypermethrin	3-PBA	Cis/trans-DCVA	Mancozeb	ETU
V_u-urine volume from t_l to t_c (ml) [1]	600	600	600	600	600
t_c-sample collection time	6 am	6 am	6 am	6 am	6 am
t_l-last collection time	11 pm	11 pm	11 pm	11 pm	11 pm
F_a-fraction absorbed over the skin	0.012 [2]	-	-	0.0024 [3]	-
k_a-absorption rate (per hr)	0.01-0.1	-	-	0.001-0.01	-
Half life (hr)	13 [2]	-	-	100 [4]	-
k_e-elimination rate (per hr)	0.053	-	-	0.01	-
R-stoichiometric ratio	-	1	1	-	1
S-selectivity (0-1)	-	0.8-1	0.8-1	-	0.8-1
Molecular weight	416.303	214.22	208.064	271.2	102.17
V_d-volume of distribution (l/kg)	-	4.60 [1]	3.971 [1]	-	3-8
K_f-fraction of pesticide on skin [5]					
Sprayers – using hand held methods	Minimum – 1.60×10^{-7}		Median – 1.91×10^{-6}		Maximum – 1.44×10^{-3}
– using "other"[6] methods	Minimum – 3×10^{-10}		Median – 5×10^{-8}		Maximum 2.07×10^{-5}
Bystanders	Minimum – 0.0003		Median – 0.002		Maximum – 0.032

1 - Tornero-Velez et al. (2006)
2 - Woollen et al. (1992)
3 - EC (2005). Review report for the active substance mancozeb
4 - Kurttio et al. (1990), EUROPOEM
5 - http://europoem.csl.gov.uk/
6 – boom and air-assisted methods

4 RESULTS

4.1 NUMBER PARTICIPATING

4.1.1 Recruitment during the first year: cypermethrin

Sprayers

Twenty two sprayer companies were recruited. Two of the spraying companies were recruited through a manufacturer, one from the list of farm tenants supplied by the Crown Estates Office and the remainder were recruited from the list obtained from the NAAC website. Since some companies had more than one sprayer, a total of 37 sprayers were recruited. On receipt of the sampling pack, one sprayer decided not to participate, citing the problem of keeping the daily diary as the reason for withdrawing. Despite repeated and frequent reminders, only 10 of these sprayers provided samples. In addition, one subject recruited as a post-application worker was identified as a sprayer from his diary, giving a total of 11 sprayers.

Post-application workers

Thirteen potential post-application workers were recruited. Five were recruited through one of the recruited sprayers and eight from the lists provided by the Crown Estate Offices. Samples were eventually received from three post-application workers, again only after repeated reminders.

A further two samples were obtained from two subjects who had indicated that they were post-application workers, however, on examination of their diaries it was clear that one was a sprayer and one a bystander/neighbour and they were reallocated to the appropriate group and are included in the final numbers for sprayers and bystanders/neighbours. It should be noted, that these two subjects were exposed to deltamethrin rather than cypermethrin.

Bystanders or neighbours

Eighteen potential bystanders were recruited. Four were recruited through one of the recruited sprayers, nine from the lists provided by the Crown Estate Offices and the remaining five through one of the spraying companies that participated in the study. Samples were eventually received from six bystanders, including two who were recruited on the day of spraying.

Consumers

Initially 30 names and numbers were selected from the Manchester and from the Sheffield telephone directories in order to recruit consumers. Two or three attempts were made to contact each number on the list. The response rate is shown in Table 11.

Table 11 Response rate from contacting numbers selected from the telephone directories

Directory	Response (%)				
	Willing to look over information	Not available	Refused	Unsuitable	No. not recognised
Sheffield	13	57	20	10	0
Manchester	7	73	17	0	3
Overall	10	65	18	5	2

There was no answer at over 60% of phone numbers. Where contact was made, almost 20% refused outright to participate, with very small percentages being either unsuitable or the number not recognised. Only 10% expressed an interest and agreed to look at information about the study. Each phone list was extended to 100 numbers and overall 10% were willing to receive further information about the study. However, only one person actually participated in the study via this route. Although the others were followed up by telephone, they either intimated that they no longer wished to participate or said that they would but failed to return a sample. Individuals were contacted both during the day and in the evening.

The recruiting strategy was later modified, with individuals being recruited from the English offices of the IOM and HSL, with a total of nine individuals (including one child) being recruited via this route. Fourteen were recruited from England through personal contacts of IOM staff, though only seven (50%) actually returned samples. Later, the study was extended to IOM staff and relatives living in Scotland, with 10 individuals (including four children) being recruited by this route.

Recruitment summary year 1, cypermethrin

A total of 47 subjects participated in the study. Twenty seven consumers participated (11 male and 16 female) of which five were children (two male and three female). All sprayers and post-application workers were male. Of the six bystanders, five were male and one female. If should be noted that not all subjects completed every part of the study. A breakdown of the parts completed for each group is shown in Table 12.

Table 12 Breakdown of parts completed by subjects in each group

	No. completed		
	Questionnaire	**Diary**	**Urine sample**
Sprayers	9	6	11
Workers	3	3	3
Bystanders	6	4	6
Consumers	23	23	27

Three sprayers returned samples, but gave no details of what was sprayed or completed questionnaires or diaries. Four consumers, including two children, from the same family provided samples but did not complete questionnaires or diaries. In all cases, despite repeated attempts, it was not possible to obtain further information. It should be noted that one bystander and one sprayer were exposed to deltamethrin rather than cypermethrin.

4.1.2 Recruitment during the second year: mancozeb

Sprayers

At least two or three attempts were made to contact orchard and vineyard owners whose details were listed on various sites on the internet (see section 3.3.2). Twenty nine individuals indicated that they were interested in participating and were sent further information. Of these, four refused to participate (one owner said their vineyard was too small, another owner was concerned that it may lead to further red tape, others had no time). Of the remainder, nine requested the full sampling kit, although only six returned samples. One sprayer was recruited through a personal contact from an IOM member of staff. The final three sprayers were recruited from a company that participated in the first year of the study. Of these three, two

returned samples with one sprayer participating twice. In addition, one subject recruited as a post-application worker was identified as a sprayer from his diary giving a total of 11 sprayers.

Post-application workers

Four potential post-application workers were recruited, all through the recruited spraying company that had participated previously. Only two samples were returned. A further sample was obtained from a subject who had indicated that he was a post-application worker, however, on examination of his diary it was clear that he was a sprayer and hence he was reallocated to the sprayers group.

Bystanders or neighbours

Eleven potential bystanders were recruited. Two were associated with a sprayer recruited from the English wine producer's website and two were associated with the sprayer recruited through an IOM personal contact. Six bystanders were recruited from IOM staff, three of whom were participating in another study associated with exposure to pesticides. The remaining three were instructed to walk in the countryside near to the field being sprayed during the spraying. Two of the IOM staff provided more than one sample.

Consumers

No further consumers were recruited.

Recruitment summary year 2, mancozeb

In summary, a total of 13 subjects provided 16 mancozeb samples. The breakdown by group is shown in Table 13. The eight sprayers, the post-application worker and one bystander were male.

Table 13 Breakdown of parts completed by subjects in each group

	No. completed		
	Questionnaire	**Diary**	**Urine sample**
Sprayers	8	5	8
Workers	1	1	1
Bystanders	7	5	7

The remaining subjects used pesticides containing active ingredients that could not be analysed using standard techniques and so had to be excluded.

4.2 BRIEF SUMMARY OF QUESTIONNAIRES AND DIARIES

4.2.1 Year 1: cypermethrin

A brief summary of the participant ages and their daily consumption of portions of fruit and vegetables, reported in the questionnaires is shown in Table 14 below.

Table 14 Summary of completed questionnaires

	No.	Age			No. of portions of fruit and vegetable consumption		
		Average	SD	Range	Average	SD	Range
Sprayers	8	52	7.3	40-60	5	2.0	2-8
Post-application workers	3	32	4.6	27-36	6	5.9	2-13
Bystanders	6	40	9.0	24-50	5	3.6	1-11
Consumers	23	37	12.9	13-55	6	2.1	4-12

SD – standard deviation

The average age for sprayers was higher than that of the other three groups. Post-application workers had a lower average age, although there were only three subjects in this group. On average, all groups consumed between five and six portions of fruit and vegetables per day, with a few subjects reporting that they consumed more than 10 portions per day. Further, the majority of subjects reported that they consumed few or no portions of organic fruit, vegetables or bread (Table 15).

Table 15 Amount of organic produce consumed

	Few or none	Some	Not sure
Sprayers	6	1	1
Post-application workers	2	1	0
Bystanders	4	1	1
Consumers	13	8	2

The above figures for fruit and vegetable consumption and the amount of organic produce consumed are reflected in the information provided by those subjects who completed the diaries.

Sprayer diaries

Details of the cypermethrin products used for spraying and other related details are recorded in Table 16.

Table 16 Spraying information

Study no.	Cypermethrin product	Field size (ha)	Amount used (kg)	Time (mins)	Crop treated
SP12	Cypermethrin[1]	21.5	0.025	300	Brussel sprouts
SP14	Toppel 10	47	1.16	270	Winter wheat
SP15	Toppel 10	80	1.60	390	Oilseed rape
SP17	Permasect C	51	1.27	375	Winter wheat
SP19	Permasect C	12	0.015	45	Winter wheat
W4	Bandu[2]	93	-	300	Oilseed rape

1-product name not given, 2 – product contains deltamethrin, not cypermethrin

One sprayer simply recorded the product used as "cypermethrin". However, the manufacturer was recorded as NuFarm Whyte and so it is likely that the product was Cyperguard 100 EC. One sprayer used Bandu, which contains deltamethrin rather than cypermethrin. It should be noted that in all cases more than one product was used for spraying.

Since one subject was originally recruited as a post-application worker rather than a sprayer, there were no details about, whether he cleaned the sprayer after use, got concentrate or diluted product on his skin or had been spraying prior to the day of interest. Of the remaining five sprayers, all applied pesticide using a boom sprayer and all had been spraying cypermethrin on at least one day in the four days prior to the day of interest.

The details of personal protective equipment worn for the various tasks associated with spraying are shown in Table 17.

Table 17 Personal protective equipment worn by sprayers

Study no.	Mixing/loading	Spraying	Cleaning
SP12	Gloves	Overalls	
	Overalls	Rubber boots	
	Rubber boots		
	Face shield/mask		
SP14	Gloves	Overalls	
	Overalls		
	Rubber boots		
	Face shield/mask		
SP15	Gloves	Overalls	
	Overalls		
	Face shield/mask		
SP17	Gloves	None	
	Overalls		
	Safety boots		
	Face shield/mask		
SP19	Gloves	Overalls	Gloves
	Overalls	Safety boots	Overalls
	Safety boots		Rubber boots
	Face shield/mask		Face shield/mask

The sprayer originally recruited as a post-application worker recorded that gloves and overalls were worn, but this cannot be matched with the task. The remaining five sprayers all wore gloves, overalls and a face shield or mask while mixing and loading pesticide. Much less protection was worn while spraying, but since all were in an enclosed cab less exposure is to be expected. Only one sprayer cleaned equipment after use (SP19) and he did this while wearing gloves, overalls, rubber boots and a face shield or mask.

Unsuccessful attempts were made to contact sprayers who did not complete a diary in order to obtain further details about spraying.

Post-application worker diaries

Two subjects were exposed to cypermethrin containing pesticides, no details as to what the third subject was exposed to were provided. All three reported spending 15 minutes in the field. Details about post-application workers are shown in Table 18. Since one subject was originally classified as a bystander there were no details about what was worn. None of the post-application workers used household or garden products containing the pesticides of interest prior to providing a urine sample.

Table 18 Information about post-application workers

Study no.	Clothing	Time in fields (mins)
B13	n/a	15
W9	Rubber boots Normal clothing including jacket	15
W11	Overalls Safety boots Normal clothing including jacket	15

Bystander or neighbour diaries

Four of the six recruited bystanders completed diaries; all six completed questionnaires. The two who did not complete diaries had not done so because they were recruited on the day of spraying. From the questionnaires, five bystanders were exposed to Cyperguard 100 EC. The remaining bystander was exposed to deltamethrin rather than cypermethrin.

All five bystanders who reported proximity to the field were within 20 m of the field while it was being sprayed. Details about bystanders are shown in Table 19. Conditions were mild and sunny, with a slight wind on all occasions. There were no reports of spray droplets landing on exposed skin or clothing. None of the bystanders used household or garden products containing the pesticides of interest prior to providing a urine sample.

Table 19 Information about bystanders

Study no.	Clothing	Proximity to field (m)	Time potentially exposed (mins)[1]
B15	Normal clothing, including jacket Fluorescent jacket for part of time	< 20	220
B17	Normal clothing, including jacket	< 20	60
B18	Normal clothing, including jacket	< 20	60
B19	Normal clothing	< 20	60
B20	Normal clothing	< 20	60

1 – time includes periods between spraying events

Consumer diaries

In addition to exposure from the ingestion of foodstuffs containing pesticide residues, consumers may be exposed through the use of pesticides within the home or garden. Of the 23 consumers completing diaries, four used such pesticides. Two were insecticides containing pyrethroids, which could potentially influence the levels of the metabolite 3-PBA in the urine. One consumer used a dog flea powder containing diazan and the remaining consumer used slug pellets but did not provide any further information.

4.2.2 Year 2: mancozeb

The participant ages and their daily consumption of portions of fruit and vegetables per day are summarised in Table 20.

Table 20 Summary of completed questionnaires - 2006

	No.	Age			No. of portions of fruit and vegetable consumption		
		Average	SD	Range	Average	SD	Range
Sprayers	7	48	13.1	30-67	6	3.0	4-11
Post-application workers	1	49	-	-	8	-	-
Bystanders	5	37	5.1	31-43	5	1.9	3-8

SD – standard deviation

The average age for bystanders was slightly younger than that of sprayers. On average, sprayers and bystanders consumed between five and six portions of fruit and vegetables per day. Overall consumption ranged from three to eight portions per day. The majority of subjects reported that they ate few or no portions of organic fruit, vegetables or bread (Table 21).

Table 21 Amount of organic produce consumed - 2006

	Few or none	Some	Not sure
Sprayers	3	4	0
Post-application workers	1	0	0
Bystanders	4	1	0

Sprayer diaries

Details of the mancozeb products used for spraying and other related details are recorded in Table 22. It should again be noted that more than one product was used for spraying and only those containing mancozeb are shown.

Table 22 Spraying information

Study no.	Mancozeb product	Field size (ha)	Amount used (kg)	Time (mins)	Method of application	Crop treated
SP35	Karamate	1.2	0.432	240	Hand-held	Vines
SP39	Karamate	1.87	135	155	Air-assisted	Vines
SP43	Unikat 75 WG	7	1.8	180	Air-assisted	Vines
SP55	Karamate	2	0.68	70	Air-assisted	Vines
SP61	Karamate	4	0.562	390	Air-assisted	Vines
SP62	Electis 75 WG/ Rhapsody	45.75	42.74	615	Boom	Potatoes
SP64	Rhapsody/ Electis 75 WG	27.95	35.74	285	Boom	Potatoes
SP66	Matilida	49	24.48	150	Boom	Potatoes

Details of personal protective equipment worn for the various tasks associated with spraying are shown in Table 23.

Table 23 Personal protective equipment worn

Study no.	Mixing/loading	Spraying	Cleaning
SP35	Overalls	Overalls	Overalls
	Rubber boots	Rubber boots	Rubber boots
SP39	Gloves	Gloves	Gloves
	Overalls	Overalls	Overalls
	Rubber boots	Rubber boots	Rubber boots
	Face shield/mask		
SP43	Gloves		Gloves
	Overalls		Overalls
	Rubber boots		Rubber boots
	Dust mask		Dust mask
SP55	Gloves	Gloves	
	Overalls	Overalls	
	Rubber boots	Rubber boots	
	Battery powered spray helmet	Battery powered spray helmet	
SP61	Gloves	Overalls	Overalls
	Overalls	Safety boots	Safety boots
	Safety boots	Moldex 8000 P3D and goggles	Moldex 8000 P3D and goggles
	Moldex 8000 P3D and goggles		
SP62	Gloves	Safety boots	
	Safety boots		
SP64	Gloves	Safety boots	
	Safety boots		
SP66	Gloves	Safety boots	Gloves
	Safety boots		Safety boots

Seven of the eight sprayers wore gloves while mixing and loading and five wore overalls. Generally less protective equipment was worn while spraying, especially when the sprayer was in an enclosed cab. Five sprayers recorded cleaning equipment after use and wore a variety of protective equipment.

Post-application worker diaries

One subject was exposed to mancozeb containing pesticides. Normal clothing was worn when entering the fields which had been sprayed 10 days previously. He reported spending 20 minutes in sprayed fields (Table 24). No household or garden products containing the pesticides of interest were used prior to providing the urine sample.

Table 24 Information about post-application workers

Study no.	Clothing	Time in fields (mins)
B43	Normal clothing	20

Bystander or neighbour diaries

All seven bystanders were within 20 m of the field while it was being sprayed (Table 25). Conditions were warm and sunny on all occasions. A very light NW wind was observed when bystanders B45 and B46 were potentially exposed, on all other occasions there was no wind. There were no reports of spray droplets landing on exposed skin or clothing. None of the bystanders used household or garden products containing the pesticides of interest prior to providing a urine sample.

Table 25 Information about post-application workers

Study no.	Clothing	Proximity to field (m)	Time potentially exposed (mins)[1]
B38	Trousers and long sleeved top	< 20	265
B39	Trousers and t-shirt	< 20	195
B40	Trousers and t-shirt	< 20	284
B45	Trousers and long sleeved top	< 20	150
B46	Trousers and long sleeved top	< 20	150
B49	Trousers and long sleeved top	< 20	91
B50	Trousers and sleeveless top	< 20	91

1 – time includes periods between spraying events

4.3 SUMMARY OF RESULTS FROM URINE SAMPLES

4.3.1 Cypermethrin

Table 26 summarises the concentrations of 3-PBA and DCVA (cis- and trans-DCVA combined) for each group of subjects, normalised for creatinine. Individual results, including creatinine concentrations are presented in Appendix 12 for each group of subjects.

Table 26 Pyrethroid metabolites in urine samples (μg/l) for each group

Metabolite	N	n>LOD	Median	90th %ile	Range
3-PBA					
Consumers	27	0	<LOD	<LOD	<LOD
Bystanders	5	2	<LOD	2.73	<LOD-3.30
Post-application workers	3	2	1.18	-	<LOD-1.91
Sprayers	10	8	1.32	4.21	<LOD-5.85
DCVA					
Consumers	27	1	<LOD	<LOD	<LOD-1.27
Bystanders	5	0	<LOD	<LOD	<LOD
Post-application workers	3	0	<LOD	<LOD	<LOD
Sprayers	10	2	<LOD	6.08	<LOD-6.49

LOD – limit of detection, 1.07 μg/l for 3-PBA, 2.08 μg/l for DCVA ½ LOD was used in the calculation of the 90th percentile

The most commonly detected metabolite was 3-PBA, which is to be expected since 3-PBA reflects pyrethroid exposure in general, whereas DCVA is more specific to cypermethrin. The measured metabolite levels are very low and may be influenced by dietary and environmental exposures which may arise from all pyrethroids, not just cypermethrin or permethrin.

None of the consumers had detectable concentrations of 3-PBA in their urine sample. Only one consumer had a detectable amount of DCVA in their urine (1.27 μg/l or 0.77 μmol/mol creatinine) which was within background levels observed in another UK study (Table 36). A review of the diary for this subject revealed no obvious reason for this. This subject consumed a total of seven portions of fruit and vegetables of which very few or none were organic. The majority of the subject's time was spent indoors either at home (15 hours) or elsewhere (six hours) and the remaining hours were spent outside at home. There was therefore no obvious difference between this consumer and other consumers with respect to fruit and vegetable consumption, or any other noticeable difference, though she was one of only eight (out of 22) subjects who reported eating raw and unpeeled vegetables. No pesticide use in the home or garden was recorded. It is possible that this level of DCVA is from "environmental" exposure, most probably the diet.

Two bystanders and two post-application workers had detectable amounts of 3-PBA in their urine, but none had detectable levels of DCVA. Eight sprayers had detectable levels of 3-PBA, two had detectable levels of DCVA. Levels of both metabolites were higher for sprayers than for the other groups. Since such small numbers of bystanders and post-application workers were involved, it is not possible to make comparison between these and other groups. Two sprayers had no detectable levels of any of the three metabolites, however, as they failed to return diaries there is no certainty that they did in fact spray using cypermethrin.

In addition, two subjects (a sprayer and bystander/neighbour) were exposed to deltamethrin rather than cypermethrin. No deltamethrin was found in the urine of either of these workers.

Interestingly, 13.1 μg/l deltamethrin was found in the urine of one of the bystanders exposed to permethrin. This subject also had a detectable amount of 3-PBA in his urine. Since this subject worked for one of the spraying companies it is possible that he had experienced exposure previous to this. However, as he was recruited on the day of spraying there is no diary that could have provided further information and an explanation.

A urine sample was also collected and analysed from an IOM employee who was shadowing a sprayer as part of another project studying exposure to pesticides. Although mostly just outside the field during spraying, this subject also spent approximately 30 minutes with the sprayer in his cab while spraying. No DCVA was detected in the urine sample, but 1.99 μg/l 3-PBA was detected.

4.3.2 Mancozeb

The data for ETU in those exposed individuals is shown in Table 27. Individual results, including creatinine concentrations are presented in Appendix 13 for each group of subjects.

Table 27 ETU in urine samples (μg/l) for each group

	N	n>LOD	Median	90th %ile	Range
Bystanders	7	3	<LOD	0.65	<LOD-0.72
Post-application workers	1	0	<LOD	<LOD	<LOD
Sprayers	8	7	4.75	6.65	<LOD-7.15

LOD – limit of detection, 0.20 μg/l, ½ LOD was used in the calculation of the 90th percentile

There were detectable levels of ETU in three of the samples from bystanders and seven of the samples from sprayers. The post-application worker had no detectable ETU in his sample. Not surprisingly, levels of ETU were higher for sprayers than for bystanders.

A urine sample was also collected and analysed from an IOM employee who was shadowing a sprayer as part of another project studying exposure to pesticides. Although mostly just outside the field during spraying, this subject also spent approximately 30 minutes with the sprayer in his cab while spraying. No ETU was detected in the urine sample.

4.4 PHARMACOKINETIC MODELLING

4.4.1 Cypermethrin

Pharmacokinetic modelling was carried out for both 3-PBA and DCVA for sprayers and for 3-PBA only for bystanders and post application workers. The results for sprayers are presented in Tables 28 and 29 for 3-PBA and DCVA respectively. It is immediately apparent that in the majority of cases, both exposure levels estimated from EUROPOEM data and the regulatory risk assessments lead to a gross over estimation of urinary concentrations. Using data from EUROPOEM, the estimated median values are comparable for SP12 and SP19, where less than 1 kg of active ingredient was used. However, estimated median urinary concentrations were two orders of magnitude higher than the observed urinary concentrations for the other sprayers where more active ingredient was used. Where the exposure assessments from the regulatory risk assessments were used, urinary concentrations were generally two to three orders of magnitude higher than the observed concentrations.

DCVA was only detected in the urine of two of the five operators exposed to cypermethrin. A similar pattern to that for 3-PBA was observed, with estimated urinary levels using EUROPOEM data being much higher than the observed levels and higher still using the exposures from the regulatory risk assessments.

Since DCVA was not detected in the urine of either bystanders or post application workers the pharmacokinetic model was only applied for 3-PBA (Tables 30 and 31). Very low levels of 3-PBA in urine were predicted using estimated exposures from EUROPOEM or the regulatory risk assessment, though those predicted using the regulatory risk assessment exposures were generally higher.

Four of the exposed bystanders worked for the crop chemical company, the fifth (B15) was an IOM employee who was engaged in recruiting subjects. Bystanders B17 and B18 were potentially exposed to spray from one field in the morning and bystanders B19 and B20 were potentially exposed to spray from one field in the afternoon, B15 was exposed to spray from both fields. Only two bystanders, B17 and B19, had detectable levels of 3-PBA in their urine. They worked for a crop services company and it is likely that they had previous exposure to cypermethrin or other pyrethroids which may have influenced the observed urinary concentrations. Since the pharmacokinetic model does not take account of repeated exposures to cypermethrin, it is not surprising that the predicted urinary concentrations for these two bystanders are lower than the corresponding observed values.

The results for post application workers are presented in Table 31. Only the results for exposure estimated using the regulatory risk assessment are used. Median estimated urinary concentrations are two orders of magnitude higher than the observed values. The predicted values are identical, due to all three post application workers spending the same amount of time in sprayed fields.

Table 28 Predicted exposure of sprayers to cypermethrin and concentration of 3-PBA in urine

Study no.	Source	Estimated exposure (mg)				Estimated concentration in urine (μg/l)				Observed concentration in urine (μg/l)
		Minimum	Estimated[1]	90th %ile	Maximum	Minimum	Median	90th %ile	Maximum	
SP12	IOM	<0.01	0.15	0.37	0.51	0.02	9.9	28	49	2.1
	RRA		11			280	1100	1500	1700	
SP14	IOM	0.05	6.9	16	24	2.1	400	1200	2100	1.1
	RRA		11			250	1100	1500	1700	
SP15	IOM	0.05	9.6	22	32	3.2	590	1800	3400	5.9
	RRA		20			450	2000	2700	3000	
SP17	IOM	0.05	8.3	18	26	0.15	490	1300	2600	1.3
	RRA		11			240	1000	1400	1600	
SP19	IOM	<0.01	0.09	0.22	0.30	0.01	1.8	5.2	9.5	4.0
	RRA		11			250	960	1200	1300	

1 – IOM uses EUROPOEM data, RRA uses data from the regulatory risk assessment

Table 29 Predicted exposure of sprayers to cypermethrin and concentration of DCVA in urine

Study no.	Source	Exposure exposure (mg)				Estimated concentration in urine (μg/l)				Observed concentration in urine (μg/l)
		Minimum	Estimated[1]	90th %ile	Maximum	Minimum	Median	90th %ile	Maximum	
SP12	IOM	<0.01	0.15	0.35	0.50	0.02	9.3	27	49	ND
	RRA		11			280	1100	1400	1600	
SP14	IOM	0.01	6.9	16	23	0.40	410	1100	2200	ND
	RRA		11			250	1100	1400	1600	
SP15	IOM	0.07	9.6	23	32	2.3	610	1900	3300	6.5
	RRA		20			460	2000	2000	3000	
SP17	IOM	0.04	8.3	18	26	1.7	510	1400	2600	ND
	RRA		11			250	1000	1400	1600	
SP19	IOM	<0.01	0.09	0.21	0.31	<0.01	1.8	5.0	8.9	6.0
	RRA		11			240	900	1200	1300	

1 – IOM uses EUROPOEM data, RRA uses data from the regulatory risk assessment

Table 30 Predicted exposure of bystanders to cypermethrin and concentration of 3-PBA in urine

Study no.	Source	Estimated exposure (mg)				Estimated concentration in urine (μg/l)				Observed concentration in urine (μg/l)
		Minimum	Estimated[1]	90th %ile	Maximum	Minimum	Median	90th %ile	Maximum	
B15	IOM	<0.01	0.03	0.07	0.10	0.03	1.8	4.7	8.8	ND
	RRA		0.01			0.28	1.2	1.6	1.8	
B17	IOM	<0.01	0.02	0.03	0.05	0.02	0.41	1.1	2.0	3.3
	RRA		0.01			0.26	1.1	1.4	1.6	
B18	IOM	<0.01	0.02	0.03	0.05	0.01	0.38	1.1	1.8	ND
	RRA		0.01			0.26	1.1	1.4	1.6	
B19	IOM	<0.01	0.02	0.04	0.05	0.01	0.41	1.1	2.1	1.9
	RRA		0.01			0.22	1.0	1.4	1.6	
B20	IOM	<0.01	0.02	0.04	0.05	0.01	0.40	1.1	2.1	ND
	RRA		0.01			0.23	0.97	1.4	1.6	

1 – IOM uses EUROPOEM data, RRA uses data from the regulatory risk assessment

Table 31 Predicted exposure of post application workers to cypermethrin and concentration of 3-PBA in urine, using exposures from the regulatory risk assessment

Study no.	Estimated exposure (mg)	Estimated concentration in urine (μg/l)				Observed concentration in urine (μg/l)
		Minimum	Median	90th %ile	Maximum	
B13	0.38	27	120	170	190	1.2
W9	0.38	27	120	170	190	ND
W11	0.38	27	120	170	190	1.9

1 –using data from the regulatory risk assessment

4.4.2 Mancozeb

Pharmacokinetic modelling was carried out for ETU for sprayers, bystanders and a post application worker. The results for sprayers are presented in Table 32. Generally, predicted urinary concentrations are much higher than the observed values whether exposure is estimated using data from EUROPOEM or using the regulatory risk assessment, by one to two orders of magnitude. The effects of method of application (which in turn affects estimation of exposure) can clearly be observed. Sprayer SP35 used a hand held method and the estimated dermal exposure and hence predicted urinary concentrations are more similar, with the medians being two to three orders of magnitude higher than the observed value. Sprayers SP39, SP43, SP55 and SP61 used air assisted methods. For sprayer SP39 predicted urinary concentrations were two orders of magnitude higher than that observed. This sprayer used a much larger amount of active ingredient than the other three sprayers (Table 22). Using the regulatory risk assessment estimates, predicted urinary concentrations were an order of magnitude higher for the remaining three sprayers using air assisted methods. Using EUROPOEM data, predicted urinary concentrations were an order of magnitude lower for SP55 and SP61. In these two cases less than 1 kg of active ingredient was used, resulting in relatively low estimated exposures and hence predicted urinary concentrations. The predicted urinary concentration for SP43 using EUROPOEM data was comparable to the observed value, whereas the predicted value using the risk assessment data was an order of magnitude higher. Where boom sprayers were used for application (SP62, SP64 and SP66), predicted urinary concentrations using regulatory risk assessment data were closer to the observed values than those predicted using EUROPOEM data.

The results for bystanders are presented in Table 33. Using exposures estimated using data from EUROPOEM, all predicted urinary concentrations were very low. Higher urinary concentrations were predicted using data from the regulatory risk assessment.

The bystanders potentially exposed to mancozeb were IOM employees who were either working with the crop services company as part of another research project or who were asked to be in the area when spraying was taking place. Bystanders B38, B40, B49 and B50 were potentially exposed on the same day and to two of the same fields. Interestingly B49 and B50, who were only potentially exposed to spray from two fields had detectable levels of ETU in their urine whereas B38 and B40 who were potentially exposed to spray from four and five fields respectively. For one of the fields where all four bystanders were potentially exposed, bystanders B49 and B50 walked along and road adjacent to the field and then an overgrown track adjacent to the field, separated from the field by a line of trees, bystander B38 was near the entrance to the field and bystander B40 was near the entrance of the field, walked along the road and the first part of the overgrown track. While walking on the track it was estimated that although less than 20 m from the field, the walkers were about 50 m from the sprayer. In total bystanders B49 and B50 were only potentially exposed to spray for approximately 30 minutes. Further, although bystanders B38, B39, B40, B45 and B46 will have at some point been only 3-5 m from spaying, B49 and B50 will have been no closer than 10 m from spraying.

Only one post application worker entered fields that had been sprayed with mancozeb and no ETU was detected in his urine (Table 34). The median predicted urinary concentration was approximately 6 µg/l.

It should be noted that the present model does not account for previous exposure to pesticide. This is particularly relevant for mancozeb which has a half life of 100 hours and hence the body burden and urinary concentrations would be expected to increase with repeated exposures. This is particularly relevant for sprayers SP62, SP64 and SP66 who reported spraying using mancozeb for the previous two weeks, 10-12 hours a day, seven days a week. Since the

predicted urinary concentrations were already generally too high, it was not considered useful to account for repeated exposure.

Table 32 Predicted exposure of sprayers to mancozeb and concentration of ETU in urine

Study no.	Source	Estimated exposure (mg)				Estimated concentration in urine (μg/l)				Observed concentration in urine (μg/l)
		Minimum	Estimated[1]	90th %ile	Maximum	Minimum	Median	90th %ile	Maximum	
SP35	IOM	0.45	180	430	610	0.09	77	230	500	0.92
	RRA		250			24	130	210	250	
SP39	IOM	1.1	840	1900	2700	0.23	270	870	1800	7.2
	RRA		290			20	110	180	220	
SP43	IOM	0.09	11	26	36	0.04	4.4	15	27	6.4
	RRA		150			13	71	110	140	
SP55	IOM	0.01	4.3	9.9	14	<0.01	0.56	1.8	3.7	1.3
	RRA		290			8.6	47	76	92	
SP61	IOM	0.01	3.2	8.0	11	<0.01	0.92	3.3	7.1	4.7
	RRA		290			19	110	180	210	
SP62	IOM	0.11	250	590	874	0.12	150	480	1000	4.8
	RRA		41			5.2	30	49	58	
SP64	IOM	0.48	220	510	721	0.13	100	310	680	6.1
	RRA		41			3.9	23	36	43	
SP66	IOM	0.13	150	340	510	0.04	40	130	260	ND
	RRA		49			2.9	17	26	31	

1 – IOM uses EUROPOEM data, RRA uses data from the regulatory risk assessment

Table 33 Predicted exposure of bystanders to mancozeb and concentration of ETU in urine

Study no.	Source	Estimated exposure (mg)				Estimated concentration in urine (μg/l)				Observed concentration in urine (μg/l)
		Minimum	Estimated[1]	90th %ile	Maximum	Minimum	Median	90th %ile	Maximum	
B38	IOM	<0.01	0.03	0.07	0.09	<0.01	0.01	0.04	0.08	ND
	RRA		0.68			0.06	0.36	0.57	0.67	
B39	IOM	<0.01	0.03	0.07	0.10	<0.01	0.01	0.03	0.06	ND
	RRA		0.60			0.05	0.25	0.41	0.49	
B40	IOM	<0.01	0.04	0.08	0.11	<0.01	0.02	0.05	0.12	ND
	RRA		0.60			0.06	0.33	0.52	0.63	
B45	IOM	<0.01	0.01	0.03	0.04	<0.01	0.01	0.02	0.03	ND
	RRA		0.68			0.06	0.36	0.56	0.67	
B46	IOM	<0.01	0.01	0.03	0.04	<0.01	0.01	0.02	0.03	0.72
	RRA		0.68			0.06	0.35	0.56	0.67	
B49	IOM	<0.01	0.02	0.03	0.05	<0.01	<0.01	0.01	0.01	0.31
	RRA		0.60			0.02	0.12	0.18	0.22	
B50	IOM	<0.01	0.02	0.03	0.05	<0.01	<0.01	0.01	0.01	0.61
	RRA		0.60			0.02	0.11	0.18	0.22	

1 – IOM uses EUROPOEM data, RRA uses data from the regulatory risk assessment

Table 34 Predicted exposure of post application workers to mancozeb and concentration of ETU in urine, using exposures from the regulatory risk assessment

Study no.	Estimated exposure (mg)	Estimated concentration in urine (μg/l)				Observed concentration in urine (μg/l)
		Minimum	Median	90th %ile	Maximum	
B43	18	1.0	6.2	9.8	12	ND

1 –using data from the regulatory risk assessment

4.4.3 Testing of the pharmacokinetic model

It would appear that the estimated dermal exposure (both from EUROPOEM and the regulatory risk assessments) and the pharmacokinetic model we developed are conservative in the majority of cases, but as a consequence does not predict urinary concentrations of either cypermethrin or mancozeb metabolites with any accuracy. Although uncertainty in defining parameters such as the absorption rate constant for both cypermethrin and mancozeb and the volume of distribution for mancozeb could contribute to inaccurate estimates, it is likely that the main reason for the overestimation is overestimation in exposure, both using data from EUROPOEM and the regulatory risk assessment.

One of the difficulties in using this type of model is being able to assess its validity. Very little information exists in the literature that provides information on both exposure and urinary concentrations, with no information being found for cypermethrin metabolites. A study published in 2002 by Colosio *et al.*, investigated ETU in urine as an indicator of exposure to mancozeb in vineyard workers who had not previously been exposed to mancozeb and/or other pesticides. Of the 13 workers, 12 weighed and mixed mancozeb before applying it, while one worker was involved in re-entry. Sprayers used an average of 5.3 kg of pesticide. Summary statistics for both dermal exposure as mg/hour and end-shift ETU as µg/g creatinine were presented. Exposure was adjusted to full shift exposure by multiplying the dermal exposure rate by eight hours, which was the approximate time of exposure (actual range six to nine hours). We used a creatinine level of 1.55 µg/l to adjust the ETU values reported by Colosio *et al.* to concentrations in µg/l, in order for comparison with those reported by the pharmacokinetic model. This value was the average of the creatinine levels reported in the samples provided by the eight sprayers participating in this study who used mancozeb. The dermal exposure values and urinary concentrations are reported in Table 35.

Table 35 Exposure to mancozeb determined on the skin and ETU excretion in study subjects (Colosio *et al.*, 2002)

	Dermal exposure		End-shift ETU	
Study no.	**mg/h**	**Full shift (mg)**	**µg/g creatinine**	**µg/l**
Median	0.14	1.12	2.5	3.88
Minimum	0.01	0.08	<0.5	<0.78
Maximum	1.40	11.2	95.1	147.41

It was assumed that exposure took place between 08:00 and 16:00 hours and that the sample was collected at 16:30 since it is stated in the paper that an end-shift sample was taken. The last urine void was assumed to be at 13:00 hours and the volume of urine to be 300 ml. Colosio *et al.* assumed that 1.5% of exposure penetrated the skin and so the fraction absorbed was assumed to be 0.015 compared with a fraction of 0.0024 which was used in our earlier calculations. All other parameters were the same as those reported in Table 10. The model was run for 100 iterations.

A median urinary concentration of 1.13 µg/l, range 0.09-4.79 µg/l was predicted. These predicted values are comparable to those reported in Table 31, although the maximum value is much lower than that of the observed values, even allowing for any discrepancies introduced as a result of adjusting the data to a suitable format. This suggests that the model predicts reasonable estimates for the range of dermal exposures reported by Colosino *et al.* and hence it is possible to be confident that the pharmacokinetic model is operating correctly.

The model was re-run calculating dermal exposure using the parameters from EUROPOEM to estimate exposure. The estimated median exposure was 35.13 mg, range 1.26-102.43 mg, which is substantially higher than the actual exposure measured by Colosino *et al.* Using

these exposures in the pharmacokinetic model resulted in a median predicted urinary concentration of ETU of 8.61 μg/l (0.42-56.36 μg/l). If it is assumed that only 0.24% mancozeb is absorbed over the skin, as assumed in the current study, a median predicted urinary concentration of ETU of 2.47 μg/l (0.01-19.70 μg/l) is obtained. These results tend to suggest that exposures estimated using information from EUROPOEM, where a proportion of the amount sprayed is used or using the regulatory risk assessment methodology provide exposure estimates that are too high for sprayers. Colosino *et al.* (2002) reported that end-shift urinary ETU was positively correlated with the mancozeb exposure rate determined on both clothes and skin, whereas there was no correlation between dermal exposure and the amount of mancozeb sprayed.

5 DISCUSSION

5.1 CYPERMETHRIN

In order to put the above results into perspective, a summary of data from studies carried out by two of the authors at the Health and Safety Laboratory (JC and KJ) is shown in Table 36. Subjects had exposure to pyrethroids, which in addition to cypermethrin includes permethrin, deltamethrin and cyfluthrin. Comparable results from the current study (converted to μmol/mol creatinine and split into cis-DCVA and trans-DCVA) are appended to the bottom of each section of the table.

Table 36 Pyrethroid metabolites in urine samples (μmol/mol creatinine) for each group

Metabolite	N	90th percentile	Maximum
3-PBA			
None[1]	80	1.02	2.59
Wool gatherers	128	7.2	42.6
Pest control	59	4	17.4
Timber/forestry	105	3.4	8
Consumers[2]	27	<LOD	<LOD
Bystanders[2]	5	0.91	1.20
Post-application workers[2]	3	-	0.46
Sprayers[2]	10	1.61	1.76
cis-DCVA			
None[1]	80	0.18	1.5
Wool gatherers	128	<LOD	0.7
Pest control	59	0.3	1.7
Timber/forestry	105	4.9	8.3
Consumers[2]	27	<LOD	<LOD
Bystanders[2]	5	<LOD	<LOD
Post-application workers[2]	3	<LOD	<LOD
Sprayers[2]	10	0.85	1.16
trans-DCVA			
None[1]	80	0.40	1.59
Wool gatherers	128	<LOD	0.8
Pest control	59	1.5	16.5
Timber/forestry	105	9.6	14.8
Consumers[2]	27	<LOD	0.77
Bystanders[2]	5	<LOD	<LOD
Post-application workers[2]	3	<LOD	<LOD
Sprayers[2]	10	1.06	1.55

1 – no known occupational exposure, 2 – current study

For consumers, bystanders and post-application workers, the 90th percentile and maximum values observed in this study are well below those reported for persons with no known occupational exposure. The 90th percentile and maximum 3-PBA levels for sprayers are lower than those observed for persons with no known exposure. However, the 90th percentile cis-DCVA and trans-DCVA values are comparable with the values for persons working in the pest control industry. The maximum values for all four groups in this study are lower than those with no known exposure. However, since small numbers of subjects are involved, the observed levels are unlikely to be representative of sprayers in general. HSL have previously estimated an expected urine level of 250 μmol/mol creatinine, from intake of the acceptable daily intake (ADI) for cypermethrin (0.05 mg/kg/day). Using these figures, the maximum

urine result, 1.76 μmol/mol creatinine for 3-PBA amongst the sprayers (Appendix 12, Table12.4) is approximately 150 times lower than that expected from the ADI.

Hardt and Angerer (2003) investigated exposure to pyrethroid metabolites by measuring urinary levels in workers applying various pyrethroid pesticides and controls (only DCVA), including cypermethrin, permethrin and deltamethrin etc. Results were reported as μg/g creatinine and the results from the present study were converted to these units for the purposes of comparison (Table 37).

Table 37 Pyrethroid metabolites in urine samples (μg/g creatinine) for each group

Metabolite	N	n>LOD	Median	Range
3-PBA				
Agriculture	24	16	0.6	<LOD-28.5
Pest control	29	26	1.4	<LOD-57.5
Greenhouse	7	7	2.9	n/a -26.0
Sprayers[1]	10	8	1.17	<LOD-3.33
DCVA				
Agriculture	24	11	<LOD	<LOD-1.9
Pest control	29	21	1.8	<LOD-92.4
Greenhouse	27	7	2.9	n/a-15.1
Controls	45	40	0.5	<LOD-9.1
Sprayers[1]	10	2	<LOD	<LOD-2.13

1 – current study

The median 3-PBA values for sprayers in this study are comparable with those reported by Hardt and Angerer, however, the maximum 3-PBA level is much lower for sprayers. For DCVA, the levels for sprayers are comparable with those of the agricultural workers.

A number of papers have been published which have investigated exposure to pyrethroid metabolites by measuring urinary levels of pyrethroid metabolites in persons with no known exposure (Heudorf and Angerer, 2001, Schettgen *et al.*, 2002, Hardt and Angerer, 2003, Heudorf *et al.*, 2004, Saieva *et al*., 2006). In addition, Bouvier *et al.* published a review article in 2005 summarising studies that measured urinary metabolites to assess the exposure of populations with no occupational exposure to pesticides, including pyrethroids. Heudorf *et al.* (2006) have summarised urinary concentrations of pyrethroid metabolites in non-occupationally exposed adults and children from studies carried out on the German population. As a result of their study, Heudorf *et al.* (2006) have derived reference values of 2 μg/l, 1 μg/l and 2 μg/l for 3-PBA, cis-DCVA and trans-DCVA respectively. However, the authors stress that these reference values are derived statistically and cannot be used for health related evaluation of biomonitoring data. In the present study, only one consumer had detectable levels of trans-DCVA in urine at a level of 1.27 μg/l. The results presented in the papers mentioned above have been presented in different units, however, what is apparent is that the one value observed above the detection limit in this current study is lower than the maximum values presented in the above studies.

5.2 MANCOZEB

Since such a small number of subjects was involved, it is unlikely that the levels observed for sprayers, bystanders and the post-application worker are representative of these groups in general. The ADI of mancozeb is 0.03 mg/kg bw/day, which is approximately equivalent to a urine level of 30 μmol/mol creatinine. The maximum urine result, 5.95 μmol/mol (Appendix 13, Table 13.3) is therefore approximately five times lower than that expected from the ADI.

A number of papers have been published which report urinary levels of ETU. Kurttio *et al.* (1990) studied the exposure of 29 pesticide operators who sprayed potato fields with ethylenebisdithiocarbamate fungicides (EBDCs) maneb or mancozeb. The concentration of ETU was measured for 22 days after exposure using 24 hour urine samples. On the first day after sampling concentrations of 0.09 to 2.5 µg/mmol were measured. In the present study, two samples had higher concentrations than the maximum value of 2.5 µg/mmol. However, in the study reported by Kurttio *et al.* urine concentrations represented the exposure to a single application, whereas that was not necessarily the case in this study, with concentrations from at least two of the samples representing multiple exposures. Kurttio and Salonlainen (1990) reported urinary concentrations for five pine nursery workers applying maneb. All urine samples were collected from each worker over a period of up to 50 hours and concentrations of <0.2 and 5.6 µg/l were reported which compares with concentrations for the eight sprayers of between <0.2 and 7.15 µg/l in the current study.

It should be noted that concentrations of ETU in the urine of the three bystanders who had detectable levels, reflected only single exposures. Two studies relating to ETU concentrations in the urine of the general population in Italy, where the diet can be assumed to be the main source of exposure, have been carried out (Saieva *et al*., 2004, Colosio *et al*., 2006). One study investigated ETU concentrations in urine samples in the general urban population of four regions of Italy and also in a rural population in Italy during aerial spraying of vineyards with ethylenebisdithiocarbamates (EBDCs) and who were therefore potentially exposed to EBDCs (Aprea *et al.*, 1996) from this source in addition to dietary sources and who are effectively bystanders. The results from these studies and the bystanders in the present study are presented in Table 38.

Table 38 ETU in urine samples (µg/g creatinine)

Reference	N	LOD	n>LOD (%)	Range (µg/g [1])
Present study – bystanders	7	2 nmol/l	3 (43)	<LOD-1.01
Aprea *et al.* (1996)				
Urban population	167	1 µg/l	40 (24)	<LOD-8.3
Rural population [2]	97		36 (37)	<LOD-61.4
Saieva *et al.* (2004)	69	5 nmol/l	15 (22)	<LOD-3.90
Colosio *et al.* (2006)	95	0.5 µg/g [1]	56 (59)	<LOD-11.6

1 – µg/g creatinine, 2 – bystanders

It is apparent that the maximum ETU concentration detected for bystanders in this present study is lower than that detected for the urban/general populations in the other studies and considerably lower than that detected in the rural population (bystanders).

Aprea *et al.* (1996) demonstrated that wine consumption and smoking were significantly associated with ETU detected in the urine. Residence, i.e. urban v rural area was not significantly associated, which is interesting, given that place of residence will be a surrogate for potential exposure to EBDCs (dietary v bystander plus dietary). Of the three bystanders in this present study with detectable urinary ETU, only one (B46) consumed alcohol (2 glasses each night) in the two days before the sample was taken. None of the bystanders were smokers. However, it is not advisable to make comparisons between two diverse populations in different countries, since it is likely that diet differs markedly from one country to another. In the present study, urine samples of consumers were not tested for ETU, making it more difficult to interpret the bystander data. Although ETU detected in the urine of bystanders may have resulted from dietary sources, the possibility of potential exposure to mancozeb during spraying cannot be discounted and further work is necessary to establish the relationship between potential exposure to pesticides and urinary excretion of associated metabolites.

Although the post-application worker had had multiple exposures to mancozeb, no ETU was detected in his urine. Kurttio and Salonlainen (1990) also studied a group of 15 women who carried out weeding in a pine nursery, i.e. the equivalent of post-application workers. These women carried out weeding two weeks after treatment of the pine nurseries with maneb and were studied after a single exposure period. Twenty four hour urine samples were collected on four occasions after possible exposure – on days 1, 8, 15 and 22. Concentrations of ETU exceeded the limit of detection for only four of the 60 urine samples collected. No ETU was detected on the clothes or skin of the subjects.

5.3 PHARMACOKINETIC MODELLING

Testing of the pharmacokinetic model using dermal exposure data indicated that the model predicts reasonable estimates of urinary concentrations for the range of dermal exposure data presented by Colosino *et al.* (2002). However, in our study predicted urinary concentration are in the majority of cases higher than the observed concentrations, particularly for sprayers and post-application workers.

It should be remembered that the metabolite 3-PBA is not specific to cypermethrin and so its presence may indicate exposure to other pyrethroids and chemicals. For example, 3-PBA is also a metabolite of deltamethrin and one of the bystanders in this study (B17) had clearly been exposed to deltamethrin since in addition to 3-PBA, deltamethrin was also detected in his urine. This will result in an inflated 3-PBA level in the urine and hence it is not surprising that in this case the predicted median urinary concentration was lower than that observed.

In addition to making assumptions about toxicokinetic parameters, it was also assumed that once spraying stops, exposure immediately ceased, which is highly unlikely since pesticide will remain on the skin until washed off. This assumption means that predicted urinary concentrations will be decreased. However, since predicted exposures here are in the majority of cases higher than the observed, this was not a problem. In addition, the effects of repeated exposures to pesticide over a number of days have not been accounted for, which is particularly relevant for mancozeb because it has a half life of about 100 hours. This limitation means that the predicted urinary concentrations will result in lower predicted urinary concentrations.

In order for model predictions to be improved, sufficient toxicokinetic information on pesticide and metabolite(s) is required. For this study there was no information on the absorption rate for both pesticides and the volume of distribution for ETU. Further, it was obvious that exposure estimation was generally an overestimate of the exposure experienced by the subjects in our study, whether data from EUROPOEM or the regulatory risk assessments was used.

It is possible to use pharmacokinetic models inversely, such that exposure concentrations are calculated using urinary concentrations. Such modelling is complex and requires the use of optimisation methods such as maximum likelihood estimation. This approach could not be applied in this study because an insufficient number of measurements were collected. However, even if sufficient data were available there is a lack of information on the toxicokinetics of 3-PBA, DCVA and ETU excretion, particularly ETU.

For future studies of this type it is recommended that the pesticide selected for study should have adequate toxicokinetic information available, both for the pesticide and its metabolite(s). One such pesticide is chlorpyrifos.

5.4 RECRUITMENT ISSUES

A number of difficulties with recruitment have already been described above. For consumers, random selection was not successful. Recruitment from IOM and HSL staff and through personal contacts of IOM staff proved successful. Subjects recruited through such sources are likely to be well motivated. However, they may not be representative of the population as a whole; such subjects are more likely to be motivated to have healthy diets, for example, have a higher consumption of fresh fruit and vegetables, which is supported by the completed questionnaires and diaries. Levy *et al.* (2007) used postal recruitment to background survey of urinary cadmium and mercury concentrations within the UK general population and achieved a response rate of 16% (out of a random selection of 488). An incentive of a £5 gift voucher was offered. The authors concluded that postal recruitment may be a useful and cost effective method for carrying out biomonitoring studies using urine in the general population.

Although spraying companies and sprayers were generally willing to participate, a major problem was that they could not be certain when they would be spraying. This is partly influenced by the weather and spraying is postponed in wet, windy or frosty conditions. Due to the nature of their work, it was often difficult to contact sprayers. Spraying often occurred at very short notice, making it very difficult to coordinate keeping a diary with the timing of spraying and providing the urine sample. Further, although cypermethrin was initially chosen because of its wide-spread use, during the course of this study it became evident that this was no longer the case. Other active ingredients, for example, lambda cyhalothrin, which is often found in an encapsulated product form, were being more frequently used, particularly since it was perceived that there was less chance of exposure to the active ingredient and so would be safer for the spraying operator to use.

In general, potential sprayers found keeping a diary to be off putting, partly due to timing and partly because they felt there were too many irrelevant questions to be answered. This is clearly evidenced by the fact that only six out of 12 sprayers returned completed diaries. In addition, three sprayers were unwilling to fill in even the questionnaire. A number of sprayers studied in 2006 were typically working 10-12 hours a day, seven days a week during the summer months. It is not surprising that in such cases keeping a diary is perceived to be too time consuming. However, one sprayer intimated that, despite the problems of filling in a questionnaire and keeping a diary, he would be willing to participate in any further studies since he felt the issue of exposure to pesticides was very important. One potential sprayer suggested that a more effective strategy would be to target farmers who would have a better idea of their spraying schedule since, as a contractor, he only receives the schedule at the last minute and often doesn't come into contact with the land owner.

Farmers may be eligible to participate as sprayers, post-application workers or bystanders. Three farmers were recruited as post-application workers, but on receipt of their diaries, two proved to be sprayers and one a bystander and so were reallocated accordingly.

At the outset of this study it was intended to recruit sprayers, post-application workers and bystanders associated with the same spraying event. However, due to the recruitment problems, this was generally not possible. One potential sprayer was uncomfortable about providing details about customers without their permission and generally potential subjects were reluctant to provide details of neighbours who may have been exposed as bystanders.

Another issue was the completeness and quality of the information recorded in diaries and spraying sheets. This was particularly apparent where one sprayer was shadowed by an IOM employee. Both IOM employee and sprayer recorded details about spraying. The extent of the dissimilarity between the two spraying sheets was remarkable, with agreement only on the location of spraying, the crop and active ingredient used. Inaccurate recording of information will obviously affect model predictions.

For future studies of this type, consideration should be given to providing an incentive for participants. On a number of occasions, the question of payment arose during recruitment of subjects. Any incentive should be proportional to the amount of involvement and effort required by the subject, with a higher incentive being offered to sprayers since they are required to provide detailed information about spraying. A sliding scale of payment could be implemented depending on the completeness of the information provided. An example of the success of this approach is illustrated by the Farm Family Exposure Study (FEFS), which was carried out in the U.S. during 2000 and 2001 (Baker *et al.*, 2005). This study recruited both farmers and their families. Farmers and their spouses were asked to complete enrolment questionnaires and follow-up questionnaires for each pesticide application. Five 24 hour urine samples associated with each application event were also collected. Each family participating in the study received $250 plus $50 for each participating child if all parts of the study were completed. The costs of the pesticides used were also reimbursed up to $1000. Participants who completed part of the study were paid a pro-rated incentive. Compliance with the 24 hour urine collection was very good, generally over 99%.

5.5 PUBLIC CONCERNS AND RECENT PUBLISHED INFORMATION

The use of pesticides and their possible health effects is a subject which gives rise to much public concern and discussion. There have been many reports of ill health associated with pesticide exposure. In particular, people living next to fields that are regularly sprayed often tend, rightly or wrongly, to attribute any health problems to the spraying of pesticides. Such issues contributed to the reluctance of farmers to participate in the study.

Further, the publication in September 2005 of a report by the Royal Commission on Environmental Pollution (RCEP) on "Crop Spraying and the Health of Residents and Bystanders" generated considerable interest and comment and ensured that the issue has remained in the public eye. The Royal Commission report makes a number of recommendations including the collection of population data on biomarkers for pesticides, which could be used to establish a national database of exposure measurements. It also recommends that a computational probabilistic model should be used to assess resident and bystander exposure by a wide range of exposure routes and better reflect worst-case scenarios. This model should then be validated by monitoring representative pesticides under a range of field conditions. The introduction of a compulsory 5 m buffer zone alongside residential property and other buildings such as schools, hospitals and retirement homes to protect against spray drift was also recommended.

In December 2005, the Advisory Committee on Pesticides (ACP) published a commentary on the report by the RCEP. While agreeing with parts of the report, it also expressed reservations, particularly with respect to the proposed introduction of a compulsory 5 m buffer zone which they consider unnecessary and unwarranted on the basis of current available scientific information. The ACP further points out that the RCEP report provides no scientific support for choosing a distance of 5 m or any assessment of the reduction in exposure which would result from this. However, they do concede that it may be justifiable on the basis that many people do not like pesticides being sprayed right up to the boundary of their property. It is supportive of the RCEP's proposal to measure biomarkers for exposure to pesticides in the general public and suggests that information on factors which may affect exposure should also be collected in order to assess their importance. Although commenting that it would be difficult to develop a probabilistic model to estimate exposure, the ACP stressed that all potential routes of exposure should be considered when carrying out risk assessment for bystanders. It also supported the RCEP's suggestion to monitor pesticides under a range of field conditions since that would allow the identification of potential worst-case scenarios.

5.6 CONCLUSIONS

A pharmacokinetic model was used to predict urinary metabolite levels using dermal exposure levels estimated through the regulatory risk assessment process or using data from EUROPOEM. Predicted median urinary metabolite levels were generally much higher than observed values for both sprayers and post-application workers for both cypermethrin and mancozeb containing pesticides. Such predicted values are therefore conservative and provide reassurance that the current regulatory risk assessment is likely to be protective for sprayers and post-application workers.

Where metabolites of cypermethrin or mancozeb were detected in the urine of bystanders, predicted median levels were lower than the observed values regardless of how dermal exposure was estimated. However, the two bystanders exposed to cypermethrin who had detectable levels of 3-PBA in their urine worked for a crop spraying company and it is likely that previous occupational exposure, not only to cypermethrin but to other pyrethroid pesticides, contributed to the urinary concentrations observed. This is supported by the presence of deltamethrin, which was not used during the spraying day monitored, in the urine of one of the bystanders. None of the three bystanders who had detectable levels of ETU in their urine as a result of exposure to mancozeb had any previous exposure. It could be argued that these bystanders, who were IOM employees, were generally closer to the spraying source than might normally be the case, however, it is quite possible that members of the general public could be in such close proximity and so estimated exposures should take account of this. Although the results suggest that exposures estimated using the regulatory risk assessment or data from EUROPOEM may not provide the same safety margin as for sprayers or workers, only a small number of bystanders were studied and the possibility of exposure via dietary sources cannot be completely discounted. However, it would be ill-advised to dismiss these results and it is recommended that consideration be given to how dermal exposure is estimated for bystanders in order to ensure that bystanders are protected with the same degree of overestimation of exposure as other exposed groups. Despite these observations it is reassuring that the actual exposure measured for bystanders in this study were below the ADI or the Allowable Operator Exposure Level (AOEL).

We are well aware that the numbers of subjects involved was small and we believe that further field work is desirable, particularly with respect to bystanders. It is recommended that studies focus on active ingredients for which adequate toxicokinetic information is available, for example chlorpyrifos. Finally, the establishment of a biomonitoring data collection programme as suggested by the RCEP and the ACP is recommended.

6 STATEMENT OF QUALITY

IOM recognise and adopt accepted UK guidelines for good survey practice

This project was carried out under the IOM project management system, which includes preparation of a written protocol for the research and periodic auditing of the work by experienced senior scientists not actively involved in the study.

IOM has UKAS accreditation for several measurement techniques. While the laboratory analysis of all samples collected under this study is covered by the UKAS accreditation, the sampling protocol is a non-standard research procedure and cannot easily be accredited. However, the sampling procedures followed the general quality procedures required by the overall quality management system. Sampling and analytical quality assurance included appropriate calibration checks, replicate analyses and blank samples.

Data processing and reporting was subject to the internal data processing control procedures. Raw data is stored for five years and can be audited by the sponsor.

7 ACKNOWLEDGEMENTS

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Finally, we would like to thank all those who participated in the study as sprayers, post-application workers, bystanders and consumers. We are particularly grateful to the crop services company in Scotland that allowed us repeated sampling of their employees as sprayers, post-application workers and bystanders.

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APPENDIX 1 – QUESTIONNAIRE COMPLETED BY CONSUMERS, BYSTANDERS AND POST-APPLICATION WORKERS

Institute of Occupational Medicine

Study no.

BIOLOGICAL MONITORING OF PESTICIDE EXPOSURES

LIFESTYLE QUESTIONNAIRE

All information we collect will be strictly confidential and no names or identifying information will be published or given to the DEFRA.

INSTRUCTIONS

1. For some of the questions there is a list of possible answers with a box printed beside each one. Please choose your answer and put a tick in the box beside it, for example:

	Yes	No
Do you have any pets?	✓	

2. Some questions will ask you for a number, for example:

How many glasses of water do you drink each day? 2

3. There are spaces in some questions for you to write your answer.

4. Some questions are followed by instructions, which allow you to miss out certain questions. Please follow these instructions carefully.

Section A: Personal details

Name:	
Address:	
Post code:	☎:

1. Gender — Male ☐ Female ☐

2. Date of birth — dd mm yyyy ☐☐☐☐☐☐☐☐

3. Height — ft inches ☐ or cm ☐

4. Weight — st lbs ☐ or kg ☐

5.	Occupation:
6.	Address:
	Post code:

7. Is this your normal place of work? Yes ☐ No ☐

8.	If **no**, please state the address of where you work:
	Postcode:

Section B: The food you eat

9. How many portions of the following do you normally eat every day?

Fruit - *a portion is 1 medium fruit, e.g. apple, pear, orange, 1 cupful of small fruit e.g. grapes, strawberries, 1 large slice of large fruit e.g. melon, pineapple or 2 tablespoons for frozen or tinned fruit*

Peeled	Unpeeled	Frozen or tinned
☐	☐	☐

Vegetables - *a portion is 2 tablespoons*

Raw & peeled	Raw & unpeeled	Cooked (fresh, tinned or frozen)
☐	☐	☐

Salad - *a portion is 1 cereal bowl full*

Washed/pre-packed	Unwashed
☐	☐

Bread - *a portion is 2 medium slices of bread or 1 bread roll*

Number of portions
☐

10. How many of the above foods were organic?

All or most	Some	Very few or none	Not sure
☐	☐	☐	☐

Section C: Drinks

11. How much of the following do you normally drink each day?

	Number of glasses/cups
Tap water	
Bottled still water	
Bottled fizzy water	
Fruit juice	
Bottled fizzy drinks	
Tea	
Coffee	
Alcohol	

Other, please give details of beverages and amounts normally drunk

Section D: Pets

12. Do you have any pets? Yes ☐ No ☐

If **yes**, please state what pet(s) you have

Section E: Smoking

13. Do you smoke:

	Yes	No
Cigarettes?		
A pipe?		
Cigars?		

If you answered **yes** to any of the above please state the approximate quantity that you smoke each day. If you are an occasional smoker, please enter <1 (cigarettes or cigars) or < an amount (tobacco)

Cigarettes	
Tobacco	
Cigars	

Section F: Medication

14. Please list any medications normally taken

That is the end of the questionnaire. Thank you very much for filling it in. Please send it back to us as soon as possible in the reply-paid envelope supplied.

All of your responses will be treated in the strictest confidence.

If you have any questions about this study please contact Peter Murray, Anne Soutar or Dr John Cherrie at the address below:

Institute of Occupational Medicine
Research Park North
Riccarton
Edinburgh
EH14 4AP

Tel: 0870 850 5131 (switchboard)
0131 449 8072 or 07818 426614 (Peter Murray)
0131 449 8049 or 07779 991643 (Anne Soutar)
0131 449 8032 (Dr John Cherrie)

APPENDIX 2 – QUESTIONNAIRE COMPLETED BY SPRAYERS

Institute of Occupational Medicine

Study no.

BIOLOGICAL MONITORING OF PESTICIDE EXPOSURES

QUESTIONNAIRE

All information we collect will be strictly confidential and no names or identifying information will be published or given to the Pesticide Safety Directorate or DEFRA.

INSTRUCTIONS

1. For some of the questions there is a list of possible answers with a box printed beside each one. Please choose your answer and put a tick in the box beside it, for example:

	Yes	No
Do you have any pets?	✓	

2. Some questions will ask you for a number, for example:

How many glasses of water do you drink each day? 2

3. There are spaces in some questions for you to write your answer.

4. Some questions are followed by instructions, which allow you to miss out certain questions. Please follow these instructions carefully.

Section A: Personal details

Name:	
Address:	
Post code:	☎:

1. Gender — Male ☐ Female ☐

2. Date of birth — dd mm yyyy ☐☐☐☐☐☐☐☐

3. Height — ft inches ☐☐☐☐ or cm ☐☐☐

4. Weight — st lbs ☐☐☐☐ or kg ☐☐☐

5.	Occupation:
6.	Address:
	Post code:

7. Is this your normal place of work? Yes ☐ No ☐

8.	If **no**, please state the address of where you work:
	Postcode:

Section B: The food you eat

9. How many portions of the following do you normally eat every day?

Fruit - *a portion is 1 medium fruit, e.g. apple, pear, orange, 1 cupful of small fruit e.g. grapes, strawberries, 1 large slice of large fruit e.g. melon, pineapple or 2 tablespoons for frozen or tinned fruit*

Peeled	Unpeeled	Frozen or tinned
☐	☐	☐

Vegetables - *a portion is 2 tablespoons*

Raw & peeled	Raw & unpeeled	Cooked (fresh, tinned or frozen)
☐	☐	☐

Salad - *a portion is 1 cereal bowl full*

Washed/pre-packed	Unwashed
☐	☐

Bread - *a portion is 2 medium slices of bread or 1 bread roll*

Number of portions
☐

10. How many of the above foods were organic?

All or most	Some	Very few or none	Not sure
☐	☐	☐	☐

Section C: Drinks

11. How much of the following do you normally drink each day?

	Number of glasses/cups
Tap water	
Bottled still water	
Bottled fizzy water	
Fruit juice	
Bottled fizzy drinks	
Tea	
Coffee	
Alcohol	

Other, please give details of beverages and amounts normally drunk

Section D: Pets

12. Do you have any pets? Yes ☐ No ☐

If **yes**, please state what pet(s) you have

Section E: Smoking

13. Do you smoke:

	Yes	No
Cigarettes?		
A pipe?		
Cigars?		

If you answered **yes** to any of the above please state the approximate quantity that you smoke each day. If you are an occasional smoker, please enter <1 (cigarettes or cigars) or < an amount (tobacco)

Cigarettes	
Tobacco	
Cigars	

Section F: Medication

14. Please list any medications normally taken

Section G: General information about spraying

14. How many years approximately have you been working with pesticides? ☐

15. Please record all qualification, the approximate dates of each course and the levels achieved, e.g. NPTC, LANTRA, courses run by ARC, training given by agronomists

16. How many days a year on average do you spray? ☐

17. Under what circumstances (e.g. weather, ground conditions) would you not spray pesticides?

Section H: Description of clothing and equipment typically used

18. Which of the following items of clothing and equipment do you wear?

	Mixing/loading	Spraying	Cleaning
Gloves	☐	☐	☐
Overalls	☐	☐	☐
Safety boots	☐	☐	☐
Rubber boots	☐	☐	☐
Face shield or mask	☐	☐	☐
Dust mask	☐	☐	☐

19. Do you wear any other items of clothing or equipment? Yes ☐ No ☐

If 'yes', please specify what you wear and what tasks it is worn for

20. How often are disposable items changed?

Gloves	
Disposable overalls	
Dust mask	

21. How often are non disposable items cleaned?

Overalls	
Respirators	
Gloves	
Boots	

22. If you answered 'yes' to question 19 above, please state how often the items specified are cleaned and/or changed

Section I: Spraying equipment

23. Manufacturer's name and model

Tractor mounted		Fixed boom	
Trailed		Suspended boom	
Self propelled		Boom width	

Main tank capacity

Auxiliary tank capacity

	Yes	No
24. Is a cab fitted to the tractor or sprayer during use?		
25. Are windows open during spraying?		
26. Are cab controls fitted		
27. (e.g. for turning off sections of the boom)		

28. If fitted, are cab controls:

Manual		Electric		Computer	

	Yes	No
29. Does your sprayer have a variable rate system?		
If yes, is this linked to GPS?		

30. Pesticide filling systems:

Direct pour		Induction bowl		Suction lance	

Closed transfer system (please specify)

Other (please specify)

31. Method of delivery:

Downward air assistance (air bag)		Twin fluid (Airtec/Airjet)	
Conventional		Other	

32. Nozzle type:

Flat fan		Air inclusion	
Twin jet		Hollow cone	

Make

33. How often are nozzles replaced?

34. Are nozzles fitted with an anti drip device? Yes ☐ No ☐

35. How often is each sprayer checked for:

	During use	Weekly	Annually	Never	Other
Output					
Leaks					
Spray pattern					
Nozzle drip					
Calibration					

36. Cleaning the sprayer

	Yes	No
Is the sprayer stored under cover?		
Does the sprayer have an external washing device?		
Does the sprayer have an internal rinsing device		

37. Where, when, how and with what are your sprayers externally cleaned down? – please specify location and frequency of cleaning

That is the end of the questionnaire. Thank you very much for filling it in. Please send it back to us as soon as possible in the reply-paid envelope supplied.

All of your responses will be treated in the strictest confidence.

If you have any questions about this study please contact Peter Murray, Anne Soutar or Dr John Cherrie at the address below:

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Research Park North
Riccarton
Edinburgh
EH14 4AP

Tel: 0870 850 5131 (switchboard)
0131 449 8072 or 07818 426614 (Peter Murray
0131 449 8049 or 07779 991643 (Anne Soutar)
0131 449 8032 (Dr John Cherrie)

APPENDIX 3 – DAILY DIARY SHEETS COMPLETED BY CONSUMERS

Day ☐ Date ☐☐☐

Number of portions of food eaten

Fruit - *a portion is 1 medium fruit, e.g. apple, pear, orange, 1 cupful of small fruit e.g. grapes, strawberries, 1 large slice of large fruit e.g. melon, pineapple or 2 tablespoons for frozen or tinned fruit*

Peeled ☐ Unpeeled ☐ Frozen or tinned ☐

Vegetables - *a portion is 2 tablespoons, cooked vegetables can be fresh, tinned or frozen*

Raw & peeled ☐ Raw & unpeeled ☐ Cooked ☐

Salad - *a portion one is one cereal bowl* Washed/prepacked ☐ Unwashed ☐

Bread – *a portion is 2 medium slices of bread or one bread roll* ☐

How many of the above foods were organic?

All or most ☐ Some ☐ Very few or none ☐ Not sure ☐

How many glasses of alcohol did you drink today? ☐

Approximate number of hours spent in different places during the day

Home inside ☐ Home outside ☐
Other inside ☐ Other outside ☐

Did you notice any unusual symptoms today?

Excessive tiredness ☐
Headaches/dizziness ☐
Nausea or vomiting ☐
Breathing problems ☐
Skin irritation ☐
Nervousness or depression ☐
Any other symptoms ☐

If 'yes' to 'Any other symptoms', please list them

Please list any medications other than those you normally take, which you have taken today e.g. paracetamol, ibuprofen etc

Please list the names of any of the following treatments which you or anyone in your household have used in the past 2 days

Pets	
Head lice shampoo or treatment (you or anyone in your household)	
Sprays for aphids, greenfly, black fly, whitefly, insect eggs	
Sprays for ants, cockroaches, vine weevils, woodlice, other insects	
Sprays for wasp/fly killer	
Other pesticides (please specify)	

If you have any further information which you think is relevant or any comments, please write them below

APPENDIX 4 – DAILY DIARY SHEETS COMPLETED BY BYSTANDERS

Day ☐ Date ☐☐☐

Number of portions of food eaten

Fruit - *a portion is 1 medium fruit, e.g. apple, pear, orange, 1 cupful of small fruit e.g. grapes, strawberries, 1 large slice of large fruit e.g. melon, pineapple or 2 tablespoons for frozen or tinned fruit*

Peeled ☐ Unpeeled ☐ Frozen or tinned ☐

Vegetables - *a portion is 2 tablespoons, cooked vegetables can be fresh, tinned or frozen*

Raw & peeled ☐ Raw & unpeeled ☐ Cooked ☐

Washed/prepacked ☐ Unwashed ☐

Salad - *a portion one is one cereal bowl*

Bread – *a portion is 2 medium slices of bread or one bread roll* ☐

How many of the above foods were organic?

All or most ☐ Some ☐ Very few or none ☐ Not sure ☐

How many glasses of alcohol did you drink today? ☐

Approximate number of hours spent in different places during the day

Home inside ☐ Home outside ☐

Other inside ☐ Other outside ☐

If the field was sprayed today

Were you near (< 20 m) while it was being sprayed? Yes ☐ No ☐

If the field was sprayed before today

Did you enter the field? Yes ☐ No ☐

If yes, how long did you spend in the field? ☐

Did you notice any unusual symptoms today?

Excessive tiredness ☐
Headaches/dizziness ☐
Nausea or vomiting ☐
Breathing problems ☐
Skin irritation ☐
Nervousness or depression ☐
Any other symptoms ☐

If 'yes' to 'Any other symptoms', please list them

Please list any medications other than those you normally take, which you have taken today e.g. paracetamol, ibuprofen etc

Please list the names of any of the following treatments which you or anyone in your household have used in the past 2 days

Pets	
Head lice shampoo or treatment (you or anyone in your household)	
Sprays for aphids, greenfly, black fly, whitefly, insect eggs	
Sprays for ants, cockroaches, vine weevils, woodlice, other insects	
Sprays for wasp/fly killer	
Other pesticides (please specify)	

If you have any further information which you think is relevant or any comments, please write them below

APPENDIX 5 – DAILY DIARY SHEETS COMPLETED BY POST-APPLICATION WORKERS

Daily food and location diary

1. Number of portions of food eaten

Fruit - *a portion is 1 medium fruit, e.g. apple, pear, orange, 1 cupful of small fruit e.g. grapes, strawberries, 1 large slice of large fruit e.g. melon, pineapple or 2 tablespoons for frozen or tinned fruit*

Peeled	Unpeeled	Frozen or tinned
☐	☐	☐

Vegetables - *a portion is 2 tablespoons*

Raw & peeled	Raw & unpeeled	Cooked (fresh, tinned or frozen)
☐	☐	☐

Salad - *a portion is 1 cereal bowl full*

Washed/pre-packed	Unwashed
☐	☐

Bread - *a portion is 2 medium slices of bread or 1 bread roll*

No. of portions ☐

2. How many of the above foods were organic?

All or most	Some	Very few or none	Not sure
☐	☐	☐	☐

3. How many glasses of alcohol did you drink today? ☐

4. Approximate number of hours spent in different places during the day

Home inside		Home outside	
Other inside		Other outside	

5. Please list the names of any of the following treatments which you have used

Pets	
Head lice shampoo or treatment (you or anyone in your household)	
Sprays or pesticides for ants, cockroaches, vine weevils, woodlice, other insects	
Sprays or pesticides for wasp/fly killer	
Other pesticides (please specify)	

6. Did you notice any unusual symptoms today?

Excessive tiredness	☐
Headaches/dizziness	☐
Nausea or vomiting	☐
Breathing problems	☐
Skin irritation	☐
Nervousness or depression	☐
Any other symptoms	☐

7. If 'yes' to 'Any other symptoms', please list them

8. Please list any medication, apart from your regular medication, which you have taken today, e.g. paracetamol, ibuprofen etc

Entry to field after spraying

		Yes	No
9.	Did you enter any field today which has been sprayed with pesticide in the last 5 days?	☐	☐

10. If yes:

Where were these fields?

11. How long did you spend in each of these fields?

12. What clothing and equipment did you wear?

Gloves	☐
Overalls	☐
Safety boots	☐
Rubber boots	☐
Face shield or mask	☐
Dust mask	☐

If you wore any other clothing or equipment, please state what

If your field was sprayed today, please complete one of the enclosed separate sheets about spraying

APPENDIX 6 – DAILY DIARY SHEETS COMPLETED BY SPRAYERS

Daily food and location diary

1. Number of portions of food eaten

Fruit - *a portion is 1 medium fruit, e.g. apple, pear, orange, 1 cupful of small fruit e.g. grapes, strawberries, 1 large slice of large fruit e.g. melon, pineapple or 2 tablespoons for frozen or tinned fruit*

Peeled	Unpeeled	Frozen or tinned
☐	☐	☐

Vegetables - *a portion is 2 tablespoons*

Raw & peeled	Raw & unpeeled	Cooked (fresh, tinned or frozen)
☐	☐	☐

Salad - *a portion is 1 cereal bowl full*

Washed/pre-packed	Unwashed
☐	☐

Bread - *a portion is 2 medium slices of bread or 1 bread roll*

No. of portions ☐

2. How many of the above foods were organic?

All or most	Some	Very few or none	Not sure
☐	☐	☐	☐

3. How many glasses of alcohol did you drink today? ☐

4. Approximate number of hours spent in different places during the day

Home inside		Home outside	
Other inside		Other outside	

5. Please list the names of any of the following treatments which you have used

Pets	
Head lice shampoo or treatment (you or anyone in your household)	
Sprays or pesticides for ants, cockroaches, vine weevils, woodlice, other insects	
Sprays or pesticides for wasp/fly killer	
Other pesticides (please specify)	

6. Did you notice any unusual symptoms today?

Excessive tiredness	☐
Headaches/dizziness	☐
Nausea or vomiting	☐
Breathing problems	☐
Skin irritation	☐
Nervousness or depression	☐
Any other symptoms	☐

7. If 'yes' to 'Any other symptoms', please list them

8. Please list any medication, apart from your regular medication, which you have taken today, e.g. paracetamol, ibuprofen etc

Information about spraying

9. Farm name:
Address:

Post code:

10. Precise location of field:

11. What crop was sprayed and what were the dimensions (please state units) of the field sprayed?

12. Time started _____ Time finished _____

13. What was the sprayer speed? _____

14. Environmental conditions
Temperature (°C) _____
Relative humidity (%) _____

Wind speed _____ Direction _____

15. Number of people involved in spraying _____

16. What tasks did you carry out?

Mixing ☐ Spraying ☐ Cleaning ☐

Other ☐ Please specify _____

17. Pesticide application
Product used _____
Manufacturer _____
Active ingredients (please list) _____
Quantity of concentrate used _____

18. What clothing and equipment did you wear?

	Mixing/loading	Spraying	Cleaning
Gloves	☐	☐	☐
Overalls	☐	☐	☐
Safety boots	☐	☐	☐
Rubber boots	☐	☐	☐
Face shield or mask	☐	☐	☐
Dust mask	☐	☐	☐

If you wore any other clothing or equipment, please state what

19. Did you get any concentrate on your skin? Yes ☐ No ☐

20. How long were any spillages on your skin for before washing off?

<Less than 30 minutes ☐ 30 – 60 minutes ☐ More than 60 minutes ☐

21. Were there any problems during spraying today? Yes ☐ No ☐
e.g. a hose break, accidental drift of spray, tearing of clothing on a sharp object

If 'yes', please describe the problem

Entry to field after spraying

		Yes	No
22.	Did you enter any field today which has been sprayed with pesticide in the last 5 days?		

23. If yes:

Where were these fields?

24. How long did you spend in each of these fields?

25. What clothing and equipment did you wear?

Gloves	
Overalls	
Safety boots	
Rubber boots	
Face shield or mask	
Dust mask	

If you wore any other clothing or equipment, please state what

APPENDIX 7 – EXAMPLE OF SPRAYING SHEET COMPLETED BY SPRAYERS AND POST-APPLICATION WORKERS IN 2005

Institute of Occupational Medicine

Details of Pesticide used

To be completed on the day of spraying

1.	Farm name: Address: Post code:			
2.	Date			
2.	Precise location of field:			
3.	What crop was sprayed and what were the dimensions (please state units) of the field sprayed?			

5. Pesticide application

Product used	
Manufacturer	
Active ingredients (please list)	

6.	Weather conditions

APPENDIX 8 – EXAMPLE OF SPRAYING SHEET COMPLETED BY SPRAYERS IN 2006

Study no.
Name
Date

Details of pesticide used

To be completed on the day of spraying

1. Farm name:
   Address:
   Post code:

2. Precise location of field:

3. What crop was sprayed and what were the dimensions (please state units) of the field(s) sprayed?

4. Time started _____ Time finished _____

5. What was the sprayer speed? _____

6. Environmental conditions

   Temperature (°C) _____

   Wind speed _____ Wind direction _____

7. What tasks did you carry out?

   Mixing ☐ Spraying ☐ Cleaning ☐

   Other ☐ Please specify _____

8. Pesticide application

Product	Manufacturer	Active ingredient	Amount used (state units)

9. How many much water did you use? _____ litres

10. How much pesticide did you spray? _____ litres

PTO to complete details about protective clothing and equipment used

11. What clothing and equipment did you wear?

	Mixing/loading	Spraying	Cleaning
Gloves	☐	☐	☐
Overalls	☐	☐	☐
Safety boots	☐	☐	☐
Rubber boots	☐	☐	☐
Face shield or mask	☐	☐	☐
Dust mask	☐	☐	☐

If you wore any other clothing or equipment, please state what

12. Did you get any concentrate on your skin? Yes ☐ No ☐

13. How long were any spillages on your skin for before washing off?

<Less than 30 minutes ☐ 30 – 60 minutes ☐ More than 60 minutes ☐

14. Were there any problems during spraying today? Yes ☐ No ☐
e.g. a hose break, accidental drift of spray, tearing of clothing on a sharp object

If 'yes', please describe the problem

APPENDIX 9 – EXAMPLE OF SPRAYING SHEET COMPLETED BY BYSTANDERS IN 2006

Study no.
Name
Date

To be completed on the day of spraying

Questions 1 and 2 can be omitted if they have been filled in on the sprayer's sheet

1. Farm name:
   Address:
   Post code:

2. Precise location of field:

3. Roughly how close were you to the field when it was being sprayed?

   < 20 m ☐ < 20 – 50 m ☐ 50 – <100 m ☐ >100 m ☐

4. Please draw a rough diagram to indicate where you were in relation to the field

5. Were you inside or outside when the field was sprayed? Inside ☐ Outside ☐

If you were outside

6. How long were you near the field? ☐ Minutes

APPENDIX 10 – EXAMPLE OF SPRAYING SHEET COMPLETED BY POST-APPLICATION WORKERS IN 2006

Study no.
Name
Date

To be completed on the day you entered a sprayed field

1.

Farm name:
Address:
Post code:

2.

Precise location of field(s):

3. Please list the pesticides which were used on the fields

Product	Manufacturer	Active ingredient	Amount used (state units) – if known

4. When were the fields sprayed? ☐☐☐

5. How long were you in the field(s)? ☐☐☐ Minutes

6. Did you wear any special clothing? Yes ☐ No ☐

7. What clothing and equipment did you wear (please tick all that apply)?

Gloves	☐
Overalls	☐
Safety boots	☐
Rubber boots	☐
Face shield or mask	☐
Dust mask	☐

If you wore any other clothing or equipment, please state what

APPENDIX 11 – EXAMPLE OF MATLAB PROGRAM USED FOR PHARMACOKINETIC MODELLING

```
clear;
% Define variables for cypermethrin

St=8; % start time
Fin=13; % end time
T=17; % time between end of spraying and sample taken (hours)
L=23; % time of last void at night (hours)
Samp=30; % time sample taken
H=Fin-St; % Time exposed, from time spraying starts till time sample is taken

h=13; % half life (hours) Perhaps should vary this

Amt=0.025; % amount used (kg)

BW=77; % Body weight of individual

ke=log(2)/(h*60); % elimination rate constant in minutes
dt=1; % interval of 1 minute

Mo=416.033; % Molecular weight of original substance (cypermethrin)
Mm=214.22; % Molecular weight of metabolite (3PBA)

R=1; % Stoichiometric ratio
Vd=4.60; % Volume of distribution for 3PBA (l/kg)

Vu=0.6; % urine volume (l) collected between last void and time sample taken

Fa=0.012; % fraction absorbed through skin

fid=fopen('Example.dat','w');

for j=1:1000

% Set limits for absorption rate constant (/hr)
a1=0.01;
b1=0.1;
ka=unifrnd(a1,b1)/60; % absorption rate constant in minutes

% Set limits for selectivity
a2=0.8;
b2=1;
S=unifrnd(a2,b2);

phi=(S*R)/(Vd*Mo/Mm);

% Set fraction of the amount sprayed with gets onto the skin
a3=3E-10;
b3=5E-8;
c3=2.08E-5;
```

```
kf=trianglerandm(a3,b3,c3);

E=kf*Amt*1000000000; % actual dermal exposure (microg)
Emin=E/(H*60); % actual new dermal exposure per minute (microg)

Tbegin=St*60;
Tend=Fin*60;
Tsamp=Samp*60; % time in minutes
Tlast=L*60;

for i=1:Tsamp+1
   t(i)=i-1;

   if i<Tbegin
      Ex(i)=0;
      Dx(i)=0;
   end

   if i>=Tbegin & i<Tend
      Ex(i)=Emin+(Ex(i-1)-Fa*Ex(i-1))*dt;
      U(i)=Fa*Ex(i);
      Dx(i)=U(i)/BW;
   end

   if i>=Tend
      Ex(i)=0;
      Dx(i)=0;
   end
end

Ca(1)=0;
Cb(1)=0;

for i=1:Tsamp
   Ca(i+1)=Ca(i)+(-ka*Ca(i)+phi*Dx(i))*dt;
   Cb(i+1)=Cb(i)+(ka*Ca(i)-ke*Cb(i))*dt;
 end

Sum=0;
for i=Tlast:Tsamp
   Sum=Sum+Cb(i)*dt;
end

UER=((Vd*ke)/(Tsamp-Tlast))*Sum*BW;

Cu=(UER*(Tsamp-Tlast))/Vu;
Curine(j)=Cu;

Exp(j)=E;

fprintf(fid,'%8.3f %12.6f\n',Exp(j),Curine(j)');

end
```

```
fclose(fid);

figure(1)
hist(Exp)
xlabel('Mass on skin (\mug)')
ylabel('Count')

figure(2)
hist(Curine)
xlabel('Concentration (\mug/l)')
ylabel('Count')
```

APPENDIX 12 – INDIVIDUAL RESULTS FOR CONSUMERS, BYSTANDERS, POST-APPLICATION WORKERS AND SPRAYERS FOR 3-PBA AND DCVA

Table A12.1 Pyrethroid metabolites in urine samples and creatinine concentrations for consumers

Study no.	3-PBA			DCVA			Creatinine
	µmol/mol	µg/l	µg/g	µmol/mol	µg/l	µg/g	(g/l)
C01	ND	ND	ND	ND	ND	ND	1.30
C04	ND	ND	ND	ND	ND	ND	1.81
C06	ND	ND	ND	ND	ND	ND	0.54
C08	ND	ND	ND	ND	ND	ND	1.96
C10	ND	ND	ND	ND	ND	ND	1.31
C11	ND	ND	ND	ND	ND	ND	1.44
C12	ND	ND	ND	ND	ND	ND	0.59
C18	ND	ND	ND	ND	ND	ND	0.75
C19	ND	ND	ND	ND	ND	ND	1.54
C20	ND	ND	ND	ND	ND	ND	0.94
C21	ND	ND	ND	ND	ND	ND	0.40
C23	ND	ND	ND	ND	ND	ND	0.70
C28	ND	ND	ND	ND	ND	ND	1.15
C29	ND	ND	ND	ND	ND	ND	0.94
C30	ND	ND	ND	ND	ND	ND	1.97
C31	ND	ND	ND	ND	ND	ND	1.53
C32	ND	ND	ND	ND	ND	ND	0.64
C33	ND	ND	ND	ND	ND	ND	1.11
C34	ND	ND	ND	ND	ND	ND	1.90
C35	ND	ND	ND	ND	ND	ND	2.19
C36	ND	ND	ND	ND	ND	ND	1.27
C37	ND	ND	ND	ND	ND	ND	1.18
C38	ND	ND	ND	ND	ND	ND	0.76
C39	ND	ND	ND	ND	ND	ND	0.71
C40	ND	ND	ND	0.77	1.27	1.42	0.89
C41	ND	ND	ND	ND	ND	ND	2.43
CS1	ND	ND	ND	ND	ND	ND	0.75
Minimum							0.40
Median							1.15
90th %ile							1.97
Maximum				0.77	1.27	1.42	2.43

Table A12.2 Pyrethroid metabolites in urine samples and creatinine concentrations for bystanders

Study no.	3-PBA			DCVA			Creatinine
	µmol/mol	µg/l	µg/g	µmol/mol	µg/l	µg/g	(g/l)
B15	ND	ND	ND	ND	ND	ND	1.42
B17	1.20	3.30	2.28	ND	ND	ND	1.45
B18	ND	ND	ND	ND	ND	ND	1.19
B19	0.47	1.89	0.89	ND	ND	ND	2.12
B20	ND	ND	ND	ND	ND	ND	1.86
Minimum	<LOD	<LOD	<LOD				1.19
Median	<LOD	<LOD	<LOD				1.45
90th %ile	0.91	2.73	1.72				2.01
Maximum	1.20	3.30	2.28				2.12

Table A12.3 Pyrethroid metabolites in urine samples and creatinine concentrations for post-application workers

Study no.	3-PBA			DCVA			Creatinine
	µmol/mol	µg/l	µg/g	µmol/mol	µg/l	µg/g	(g/l)
B13	0.31	1.18	0.59	ND	ND	ND	1.99
W9	ND	ND	ND	ND	ND	ND	1.24
W11	0.46	1.91	0.88	ND	ND	ND	2.17
Minimum	<LOD	<LOD	<LOD				1.24
Median	0.31	1.18	0.59				1.99
90th %ile	-	-	-				2.13
Maximum	0.46	1.91	0.88				2.17

Table A12.4 Pyrethroid metabolites in urine samples and creatinine concentrations for sprayers

Study no.	3-PBA			DCVA			Creatinine
	µmol/mol	µg/l	µg/g	µmol/mol	µg/l	µg/g	(g/l)
SP06	ND	ND	ND	ND	ND	ND	1.10
SP08	0.67	3.28	1.26	ND	ND	ND	2.59
SP12	0.56	2.08	1.07	ND	ND	ND	1.94
SP14	0.61	1.09	1.16	ND	ND	ND	0.94
SP15	1.59	5.85	3.01	1.82	6.49	3.35	1.94
SP16	ND	ND	ND	ND	ND	ND	1.38
SP17	0.44	1.26	0.82	ND	ND	ND	1.53
SP19	1.76	4.03	3.33	2.71	6.03	4.99	1.21
SP23	0.75	1.37	1.43	ND	ND	ND	0.96
SP28	0.62	1.16	1.18	ND	ND	ND	0.98
Minimum	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.94
Median	0.62	1.32	1.17	<LOD	<LOD	<LOD	1.29
90th %ile	1.61	4.21	3.05	1.91	6.08	3.51	2.01
Maximum	1.76	5.85	3.33	2.71	6.49	4.99	2.59

APPENDIX 13 – INDIVIDUAL RESULTS FOR BYSTANDERS, POST-APPLICATION WORKERS AND SPRAYERS FOR ETU

Table A13.1 ETU metabolites in urine samples and creatinine concentrations for bystanders

Study no.	ETU			Creatinine (g/l)
	µmol/mol	µg/l	µg/g	
B38	ND	ND	ND	0.98
B39	ND	ND	ND	1.88
B40	ND	ND	ND	1.70
B45	ND	ND	ND	3.75
B46	1.12	0.72	1.01	0.71
B49	0.19	0.31	0.17	1.78
B50	0.33	0.61	0.30	2.04
Minimum	<LOD	<LOD	<LOD	0.71
Median	<LOD	<LOD	<LOD	1.78
90th %ile	0.65	0.65	0.58	2.72
Maximum	1.12	0.72	1.01	3.75

Table A13.2 ETU metabolites in urine samples and creatinine concentrations for post-application workers

Study no.	ETU			Creatinine (g/l)
	µmol/mol	µg/l	µg/g	
B43	ND	ND	ND	1.04

Table A13.3 ETU metabolites in urine samples and creatinine concentrations for sprayers

Study no.	ETU			Creatinine (g/l)
	µmol/mol	µg/l	µg/g	
SP35	0.91	0.92	0.82	1.12
SP39	4.31	7.15	3.90	1.84
SP43	5.95	6.44	5.38	1.20
SP55	1.27	1.33	1.15	1.16
SP61	3.63	4.70	3.28	1.43
SP62	4.34	4.80	3.92	1.22
SP64	5.86	6.13	5.29	1.16
SP66	ND	ND	ND	3.25
Minimum	0.03	0.10	0.03	1.12
Median	3.97	4.75	3.59	1.21
90th %ile	5.89	6.65	5.32	2.26
Maximum	5.95	7.15	5.38	3.25

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