General Enquiries on the form should be made to:
Defra, Procurements and Commercial Function (Evidence Procurement Team)
E-mail: research.competitions@defra.gsi.gov.uk

Evidence Project Final Report

- **Note**
  In line with the Freedom of Information Act 2000, Defra aims to place the results of its completed research projects in the public domain wherever possible.
  The Evidence Project Final Report is designed to capture the information on the results and outputs of Defra-funded research in a format that is easily publishable through the Defra website
  An Evidence Project Final Report must be completed for all projects.
- This form is in Word format and the boxes may be expanded, as appropriate.
- **ACCESS TO INFORMATION**
  The information collected on this form will be stored electronically and may be sent to any part of Defra, or to individual researchers or organisations outside Defra for the purposes of reviewing the project. Defra may also disclose the information to any outside organisation acting as an agent authorised by Defra to process final research reports on its behalf. Defra intends to publish this form on its website, unless there are strong reasons not to, which fully comply with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.
  Defra may be required to release information, including personal data and commercial information, on request under the Environmental Information Regulations or the Freedom of Information Act 2000. However, Defra will not permit any unwarranted breach of confidentiality or act in contravention of its obligations under the Data Protection Act 1998. Defra or its appointed agents may use the name, address or other details on your form to contact you in connection with occasional customer research aimed at improving the processes through which Defra works with its contractors.

Project identification

1. Defra Project code: PS2620

2. Project title:
   Biological monitoring of pesticide exposure in residents

3. Contractor organisation(s):
   Institute of Occupational Medicine (IOM), Edinburgh
   KS Galea, L MacCalman, JW Cherrie, M van Tongeren

   Health and Safety Laboratory (HSL), Buxton
   K Jones, J Cocker

   Glasgow Caledonian University, Glasgow.
   P Teedon

4. Total Defra project costs (agreed fixed price): £ 753,663

5. Project: start date ..... 1st October 2010

   end date ..... 31st March 2015

6. It is Defra's intention to publish this form.

   Please confirm your agreement to do so.....**YES** ☒ **NO** ☐

   (a) When preparing Evidence Project Final Reports contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.

   Defra recognises that in a small minority of cases there may be information, such as intellectual property or commercially confidential data, used in or generated by the research project, which should not be disclosed. In these cases, such information should be detailed in a separate annex (not to be published) so that the Evidence Project Final Report can be placed in the public domain. Where it is impossible to complete the Final Report without including references to any sensitive or confidential data, the information should be included and section (b) completed. NB: only in exceptional circumstances will Defra expect contractors to give a "No" answer.

   In all cases, reasons for withholding information must be fully in line with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.

   (b) If you have answered NO, please explain why the Final report should not be released into public domain

Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

Background

In the United Kingdom the use of pesticides in agriculture, horticulture, forestry, food storage and the home or garden is regulated to protect human health and the environment. Approval of pesticides involves the comparison of estimated potential human exposure with toxicological reference value levels. These are levels, at and below which there is considered to be high confidence that there will be no adverse health effects. Biological monitoring of pesticide biomarkers in the urine of participants in an earlier study showed that the methods currently used in the UK for assessing pesticide exposure for regulatory purposes were likely to be appropriate for farm workers. However this earlier study but did not collect data for residents living near agricultural land.

Aims

Our study aimed to assess exposure to pesticides for adults and children (4-12 years old) living within 100m from agricultural land and investigate if exposures were elevated following pesticide spray events. In addition it aimed to determine whether the methods used in the UK pesticides approval process are appropriate for assessing exposure of residents living near fields.

Methods

The study was approved by the NHS South East Scotland Research Ethics Committee (SESREC) 3 (study number 10/S1103/63). Arable farms and orchards (hereafter referred to as farms) likely to spray relevant pesticides (captan, chlormequat, chlorpyrifos, cypermethrin and penconazole), and which had residents living within 100m of treated fields were recruited into the study. Pesticides of relevance to the study were restricted to those for which analytical methods were available for associated urinary biomarkers and likelihood of application, not because of any potential health concerns. Recruited farmers provided details of their spray events throughout the year. Households within 100m of the relevant farmers' field were approached to provide urine samples during the 48 hours after relevant spray events and additional samples both within and outwith the spray season. The spraying season was considered to be between March and August, coinciding with the main crop growing period, and stopping prior to harvest.

Selected urine samples (those collected first thing in the morning one or two days after a relevant spray event and, in addition, up to 3 within and 3 outwith spray season as background samples) from each participant providing a spray event sample were analysed to establish the levels of pesticide biomarkers (*measured concentrations*). We compared the pesticide biomarker concentrations in urine samples collected after spray days with concentrations in samples collected when no spraying occurred (background samples). We also analysed information provided by participants (e.g. time spent at home / elsewhere, use of pesticides in the home, consumption of home grown food within the previous 2 days of providing the urine sample) that might explain any differences between the urinary biomarker concentrations.

Separately, spray information obtained from participating farmers was used to predict the pesticide exposure using the approaches in place at the time of the project. These exposure predictions were completed independently by a member of the government agency and without knowledge of the corresponding spray-event urine sample results. Predictions of the levels of pesticide biomarkers expected in the urine given this exposure were calculated, using a series of mathematical equations based on current knowledge of the breakdown process of pesticides in the human body (*predicted concentrations*). The measured and predicted urinary biomarker concentrations were then compared to provide information on whether the regulatory exposure assessment methods used are sufficiently protective.

Results

Recruitment and data collection

Recruitment, data and sample collection took place in three UK geographic regions - East Lothian and Kent during both 2011 and 2012 and Norfolk only in 2012. Twenty one farms in total participated in the study (13 in 2011 and 17 in 2012) and sprayed pesticide products containing one or more of the pesticides of interest. A total of 156 households and 296 participants were recruited to the study (238 adults, 58 children) and 3,275 urine samples were obtained. A total of 149 participants provided at least one urine sample that was related to a relevant spray event involving the pesticides captan, chlormequat, cypermethrin, chlorpyrifos and penconazole. Following the application of necessary exclusion criteria, 1,587 samples results from 149 people were used in the data analysis, consisting of 542 spray event samples and 1045 background samples.

Urinary biomarker levels
For the three pesticides captan, cypermethrin and penconazole, over 80% of biomarker concentrations were below the laboratory analysis limit of detection, regardless of whether the urine samples were spray event related, or backgrounds. For chlormequat and chlorpyrifos, the geometric mean urinary biomarker concentrations following spray events were lower than the within season background concentrations for these two pesticides. The mean outwith spray season biomarker concentrations for chlorpyrifos were the same as within season backgrounds. For chlormequat, lower levels were observed outwith the spray season. There were no statistically significant differences in pesticide biomarker concentrations following spray events between males and females or adults and children participants.

Comparisons with regulatory exposure assessment predictions
Regulatory exposure assessments were conducted for each of the relevant spray events. These assessments considered exposure via three pathways: (1) spray drift at the time of application; (2) exposure following evaporation of the pesticide from the treated crop or soil surfaces after application; as well as (3) direct contact with treated surfaces after application. For each spray event, the pathway providing the highest predicted exposure was used for comparison with the measured urinary biomarker concentrations.

As a high proportion of urinary biomarker results for captan, cypermethrin and penconazole were below the laboratory's limit of detection, only the number and percentage of samples above the predicted exposures are reported. For cypermethrin the measured urinary biomarker levels were all found to be lower than the predicted concentrations. Over 98% (n=81) of the 82 measured urinary biomarker concentrations for penconazole were less than the predicted exposures. For captan, 97% (n=227) of the 234 measured urinary biomarker concentrations were lower than the predicted exposures.

A greater number of measured urinary biomarker results were above the analytical limit of detection for chlorpyrifos and chlormequat. However, no statistically significant differences in pesticide biomarker concentrations following spray events were found compared to background urinary biomarker concentrations for these pesticides. Initial comparisons of the measured urinary biomarker concentrations with the predicted exposures found that 20% of chlorpyrifos and 40% of chlormequat urinary biomarker concentrations were greater than the predicted concentrations. We compared the background urinary biomarker concentrations with the predicted exposures to further understand these results. Overall, the proportion of measured urinary biomarker concentrations exceeding the level predicted for the relevant spray event was no different from what would be expected if no spray event had taken place.

Discussion
This study reports on the exposure to pesticides experienced by residents living near agricultural land. This has not previously been investigated and reported on such a scale in the UK. The study did not aim to investigate whether exposure to pesticides was associated with any potential adverse health effects.

For captan, cypermethrin and penconazole over 80% of the urinary biomarker measurements were below detectable levels, whether or not these samples were collected following spray events. The levels of pesticide urinary biomarkers detected are generally comparable to other population studies, where such data are available. For chlormequat, there is only one other relevant study to compare our results with and this suggests that our population experienced greater exposure to chlormequat than a sub-set of the Swedish population. It is possible that this may be due to different farming practices and consumption of foods and beverages containing cereal crops for which this growth regulator is typically applied.

The predicted regulatory exposure assessments estimate residents' exposures for three pathways separately for the relevant spray events with the pathway with the highest predicted value being used in the comparison. In contrast, the measured urinary biomarker concentrations indicate the participants' exposure from all sources of exposure and therefore may not be directly comparable. All measured urinary biomarker concentrations in the spray event urine samples for cypermethrin and over 97% of captan and penconazole biomarker concentrations were below the predicted exposures. There appeared to be a number of chlormequat (82 from a total of 195) and chlorpyrifos (12 from a total of 54) measured urinary biomarker levels that were higher than predicted, however it is considered that this is due to variability in background levels of exposure from other sources of exposure, e.g. food and beverage consumption.

Overall this study concludes that, for the pesticides considered in this study and the spray practices assessed, there was no evidence of increased pesticide exposure in the study population following a spray event within 100m of their home, when compared to the exposure at times when spraying does not occur. The study also concludes that the regulatory exposure assessment methods currently used generally provide sufficiently conservative estimates of residents' exposure. The study findings may impact on future developments of methods used to determine expected urinary biomarkers from pesticide spray activities.

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
   - the objectives as set out in the contract;
   - the extent to which the objectives set out in the contract have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Exchange).

Background

In the United Kingdom (UK) 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 Chemical Regulation Directorate (CRD) of the Health and Safety Executive (HSE). The health regulatory risk assessment (RRA) underpinning the approval of pesticides involves the comparison of estimates of potential human exposure with toxicological reference values levels. These are levels below which there is considered to be high confidence that there will be no adverse health effects. The system is generally considered to be conservative such that estimated exposures represent some multiple of the likely actual exposure. 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 residents living nearby. 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 to estimate exposure (HSE, 2012).

There is generally a lack of actual pesticide exposure measurement data available for residents and there are no specific studies on the pesticide exposures of people living near agricultural land in the UK. Therefore the exposure assessment will generally rely on simple exposure assessment tools. Due to the inherent nature of these tools, there is considerable uncertainty associated with estimates obtained. To account for this uncertainty the tools are designed to provide conservative estimates; however, they have not been comprehensively evaluated to determine if they are truly conservative, in particular for residents. Work undertaken in previous studies of pesticide exposure suggests that the current RRA methods are sufficient for farm workers and pesticide applicators (Cooper and Dobson, 2007; Sleeuwenhoek et al. 2007; Colosio et al. 2011). However, there is a lack of information on the potential exposures experienced by non-occupational groups, such as bystanders and residents. Sleeuwenhoek et al. (2007) reported that exposures of bystanders may sometimes be underestimated using the exposure models when compared to their measured urinary biomarker levels. This earlier study did not collect data for residents.

Aims

The aims of this research were to assess exposure to pesticides for adults and children living within 100m from agricultural land and investigate if exposures were elevated following spray events. In addition the project aimed to assess whether the methods used in the UK pesticides regulatory approval process are appropriate for assessing exposure to pesticides amongst residents living near agricultural fields.

Objectives as set out in the project contract and extent to which these have been met

To achieve these aims, the project had the following specific objectives:

1. To obtain ethical approval;
2. To select appropriate pesticides and active ingredients;
3. To identify and recruit suitable arable farms and orchards and residents;
4. To collect and analyse urine samples during two spraying seasons (2011-2012);
5. To predict the level of biomarkers in urine using a pharmacokinetic (PK) model;
6. To predict resident exposure to pesticides using the exposure models used in the RRA approach;
7. To compare predicted and measured pesticide exposure; and
8. To report the study results, including preparation of peer-reviewed publications.

These objectives are reported in the proceeding sections of this report. With respect to objective 8, details of the peer-reviewed publications are detailed in Section 9, which will be updated as necessary.

Methods

Ethical approval and independent review

The study received full approval by the NHS South East Scotland Research Ethics Committee (SESREC) 3 (study number 10/S1103/63). An Advisory Committee comprising of four independent experts (Prof. David Coggon (University of Southampton), Paul Hamey (CRD, HSE), Prof. Len Levy (University of Cranfield) and Dr. Sean Semple (University of Aberdeen)) monitored the progress of the research throughout the project, providing advice and guidance. Our published study protocol (Galea et al. 2011) provides detail of the methodology and is briefly summarised below.

Geographical areas

Surveys were planned to be carried out in three locations in the UK: East Lothian and Kent both in years 2011 and 2012, with the study being expanded to include Norfolk in 2012. East Lothian and Norfolk are major arable crop growing areas, while most of the orchards in the UK are located in South East England (Garthwaite et al. 2013). Orchard spraying is likely to result in higher exposures and is considered the worst-case exposure scenario (Lloyd and Bell, 1983; Lloyd et al. 1987). Recruitment of, and liaison with, participants in the study was carried out by community researchers. The community researchers were recruited from the UK geographical areas of interest to the study to have detailed knowledge of the local area and ideally knowledge of local farming practices.

Farmer recruitment and data collection

The community researchers recruited owners/managers of farms and orchards (hereafter both referred to as farms) that reported they were likely to spray their crops with the active ingredients in Table 1 and had residential areas within 100m of their fields. Farmers were initially identified through publicly available resources and contacted via letter explaining the aims and objectives of the study. This was then followed by telephone contact and if the farmer was willing, an in-person meeting at the farm. Consenting farmers were asked to provide details of their pesticide usage throughout the spray season, including information on the start and finish times of spray events, products and active ingredients used, spraying methods and weather conditions. Where farmers maintained comprehensive records of their pesticide usage, the community researcher requested copies of these. Where detailed records were not maintained, farmers were asked to record the relevant information prospectively using an adaptation of the spray record form recommended in the code of practice for using pesticide products (DEFRA, 2006).

Pesticides relevant to the study

Table 1 provides details of the pesticides originally considered in the study. Inclusion was restricted to those for which analytical methods were available for urinary biomarkers and was not influenced by health concerns. Following discussions with farmers and agronomists, it emerged that several of the pesticides listed in Table 1 were no longer regularly used and so the study focused on captan, chlormequat, chlorpyrifos, cypermethrin and penconazole due to anticipated spray practices.

Table 1: Pesticides of interest to the study (Galea et al. 2011)

Pesticide	Function	Relevant crops approved[a] for use
Captan	Fungicide	Apple, pear
Chlormequat	Growth regulator	Cereals
Chlorpyrifos	Insecticide	Apple, cereals, veg incl. potato
Cypermethrin	Insecticide	Apple and various arable crops incl. potato
Deltamethrin	Insecticide	Apple and various arable crops (not potato)
Diquat	Herbicide/desiccant	Various arable crops incl. potato
Iprodione	Fungicide	Field beans, oil seed rape
Penconazole	Fungicide	Apple, blackcurrant, hops
Pririmicarb	Insecticide	Apple and various arable crops incl. potato
Thiophanate-methyl	Fungicide	Wheat, triticale, field beans, oil seed rape

[a]approval in 2011

Resident recruitment and data collection

Residents (adults aged 18 years and over and children in their care aged 4-12 years) living within 100m of the edge of a field belonging to a recruited farm were approached to participate in the study. Residents involved in occupational pesticide application or re-entry activities were excluded from participation. Letters introducing the study, enclosing a participant's information leaflet and reply form to indicate interest were issued. Where telephone numbers were available from publicly available resources, attempts to contact residents were also made in this manner. Care was taken to ensure the number of attempted contacts was restricted to an acceptable level. Given the nature of the recruitment strategy it was not always possible to obtain a response (either positive or negative) to the invitation to participate.

Informed consent was obtained (participating adults consenting on behalf of the children in their care). Participants completed a background questionnaire. This included questions concerning their age, sex, weight, lifestyle (e.g. leisure activities), occupational and para-occupational exposure to pesticides (exposure via, for example, family members who work with pesticides and live in the same home) and pesticide usage within the home. The child questionnaire was completed by the consenting adult.

Consenting adults and children were then asked to provide urine samples and complete accompanying questionnaires during the spraying season (considered to be between March – August, coinciding with the main crop growing season and stopping prior to harvest) and also for 3 weeks outwith the spraying season to allow background pesticide biomarker levels to be determined. In instances when the community researchers' were advised of a relevant spray event occurring on a given field, participants residing within 100m of the field were contacted and asked to provide a urine sample one and two days following the day of the spray event. For this sample collection strategy, participants served as their own controls (rather than using another individual or group with which to compare), allowing comparison of spray event and background pesticide urinary biomarker levels.

In all instances, first morning void samples (~70ml) were collected in polypropylene containers (Starplex Scientific Inc., Canada) along with details of the time of collection. Appendix 1 provides details of the methodology used to determine this optimal time for urine sample collection, the results of which agree with a study by Kissel et al. (2005). Participants were also asked to complete a questionnaire at the time of providing each urine sample, providing information on home and para-occupational pesticide usage, outdoor and indoor activities and their duration as well as their consumption of home grown produce within the previous 48-hour period. The questionnaire completed by the adult on behalf of the child was shorter and included questions concerning the time spent by the child in both outdoor and indoor environments.

Urine sample storage, selection and analysis

Upon collection by the community researchers on the day of sample provision, urine samples were frozen (−15 to −20°C) as soon as possible and typically within a 6-hours of collection. Urine samples fulfilling the following three criteria were selected for analysis:

1. Urine samples collected within 2 days of relevant spray events taking place in fields within 100m of the participant's residence.
2. For each participant providing at least one relevant spray event sample, up to 3 background samples obtained within the spraying season (randomly selected) which did not coincide with a relevant spray event.
3. For each participant providing at least one relevant spray event sample, up to 3 background samples collected outwith the spraying season (during Nov-Dec) and which did not coincide with a relevant spray event.

Urine samples collected within 2 days after a relevant spraying event were analysed only for the relevant pesticides sprayed during the event. Background samples, both within and outside the spray season, were analysed for all five relevant pesticides selected for investigation for which urine samples were collected for spray events.

Laboratory analysis

Samples were analysed by HSL analysts blind to whether the urine samples were spray event related or background samples. Table 2 provides a summary of the urinary biomarkers analysed for the relevant active ingredients sprayed and the analytical methods used. For all pesticides except penconazole, established methods were available.

Table 2: Spray event related urine samples collected and measured pesticide exposure biomarkers

Pesticide	Analyte(s)	Analytical Method	Detection limit (µg/l)	Method Reference
Captan	*cis*-1,2,3,6-Tetrahydrophthalimide (THPI)	SPE LC-MS/MS	0.1	Berthet et al. (2011)
Chlormequat	Chlormequat (parent)	SPE LC-MS/MS	0.6.	Lindh et al. (2011)
Chlorpyrifos	3,5,6-Trichlorpyridinol (TCP)	Acid hydrolysis Solvent extraction GC-MS	0.8	Sams et al. (2011)
Cypermethrin	*cis*- & *trans*-2,2-Dichlorovinyl-3,3-dimethylcyclopropane-1-carboxylic acid (DCVA)	Enzyme hydrolysis SPE LC-MS/MS	1.0	Jones et al. (2009)
Penconazole	4-(2,4-Dichlorophenyl)5-(H-1,2,4-triazol-1-yl)pentoic acid (Pen-COOH)	Solvent extraction LC-MS/MS	0.25	Jones et al. (in draft)

SPE: solid phase extraction; LC-MS/MS:liquid chromatography tandem mass spectrometry; GC-MS: gas chromatography mass spectrometry

A novel method was developed by HSL during the study for penconazole biomarkers which will be described in a separate publication. This was based on earlier work completed by HSL to develop a method based on a major animal biomarker (Pen-COOH) (Jones et al. 2009), the presence of which being confirmed in potentially exposed Italian farmers (Silva Fustinoni, personal communication).

HSL participates in external quality assurance schemes for chlorpyrifos and cypermethrin (G-EQUAS, www.g-equas.de). Detection limits were comparable to previous reported studies looking at general population levels. Aliquots of positive samples were reanalysed throughout the project to evaluate sample stability. There was no evidence of sample degradation for any biomarker studied throughout the assessment period. Further analytical methodology details are available in Appendix 2.

Data analysis: ***Comparison of spray event and background pesticide urinary biomarker concentrations***

The characteristics of the participants were summarised, along with summaries of their responses to their background questionnaires, in terms of percentage in each category or mean and range for continuous variables. Here we report only those participants who provided at least one spray-event related sample.

Before any data analysis of the urinary biomarker concentrations took place, a number of necessary exclusions were made based on their pre-defined eligibility for inclusion in the study (at least one spray event related sample and at least one background). Urine samples where the creatinine level was below 2 or greater than 30 mmol/l (0.23 g/l and 3.39 g/l respectively) were excluded (Cocker et al. 2011, EWDTS, 2002).

All the results reported are in terms of the creatinine corrected levels. These were obtained by dividing the urinary biomarker concentration by the creatinine concentration and are reported as µg biomarker per g creatinine.

A very high proportion of samples was below the analytical limit of detection (LOD) for captan (89%), cypermethrin (93%) and penconazole (89%) and for these three pesticides only the proportion of detects, 95th percentile and the maximum levels are reported. For chlormequat and chlorpyrifos a random imputation procedure was used to replace the values below the LOD. The geometric mean (GM) and geometric standard deviation (GSD) of concentrations above the LOD were determined, assuming a log-normal distribution. It should be noted that a number of different methods were originally considered to identify the best procedures for replacing values below the LOD, and a sensitivity analysis (not reported here) indicated there was little effect on the overall results. Each of the values below LOD was then replaced by a value between 0 and the LOD which was randomly generated from the log-normal distribution. As is typical of data of this nature, concentrations were not normally distributed. Transforming by taking the natural logarithm improved the normality of the data significantly and therefore the sample results were summarised in terms of GM and GSD (Helsel, 2005).

For each individual the GM of their urinary biomarker concentrations was determined separately for samples provided after a spray event and for within and outwith spray season backgrounds. The GM ratio of the spray event sample to the backgrounds was then calculated by taking the log of each individual ratio, averaging and then exponentiation. Results are reported as GM and associated 95% confidence interval. A confidence interval containing the value 1 means that the ratio is not significantly different from 1, i.e. spray event samples are not significantly higher or lower than backgrounds.

The data were further examined to investigate factors from either the background or sample-related questionnaires that might explain any differences between the biomarker concentrations. General Linear Mixed Models (GLMM) were used with the log of the biomarker level as the response, where the participant was treated as a random variable and sample type as a fixed effect of *a priori* interest. The levels of sample type were defined as; outwith and within spray season backgrounds, samples provided the day after spraying (24 hours), samples provided the second day after a spray event (48-hours) or where the sample was provided after 2 consecutive days of spraying (24 and 48 hours). Variables were considered in a step-wise manner for inclusion in the model where they significantly improved the fit of the model (based on the likelihood ratio test) or significantly affected the coefficients of terms previously included (by > than 10%).

Imputation was carried out using R v3.1.0 (R Core Team, 2014), while all statistical analyses were carried out using Genstat v16 (VSN International, 2013) and plots were prepared using Sigmaplot v10 (Systat Software, San Jose, CA).

Predicting residents exposure using regulatory exposure assessment (REA) tools

Data for all spray events that involved products containing the relevant pesticides and for which urine samples were collected from participants were entered into a Microsoft Excel file in an anonymised format. This file was forwarded to a member of the Chemicals Regulation Directorate (CRD) (the body responsible

for regulating pesticide products in the UK). The representative used the information provided to predict the residents' potential exposures using the approaches to assess exposures in the regulatory process at the time of reporting (2013-2014) (HSE, 2012). These independent predictions where made without any knowledge of the urinary biomarker results obtained from the participants.

The regulatory exposure assessments (REA) consider three pathways of exposure (HSE, 2012). The first of these is direct exposure to spray drift at the time of application. Based on values derived from generic field trials, estimates are made of the amount of pesticide that could be deposited on the skin and enter the breathing zone of individuals 8 metres from the sprayer. From these amounts, using dermal absorption data and information on inhaled air volumes, estimates of the total systemic amounts were calculated. As the generic field data were limited to measurements on adults estimates of direct drift were only made for adults. The second pathway is inhalation of pesticide vapour following volatilisation from plant or soil surfaces after the application. In this case estimates of potential daily inhalation were made using worst-case estimates of daily concentrations of residues in air based on field monitoring data. The estimates of vapour exposure were made for both adults and children, assuming they are continuously sited next to the treated crop. The third pathway is that of exposure via contact with treated surfaces after application. This scenario is conceptualised by considering exposure of a young child playing on a lawn in a garden next to a sprayed crop where pesticide spray has drifted onto the grass. In this case direct dermal transfer and uptake following contact with pesticide residues on turf, hand-to-mouth transfer and object-to-mouth transfer of pesticide residues were the routes of exposure assessed. The contributions of the different routes in each of these pathways were summed to provide total estimates for each pathway. For each spray event, the pathway providing the greatest predicted estimate of exposure for the participant (adult or child) was used for comparison with the measured urinary biomarker concentrations.

When considering proposed uses of pesticides the regulatory exposure assessment (REA) considers the worst-case directions for use which are expected to produce the highest exposures. However, actual uses are often less testing, for example pesticides are often used at lower doses. Therefore, individual estimates were made reflecting the reported use details for each of the application events as provided by the farmers. Data on method of application, applied dose (weight of active substance/ha), and spray volume (litres of spray/ha) were used. In a small number of cases spray volume data were not reported. Missing spray volume data for 38 broadcast air assisted applications were assigned a value of 250 l/ha; this was the mode for the remaining 252 applications with a mean of 279 l/ha. Four missing ground boom values were assigned a value of 200 l/ha; the mode of 51 remaining, with an arithmetic mean of 156 l/ha. Dermal absorption values applied for the individual products were taken from current regulatory assessments (Table 3), with some of the data originating from unpublished studies submitted by applicants to support their products (P. Hamey, personal communication).

Table 3: Dermal absorption values used in regulatory exposure estimates

Active substance	Dermal Absorption (%)
Captan	2
Chlormequat[1]	4
Chlorpyrifos[2]	1
Cypermethrin[3]	10
lambda-cypermethrin	0.3
Zeta-cypermethrin	7

[1] EFSA (2008) [2] EC (2005a) [3] EC (2005b)

There are a number of mathematical models that could be used to predict the urinary pesticide output, ranging from a simple one compartment model (which treats the body as a kinetically homogenous unit, with plasma or serum as the anatomical reference compartment) to more complex physiologically-based pharmacokinetic (PBPK) models which make use of physiological and biochemical information to quantify pharmacokinetic processes influencing distribution and disposition of chemicals within an organism. We used a simple PK or toxicokinetic model (hereafter referred to as TK model) to predict the urinary biomarker concentration after a specified exposure. The chosen model was used in a previous study of pesticide exposure in bystanders (Sleeuwenhoek et al. 2007) and is based on that of Rigas et al. (2001).

The model assumes that the pesticide is absorbed into a single compartment within the body, as a single bolus dose, D. Equation [1] describes how this dose of pesticide is then converted into levels of biomarkers of interest. The parameters used in this equation are described in Appendix 3.

The model then describes the movement of this biomarker out of the body and into the urine through two equations one to describe the concentration of the biomarker in the body [2], the other to describe the concentration in the urine [3]. k_e is calculated from the half-life (HL).

$$D_M = \frac{S.R}{V_d \frac{M_o}{M_m}} D \quad [1]$$

$$\frac{dC_B}{dt} = -k_e C_B \quad [2]$$

$$\frac{dC_U}{dt} = k_e C_B \quad [3]$$

$$k_e = \frac{\ln(2)}{HL} \quad [4]$$

The concentration of biomarker in the urine was then obtained by calculating the concentration in the urine at the time of sampling, minus the concentration in the urine at the time of last urinary void.

In order to predict the urinary concentration the dose (D), as determined via the REA (in µg/kg BW/day), was assumed to be received as an instantaneous systemic dose at four different time points (8am, 12pm, 4pm and 8pm) on the day of spraying. The different time points were used as it is not possible to know exactly when exposure may have occurred following the spray activity and were chosen to reflect both the typical earliest and latest spray times reported by the participating farmers. The TK model was run to predict the urinary biomarker concentration on the morning of day 2 (24-hours) and day 3 (48-hours), where day 1 was assumed to be the day that the spray application took place. Assumptions were made about the timing of the last urine void the previous evening, these being between 8pm and 12am for adults, and 6pm and 9pm for children, with a time chosen randomly for each run of the model. Appendix 3 outlines the information used by the model for each pesticide and its associated biomarker. Oral half-lives and selectivity ratios were used for the modelling carried out here but, where available the dermal parameters are given in the table for completeness. Where a range was available for a given modelling parameter (selectivity ratio (the ratio of biomarker of interest to other biomarkers formed), stoichiometric ratio (optimum amount where all the reagent is consumed in the reaction, i.e. metabolism), time of last void, volume of distribution) the value used was randomly generated from this range. The model was then run 1000 times for each to obtain an average REA-based prediction for each sample result.

Based on statistical analysis of the measured urine sample results there was very little difference in the background concentrations of chlorpyrifos biomarker regardless of whether the samples were obtained within spray season or outwith and regardless of area. There were apparent differences in the outwith season background levels for chlormequat, with East Lothian samples being significantly lower than those in Kent and Norfolk. It is evident that background levels of both pesticides are measured in the urine and as the TK model assumes complete elimination over time it is clear that adjustments of the predictions are required. To account for this we obtained background corrected predicted ($Predicted_{BC,}$ equation [5]) estimates of exposure by adding the individual's average within season background (BG_j) urinary biomarker concentration to the model predicted exposure ($P_{i,j}$), rather than subtracting from the measured urinary biomarker concentration, so as to avoid negative values.

So for individual j, sample i, the $Predicted_{BC}$ was calculated by:

$$\text{Predicted}BC_{i,j} = P_{i,j} + BG_j \quad [5]$$

GM and GSD are presented for both the background-corrected REA predicted exposure concentrations and the measured urinary biomarker concentrations. In addition the GM ratio of the background-corrected REA predicted exposures to the measured urinary biomarker concentrations and the proportion of measurements above the associated predicted concentrations, shown separately for 24 and 48-hour samples and for the four initial assumed exposure times (8am, 12pm, 4pm and 8pm) are given. Due to the high level of non-detects in the captan, cypermethrin and penconazole samples, we present only the proportion where the measured urinary biomarker concentration was greater than the REA predicted exposure. Plots of the $predicted_{BC}$ against the measured are also shown for chlorpyrifos and chlormequat.

Further comparisons were then undertaken using the background urinary biomarker samples. For each individual the average REA-based prediction of their associated spray events was calculated (REA_j), this was then corrected, as for the predictions above, by adding the individual's average within season background (BG_j) urinary biomarker concentration, to obtain the background corrected average REA-based prediction for each individual.

$$\text{Predicted}BC_j = REA_j + BG_j \qquad [6]$$

The proportion of background urinary biomarker results above this average background corrected REA-based prediction was then compared to the proportion of the spray event urinary biomarker results above the REA-based predictions by carrying out a binomial test of proportions.

The model was coded and run in Matlab (Mathworks); all statistical analysis was done using Genstat (VSN International, 2013) and R (R Core Team) and all plots were generated using Sigmaplot (Systat Software).

Results

Study population

A total of 13 farms participated during 2011 and 17 during 2012 and sprayed pesticides containing at least one of the relevant active ingredients. All participating Kent farms were orchards whereas the remaining farms were all arable. Chlormequat and cypermethrin were applied by the East Lothian and Norfolk participating farms whereas captan, chlorpyrifos and penconazole were applied by the Kent farms (Table 4). The number of spray days indicated was derived by adding the number of days each pesticide was applied by each participating farm on a field within 100 m of the participants. It does not take into account whether any spray event related urine samples was collected coinciding with these events. It is clear however that captan (a fungicide) was the most frequently applied for the five pesticide products and cypermethrin the least.

Table 4: Number of participating farms and relevant spray days by pesticide and geographical area

		Spray days (n)				
Area	**Farms (N)**	**Captan**	**Chlormequat**	**Chlorpyrifos**	**Cypermethrin**	**Penconazole**
East Lothian	7	0	22	0	2	0
Kent	9	118	0	27	0	33
Norfolk	4	0	9	0	1	0
Total spray days		118	31	27	3	33

Table 5 provides a breakdown of the number of households contacted by introductory letter to participate in the study, by area, and the number and percentage of those contacted where a household resident agreed to participate.

Table 5: Households approached and agreeing to participate in the study

Location	Contacted (N)	Participated (N)	Participation rate (%)
East Lothian	335	50	15
Kent	302	58	19
Norfolk	231	48	21
Total	868	156	18

A total of 296 participants were recruited to the study (238 adults, 58 children) (Figure 1). Of these, just over half (149) provided at least one sample result that was related to a relevant spray event for captan, chlormequat, chlorpyrifos cypermethrin or penconazole.

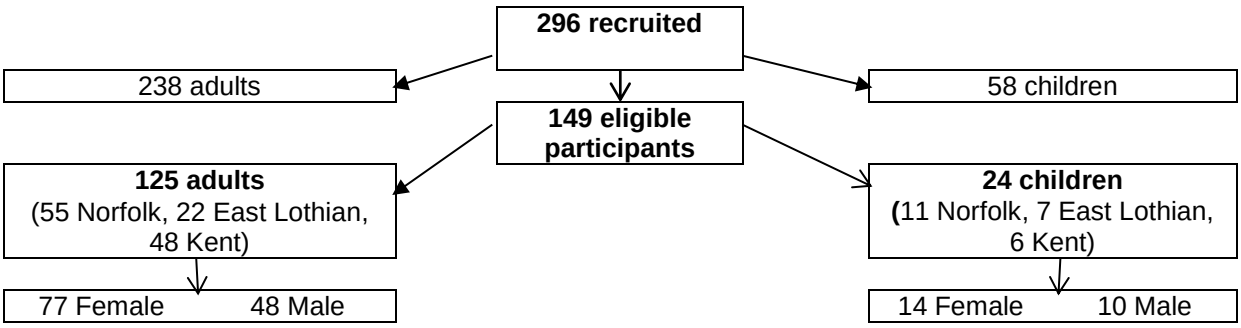

Figure 1: Participant numbers by area, sex

Table 6 summarises the characteristics of the participants who provided at least one relevant spray event related sample and the information provided in their background questionnaire. The majority of the participants were adults and female. A greater proportion of these participants were from Norfolk with fewer being from East Lothian. Just over half of the eligible participants owned a pet and 80% reported using pesticides in their home at least once a year.

Table 6: Description of the participants (background questionnaire responses)

Descriptor	Number	%
Adult	125	84
Child	24	16
Sex		
Male	58	39
Female	91	61
Location		
East Lothian	29	19
Kent	54	36
Norfolk	66	44
Employment (Adults)		
Full Time Employment	32	21
Part Time Employment	38	26
Full Time Education	1	1
Part Time Education	41	28
Retired	9	6
Non-Paid Employment	1	1
Missing or No Answer	27	18
Education (Children)		
Secondary	2	1
Primary	20	13
Full Time Nursery	0	0
Part Time Nursery	2	1
No Answer	125	84
Smoke	6	4
Own a pet	85	57
Job involves travelling around the local area	22	15
Use pesticides at work	6	4
Family use pesticides at work	7	5
Use pesticides in the home at least once a year	119	80
	Mean	Range
Age at time of recruitment (Adult)	56	(18 - 83)
Age at time of recruitment (Child)	8	(4 - 12)

Urine samples

3,275 urine samples were obtained but a high proportion of these were not linked to a relevant, or any spray event. Following the application of necessary creatinine exclusion criteria, the final dataset for statistical analysis was obtained from 1,587 urine sample results for 149 participants, consisting of 1045 background samples (484 outwith and 561 within spray season) and 542 spray-event samples.

Urinary biomarker results

Table 7 summarises the urine sample results for the five relevant pesticides for spray events and backgrounds, both within and outwith the spray season.

Table 7: Urinary biomarker concentrations (µg/g creatinine), for spray events and backgrounds, both within and outwith the spray season

Pesticide	N	N<LOD	%<LOD	Max	GM	GSD	95th percentile
Captan							
Outwith	484	422	87	3.5	**	**	0.4
Within	559	495	89	3.9	**	**	0.5
Spray	255	232	91	1.2	**	**	0.2
Chlormequat							
Outwith	484	17	4	281.6	13.3	3.1	75.0
Within	561	4	1	388.2	16.6	2.8	89.5
Spray	197	3	2	248.1	15.4	2.7	72.4
Chlorpyrifos							
Outwith	484	63	13	22.7	3.0	2.2	9.5
Within	560	69	12	76.4	3.0	2.4	10.7
Spray	63	7	11	14.8	2.5	2.1	7.9
Cypermethrin							
Outwith	387	372	96	15.4	**	**	***
Within	355	318	90	10.8	**	**	5.2
Spray	46	45	98	7.0	**	**	***
Penconazole							
Outwith	483	427	88	4.8	**	**	0.6
Within	556	500	90	3.3	**	**	0.8
Spray	89	72	81	5.1	**	**	0.9

N=number; LOD=Limit of Detection; Max=Maximum; GM=Geometric Mean; GSD=Geometric Standard Deviation **GM and GSD not calculated due to the high proportion of values below LOD. *** not calculated due to the proportion of values below LOD > than 95%

Summarising the sample results for each active ingredient of interest shows that for captan, cypermethrin and penconazole the proportion of values below the LOD was over 80%, regardless of whether the samples were spray event related, or backgrounds.

There were a number of spray events where both captan and penconazole were applied in the same spray tank mix. From Figure 2 it is clear that there is no relationship between penconazole and captan urinary biomarker concentrations, All points shown below the LOD lines were randomly imputed values. It is also clear that spraying both at the same time has not resulted in the levels of captan and penconazole being particularly high with only 2 measurements being above the LOD for both captan and penconazole and the majority (53) being below LOD for both pesticides.

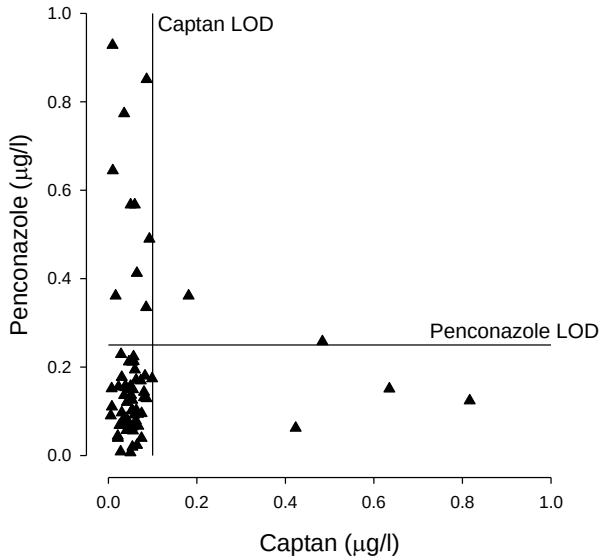

Figure 2: Creatinine adjusted urinary biomarker levels for captan and penconazole following spray events involving both pesticides

The remainder of the urinary biomarker analysis focussed on the chlormequat and chlorpyrifos samples

Figure 3 shows the creatinine adjusted urinary biomarker concentrations (µg/g) for chlorpyrifos and chlormequat for the spray event related samples as well as the within and outwith spray season background samples. These show that spray event related samples did not appear to contain elevated concentrations of urinary biomarkers compared to the background samples.

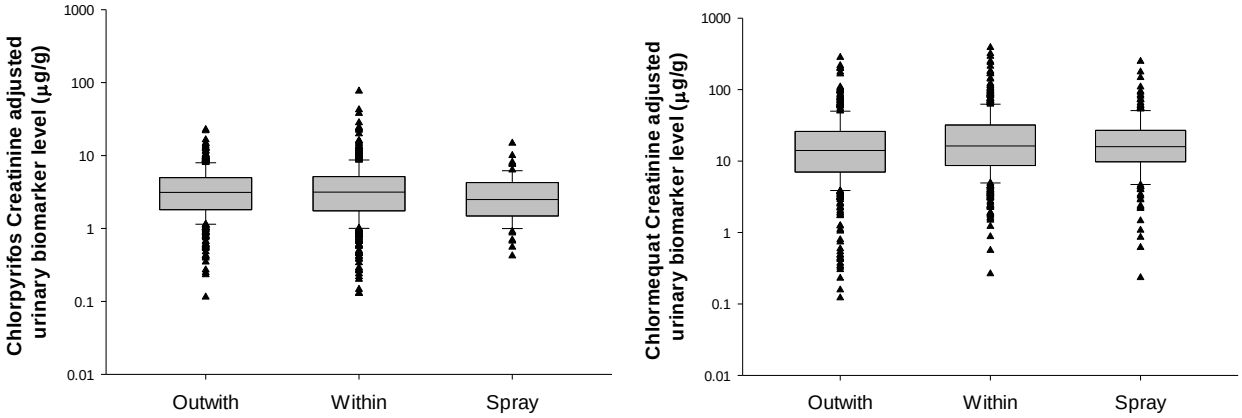

Figure 3: Creatinine adjusted urinary biomarker levels for spray event and within and outwith spray season background samples for (a) Chlorpyrifos and (b) Chlormequat

Spray event sample urinary biomarker concentrations for chlormequat were statistically significantly higher than outwith spray season backgrounds (95% CI does not include 1) but there was no significant difference between spray event-related urinary biomarker concentrations and within spray season backgrounds (Table 8). The average spray event related chlorpyrifos urinary biomarker concentrations were lower than both the outwith and within spray season background sample results.

Table 8: GM ratio of creatinine corrected concentrations following spray events to the GM of backgrounds

Pesticide	Outwith spray season			Within spray season		
	GM ratio	95% CI		GM-ratio	95% CI	
Chlormequat	1.27	1.06	1.54	1.0	0.8	1.1
Chlorpyrifos	0.85	0.63	1.16	1.0	0.7	1.3

GM=Geometric Mean; 95% CI =95 percentile Confidence Interval

Males were observed to have slightly higher creatinine adjusted urinary chlormequat biomarker levels than females (Table 9). This difference was not significant for outwith season backgrounds and spray event related samples but was significant for within spray season backgrounds (p=0.036). No statistically significant differences in the chlorpyrifos concentrations were observed between the sexes.

Table 9: Urinary biomarker concentrations (µg/g creatinine) for males and females for chlormequat and chlorpyrifos, for spray events and backgrounds, both within and outwith the spray season

Pesticide	Female				Male			
	N	N<LOD	GM	GSD	N	N<LOD	GM	GSD
Chlormequat								
Outwith	284	11	12.8	3.3	200	6	13.9	2.9
Within	338	4	15.5	2.8	223	0	18.3	2.7
Spray	120	1	14.4	2.6	77	2	17.1	2.7
Chlorpyrifos								
Outwith	284	41	3.2	2.1	200	22	2.7	2.3
Within	41	7	2.5	2.2	22	0	2.5	1.9
Spray	337	42	3.0	2.4	223	27	2.9	2.4

N=number; LOD=Limit of Detection; GM=Geometric Mean; GSD=Geometric Standard Deviation.

There were no statistically significant differences in the urinary biomarker concentrations of chlormequat and chlorpyrifos between adults and children (Table 10) although the concentrations of creatinine adjusted urinary biomarkers were higher in samples provided by children. The variation in the children's sample results also tended to be greater. To ensure these patterns were not the result of variation in creatinine levels, which tend to be higher in males than females and in adults than children, the uncorrected creatinine urinary biomarker concentrations were analysed and similar patterns were observed.

Table 10: Urinary biomarker concentrations (µg/g creatinine) for adults and children for chlormequat and chlorpyrifos for spray events and backgrounds, both within and outwith the spray season

Pesticide	Child				Adult			
	N	N<LOD	GM	GSD	N	N<LOD	GM	GSD
Chlormequat								
Outwith	68	3	13.2	4.2	416	14	13.3	3.0
Within	40	0	17.7	2.8	157	3	14.9	2.6
Spray	81	3	19.7	3.3	480	1	16.1	2.7
Chlorpyrifos								
Outwith	68	4	3.6	2.1	416	59	2.9	2.2
Within	8	1	2.2	2.3	55	6	2.6	2.0
Spray	81	4	3.8	2.5	479	65	2.8	2.4

N=number; LOD=Limit of Detection; GM=Geometric Mean; GSD=Geometric Standard Deviation.

There were no statistically significant differences in chlormequat or chlorpyrifos concentration for the first morning void samples collected the day or two days after the spray events (Table 11).

Table 11: Description of spray sample results by whether the sample was the day after a spray event (24 hours) or two days after (48 hours)

Pesticide	24 hour spray samples					48 hour spray samples				
	N	N <LOD	GM	GSD	95th %	N	N <LOD	GM	GSD	95th %
Chlormequat	100	1	13.7	2.9	83.0	97	2	14.2	3.0	57.4
Chlorpyrifos	27	4	2.6	2.4	11.1	33	2	2.8	2.1	7.7

N=number; LOD=Limit of Detection; GM=Geometric Mean; GSD=Geometric Standard Deviation; 95% = 95th percentile.

Hierarchical, multivariate statistical analyses were run to explain any differences between the biomarker concentrations (Table 12). For chlormequat, after including sample type in the model the only significant factor, based on Wald test for addition to the model, was the level of organic food consumption reported. However this factor did not significantly improve the fit of the model and so was not included. The statistically significant differences in the urinary biomarker levels between sample type is driven by the outwith season backgrounds being lower than spray event related samples and within season backgrounds. For chlorpyrifos there is no significant difference between spray event and backgrounds. Although some

other factors such as sex, age, time spent on/outdoors, were significant, according to the Wald test, they did not significantly improve the fit of the model and were not included.

Table 12: The optimal mixed effects model for chlormequat and chlorpyrifos, considering all available factors, after inclusion of sample type in the model. The exponential of the coefficient is shown as the analysis was carried out on the log scale. The p-value for the inclusion of each factor is given in the table.

	Chlormequat		Chlorpyrifos	
	Exp(coeff)	P-value	Exp(coeff)	P-value
Constant	13.9		2.97	
Sample Type		<0.001		0.764
Outwith	1.0		1.0	
Within	1.3		1.0	
24 Hours	1.3		0.9	
48 Hours	1.2		1.0	

Comparisons with regulatory exposure assessment estimates

The regulatory exposure assessments (REA) consider three pathways of exposure: (1) direct exposure to spray drift at the time of application (dermal and inhalation), (2) inhalation of pesticide vapour following exposure following volatilisation of the pesticide from the treated crop or soil surfaces after application and (3) direct dermal contact and uptake from contact with treated surfaces after application. Table 13 details for each pesticide, separately for adults and children, the pathway which resulted in the highest exposure estimate as well as the range of predicted exposures for this pathway based on the spray event information provided by the farmers. In addition the Table 13 provides details of the Acceptable Operator Exposure Level (AOEL) for each of the pesticides.

Table 13: REA pathways resulting in the highest predicted exposure (and the range of estimates) based on farmers spray event information and the AOEL for each pesticide assessed

Pesticide	**AOEL (µg/kg BW)**	**Adult**		**Child**	
		Pathway	**Predicted exposure (µg/kg BW)**	**Pathway**	**Predicted exposure (µg/kg BW)**
Captan	100	3	8.0-24.0	2	8.30
Chlormequat	40	3	5.0-21.6	2	0.53
Chlorpyrifos	10	3	4.8	2	8.30
		2	3.8		
Cypermethrin	20	3	0.5-1.3	2	0.53
Penconazole	30	2	3.8	2	8.30

Pathway 2- inhalation following volatilisation of the pesticide after spray event; Pathway 3 – direct contact with treated surfaces and plants; BW- body weight

For adults, in most instances, (3) direct dermal contact and uptake following contact with treated surfaces was the pathway that resulted in the highest exposure estimate although for chlorpyrifos and penconazole the (2) volatisation of pesticides from the treated crop and surfaces was also used. (For chlorpyrifos the selection of either of these pathways in the comparisons was dependent on the highest exposure estimate generated for the particular spray event). Pathway 2 which predicts exposure following the volatisatision of the pesticides from the treated crop / soil surfaces was used in all the child comparisons. It was evident that the highest estimated pathway of exposure for the spray events assessed for the various pesticides was well below the AOEL.

Due to the high level of non-detects in the captan and cypermethrin samples only the number of samples above the REA-based predicted exposure concentrations are reported. Table 14 reports the number and percentage of captan and cypermethrin urinary biomarker results greater than the REA-based predicted exposures, assuming exposure occurred at 8am for samples collected one (24-hours) and two days (48-hours) after the spray event.

All measured 24-hour urinary biomarker concentrations for captan were below the exposure estimated to be present in their urine using the REA-TK models. 5% of the 48-hour urinary biomarker results were found to be above predicted urinary biomarker concentration. All cypermethrin urinary biomarker results were found to be below the level estimated to be present in their urine based on the REA-TK model predictions. There was one penconazole measured urinary biomarker result above the predicted exposure.

Table 14: Number and percentage of captan, cypermethrin and penconazole urinary biomarker results higher than the REA-TK- based predicted exposure, assuming exposure occurred at 8am

Pesticide	Samples collected day (24-hour) after spray event			Samples collected two days (48-hour) after spray event		
	N	N Measured > Predicted exposure	% Measured > Predicted exposure	N	N Measured > Predicted exposure	% Measured > Predicted exposure
Captan[#]	94	0	0	140	7	5
Cypermethrin	22	0	0	24	0	0
Penconazole	29	0	0	53	1	1.9

#25 samples were both 24 and 48 hour samples due to spraying on two consecutive days

For chlorpyrifos, there were 3-4 measured urinary biomarker concentrations above the predicted exposures at 24 hours, and 6-8 measured concentrations above the predicted exposures at 48 hours (depending on estimated timing that exposure occurred (Table 15). Figure 4 shows that in all instances the predicted urinary biomarker concentrations (uncorrected) are below those concentrations estimated if exposure to the AOEL had occurred. Children seem to have higher concentrations than adults, due to the REA predicted exposures being higher (8.3 V 3.8-4.8 µg/kg body weight).

Table 15: Comparison of measured with predicted (based on REA-TK exposure predictions) biomarker levels for chlorpyrifos. The GM predicted levels is provided along with the GM ratio of the predicted to the measured.

	Day after spray					2 Days after spray				
	N	N Measured> Predicted exposure	GM (µg/l)	GSD	GM Ratio	N	N Measured > Predicted exposure	GM (µg/l)	GSD	GM Ratio
Measured spray event biomarker conc.			2.5	2.4				2.6	2.2	
Measured background biomarker conc.			3.0	2.2				3.0	2.2	
Predicted exposure										
8am	24	4	3.6	1.3	0.4*	30	8	1.9	1.3	0.5*
12pm	24	4	4.0	1.3	0.4*	30	8	2.1	1.3	0.5*
4pm	24	4	4.5	1.3	0.4*	30	8	2.3	1.4	0.5*
8pm	24	3	4.9	1.3	0.3*	30	6	2.6	1.4	0.5*

*Predicted significantly higher than measured; N=number; GM=Geometric Mean; GSD=Geometric Standard Deviation

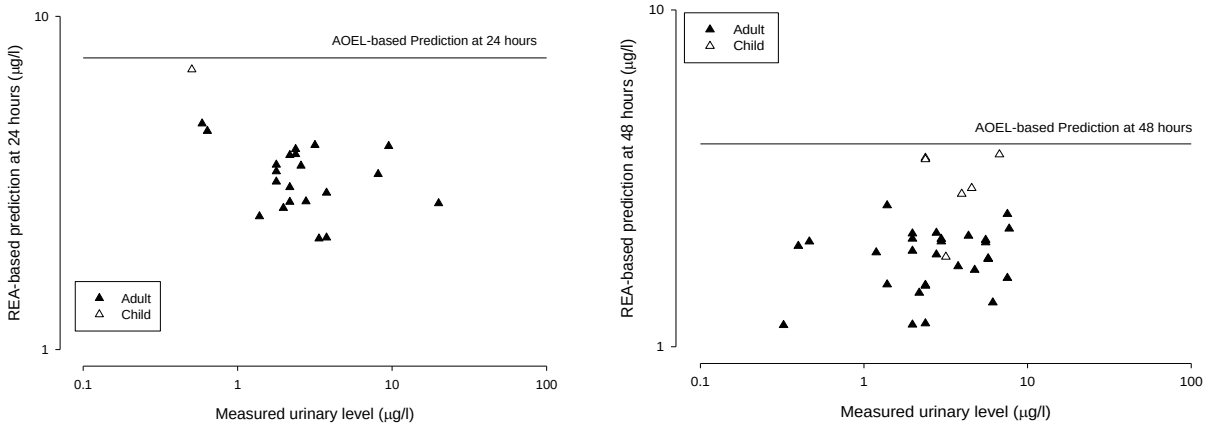

Figure 4: The measured urinary biomarker concentration for chlorpyrifos spray event related samples vs the REA-TK based-prediction at 24 hours (left) and 48 hours (right). The solid line is the prediction obtained from the model where the AOEL is used to determine the dose.

For chlormequat, there were 12-39 measured urinary biomarker concentrations above the predicted REA-TK exposures at 24 hours, and 41-43 measured urinary biomarker concentrations above the predicted REA-TK exposures at 48 hours (Table 16). This equates to around 40% for measured urinary biomarker concentrations being in excess of the predicted exposures.

Table 16: Comparison of measured with predicted (based on REA-TK exposure predictions) for chlormequat. The GM predicted levels is provided along with the GM ratio of the predicted to the measured

	Day after spray					2 Days after spray				
	N	N Meas>Est	GM (µg/l)	GSD	GM Ratio	N	N Meas>Est	GM (µg/l)	GSD	GM Ratio
Measured spray event biomarker conc.			13.7	2.9				14.0	3.0	
Measured background biomarker conc.			12.3	3.1				12.3	3.1	
Predicted exposure										
8am	99	39	0.3	4.0	0.7*	96	43	0.1	4.9	0.8*
12pm	99	34	1.3	3.5	0.7*	96	43	0.1	4.9	0.8*
4pm	99	24	6.8	3.7	0.5*	96	43	0.1	4.9	0.8*
8pm	99	12	27.2	4.2	0.2*	96	41	0.2	4.9	0.8*

Predicted significantly higher than measured; N=number; GM=Geometric Mean; GSD=Geometric Standard Deviation

Figure 5 shows that in all instances the predicted urinary biomarker concentrations (uncorrected) are below those concentrations estimated if exposure at the AOEL had occurred. From Figure 5 we can see that there is a clear split between the REA-based predictions for children and adult participants. Children have a lower REA-based predicted exposure for chlormequat as their REA exposure estimate is a lot lower than for adults, as is their body weight. The measured urinary biomarker concentrations do not show any relationship with the REA-based prediction.

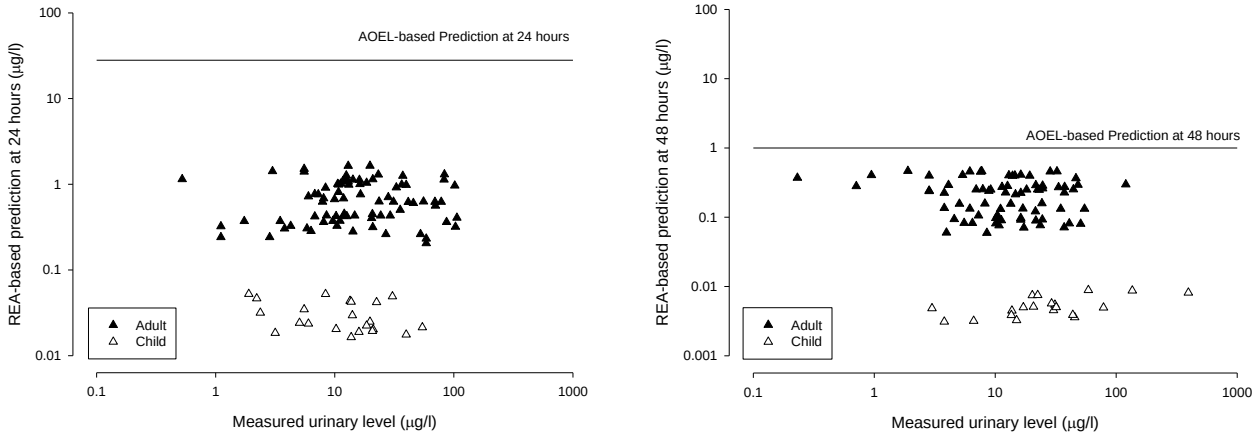

Figure 5: Measured urinary biomarker chlormequat concentration for spray event related samples vs the REA based-prediction at 24 hours (left) and 48 hours (right). The solid line is the prediction obtained from the model where the AOEL is used to determine the dose

To understand whether the number of results higher than the REA-TK based prediction differs from what would be expected, an investigation of the background levels in relation to the predicted levels was also undertaken.

Figure 6 shows the background corrected REA-TK-based prediction against the measured urinary biomarker chlormequat concentrations. The points appear to be equally scattered around the line of equality, at both 24 and 48 hours indicating that, while there are some measured levels higher than predicted, on average they are equal. Looking at the plot of the background-corrected, average REA-based prediction as compared to the measured urinary background levels (Figure 7), the pattern is similar. There is slightly more scatter when comparing the outwith season background level to the background corrected average REA-based prediction. As the levels are lower, on average, outwith the spray season, this is as you would expect.

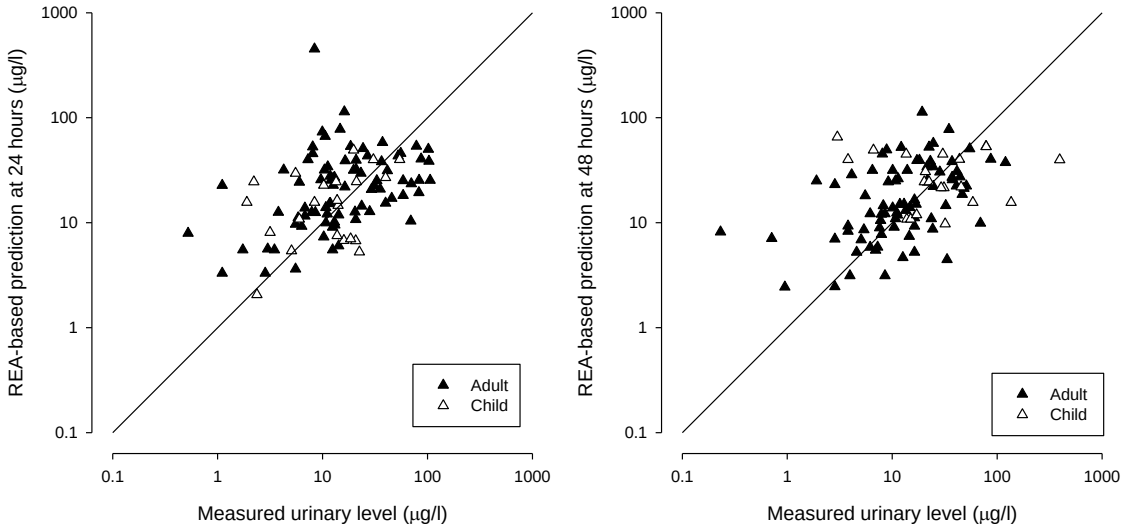

Figure 6: Scatterplot of measured urinary biomarker chlormequat concentrations against the background corrected REA-based urinary predictions. This is at 24 hours (left) and 48 hours (right). The symbols distinguish between adults and children.

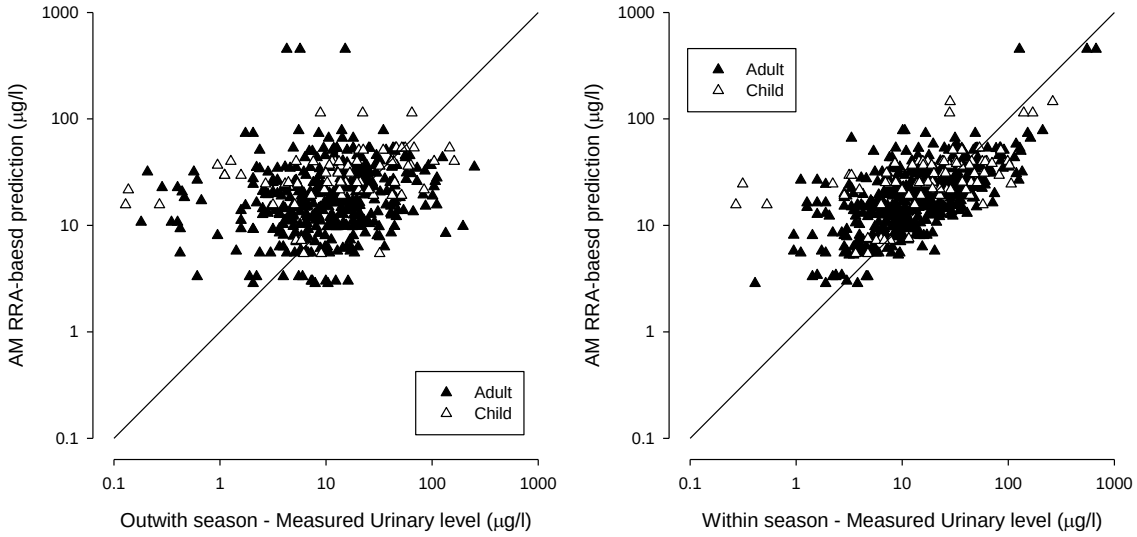

Figure 7: Scatterplot of measured urinary biomarker background chlormequat concentrations against the arithmetic mean background corrected REA-TK- based urinary biomarker predictions. Left: Outwith season backgrounds; Right: Within season backgrounds. The symbols distinguish between adults and children.

The pattern is similar for chlorpyrifos (Figures 8 and 9), although with fewer measured being higher than background-corrected predicted.

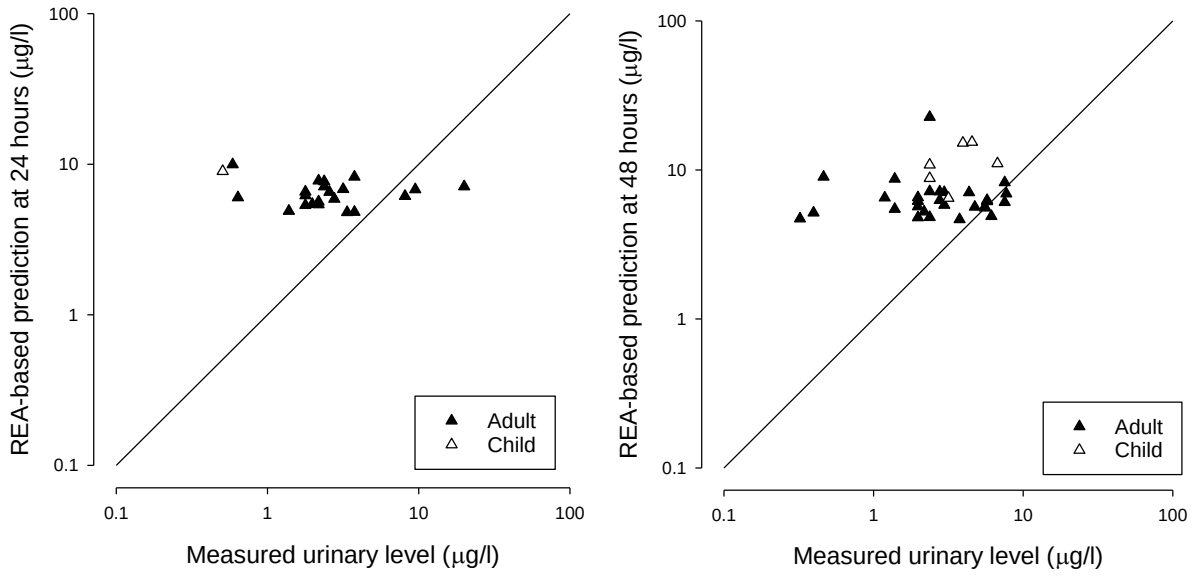

Figure 8: Scatterplot of measured urinary biomarker chlorpyrifos concentrations against the background corrected REA-based urinary predictions. This is at 24 hours (left) and 48 hours (right). The symbols distinguish between adults and children.

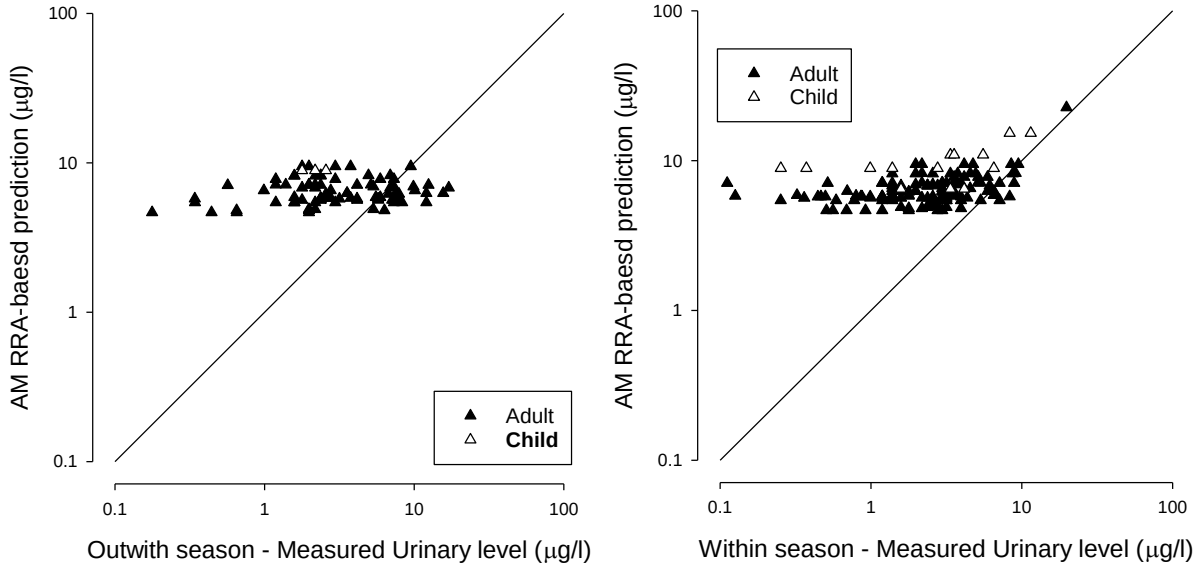

Figure 9: Scatterplot of measured urinary biomarker background chlorpyrifos concentrations against the arithmetic mean background corrected REA-TK- based urinary biomarker predictions. Left: Outwith season backgrounds; Right: Within season backgrounds. The symbols distinguish between adults and children.

Table 17 shows that the percentage of spray-event related measurements above background corrected predictions for chlorpyrifos is not significantly different to what you would expect as the p-value for the binomial test for difference is greater than 0.05. Similar for chlormequat with the exception that the percentage of spray event related measurements above predicted is significantly different to what we would expect in background samples taken outwith the spray season.

So although a number of the chlorpyrifos and chlormequat spray event related urinary biomarker concentrations were greater than the REA-TK-based predictions these were not significantly different to what would be expected had no spray event occurred.

Table 17: Comparison of measured spray and background urinary biomarker concentrations with predicted (based on REA-TK exposure predictions) exposure for chlorpyrifos and chlormequat

	Day after spray				Two days after spray event			
	N	Measured > Predicted exposure (N)	Measured > Predicted exposure (%)	P-value	N	Measured > Predicted exposure (N)	Measured > Predicted exposure (%)	P-value
Chlorpyrifos								
Spray	24	4	17		30	8	27	
Outwith	143	10	7	0.114	143	9	6	0.100
Within	88	30	34	0.100	88	30	34	0.452
Chlormequat								
Spray	99	39	39		96	43	45	
Outwith	302	91	30	0.088	302	91	30	0.008
Within	322	121	38	0.745	322	125	39	0.204

Discussion

The aims of this research were to assess exposure to pesticides for adults and children living within 100m from agricultural land and investigate if exposures were elevated following spray events. In addition it aimed to assess whether the methods used in the UK pesticides approval process are appropriate for assessing exposure amongst residents living near fields.

There were a number of issues that needed to be overcome to achieve these aims. The relatively short biological half-life of modern pesticide compounds or their biomarkers in the human body presents a major challenge to linking biological monitoring data to specific spray events and so urine samples should be collected ideally within 24 hours, and no later than 48 hours following the spray events. Farming activities are inherently unpredictable because of the changing weather and the presence of insects or other potentially damaging infestations and so there was a need for effective engagement and development of good/trusting relationships with residents to obtain the spray event samples. Through the use of community researchers, located in and knowledgeable of the geographical areas and farming practices within which they worked, over 3,000 urine samples were collected of which over half fulfilled the sample and data analysis inclusion criteria to fulfil the project aims and objectives (Teedon et al, in draft).

The methodology applied in this study was robust but was not without some limitations. The participating farmers may not have been representative of all farmers within the study areas although there is no reason to believe that their spraying practices were any different from those in the wider farming communities. The farmers were enthusiastic and willing to share their spray records. This information was used in the comparisons of the exposure estimates generated using the REA process, rather than 'worst-case' spray scenarios as typically used in the regulatory process which would have resulted in higher predicted estimates of exposure and a less robust testing of the assessment process. In addition, the REA expects operators to follow good agricultural practices when spraying pesticides. It was clear from the farmers spray records that a number of pesticide products are applied throughout the spraying season (dependent on crops, infestation, weather conditions etc) and so the number of spray events for the relevant pesticides may differ on a yearly basis. For example, in 2012 the year began with ongoing concerns over long-term drought which was followed by an abrupt shift in weather patterns bringing an exceptionally wet period for most of the country from April lasting through much of the summer. The wet weather affected the harvest of the 2012 crops (DEFRA, 2013). In June 2011 there were 'near drought' conditions in South East England, with report of the driest conditions across England and Wales since 1900 (DEFRA, 2012). Spray event start and finish times were obtained and residents were asked to provide details of their activities in the 48-hour period prior to provision of each urine sample. However it was not possible to establish from this when, where or how the residents' potential exposure to the assessed pesticides may have occurred. To allow for the comparison with the REA predictions, a number of time periods were considered to accommodate this lack of information.

All laboratory methods for the urine samples showed good day-to-day repeatability and all (except penconazole) were based on established methods. For penconazole, a novel method was developed by HSL during the study (Jones et al, draft), based on earlier work to develop a method based on a major animal biomarker. External quality assurance was available for chlorpyrifos and cypermethrin (G-EQUAS, www.g-equas.de). All chlorpyrifos, chlormequat and penconazole samples were analysed within the timeframe of the stability trials. The length of each analyte stability trial was determined by available samples within the dataset. Samples analysed outside the time frame are therefore not necessarily unstable but we were unable to test for longer due to insufficient sample volume.

The TK model used, based on that of Rigas et al (2001), was a simple one-box model which described the excretion of biomarkers in the urine, over time, given an internal dose of pesticide. There are a number of

mathematical models that could be used to predict the urinary pesticide output, ranging from the simple one compartment model, which treats the body as a kinetically homogenous unit, with plasma or serum as the anatomical reference compartment (Lu et al, 2010, Lu and Andres, 2011, Rigas et al, 2001) to more complex physiologically-based pharmacokinetic (PBPK) models, which make use of physiological and biochemical information to quantify pharmacokinetic processes influencing distribution and disposition of chemicals within an organism (Goldsmith et al, 2012; Wen et al, 1999; Lu et al, 2010). Use of a more comprehensive PBPK model would allow estimation of the distribution of biomarker(s) around all organs of the body, and excretion, over time but there is very little information available for the required parameters which would mean that a number of assumptions would have to be made and consequent high level of uncertainty around any estimates.

The values of the parameters used for the TK modelling were obtained from published literature where information such as biological half-life was often determined via small volunteer studies. In order to evaluate how the model predictions would be affected by the value of these parameter estimates we undertook a simple sensitivity analysis. Using chlormequat as an example it was found that doubling the half-life effectively doubled the 24 hour prediction and resulted in an increase in the 48 hour prediction by a factor of 9. Similarly, doubling the selectivity ratio doubled the prediction at both 24 and 48 hours. The volume of distribution had an inverse relationship with the 24 and 48 hour predictions, where doubling the volume of distribution effectively halved the predicted values. Therefore, most of the parameters were observed to have a multiplicative effect on the prediction, with the exception of half-life, which could potentially have a big impact on the prediction, particularly at 48 hours.

Level of urinary biomarker levels

The urinary biomarker levels for captan, cypermethrin and penconazole were very low in this population with over 80% of analysed samples having undetectable concentrations. For chlormequat and chlorpyrifos, the GM of urinary biomarkers following spray events were 15.4 µg/g creatinine and 2.5 µg/g creatinine, respectively, compared to 16.6 µg/g creatinine and 3.0 µg/g creatinine for within season background.

For penconazole, we have not been able to identify any previous general population studies to compare our urinary biomarker results. This is unsurprising given the very recent development of the new analytical methods to determine human urinary biomarker for this pesticide.

For chlormequat, we have only been able to identify one other, relevant, study. Lindh et al. (2011) reported a general population study in southern Sweden where the chlormequat concentrations ranged from 0.4 – 30.2 µg/g creatinine (median 2.9, 95th percentile <17.3 µg/g creatinine, N=100). These values are somewhat lower than our reported concentrations for all the chlormequat spray event and background samples combined (median 15.1, 95th percentile 79.8 µg/g creatinine). Despite these higher concentrations, UK exposures are still two orders of magnitude below the values obtained from an oral dose at half the ADI (Lindh et al. 2011). It is possible that we observed higher chlormequat urinary concentrations than the Swedish study due to different farming practices for cereal crops and consumption of foods and beverages derived from cereal crops for which this growth regulator was applied. For example, the mean daily per capita consumption for bread and rolls, bakery products, cereal and products in the UK was reported by DAFNE (2006) as 103, 44 and 36g respectively compared to 96, 21 and 25g for Sweden.

Approximately 90% of the captan urinary biomarker concentrations in this study were below the LOD, with the maximum value of 3.9 µg/g creatinine detected in a background sample. There are very few data available on environmental exposures to captan. Berthet et al. (2012) reported mean “pre-season” urine biomarker concentrations of 0.2 µg/l (~0.15 µg/g creatinine), but this was based on only four samples. Verberk et al. (1990) reported results below the LOD (8 µg/l, ~5.9 µg/g creatinine) for six unexposed controls. Recent occupational studies have determined post-exposure THPI levels of < 5 µg/l (~3.1 µg/g creatinine) (Berthet et al. 2012). No other published general population studies could be found.

There is an extensive literature on the use of urinary 3,5,6-trichloro-2-pyridinol (TCP) as a biomarker of chlorpyrifos exposure. The US CDC NHANES study reports 95th percentiles for adults as 6.4 µg/g creatinine, N=832 and 7.4 µg/g creatinine, N=1,113 for 1999/2000 and 2001/2002 respectively (CDC, 2005). A study of 100 pregnant women in the Netherlands (Ye et al. 2008) found a 95th percentile of 6.4 µg/l (~4.7 µg/g creatinine) with a maximum of 158 µg/l (~116 µg/g creatinine). Studies in Germany (Koch et al. 2001) and Italy (Aprea et al. 1999) showed similar values. For Germany, the 95th percentile was 11.3 µg/l (~8.3 µg/g creatinine, N=50) and for Italy, the estimated 95th percentile was 6.5 µg/g creatinine (N=42). Our data for all the spray and background samples combined (95th percentile 10.1 µg/g creatinine) are comparable with these data from other general population studies, despite the varied geographical sources of the data sets. A study of residents’ exposure to chlorpyrifos after treatment inside the home (Byrne et al. 1998) showed that potential exposures to the adult residents, as indicated by urinary 3,5,6-TCP biomonitoring, did not increase as a result of the application. Another study (Dai et al. 2003) reported

urinary 3,5,6-TCP concentrations of 0.1–7.8 µg/g creatinine in 41 residents from houses where chlorpyrifos had been detected. Alexander et al (2006) reported a study of farm family members (spouses, and children aged 4-17 years) from Minnesota and South Carolina. Five consecutive 24-hour urine samples were obtained from 34 families of licensed pesticide applicators from one day before to three days after a chlorpyrifos application. The spouses' GM exposure was reported as being 3.6 µg/g creatinine pre-application, 3.8 µg/g creatinine on the day of application and then 4.2 µg/g µg/g creatinine on days 1 and 2 post application. The reported children's exposure was 5.1 µg/g µg/g creatinine pre-application, 6.0 µg/g creatinine on day of application and 5 and 5.9 µg/g creatinine for days 1 and 2 post application. These are all higher than the geometric means that we observed.

A number of general population studies have been reported for cypermethrin exposure. The US CDC NHANES study reports 95th percentiles for adults as 0.9 µg/g creatinine (N=1,128) and 2.5 µg/g creatinine (N=1,123) for cis-DCVA and trans-DCVA respectively in 2001/02 (CDC, 2005). Detection rates were reported as less than 50% for cis-DCVA and less than 25% for trans-DCVA. A study of 1,149 pregnant women in China (Qi X, 2012) found median levels of 0.7 µg/g creatinine for cis-DCVA and 1.9 µg/g creatinine for trans-DCVA. In the UK (Bevan et al. 2013) the 95th percentiles for adults (on the voting register) in the general population were reported as 0.7 µg/g creatinine (N=405) and 1.8 µg/g creatinine (N=404) for cis-DCVA and trans-DCVA respectively and 2.3 µg/g creatinine combined as total-DCVA. In comparison, the 95th percentile was 5.8 µg/g creatinine for the spray event samples and 5.2 µg/g creatinine for our within spray season samples (which includes both adults and children). Whilst the 95th percentile results for our data are greater than reported by Bevan et al. (2013), the number of samples above the LOD was far lower despite the same detection limit. These differences may reflect regional differences in exposure (our study was conducted in three regions compared to across the whole of the UK for Bevan et al. (2013)) or temporal differences in pesticide use and food residues of pyrethroids (our samples were collected in 2011 and 2012 whereas those of Bevan et al. (2013) were collected in 2005/06).

Do spray events result in elevated exposures to residents living near agricultural land?
This study investigated whether residents living near agricultural land experienced elevated exposure to pesticides' following spray events by comparing urinary biomarker levels from samples coinciding with relevant spray events and background samples both within and out with the spray season. A strategy of participants serving as their own controls, rather than recruiting a separate urban population was used to eliminate any need for matching by age, sex and other potential sources of pesticide exposure. In addition, studies that have reported on urban and rural pesticide exposures present an unclear picture, with some observing significant differences (Corture et al, 2009) whereas others do not (Kimata et al, 2009; Koureas et al 2009).

The results presented in this report provide no evidence that, in this study population, the spray events resulted in elevated exposures compared to background samples taken within the season. For chlormequat, the biomarker levels (both spray event and background) were higher within the spray season compared to outwith the season. Chlormequat is a plant growth regulator, which acts by inhibiting cell elongation hence shortening and strengthening the stem producing a sturdier plant. It also influences the developmental cycle, leading to increased flowering and harvest (Tomlin, 2009). Growth regulating products containing chlormequat are widely used by the UK. The Pesticide Usage Survey Teams of the Food & Environment Research Agency (FERA) conducted surveys of pesticide usage in arable crops in 2011/12 and reported that chlormequat applied alone or in mixtures accounted for 59% of the area of arable crops treated with specific growth regulators: in addition there was a 13% increase in area treated using chlormequat since the previous survey conducted in 2010 (Garthwaite et al, 2014).

Although not the primary aim of the study and reporting on different pesticides, Jones et al (2014) reported a statistically significant difference for dialkylphosphate levels (derived from organophosphate pesticides) in young children (<5 years) during different seasons, with autumn resulting in the highest levels. No seasonal effect was however observed for pirimicarb or carbaryl. Our chlormequat findings could be due to other sources of exposure. The most recent publication presenting results of pesticide residues in food commodities (including both raw and processed) sampled during the calendar year 2010 in the 27 European Union Member States and two European Free Trade Association countries (Iceland and Norway) reported chlormequat/oats to be the pesticide/crop combination for which residue concentrations were most frequently above the reporting level (64.6% of the samples). In addition, the highest percentage of maximum residue limit (MRL) exceedances in foods was found for chlormequat in oats (8.1% of all samples). In rye, the most frequently found pesticide residue was also chlormequat (35.9%) (EFSA, 2013). Of the 178 pesticides included in the 2010 EU-coordinated programme, the most frequent MRL exceedances were detected for chlormequat residues (3.6% of the samples). Chlormequat was also detected in a small number of organic food samples analysed (13 of the 3,571 samples), with measured residue levels ranging from 0.127-0.0011 mg/kg (EFSA, 2013). Whilst in most instances these data relate to unprocessed food commodities and residue levels may decrease during food processing, it is considered that diet is the primary source of exposure. For chlorpyrifos, the biomarker levels were very similar for the

various sample types. Finally, for captan, cypermethrin and penconazole, a very large proportion of the measurements were below the limit of detection, whether or not these samples were collected following spray events.

Are the regulatory exposure assessment methods used to assess residents' exposure suitably conservative?

When considering proposed uses of pesticides the REA process considers the worst-case directions for use which are expected to produce the highest exposures. However, in reality pesticides are often used at lower doses etc. In our comparisons the predicted exposure concentrations were made reflecting the reported use details for each of the application events as provided by the farmers therefore providing a more challenging assessment of the exposure assessment process. Although the regulatory exposure assessment process allows for the separate assessment of three pathways of exposure (1) spray drift at the time of application, (2) exposure following evaporation of the pesticide from the treated crop or soil surfaces after application as well as (3) direct contact with treated surfaces after application only the pathway providing the highest predicted exposure is then used. For our adult participants this was typically the direct contact pathways whereas for the child participants this was exposure following evaporation of the pesticide following application for the spray applications reported and considered in this study. It should be considered that the measured biomarker concentrations from the collected urine samples allows for the assessment of pesticide exposure from multiple pathways of exposure, not just that used in the comparisons, and also reflects exposure via other sources such as diet.

The TK modelling used to allow the comparison of the REA predictions with the measured urinary biomarker levels considers each pesticide in isolation. It was evident from the farmers spray records that for the majority of spray events, the relevant pesticides where applied with other products. For captan and penconazole, there were a number of occasions where these products were both applied using the same tank mixture. Information concerning the metabolism of pesticides in the human body is sparse at best and it is unknown what the impact of tank mixing may have on the modelling parameters used in this study. However, no relationship between penconazole and captan biomarker concentrations, in instances where both were sprayed at the same time, was found.

Due to the high proportion of measured urinary biomarker results for captan, cypermethrin and penconazole being non-detects only the number and percentage of samples above the predicted exposures are reported. For cypermethrin the measured urinary biomarker levels were all found to be lower than the predicted concentrations. Over 98% of the measured urinary biomarker concentrations for penconazole and 97% of measured captan urinary biomarker concentrations were found to be lower than the predicted exposures.

A greater number of measured urinary biomarker results were above the analytical limit of detection for chlorpyrifos and chlormequat. Initial comparisons of the measured urinary biomarker concentrations with the predicted exposures found that 20% of chlorpyrifos and 40% of chlormequat urinary biomarker concentrations were greater than the predicted concentrations. As no statistically significant differences in pesticide biomarker concentrations following spray events were found compared to background urinary biomarker concentrations for these pesticides we compared the background urinary biomarker concentrations with the predicted exposures to further understand these results. Overall, the proportion of measured urinary biomarker concentrations in excess of the exposures predicted for the relevant spray event were no different to what would be expected if no spray event had taken place.

Main implications of the findings

This study reports urinary biomarker concentrations for a number of active ingredients both for spray event and in background samples amongst people living within 100m of agricultural land. It also compares actual measured urinary biomarker levels with the estimated exposures generated during the pesticide approval process. As far as the project team are aware, both of these aspects have not be studied as systematically anywhere else and provide a useful dataset for further investigations in residents to pesticides.

The strategy of using community researchers to recruit participants and encourage continued sample and data provision was considered highly successful. It is suggested that any future similar exposure studies should consider adopting this strategy.

For this study population, there was no evidence of increased pesticide biomarker excretion in residents following a spray event within 100m of their home. The levels of urinary biomarkers detected in the reported population are generally comparable to other available general population studies, except for chlormequat which was higher than the one other comparable (non-UK) study. It is suggested that the chlormequat levels experienced by the participants in the present study were a result of dietary intake rather than any inhalation or dermal exposure to pesticide during local spraying events. Further studies aimed at further identifying and quantifying dietary intake of this pesticide in the UK population would help

clarify this finding.

There appeared to be a significant number of chlorpyrifos and chlormequat urinary biomarker measurements that were higher than the predicted exposures. However it is considered that this is due to variability in background levels of exposure. Overall we conclude that, for the pesticides considered in this study and the spray practices assessed, that the REA methods currently used provide sufficiently conservative estimates of residents' exposure.

The background chlormequat concentrations in the UK population were previously unknown and this dataset provides a useful resource to build upon. In addition, this is the first study to report on penconazole urinary biomarker concentrations in residents and again will be a useful resource to allow comparisons with future studies.

When considering the application of the study results to other pesticides, consideration should be given to the spray techniques used and their potential to distribute the pesticide beyond the target area and the propensity for the pesticide to redistribute post application. The spray equipment and techniques used by the participating farmers were not atypical and were reportedly used when applying other pesticide products to the given crops throughout the spray season and so we consider that our study covers both the likely spectrum and worst case vapour pressures used in modern day pesticides in the UK. Orchard spray techniques are usually considered as potentially giving rise to higher levels of pesticide drift (and therefore potential exposure) in comparison to field crop spraying practices. In addition, in the 2012 pesticide usage survey report for orchards, captan was reported as the most extensively used fungicide formation with chlorpyrifos being the most extensively used insecticide (Garthwaite et al, 2013). Given that we collected in excess of 300 orchard spray event samples, the majority of which were analysed for captan and chlorpyrifos, we also consider that our study adequately considers potentially higher risk spray techniques for the most commonly reported pesticide formulations. The majority of these urinary biomarker results were observed to be less than the analytical LOD.

Our findings may impact on further iterations of exposure models used to determine expected urinary biomarkers from spraying. Consideration should be given to whether the background levels of pesticide exposures as identified in this study need to be taken into account when REA take place although further knowledge is needed to ensure robust methodologies are adopted.

Acknowledgements
We would like to thank the following individuals (in alphabetical order) for their work as community researchers in this project: S Attwood, J Cadzow, A Carr, D Dahrendorf, T Hogg, L Jewsbury and F MacIver. Their hard work was crucial to the success of the recruitment and data collection in this project.

Thanks also to the project Advisory Committee (Prof. David Coggon, Paul Hamey, Prof. Len Levy and Dr. Sean Semple) for their helpful comments throughout the project and on earlier manuscript drafts; Dr. Anne Sleeuwenhoek and David Todd (IOM) for their assistance with the storage of the urine samples and related administration tasks; IOM personnel (Shahzad Rashid, Peter Ritchie, Yvonne O'Neill, Marlyn Davis, Julie O'Neill, Selima Argoub and Araceli Sanchez Jimenez) for assistance with data storage, data input and other administrative tasks; and HSL personnel (Dr. Craig Sams, Laura Kenny, Shahwaiz Iqbal and Fiona Garner) for the urine sample analysis. Also, David Stewart, Napier University, for his assistance with the review of modelling approaches. Many thanks to Paul Hamey who also independently completed the REA for the spray events assessed in this study.

We would also like to express our sincere gratitude to all the farmers and householders who participated in the study.

References

These are listed in Appendix 4.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

Galea KS, MacCalman L, Jones K, Cocker J, Teedon P, Sleeuwenhoek A, Cherrie JW, van Tongeren M. (2011) Biological monitoring of pesticide exposures in residents living near agricultural land. BMC Public Health; 11:856. URL: http://www.biomedcentral.com/content/pdf/1471-2458-11-856.pdf.

Teedon P, Galea KS, MacCalman L, Jones K, Cocker J, Cherrie JW, van Tongeren M. (2015) Engaging with community researchers for exposure science: lessons learned from a pesticide biomonitoring study. PLOS One; 10: e0136347. DOI: 10.1371/journal.pone.0136347.
URL: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0136347.

Galea KS, MacCalman L, Jones K, Cocker J, Teedon P, Cherrie JW, van Tongeren M. (2015) Urinary biomarker concentrations of captan, chlormequat, chlorpyrifos and cypermethrin in UK adults and children living near agricultural land. J Expo Sci Environ Epidemiol. Advance online publication, 16 September 2015; doi:jes.2015.54.

Galea KS, MacCalman L, Jones K, Cocker J, Teedon P, Cherrie JW, van Tongeren M. (2015) Comparison of residents' pesticide exposure with predictions obtained using the UK regulatory exposure assessment approach. Reg Tox Pharm. DOI: 10.1016/j.yrtph.2015.09.012.

Appendix 1: Optimal time for urine sample collection

It was considered that it would be difficult for participants to provide total void samples (over 24 or 48 hours) and that it would be more practical and achievable if they were asked to provide one sample (one spot sample) on any one day. Because the half-lives of biomarker elimination are short (hours) it was important to determine the best time for collection of urine after potential exposure due to spraying; either the last void before going to bed or first void the following morning Were considered as possibilities A simple pharmacokinetic (PK) model was used to describe the elimination of biomarkers via the urine using active ingredient and biomarker specific information, such as half-life.

The model that was used to determine optimal sampling time was slightly different from that used for the comparisons with the REA. This model began with the external exposure which was then absorbed into the dose compartment, with the same model estimating excretion being used when the dose was inside the body. The model was run to predict the urinary biomarker levels for the last void (assumed to be between 8pm and 12am for adults and between 6pm and 9pm for children) and the following morning (assumed to be 7am) for specific scenarios, and the two levels were compared. Urinary biomarker levels were predicted for a young child (16kg), older child (40kg), average female (70kg) and average male (84kg), given a systemic exposure which was assumed to have occurred over an hour from either 8am, 12pm, 4pm or 8pm.

Comparing the biomarker levels predicted in the night void urine to those predicted at morning void (Table A1), it is clear that the predicted levels were significantly higher for the morning void. In Table A1 we show the ratio for an average male but the other subjects showed a similar pattern.

The difference between the two voids was more noticeable the later the exposure was assumed to have occurred, as the later the exposure occurs the lower the level of biomarkers that would be excreted in the urine. The ratio is very similar across all pesticides, with the exception of chlormequat which, due to the shorter half-life, is predicted to have lower levels in the morning void when the exposure starts at 8am the previous day. These results clearly indicate that the morning void is the better of the two to collect. This approach is also supported by data from published field studies (Kissel et al. 2005).

Table A1: Ratio of urinary level measured for night void to morning void (night/morning) assuming exposure at 8am, 12pm, 4pm or 8pm

Time of exposure	Captan	Chlormequat	Chlorpyrifos	Cypermethrin	Penconazole
8am	0.9	1.1	0.8	0.9	0.9
12pm	0.8	1.0	0.7	0.8	0.7
4pm	0.1	0.1	0.1	0.1	0.1
8pm	0.1	0.1	0.1	0.1	0.1

Appendix 2: Analytical methods used to determine urinary biomarker concentrations

The analytical method for chlormequat was based on that reported by Lindh et al. (2011) measuring chlormequat itself. Internal standard (d_4 chlormequat chloride) was added to an aliquot (200 µl) of urine. Samples were diluted ten-fold in 10 mM ammonium acetate. Samples were extracted using solid phase extraction (SPE, Isolute HCX-Q (50mg; 1ml), Biotage) and eluted with 1% formic acid in methanol. Extracts were evaporated under nitrogen and reconstituted in 100 µl of 0.1% formic acid. Analysis was by liquid chromatography tandem mass spectrometry (LC-MS/MS) using positive electrospray ionisation with multiple reaction mode (MRM). Transition 122→58 was monitored for chlormequat. Calibration was linear to at least 100 µg/l, with a detection limit of 0.6.µg/l. Coefficient of variation was 5.7% (N=263). Samples were stable for the whole stability assessment period (at least 24 months).

The analytical method for captan was based on that reported by Berthet et al. (2011) measuring cis-1,2,3,6-Tetrahydrophthalimide (THPI). Internal standard (d_6 THPI) was added to an aliquot (2 ml) of urine. Samples were extracted using SPE (HLB (30mg; 1ml), Waters) and eluted with dichloromethane. Extracts were evaporated under nitrogen and reconstituted in 100 µl of acetonitrile. Analysis was by LC-MS/MS using negative atmospheric pressure chemical ionisation with MRM. Transition 150→96 was monitored for THPI. Calibration was linear to at least 10 µg/l with a detection limit of 0.1 µg/l. Coefficient of variation was 7.3% (N=413). Samples were stable for the whole stability assessment period (at least 10 months).

The analytical method for chlorpyrifos was based on that reported by Sams et al. (2011) measuring 3,5,6-Trichlorpyridinol (TCP). Internal standard (2,3,5,6-Tetrachlorophenol) was added to an aliquot (2 ml) of urine. Samples were acidified (c.HCl) and hydrolysed for 90 minutes at 90^{o}C, then extracted into diethyl ether. Extracts were evaporated under nitrogen and derivatised with BSTFA (60^{o}C, 1 hour). Analysis was by GC-MS using negative chemical ionisation with selected ion monitoring. Ion m/z 161 was monitored for TCP. Calibration was linear to at least 500 µg/l with a detection limit of 0.8 µg/l. Coefficient of variation was 17.4% (N=206). Samples were stable for the whole stability assessment period (at least 21 months).

The analytical method for cypermethrin was based on an established HSL method (Jones et al. 2009) measuring cis- and trans- 2,2-Dichlorovinyl-3,3-dimethylcyclopropane-1-carboxylic acid (DCVA). Internal standard (4-hydroxy-3-phenoxybenzoic acid) was added to an aliquot (2 ml) of urine. Samples were hydrolysed using glucuronidase (37^{o}C, 16 hours) then acidified and extracted using SPE (C18 (100mg; 1ml)) and eluted with methanol and acetonitrile. Extracts were evaporated under nitrogen and reconstituted in 100 µl of mobile phase (50% acetonitrile containing 1% acetic acid). Analysis was by LC-MS/MS using negative electrospray ionisation with MRM. Transition 207→35 was monitored for DCVA. Calibration was linear to at least 100 µg/l, with a detection limit of 1 µg/l. Coefficient of variation was < 20% (N=211). Samples were stable for at least 9 months.

There were no reported methods for human biomarkers of penconazole at the time of starting the project. Metabolism studies in hens, rats and goats indicated that 4-(2,4-Dichlorophenyl)5-(H-1,2,4-triazol-1-yl)pentoic acid (Pen-COOH) was the primary biomarker in these species (not available information on humans) (JMPR, 1992) and so a method was developed to determine this. Internal standard (d_2 Pen-COOH) was added to an aliquot (2 ml) of urine. Samples were acidified (0.2% phosphoric acid) and extracted into ethyl acetate. Extracts were evaporated under nitrogen and reconstituted in 100 µl of mobile phase (10 mM ammonium formate+ 0.1% formic acid in 60% methanol). Analysis was by LC-MS/MS using positive electrospray ionisation with MRM. Transition 314→70 was monitored for Pen-COOH. Calibration was linear to at least 100 µg/l with a detection limit of 0.25 µg/l. Coefficient of variation was 14.9% (N=308). Samples were stable for the whole stability assessment period (at least 31 months).

Creatinine was determined in all urine samples by an automated alkaline picrate method (Jaffé reaction) using a Pentra 400 (ABX, France) (Cocker et al, 2011). The coefficient of variation for within-day analysis was 1.5% and for between-day analysis was 3% at 6 mmol/L.

Appendix 3: TK model parameters used

Table A3: Model parameters used in toxicokinetic (TK) modelling

	Parameter	Units	Penconazole	Captan	Chlorpyrifos	Cypermethrin	Chlormequat
	Biomarker		4-(2,4-Dichlorophenyl)5-(H-1,2,4-triazol-1-yl)pentoic acid	cis-1,2,3,6-tetrahydrophthalimide	3,5,6-trichlorpyridinol	cis- and trans- 2,2-Dichlorovinyl-3,3-dimethylcyclopropane-1-carboxylic acid	Chlormequat
MW_0	Molecular weight of active ingredient		284.18	300.57	350.6	416.30	158.07
MW_M	Molecular weight of biomarker		314.2	151.17	198	208.06	158.07
S	Selectivity Ratio		0.75-0.8 (female)[1] 0.45-0.6 (Male)[1]	0.0002 (dermal)[2] 0.035 (oral)[2]	0.013 (dermal)[3] 0.7 (oral)[3]	0.12 (dermal)[4,5] 0.36 (oral)[4,5]	Approx (1.0)[6]
R	Stochiometric Ratio		0.8-1	0.8-1	0.8-1	0.8-1	0.8-1
V_d	Volume Distribution	l/kg	3-8	3-8	3-8	3-8	3-8
HL	Half-life	h	Approx 15[1]	13.4 (oral)[2] 21.3 (dermal)[2]	27 (oral)[3] 27 (dermal)[3]	16.5 (oral)[4,5] 13 (derma)[4,5]	Dual half-life[7] 2-3 h 10-14h
	Limit of detection	µg/l	0.25	0.1	1	1	0.8

[2] Heredia-Ortiz and Bouchard (2012)
[3] Nolan et al (1984)
[4.] Woolen (1992)
[5] Eadsforth (1988)
[6.] As it is chlormequat it is measured in the urine
[7] Lindh et al (2011) – single volunteer study with two volunteers

Table notes

1. Data presented based on human volunteer studies, with the exception of penconazole (no human volunteer studies conducted).
2. Good agreement between animal and human data for remainder of pesticides.
3. Not every marker measured is a specific product of parent compound metabolism (TCPy, DCCA).
4. Selectivity - amount on a molar basis of absorbed material that can be collected as biomarker of interest
5. Stochiometric ratio –ratio of active ingredient to its biomarker
6. Volume distribution (or the apparent volume that accounts for the entire active ingredient burden in the body); this is taken as 3-5 for children and 8 for adults.
7. Oral half-lives and selectivity ratios were used for the TK modelling

Appendix 4: Reference list

Alexander BH, Burns CJ, Bartels MJ, Acquavella JF, Mandel JS, Gustin C, Baker BA. 2006. Chlorpyrifos exposure in farm families: results from the farm family exposure study. J Expo Sci Environ Epidemiol 16(5):447-56.

Aprea C, Betta A, Catenacci G, Lotti A, Magnaghi S, Barisano A, Passini V, Pavan I, Sciarra G, Vitalone V, Minoia C. 1999. Reference values of urinary 3,5,6-trichloro-2-pyridinol in the Italian population - Validation of analytical method and preliminary results (Multicentric study). Journal of Aoac International 82:305-12.

Berthet A, Heredia-Ortiz R, Vernez D, Danuser B, Bouchard M. 2012. A detailed urinary excretion time course study of captan and folpet biomarkers in workers for the estimation of dose, main route-of-entry and most appropriate sampling and analysis strategies. Ann Occup Hyg 56:815-28.

Berthet A, Bouchard M, Schüpfer P, Vernez D, Danuser B, Huynh CK. 2011. Liquid chromatography-tandem mass spectrometry (LC/APCI-MS/MS) methods for the quantification of captan and folpet phthalimide metabolites in human plasma and urine. Anal Bioanal Chem 399(6):2243-55.

Bevan R, Jones K, Cocker J, Assem FL, Levy LS. 2013. Reference ranges for key biomarkers of chemical exposure within the UK population. Int J Hyg Environ Health 216:170-74.

Byrne SL, Shurdut BA, Saunders DG. 1998. Potential chlorpyrifos exposure to residents following standard crack and crevice treatment. Environ Health Perspect 106:725-31.

CDC, 2005. Third National Report on Human Exposure to Environmental Chemicals. NCEH Pub. No. 05-0570, Atlanta, Georgia.

Cocker J, Mason HJ, Warren ND, Cotton RJ. 2011. Creatinine adjustment of biological monitoring results. Occup Med-Oxf 61:349-53.

Colosio C, Rubino F, Alegakis A, Ariano E, Brambilla G, Mandic-Rajcevic S, Metruccio F, Minola C, Moretto A, Somaruga C, Tsatsakis A, Turci R, Vellere F. 2011. Integration of biological monitoring, environmental monitoring and computational modeling into the interpretation of pesticide exposure data: Introduction to a proposed approach. Toxicol Lett 213:45-46.

Cooper J, Dobson H. 2007. The benefits of pesticides to mankind and the environment. Crop Protection 26:1337-48.

Couture C, Fortin MC, Carrier G, Dumas P, Tremblay C, Bouchard M. 2009. Assessment of exposure to pyrethroids and pyrethrins in a rural population of the Montérégie area, Quebec, Canada. J Occup Envrion Hyg 6(6):341-52.

DAFNE. 2006. The Pan-European food bank based on household budget surveys, National and Kapodistrian University of Athens; Athens. [http://www.nut.uoa.gr/dafnesoftweb/.

Dai H, Asakawa F, Suna S, Hirao T, Karita T, Fukunaga I, Jitsunari F. 2003. Investigation of indoor air pollution by chlorpyrifos: Determination of chlorpyrifos in indoor air and 3,5,6-trichloro-2-pyridinol in residents' urine as an exposure index. EHPM 8:139-45.

DEFRA 2013. Agriculture in the UK 2012. Department for Environment, Food and Rural Affairs. London URL: https://www.gov.uk/government/statistics/agriculture-in-the-united-kingdom-2012

DEFRA 2012. Agriculture in the UK 2011. Department for Environment, Food and Rural Affairs. London URL:https://www.gov.uk/government/statistics/agriculture-in-the-united-kingdom-2011

DEFRA 2006. Pesticides. Code of practice for using plant protection products. Department for Environment, Food and Rural Affairs. London URL: http://www.pesticides.gov.uk/Resources/CRD/Migrated-Resources/Documents/C/Code_of_Practice_for_using_Plant_Protection_Products_-_Complete20Code.pdf

Eadsforth CV, Bragt PC, van Sittert NJ. 1988. Human dose-excretion studies with pyrethroid insecticides cypermethrin and alphacypermethrin: relevance for biological monitoring. Xenobiotica; 18:603-14.

EC 2005a. Review report for the active substances chlorpyrifos. SANCO/3059/99- rev1.5. June 2005. European Commission.

EC 2005b. Review Report for the active substance cypermethrin, SANCO/4333/2000 final, February 2005. European Commission.

EFSA 2013. Scientific report of EFSA. The 2010 European Union Report on Pesticide Residues in Food. European Food Safety Authority (EFSA), EFSA Journal 11(3):3130

EFSA 2008. Conclusion on the peer review of chlormequat. EFSA Scientific Report; 179: 1-77. URL: http://www.efsa.europa.eu/en/scdocs/doc/179r.pdf

EWDTS 2002. European Laboratory Guidelines for Legally Defensible Workplace Drug Testing. www.ewdts.org

Furtaw EJ JR. 2001. An overview of human exposure modelling activities at the USEPAs National exposure Research Laboratory. Toxicol Ind Health; 17:302-314.

Galea KS, MacCalman L, Jones K, Cocker J, Teedon P, Sleeuwenhoek AJ, Cherrie JW, van Tongeren M. 2011. Biological monitoring of pesticide exposures in residents living near agricultural land. BMC Public Health 11:856. URL: http://www.biomedcentral.com/content/pdf/1471-2458-11-856.pdf.

Garthwaite DG, Hudson S, Barker I, Parrish G, Smith L, Pietravalle S. 2014. Pesticide usage survey reports 250. Arable crops in the United Kingdom 2012 (including aerial applications 2012). DEFRA, York, UK.

Garthwaite DG, Hudson S, Barker I, Parrish G, Smith L, Pietravalle S. 2013. Pesticide usage survey reports 252 Orchards in the United Kingdom 2012. DEFRA, York, UK. http://www.fera.defra.gov.uk/landUseSustainability/surveys/documents/orchards2012V2.pdf

Goldsmith M, Johnson J, Chang D, Torneo-Velez R, Knaak J, Dary C. 2012. Parameters for pesticide QSAR and PBPK/PD models to inform human risk assessments. American Chemical Society, Symposium Series. 1099, 1-15.

Helsel DR, 2005. Nondetects and Data Analysis: Statistics for Censored Environmental Data. John Wiley and Sons, New York.

Heredia-Ortiz R, Bouchard M. Toxicokinetic modeling of captan fungicide and its tetrahydrophthalimide biomarker of exposure in humans. Toxicol Lett. 2012 Aug 13;213(1):27-34. doi: 10.1016/j.toxlet.2011.09.023.

HSE, 2012. Bystander exposure guidance. HSE, UK. URL: http://www.pesticides.gov.uk/Resources/CRD/Migrated Resources/Documents/B/Bystander-exposure-guidance.pdf.

Jones K, et al (in preparation) Penconazole analysis and urinary metabolite levels in adults and children living near agricultural land.

Jones K, Everard M, Harding AH. 2014. Investigation of gastrointestinal effects of organophosphate and carbamate pesticide residues on young children Int J Hyg Environ Heal 217:392– 398

Jones K, Sams C, Patel K, Johnson P. 2009. Development of cost-effective biomarkers for herbicides and fungicides. http://www.foodbase.org.uk/results.php?f_report_id=407 (last accessed 11/06/2014).

Kimata A, Kondo T, Ueyama J, Yamamoto K, Yoshitake J, Takagi K, Suzuki K, Inoue T, Ito Y, Hamajima N, Kamijima M, Gotoh M, Shibata E. 2009. Comparison of urinary concentrations of 3-phenoxybenzoic acid among general residents in rural and suburban areas and employees of pest control firms. Int Arch Occup Environ Health 82(10); 1173-1178.

Kissel JC, Curl CL, Kedan G, Lu C, Griffith W, Barr DB, Needham LL, Fenske RA. 2005. Comparison of organophosphorus pesticide metabolite levels in single and multiple daily urine samples collected from preschool children in Washington State. J Expo Anal Epidemiol 15(2); 164-171

Koch HM, Hardt J, Angerer J. 2001. Biological monitoring of exposure of the general population to the organophosphorus pesticides chlorpyrifos and chlorpyrifos-methyl by determination of their specific metabolite 3,5,6-trichloro-2-pyridinol. Int J Hyg Environ Health 204:175-80.

Koureas M, Tsakalof A, Tzatzarakis M, Vakonaki E, Tsatsakis A, Hadjichristodoulou C. 2014. Biomonitoring of organophosphate exposure of pesticide sprayers and comparison of exposure levels with other population groups in Thessaly (Greece). OEM 71(2)126-33.

Lindh CH, Littorin M, Johannesson G, Jonsson BAG. 2011. Analysis of chlormequat in human urine as a biomarker of exposure using liquid chromatography triple quadrupole mass spectrometry. J Chromatogr B Anal Technol Biomed Life Sci 879:1551-56.

Lloyd GA, Bell GJ. 1983. Hydraulic nozzles: comparative spray drift study. MAFF/ADAS.

Lloyd GA, Bell GJ, Samuels SW, Cross JV. Berrie AM. 1987. Orchard Sprayers: Comparative operator exposure and spray drift study. UK Pesticide Registration and Surveillance Department.

Lu C, Andres L. 2011. Reconstructing organophosphorus pesticide doses using the reversed dosimetry approach in a simple physiologically-based pharmacokinetic model. J. Tox. Doi:10.1155/2012/131854.

Lu C, Holbrook C, Andres L. 2010. The implications of using a Physiologically Based Pharmacokinetic (PBPK) model for pesticide risk assessment. Envrion Health Perspect 118:125-30.

MATLAB and Statistics Toolbox Release 2012a, The MathWorks, Inc., Natick, Massachusetts, United States.

Nolan RJ, Rick DL, Freshour NL, Saunders JH. 1984. Chlorpyrifos: pharmacokinetics in human volunteers. Toxicol Appl Pharmacol; 73(1):8-15.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.

Rigas M, Okino M. Quackenboss J. 2001. Use of a pharmacokinetic model to assess chlorpyrifos exposure and dose in children, based on urinary biomarker measurements. Toxicological Sciences. 61, 374-81.

Sams C, Jones K. 2011. Human volunteer studies investigating the potential for toxicokinetic interactions between the pesticides deltamethrin; pirimicarb and chlorpyrifos-methyl following oral exposure at the acceptable daily intake. Toxicol Lett 200(1-2):41-5.

SigmaPlot version 10, from Systat Software, Inc., San Jose California USA, www.sigmaplot.com

Sleeuwenhoek A, Cocker J, Jones K, Cherrie JW: Biological monitoring of pesticide exposures. IOM Research Report TM/07/02; 2007.

Teedon P, Galea KS, MacCalman L, Jones K, Cocker J, Cherrie JW, van Tongeren M. (2015) Engaging with community researchers for exposure science: lessons learned from a pesticide biomonitoring study. PLOS One; 10: e0136347. DOI: 10.1371/journal.pone.0136347.

Tomlin C (Ed.) The Pesticide Manual: a world compendium 15th Edition. British Crop Protection Council, 2009. ISBN 81901396188.

Qi X, Z.M., Wu C, Wang G, Feng C, Zhou Z. 2012. Urinary pyrethroid metabolites among pregnant women in an agricultural area of the Province of Jiangsu, China. Int J Hyg Environ Health 215:487-95.

Verberk MM, Brouwer DH, Brouwer EJ, Bruyzeel DP, Emmen HH, Van Hemmen JJ, Hooisma J, Jonkman EJ, Ruijten MW, Sallé HJ. 1990. Health effects of pesticides in the flower-bulb culture in Holland. La Medicina del lavoro 81:530-41.

VSN International (2013). GenStat *for Windows* 16th Edition. VSN International, Hemel Hempstead, UK. URL:http://www.genstat.co.uk

Wen Y, Kalff J, Perters R. 1999. Pharmacokinetic modeling in toxicology: a critical perspective. Environmental Reviews. 7: 1-19

Woollen BH, Marsh JR, Laird WJ, Lesser JE. 1992. The metabolism of cypermethrin in man: differences in urinary metabolite profiles following oral and dermal administration. Xenobiotica; 22:983-91.

Ye X, Pierik FH, Hauser R, Duty S, Angerer J, Park MM, et al. 2008. Urinary metabolite concentrations of organophosphorous pesticides, bisphenol A, and phthalates among pregnant women in Rotterdam, the Netherlands: The Generation R study. Environ Res 108:260-67.