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Comparison of residents' pesticide exposure with predictions obtained using the UK regulatory exposure assessment approach

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ABSTRACT

The UK regulatory methods currently used for estimating residents' potential pesticide exposure were assessed to determine whether they provide sufficiently conservative estimates. A non-random sample of 149 residents living within 100 m of fields where pesticides were sprayed provided first morning void urine samples one and/or two days after spraying. Using farmers' spray information, regulatory exposure assessment (REA) models were applied to estimate potential pesticide intake among residents, with a toxicokinetic (TK) model used to estimate urinary biomarker concentrations in the mornings of the two days following the spray. These were compared with actual measured urinary biomarker concentrations obtained following the spray applications. The study focused on five pesticides (cypermethrin, penconazole, captan, chlorpyrifos and chlormequat). All measured cypermethrin urinary biomarker levels were lower than the REA-predicted concentrations. Over 98% and 97% of the measured urinary biomarker concentrations for penconazole and captan respectively were lower than the REA-predicted exposures. Although a number of the chlorpyrifos and chlormequat spray-related urinary biomarker concentrations were greater than the predictions, investigation of the background urinary biomarker concentrations suggests these were not significantly different from the levels expected had no pesticide spraying occurred. The majority of measured concentrations being well below the REA-predicted concentrations indicate that, in these cases, the REA is sufficiently conservative.

1. Introduction

Government Ministers must approve all pesticides, including those used in agriculture, horticulture, forestry, food storage and the home or garden, before they can be marketed or used in Great Britain. The regulatory health risk assessment underpinning the approval of pesticides involves the comparison of estimates of potential human exposure with toxicological reference levels; for example, Acceptable Operator Exposure Level (AOEL) or Acceptable Daily Intake (ADI), below which there is considered to be high confidence that there will be no adverse health effects.

The Chemicals Regulation Directorate (CRD) of the British Health and Safety Executive (HSE) acts as the Regulator for pesticide products and authorises their sale, supply, use and storage in Great Britain. For residents, the exposure assessment submitted to support approval must consider three scenarios; exposure at the time of application (e.g. from spray drift), exposure after the application (e.g. spray vapour) and exposure through entry into areas where spray drift fallout has occurred (e.g. children's exposure whilst playing in garden where drift has landed). Applicants for pesticide approval may provide their own assessments based on measurements made during application, other analogous measurement data or exposure models to estimate exposure, provided these produce an appropriate exposure assessment for each of these exposure scenarios (HSE, 2012).

There is a general paucity of exposure measurements, in particular for residents. Therefore the exposure assessment usually relies on simple exposure assessment tools. Due to the inherent nature of these tools, there is uncertainty associated with estimates

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obtained. To account for this uncertainty and also the true variability that will occur in individual exposures, 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 REA methods are sufficiently conservative 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 the REA model in use for Great Britain at the time, may sometimes underestimate exposure for bystanders; however, they did not collect data for residents.

The research project 'Biological monitoring of pesticide exposure in residents' funded by the Department of Environment, Food and Rural Affairs (DEFRA) aimed to assess whether the exposure assessment tools used for the REA produce sufficiently conservative estimates. Galea et al. (2015a) reported on residents' exposure to captan, chlormequat, chlorpyrifos and cypermethrin in three geographical areas of Great Britain, whilst a separate manuscript is planned reporting on residents' exposure to penconazole.

In this manuscript, we compare the biomarker concentrations in urine obtained from residents following spray events, with the estimates obtained for residents using the exposure assessment models applied in the pesticide approval process. These exposure estimates were generated using spray event information provided by participating farmers to estimate intake for our adult and child participants residing within 100 m of the treated fields. The model outputs were converted into estimated urinary biomarkers by applying a toxicokinetic model, based on that of Rigas et al. (2001).

2. Materials and methods

2.1. Overview

The study received full ethical approval by the NHS South East Scotland Research Ethics Committee (SESREC) 3 (study number 10/S1103/63). Galea et al. (2011) describes the overall study design, which is discussed in more detail in Galea et al. (2015b). In brief, sample and data collection took place in three major arable crop growing and orchard areas in Great Britain: East Lothian, Kent, and Norfolk. Farmers were recruited into the study if they were likely to spray their agricultural crops with relevant pesticides (captan, chlormequat, chlorpyrifos, cypermethrin and penconazole) and there were residential areas within 100 m of the fields being sprayed. The farmers provided details of their pesticide usage throughout the spray season. Residents (adults aged 18 years and over and children in their care aged 4–12 years) living within 100 m of the edge of a field belonging to a recruited farm were approached to participate in the study. Participants provided informed written consent. First morning void urine samples on one and/or two days after a spray event were collected from participating residents, as well as a number of first morning void samples that were not associated with spray events (background samples collected during and outwith the spray season, with the spray season being taken to be March–August). These urine samples were frozen as soon as possible, being stored at −15 to −20 °C prior to analysis.

Urine samples collected within 2 days of a relevant spraying event were analysed only for the relevant pesticide(s) sprayed during the event. Background samples, both within and outwith the spray season, were analysed for all the relevant pesticides of interest to the study. The analytical method for chlormequat was based on that reported by Lindh et al. (2011) measuring chlormequat itself. The analytical method for captan was based on that reported by Berthet et al. (2011) measuring cis-1,2,3,6-tetrahydrophthalimide (THPI). The analytical method for chlorpyrifos was based on that reported by Sams and Jones (2011) measuring 3,5,6-trichlorpyridinol (TCP). The analytical method for cypermethrin measured cis- and trans- 2,2-dichlorovinyl-3,3-dimethylcyclopropane-1-carboxylic acid (DCVA) (Jones et al., 2009). A novel method was developed during the study for penconazole biomarkers and this was based on earlier work to develop a method based on a major animal biomarker (Pen-COOH) (Jones et al., 2009). More information on analytical methods is given in Galea et al. (2015b).

The laboratory that analysed the urine samples participates in external quality assurance schemes for chlorpyrifos and cypermethrin (G-EQUAS, www.g-equas.de). The analysts were blind to whether the urine samples were related to spray events or were background samples. All analytes were quantified using multipoint matrix-matched calibration curves (including a blank) and quality control samples (matrix spikes) were run every five samples (coefficient of variation <20% for all analytes). Samples were analysed in duplicate and the mean value reported. 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.

2.2. Pesticides of interest and spray event information collected

Table 1 provides details of the pesticides considered in the study, along with a summary of participants measured urinary biomarker concentrations following the pesticide spray events. These spray event related samples were obtained from 149 eligible participants (125 adults and 24 children). Participants were considered eligible if, after excluding samples with low (below 2 mmol/L) or high (greater than 30 mmol/L) creatinine concentrations (Cocker et al., 2011; EWDTS, 2002), they provided at least one spray event related and at least one background urine sample.

Farmers were asked to provide details of their pesticide usage throughout the spray season for the fields within 100 m of participating households. This information included the start and finish times of the spray event, product and active ingredients used, quantities applied (weight of active substance/ha), dose rate, spray method as well as the size of the field, crop and weather conditions. In instances where farmers already maintained comprehensive records of their pesticide usage, the researcher requested copies of these. Where detailed records were not already maintained, participating farmers were asked to record the relevant information using an adaptation of the spray record form recommended by DEFRA (DEFRA, 2006).

2.3. Predicting residents' exposures using regulatory exposure assessment (REA) approach

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 anonymous format. This file was then forwarded to a representative of the CRD who used this information to predict the residents' exposures using the model applied in the regulatory process as described below (HSE, 2012). These independent predictions were made without any knowledge of the urinary biomarker concentrations obtained from the participants.

The REA models considered three pathways of exposure (HSE, 2012). The first of these was direct exposure to spray drift at the time of application. Based on values derived from generic field trials, estimates using the REA models were made of the amount of pesticide that might be deposited on the skin and enter the

Table 1
Pesticides of interest and urinary biomarker levels (μg/g creatinine) following spray events (Galea et al. (2015b).

Pesticide	Class	Function	LOD (μg/L)	N	% <LOD	GM	GSD	Max	95% Ile
Captan	Phthalimide	Fungicide	0.1	255	91	[a]	[a]	1.2	0.2
Cypermethrin	Pyrethroid	Insecticide	1.0	49	98	[a]	[a]	7.0	5.8
Chlormequat	Chlorocholine	Growth regulator	0.6	197	2	15.4	2.7	248.1	72.4
Chlorpyrifos	Organophosphate	Insecticide	0.8	63	11	2.5	2.1	14.8	7.9
Penconazole	Triazole	Fungicide	0.25	89	81	[a]	[a]	5.1	0.9

N = number; LOD = Limit of Detection; Max = Maximum; GM = Geometric Mean; GSD = Geometric Standard Deviation; 95% ile = 95th percentile.
[a] GM and GSD are not calculated due to the high proportion of values below LOD.

breathing zone of individuals 8 m from the sprayer. Based on these estimates, the total systemic dose was calculated, using dermal absorption data and information on inhaled air volumes. As the generic field data underlying the REA models are limited to measurements on adults, estimates of direct exposure to spray drift were made only for adults. The second pathway was inhalation of pesticide vapour following volatilisation from plant or soil surfaces after the application. In this case, estimates of potential daily inhalation exposure 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 were continuously located next to the treated crop. The third pathway was that of exposure via contact with contaminated surfaces after application. This scenario was conceptualised by considering the exposure of a young child playing on a lawn in a garden next to a sprayed crop, where pesticide spray had drifted onto the grass. Here, direct dermal transfer and uptake following contact with pesticide residues on turf along with oral exposure through hand-to-mouth transfer and object-to-mouth transfer of pesticide residues were 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 assessing proposed uses of pesticides, the REA considers the worst-case recommended use (e.g. maximum recommended dose applied) which is expected to give the highest exposures. Actual uses are often lower, for example pesticides are often used at a lower applied dose. Therefore, individual estimates were made in this study reflecting details of the reported use for each of the application events. Data on method of application, applied dose and spray volume (litres of spray/ha) were used. In a small number of cases spray volumes were not reported by the farmer. Using the data obtained from the other participating farmers, missing spray volumes for 38 broadcast air assisted applications were assigned a value of 250 l/ha; this was the mode for the remaining 252 applications (arithmetic mean 279 L/ha). Four missing ground boom spray volumes were assigned a value of 200 L/ha; the mode of the 51 similar applications (arithmetic mean 156 L/ha) by other participating farmers. Dermal absorption values applied for the individual products were taken from current regulatory assessments (Table 2), with some of the data originating from unpublished studies submitted by applicants to support their products (P. Hamey, personal communication).

Table 2
Dermal absorption values used in regulatory exposure estimates.

Active substance	Dermal absorption (%)
Captan	2
Chlormequat	4
Chlorpyrifos	1
Cypermethrin	10
lamda-cypermethrin	0.3
Zetacypermethrin	7
Penconazole	5

2.4. Toxicokinetic modelling

A simple toxicokinetic model (hereafter referred to as TK model) was used to predict what the urinary biomarker concentration would have been based on the estimated systemic dose from the REA. The chosen model was used in a previous study of bystanders' pesticide exposure (Sleeuwenhoek et al., 2007), 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 the level of biomarker of interest (D_m). The parameters used in this equation are described in Table 3.

$$D_M = \frac{S \cdot R}{V_d \frac{M_o}{M_m}} D \tag{1}$$

where:

S = selectivity ratio, amount on a molar basis of active ingredient that can be collected as biomarker of interest
R = stoichiometric ratio of the biomarker to active ingredient
M_0 = Molecular weight of active ingredient
M_m = Molecular weight of biomarker
V_d = Volume distribution, the apparent volume that accounts for all of the active ingredient in the body.

The model then describes the transfer 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, the elimination rate, is calculated from the half-life (HL) in hours.

$$\frac{dC_B}{dt} = -k_e C_B \tag{2}$$

$$\frac{dC_U}{dt} = k_e C_B \tag{3}$$

where C_B is the concentration in the body, with C_B at t = 0 being D_M, C_U is the concentration in the urine and k_e is the elimination rate calculated from the half-life:

$$k_e = \frac{\ln(2)}{HL} \tag{4}$$

The concentration in the urine at first morning void was calculated by subtracting the total amount accumulated in the urine at the time of the last void in the evening before the urine sample from the total amount accumulated in the urine at the time of urine sample, and dividing by the total urinary volume over the time since the last void. Urinary volume is assumed to accumulate

Table 3
Toxicokinetic (TK) modelling parameters.

	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
M_0	Molecular weight of active ingredient		284.18	300.57	350.6	416.30	158.07
M_M	Molecular weight of biomarker		314.2	151.17	198	208.06	158.07
S	Selectivity Ratio		0.75–0.8 (female)[a] 0.45–0.6 (Male)[a]	0.0002 (dermal)[b] 0.035 (oral)[b]	0.013 (dermal)[c] 0.7 (oral)[c]	0.12 (dermal)[d,e] 0.36 (oral)[d,e]	Approx (1.0)[f]
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	hours	Approx 15[a]	13.4 (oral)[b] 21.3 (dermal)[b]	27 (oral)[c] 27 (dermal)[c]	16.5 (oral)[d,e] 13 (derma)[d,e]	Dual half-life[g] 2–3 h 10–14 h
	Limit of detection	μg/L	0.25	0.1	1	1	0.8

Comments on table.
- Data based on human volunteer studies, exception of penconazole (no human volunteer studies conducted at the time of modelling).
- Not every marker measured is a specific product of parent compound metabolism (TCPy, DCCA).
- Selectivity ratio - amount on a molar basis of the active ingredient that can be collected as biomarker of interest.
- Stochiometric ration –ratio of active ingredient to its biomarker.
- 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.
- Oral half-lives and selectivity ratios were used for the TK modelling.

[a] ECHA (2012).
[b] Heredia-Ortiz and Bouchard (2012).
[c] Nolan et al. (1984).
[d] Woollen (1992).
[e] Eadsforth et al. (1988).
[f] As it is chlormequat it is measured in the urine.
[g] Lindh et al. (2011) – single volunteer study with two volunteers.

at a rate of 60 mL/per hour.

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 exposure at four different time points (8 am, 12 pm, 4 pm and 8 pm) 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 (up to 24-h) and day 3 (up to 48-h), 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 8 pm and 12 am for adults, and 6 pm and 9 pm for children, with a time chosen randomly for each run of the model.

Table 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 as we assumed that oral, rather than dermal or inhalation, was the predominant route 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, stoichiometric, time of last void, and volume of distribution) a value was randomly generated from the specified range. For the remaining parameters a value was randomly chosen from a uniform distribution with limits +-10% of the point estimate in Table 3, with the exception of molecular weights which are known constants. For each sample result the Monte-Carlo model was run 10,000 times and an average REA-based prediction was calculated.

2.5. Statistical analysis

To determine if the exposure estimates from the REA were conservative, we calculated the frequency that measured urinary pesticide biomarker concentrations exceeded the predicted biomarker levels based on the REA. The comparisons were made using the urinary concentrations not corrected for creatinine as correcting the model predictions for creatinine would have involved another set of assumptions. The measured urinary biomarker levels are the result of pesticide exposure from the spray event as well as any other sources of exposure (e.g. diet). In contrast, the predicted urinary biomarker levels using the REA estimates only represent the potential exposure from the spray event. Therefore, the predicted urinary levels based on the REA from the spray were adjusted by adding the individual's average within-season background levels of the urinary biomarker to the predicted urinary biomarker. So for individual j, sample i, background corrected predicted urinary biomarker concentration ($Q_{i,j}$) was calculated by:

$$Q_{i,j} = P_{i,j} + BG_j \quad (5)$$

where:

$P_{i,j}$ = predicted urinary biomarker concentration for participant j and spray event i
BG_j = average urinary biomarker concentration of all within-season background samples for participant j

This was done for day 2 as well as day 3 urinary samples and for the four initial assumed exposure times (8 am, 12 pm, 4 pm and 8pm).

Due to the short half-life of the pesticides, the predicted urinary biomarker levels will reduce rapidly following a spray event. Hence, the measured level may exceed predicted levels following a spray event due to the variability in the background levels (i.e. if the predicted level approaches 0 then the probability that background levels will exceed the background adjusted predicted levels will approach 50%). Therefore, we compared the frequency that the

measured urinary biomarker concentration exceeded the (background-corrected) predicted concentration following a spray event, with the frequency that the measured background levels exceeded the (background-adjusted) average REA predicted concentrations.

For each individual, the average REA-based prediction of their associated spray events was calculated and corrected, as for the predictions above, by adding the individual's average within season background urinary biomarker concentration, to obtain the background corrected average REA-based prediction for each individual (R_j):

$$R_j = P_j + BG_j \quad (6)$$

where:

R_j = average, background corrected, urinary biomarker concentration for all the relevant spray events for participant j
P_j = average predicted urinary biomarker for participant j
BG_j is the average within-season background level for participant j

The proportion of background urinary biomarker results above this average background corrected REA-based prediction (% $BG_{ij} > R_j$) was then compared to the proportion of the spray event urinary biomarker results above the REA-based predictions (% urinary biomarker result for spray event i and participant j > $Q_{i,j}$) 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).

3. Results

3.1. Farms and relevant spray events

A total of 13 farms participated during 2011 and 17 during 2012 where pesticides containing at least one of the relevant active ingredients were applied. All participating Kent farms were orchards whereas the remaining farms were all arable. Chlormequat and cypermethrin were applied on farms in East Lothian and Norfolk 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 on a field within 100 m of the participants. It does not take into account whether a urine sample was collected following these events. It is clear that captan (a fungicide) containing products were the most frequently applied of the five pesticides considered, and cypermethrin the least.

3.2. Urine samples and data collection

A total of 149 residents (125 adults and 24 children) provided at least one urine sample related to a relevant spray event. A total of 542 spray-event related samples were used in the REA comparisons with some samples being analysed for more than one pesticide, resulting in 255 captan, 63 chlorpyrifos, 46 cypermethrin, 197 chlormequat and 89 penconazole spray-event related results.

3.3. REA estimates

Table 5 details for each pesticide, separately for adults and children, the pathway that resulted in the highest REA 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, Table 5 provides details of the Acceptable Operator Exposure Level (AOEL) for each of the pesticides.

For adults, in most instances, direct dermal contact and uptake following contact with surfaces contaminated with pesticide residue (e.g. due to spray drift) (pathway 3) resulted in the highest exposure predictions, although for chlorpyrifos and penconazole, volatisation of pesticides from the treated crop and surfaces was also used (pathway 2). 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 was used in all the child comparisons. It was evident that the highest estimated pathway of exposure for each of the pesticides was well below the AOEL.

Due to the high level of “non-detects” in the captan and cypermethrin samples, only the numbers of samples above the REA-based predicted exposure concentrations are reported. Table 6 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 up to one day (24 h) and two days (48 h) after the spray event. The assumed time of exposure at 8 am is seen as the worst-case in these analyses, the proportion of measured urinary biomarker concentrations above the predicted decreases as assumed time of exposure increases.

All measured 24 h urinary biomarker concentrations for captan were below the exposure predicted to be present in the participants' urine using the REA-TK models. Eleven percent of the 48 h urinary biomarker results were found to be above the predicted urinary biomarker concentration. Four and seven cypermethrin urinary biomarker results were above the level estimated in the participants' urine based on the REA-TK model predictions in 24 and 48 h samples, respectively. There was one penconazole measured urinary biomarker result above the predicted exposure. Where the measured urinary biomarker concentration was higher than the background adjusted REA-TK predictions there was usually very little difference between the two values and both were often below the LOD.

For chlorpyrifos, there were only 3 measured urinary biomarker concentrations above the predicted exposures at 24 h, and 8 to 10 measured concentrations above the predicted exposures at 48 h (depending on estimated timing that exposure occurred, see Table 7). The majority of background corrected predictions were higher than measured urinary biomarker levels in both 24 and 48 h samples.

For chlormequat, there were 12–27 measured urinary

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

Area	Farms (N)	Spray days (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
AOEL for each pesticide assessed and REA pathways resulting in the highest predicted exposure (and the range of estimates) based on farmers spray event information.

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 and uptake with surfaces contaminated with pesticide residue after application; BW- body weight.

Table 6
Number and percentage of captan, cypermethrin and penconazole urinary biomarker results higher than the REA-TK-based predicted exposure, assuming exposure occurred at 8 am.

Pesticide	Samples collected one day (up to 24-h) after spray event			Samples collected two days (up to 48-h) after spray event		
	N	Meas > pre (N)	Meas > pre (%)	N	Meas > pre (N)	Meas > pre (%)
Captan[a]	94	0	0	147	11	8
Cypermethrin	22	4	18	24	7	21
Penconazole	27	0	0	55	1	2

[a] 25 samples were both 24 and 48 h samples due to spraying on two consecutive days.

biomarker concentrations above the biomarker concentrations derived from the predicted REA-TK exposures at 24 h, and 33 to 39 measured urinary biomarker concentrations above the predicted REA-TK biomarker concentrations at 48 h (Table 8). This equates to around 40% of measured urinary biomarker concentrations in excess of the predicted concentrations.

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.

Fig. 1 shows the measured urinary biomarker chlormequat concentrations versus the background corrected REA-TK-based prediction (PREDICTED$BC_{i,j}$). The points appear to be scattered around the line of equality, at both 24 and 48 h indicating that, while there are some measured levels higher than predicted, on average they are equal. Looking at the plot of the within-season background measurement ($BG_{i,j}$) versus the mean of the REA-based prediction (AM(REA)BC_j) (Fig. 2), the pattern is similar. The pattern is similar for chlorpyrifos (Figs. 3 and 4), although with fewer measured being higher than background-corrected predicted.

Table 9 shows that the percentage of spray-event related measurements above background corrected predictions for chlorpyrifos and chlormequat is not significantly different from expected as the p-value for the binomial test for difference is greater than 0.05, with the exception of chlorpyrifos at 48 h. 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 generally not significantly different to what would be expected had no spray event occurred.

4. Discussion and conclusions

The main aim of this study was to assess whether the pesticide exposure models used for the UK REA produce sufficiently conservative estimates for residents. To achieve this aim, there were a number of issues that needed to be overcome such as obtaining good quality spray information from participating farmers and the timely collection of urine samples from participants coinciding with relevant spray events. These difficulties were overcome through the use of community researchers and successful engagement with farmers and residents (Teedon et al., 2015).

The participating farmers, who volunteered to be involved, may not have been representative of all farmers within the study areas although there is no reason to suggest that their spraying practices were different to those in the wider farming communities. The farmers were enthusiastic and willing to share their spray records and these were an essential component of the study and allowed the exposure predictions to be generated using the REA tools. Spray event start and finish times were obtained and residents were asked to provide details of their activities in the 48-hr period prior to provision of each urine sample. However, it was not possible to

Table 7
Comparison of measured with predicted (based on REA-TK exposure predictions) biomarker levels for chlorpyrifos. The GM predicted levels are provided along with the GM ratio of the predicted to the measured.

	Day after spray					2 Days after spray				
	N	Meas > pre (N)	GM (μg/l)	GSD	GM ratio	N	Meas > pre (N)	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										
8 am	20	3	3.4	1.3	0.4	35	10	2.0	1.4	0.5
12 pm	20	3	3.8	1.3	0.3	35	10	2.2	1.4	0.5
4 pm	20	3	4.2	1.3	0.3	35	10	2.4	1.4	0.5
8 pm	20	3	4.7	1.3	0.3	35	8	2.7	1.4	0.5

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

Table 8
Comparison of measured with predicted (based on REA-TK exposure predictions) for chlormequat. The GM predicted levels are provided along with the GM ratio of the predicted to the measured.

	Day after spray					2 Days after spray				
	N	Meas > pre (N)	GM (μg/l)	GSD	GM ratio	N	Meas > pre (N)	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										
8 am	102	27	5.7	4.3	0.5	94	39	0.2	4.7	0.8
12 pm	102	20	9.9	4.3	0.4	94	37	0.4	4.7	0.8
4 pm	102	15	17.2	4.3	0.3	94	36	0.7	4.7	0.7
8 pm	102	12	29.3	4.3	0.2	94	33	1.3	4.7	0.7

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

establish from this when, where or how the residents' potential exposure to the assessed pesticides may have occurred, if at all. To allow for the comparison with the REA predictions, a number of time periods (start of exposure and provision of urine sample) were considered to accommodate this lack of information.

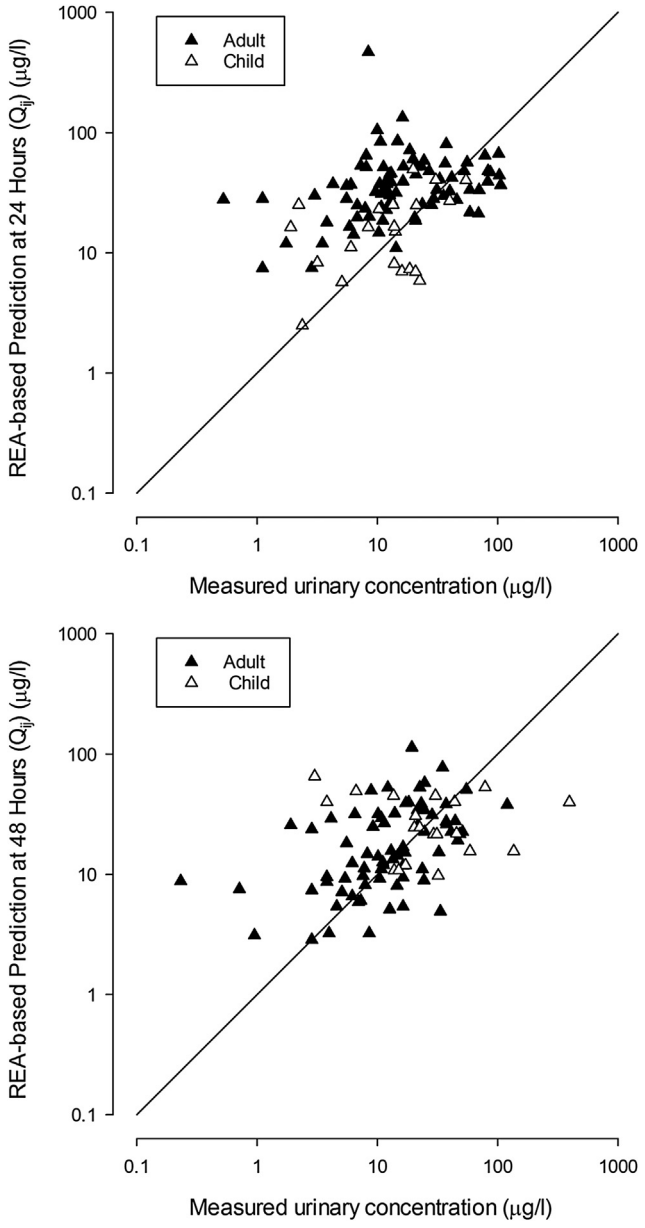


Fig. 1. Scatterplot of measured urinary biomarker chlormequat concentrations against the background corrected REA-based urinary predictions. This is at 24 h (left) and 48 h (right). The symbols distinguish between adults and children.

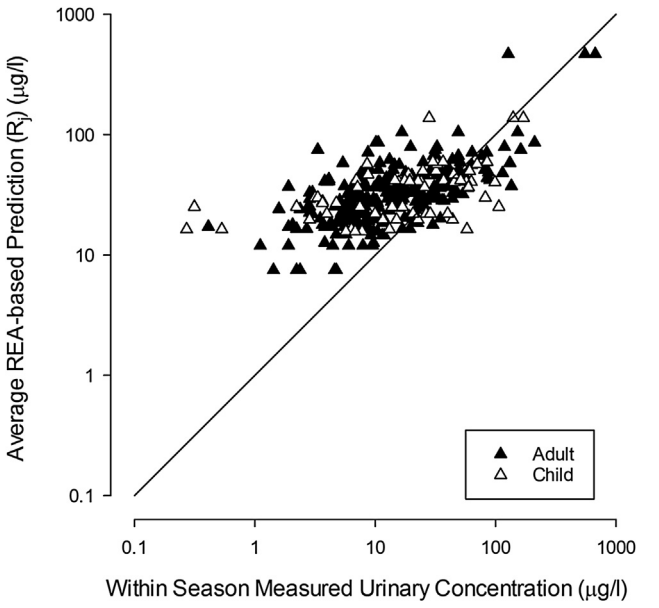
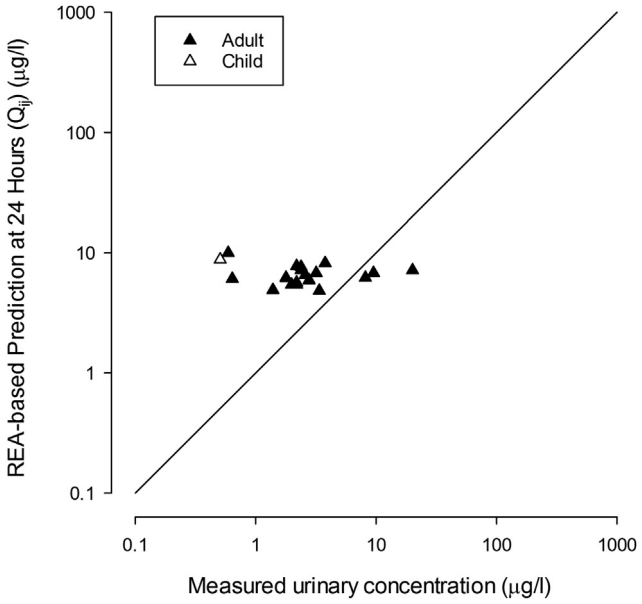


Fig. 2. Scatterplot of measured urinary biomarker within-season background chlormequat concentrations against the arithmetic mean background corrected REA-TK-based urinary biomarker predictions (AM-REA). The symbols distinguish between adults and children.

The TK model used, based on that of Rigas et al. (2001), is a simple one-compartment 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 single 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 meaning that a number of assumptions would have to be made and, consequently, there would be a 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



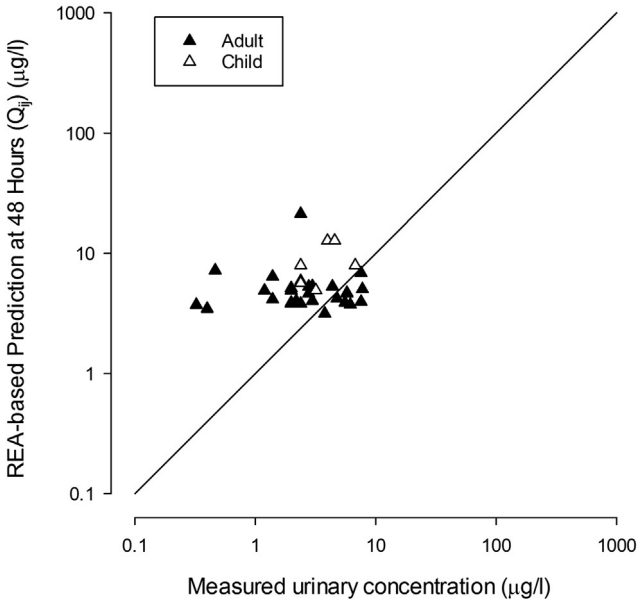


Fig. 3. Scatterplot of measured urinary biomarker chlorpyrifos concentrations against the background corrected REA-based urinary predictions. This is at 24 h (left) and 48 h (right). The symbols distinguish between adults and children.

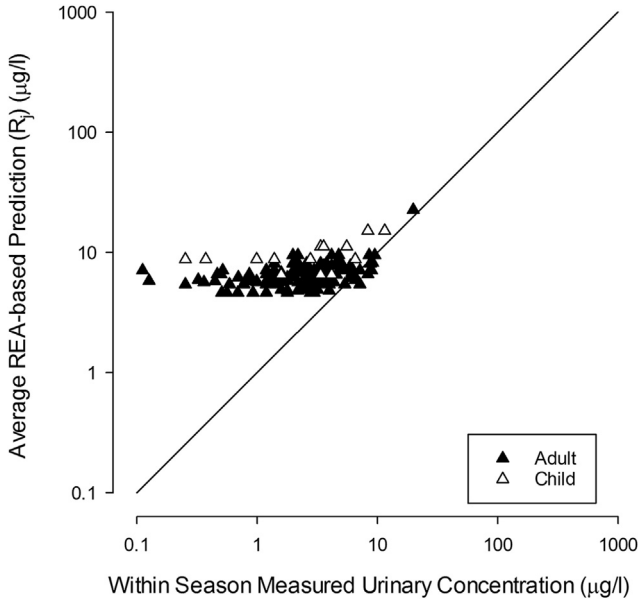


Fig. 4. Scatterplot of measured urinary biomarker within-season background chlorpyrifos concentrations against the arithmetic mean background corrected REA-TK-based urinary biomarker predictions (AM-REA). The symbols distinguish between adults and children.

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 h prediction and resulted in an increase in the 48 h prediction by a factor of 9. Similarly, doubling the selectivity ratio doubled the prediction at both 24 and 48 h. The volume of distribution had an inverse relationship with the 24 and 48 h 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 h. The predictions made by this model are sensitive to the estimates of the model parameters, which is to be expected. The model parameters used were all obtained from published literature and, while there is uncertainty about their accuracy, we can assume that the point estimates are relatively close to reality. The uncertainty in the parameter estimates has been dealt with, to some extent, by simulating values from distributions rather than using point estimates.

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. 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 accurate assessment of potential

Table 9
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	Meas > pre (N)	Meas > pre (%)	P-value	N	Meas > pre (N)	Meas > pre (%)	P-value
Chlorpyrifos								
Spray	20	3	15		35	10	29	
Within	145	11	8	0.265	145	20	14	0.035
Chlormequat								
Spray	102	27	26		94	39	41	
Within	330	62	19	0.094	330	127	38	0.599

exposure. Although the regulatory exposure assessment process allows for the separate assessment of three pathways of exposure, only the pathway providing the highest predicted exposure is then used. For our adult participants this was typically the direct contact and uptake pathways (3) following contact with contaminated surfaces whereas for the child participants this was exposure following evaporation of the pesticide following application (pathway 2) for the spray applications reported and considered in this study. It should be considered that the measured biomarker concentrations from the collected urine samples reflect pesticide exposures from multiple pathways of exposure, not just those used in the comparisons, and also includes exposure via other sources such as diet.

The REA process necessarily considers each pesticide in isolation. There is some public concern but limited data about the effects of pesticide mixtures. In this study farmers' spray records showed that for the majority of spray events, the relevant pesticides were applied with other pesticide products. For captan and penconazole, there were a number of occasions where these products were both applied using the same tank mixture. 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 concentrations 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. The majority of measured concentrations were well below the REA-based predicted concentrations indicating that for these cases the REA is sufficiently conservative.

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 those derived from 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 (Galea et al, 2015b) we compared the background urinary biomarker concentrations with those derived from the predicted exposures to further understand these results. Overall, the proportion of measured urinary biomarker concentrations in excess of the modelled concentrations for the relevant spray events were no different to what would be expected if no spray event had taken place. In conclusion, the results from this paper suggest that the contribution from spraying of the reported five pesticides to agricultural fields to the overall pesticide exposure of UK residents is low (under the conditions observed in this study) and that the REA method used to predict residential exposure to these pesticides appears to be sufficiently conservative.

Competing financial and other interests

There authors declare there are no financial conflicts of interest. Dr. Galea is a member of the UK Government Pesticide Incidents Appraisal Panel and Dr. Cocker was a member of the UK Government Advisory Committee on Pesticides until Dec 2014.

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2015.09.012.

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