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ACROPOLIS

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

SEVENTH FRAMEWORK PROGRAMME

Deliverable 3.3 Biomarker study

The Food and Environment Research Agency (Fera), Sand Hutton, York, UK
Contact person: Marc C. Kennedy, email: Marc.Kennedy@fera.gsi.gov.uk

Project team Acropolis: Marc C. Kennedy (Fera), Hilko van der Voet (Biometris), Angelo Moretto (Umil), Aida Turrini (INRAN), Andy D.M. Hart (Fera)

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Validation of a cumulative and aggregate exposure model using biomonitoring studies and dietary records for Italian vineyard spray operators.

Authors: Fera, UMIL, INRAN, DLO, RIVM

Abstract

To be completed

1. Introduction

The regulation of plant protection products (PPP) in the European Union (EU) now requires the cumulative risk to be considered according to the regulation 1107/2009 currently. Historically the risk assessments for human and environmental health have been performed by considering the active substances within a single product, which is often a single active substance (a.s.). The combined exposure to compounds within a cumulative action group (CAG) from various sources and routes needs to be considered, and not restricted to PPP. The model developed as part of the EU Acropolis project allows such exposures to be estimated, and the model predictions have been evaluated using the case study of exposure to triazole fungicides for spray operators in vineyards in the Lombardy region of Italy. Current models for individual exposures (REF) indicate the main routes of exposure to be dermal during the spray application and dietary. Therefore the case study measured dermal exposures of volunteer operators during the application of a traizole fungicide and dietary exposure was determined using a duplicate diet method and diary.

The exposure, in terms of absorbed dose can be determined in biomonitoring studies in which the whole urine sample is collected over a period which represents the time during which the parent compounds and metabolites would be eliminated from the body. With knowledge of the pharmacokinetics of the compounds under study the mass of parent compounds and metabolites collected in the urine can be related to the absorbed dose of the compound by all routes. Consequently the case study can compare the absorbed dose of a triazole compound with the measured intake in the diet and the estimated absorption via the dermal route by measuring the actual dermal exposure (ADE). (Inhalation Route add). These values can be further compared to model estimates with MCRA and the German Model or EUROPOEM database, and ultimately with predictions from the ACROPLOLIS model.

2. Material and methods

2.1 Occupational Exposure

Field studies were performed in the Lombardy region of Italy with application of triazole fungicides to wine grape vineyards to determine the potential dermal exposure (PDE) and actual dermal exposure (ADE) using whole body and patch dosimetry methods (UMIL paper submitted for publication 2013).

During the first year of the field studies in 2011 PPPs were used which contained the active substance (a.s.) tebuconazole. However during the local practice changed, with growers preferring to use PPPs with the a.s. penconazole.

UMIL to add more details of field protocol and analysis of samples etc

2.2 Dietary Exposure

2.2.1 Collection of duplicate diet sample

The volunteers in the field studies were asked to provide duplicate diet samples for the day prior to the planned spray application and dermal exposure assessments, and also the day of the application. Volunteers were also asked to provide a diary of food items consumed during these two days. A short interview when recollecting the one-day diary and the box containing the duplicated diet samples was undertaken to check the completeness of the information and gain an understanding of whether the duplicated diet is habitual or not. In particular, the interviewer asked whether the food consumed on the sample day was typical or if there were foods usually eaten but not consumed in the sample day. This check for completeness concerned the list of food items provided, the description of recipes.

Duplicate portions were collected in four categories based on the likelihood of the food containing residues of a conazole compound. In this way possible dilution of pesticides present by food items that do not contain the conazole compounds was minimised.

- All fresh vegetables and fruits, including fruit juice
- All food containing cereal products
- All liquids
- All meat products

The mass of each of the individual categories of food collected was the same as the mass consumed by the volunteer.

INRAN to add more details if required

The samples were collected at the end of the day then stored in a freezer below -18C until transported in dry ice to the UK where they were analysed by Fera.

2.2.2 Analytical technique

Fera to check details of extraction/analytical technique

Sample extraction was carried out using QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) acetonitrile extraction in combination with dispersive solid phase extraction dSPE. Residues of the conazole pesticides present in sample extracts were quantified using Ultra High Performance Liquid Chromatography tandem quadrupole mass spectrometry (UHPLC-MS/MS). Quantification was made by the use of calibration standards prepared in matrix or by the single point standard addition technique (individual samples analysed, both with and without, addition of analyte).

The method was validated by analysing a minimum of 5 samples spiked with each analyte at 1 µg/kg, with an acceptable mean recovery is in the range 70-120%, with an RSDr ≤ 20%). At least 1 sample was spiked with all of the analytes of interest, each at 1 µg/kg and analysed with each batch of samples to verify that the method is under control during routine use. A mean recovery of 60-140 % was considered to be acceptable for routine multi residue analysis in accordance with (Document N° SANCO/12495/2011).

2.3 Availability of data from existing models

In addition to the data from the field study, data from existing models were used as part of the validation process to compare predicted exposures with measured exposures for the dietary and non-dietary routes to validate the aggregate exposure model within ACROPOLIS

1. Predicted dermal exposure from EUROPOEM database (Table X)
2. Estimated dietary intake of tebuconazole residues for adult male Italian population (MCRA)
3. Estimated dietary intake of tebuconazole residues using food intake diary of volunteers

3. Results

3.1 Operator exposure data from the biomonitoring field study

A summary of all the data from the operator exposure field study is presented in Table 1 to illustrate the relationship between measured ADE and absorbed dose. The ADE for the field study described by Mandic-Rajcevic (in press) is summarised below separately for all eight operators and for the six operators which only used tractor mounted application equipment. The data are expressed as a proportion of the amount handled in in the same way in current exposure models the UK Predictive Operator Exposure Model (Martin 1986) and the German model (Lundehn et al 1992). As expected from model estimates and previous experience the use of hand held application technique resulted in above average exposure levels with ADE values of 20.92 and 7.87 ug a.s./kg a.s. applied.

Table 1. Details from the field study for operator dermal exposure and absorbed dose equivalent of tebuconazole

Individual ID	Age	Weight (kg)	Duplicate Diet Day	Potential dermal exposure (µg)	Actual dermal exposure (µg)	TEB equivalents in 24h post-exposure urine sample (µg)	Quantity of TEB used (g)	ADE mg TEB/kg Teb used	Absorbed dose ug TEB/kgBW
S01	51	92	1						
S01	51	92	2	15913	3650	180.6	99.0	36.9	2.0
S01	51	92	3	22496	1296	299.2	67.5	19.2	3.3
S02	49	100	1						
S02[h]	49	100	2	1877	251	28.2	198.0	1.3	0.3
S02B	50	100	1						
S02B	50	100	2	5829	558	41.3	594.0	0.9	0.4
S03	52	93	1						
S03	52	93	2	8657	2970	79.7	594.0	5.0	0.9
S04	41	57	1						
S04	41	57	2	6582	428	8.8	148.5	2.9	0.2
S04	41	57	3	4570	390	29.9	148.5	2.6	0.5
S04	41	57	4	6637	1152	32.9	148.5	7.8	0.6
S05	40	90	1						
S05	40	90	2						
S05	40	90	3	12585	2331	155.0	1530.0	1.5	1.7
S05	40	90	ns	15014	1503	341.8	900.0	1.7	3.8

BW = body weight
TEB= tebuconazole
ns = no sample

The data from Table 2 have been summarised below

Table 2. Actual dermal exposure to tebuconazole for operators during vine spraying (ug a.s./kg a.s. applied)

	All operators	Only tractor mounted application technique
Mean	7.19	5.75
Median	2.48	1.92
75th percentile	5.57	3.96
95th percentile	28.24	22.61

The German Model predicts an ADE value of 1.51 ug a.s./kg a.s. applied assuming use of appropriate PPE for mixing and loading and application and 2.44 ug a.s./kg a.s. applied assuming no use of gloves during the application. The use scenario selected for the German Model was tractor mounted/trailed broadcast air assisted sprayer. The product selected was a WP (wettable powder) formulation containing 250 g/kg a.s. applied at a dose rate of 1 kg/ha. The outputs from this model are 75th percentile values from the underlying database, which is not visible to the user.

Within the ACROPOLIS model a subset of data from the EUROPOEM database (Ref) was selected relating to a study involving the application scenario most similar to that used in the field study, i.e. broadcast air assisted spraying of grape vines. The data in the EUROPOEM model are visible and the data for the measured ADE in this study are shown below as 1.51 ug a.s./kg a.s. applied

Mean 6.24
Median 5.38
P75 8.59
P95 12.11

The data for ADE from the EUROPOEM database have been used with data on PPP usage for grape vines in Italy collected in EFSA funded surveys (Glass et al 2012) to generate a distribution of exposures. The use of such a distribution is demonstrated in case studies within ACROPOLIS (Kennedy et al 2013) to estimate ADE and absorbed dose of triazole PPPs, providing an example of non-dietary exposure within the aggregate model.

3.2 Data for dietary exposure

The collection of duplicate diet samples and a diary of food intake allows a comparison to be made of the tebuconazole intake on a daily basis for each f the volunteers. An example of the food diary information is shown in Table 3, which was used with MCRA to estimate the intake of tebuconazole for each of the coded food items

Table 3. Example of data information collected for the dietary diary of volunteer S01

Individual	Day Of Survey	Food item consumed	Amount Consumed (g)	Food Survey Reference
S01	1	100073	12.00	Duplicate Diet Study - IT 2011
S01	1	100083	36.50	Duplicate Diet Study - IT 2011
S01	1	100084	194.00	Duplicate Diet Study - IT 2011
S01	1	100122	50.00	Duplicate Diet Study - IT 2011
S01	1	100124	100.00	Duplicate Diet Study - IT 2011
S01	1	100321	171.00	Duplicate Diet Study - IT 2011
S01	1	100332	5.30	Duplicate Diet Study - IT 2011
S01	1	100362	2.00	Duplicate Diet Study - IT 2011
S01	1	100365	21.45	Duplicate Diet Study - IT 2011
S01	1	100476	70.00	Duplicate Diet Study - IT 2011
S01	1	100493	18.25	Duplicate Diet Study - IT 2011
S01	1	100542	64.84	Duplicate Diet Study - IT 2011
S01	1	100632	10.00	Duplicate Diet Study - IT 2011
S01	1	100644	80.00	Duplicate Diet Study - IT 2011

Analysis of the duplicate diet samples provides data for measured intake of tebuconazole by the volunteers in the field study. A total of 25% of the 64 samples analysed resulted in values below the limit of quantification (LOQ) which was 0.25ng/g, therefore the data have been collated in Table X half the LOQ value to represent values <LOQ for individual sample types. The total measured intake using zero to represent >LOQ values is also included in the table together with the estimated intake using the data from the food diary using MCRA.

Estimates of the daily intake of tebuconazole have also been calculated selecting within NCRA the adult males for the Italian population. The data shown in Figure X give an indication of how representative the food intake of the volunteers in the study is compared to the whole male population in Italy.

Table 4 Vales for measured residues of tebuconazole (ug/day) in duplicate diet samples and estimated values based on diet diary and MCRA

Sample ref	Cereals[1]	Vegetables[1]	Other[1]	Liquids[1]	Total[1]	Total[2]	MCRA Diary[3]
S1D1	0.095	0.009	0.031	0.063	0.198	0.127	0.222
S1D2	0.159	0.055	0.083	0.063	0.359	0.297	0.159
S1D3	0.140	0.059	0.112	0.063	0.374	0.312	0.165
S2b*D1	0.035	0.031	0.039	0.063	0.167	0.039	0.168
S2b*D2	0.035	0.028	0.015	0.063	0.140	0.000	0.098
S2D1	0.089	0.015	0.009	0.063	0.176	0.089	2.090
S2D2	0.039	0.013	0.014	0.063	0.128	0.000	0.492
S3D1	0.074	0.013	0.013	0.063	0.161	0.074	0.319
S3D2	0.038	0.013	0.102	0.151	0.303	0.253	0.496
S4D1	0.028	0.010	0.015	0.063	0.115	0.000	0.196
S4D2	0.053	0.008	0.030	0.063	0.153	0.053	0.701
S4D3	0.047	0.015	0.016	0.063	0.141	0.047	0.219
S4D4	0.028	0.016	0.026	0.168	0.237	0.168	0.218
S5D1	0.033	0.009	0.006	0.063	0.109	0.000	0.034
S5D2	0.048	0.021	0.013	0.063	0.143	0.000	0.216
S5D3	0.033	0.018	0.005	0.063	0.118	0.000	0.174

Figure 1. Estimates of the whole population

Insert figure from Hilko MCRA output

3.3 Combination of dermal exposure dietary intake and urinary metabolite data for tebuconazole

The data for the urinary metabolites of tebuconazole are presented in Table 5 together with the data for estimated dietary intake

Table 5.

IndividualID	Body Weight	Day	Dietary Intake µg/kgBW	Non dietary µg /kgBW	Urine TEBeq (µg)	Urine µg/kgBW	Aggregate (µg/kgBW)	Percentage non dietary
S01	92	1	0.00241					
S01	92	2	0.00173	39.67	180.6	1.963	39.68	100.00%
S01	92	3	0.00179	14.09	299.2	3.252	14.09	99.99%
S02	100	1	0.00168					
S02	100	2	0.00098	2.51	28.2	0.282	2.51	99.96%
S02B	100	1	0.02090					
S02B	100	2	0.00492	5.58	41.3	0.413	5.58	99.91%
S03	93	1	0.00343					
S03	93	2	0.00534	31.94	79.7	0.857	31.94	99.98%
S04	57	1	0.00345					
S04	57	2	0.01230	7.51	8.8	0.154	7.52	99.84%
S04	57	3	0.00384	6.84	29.9	0.525	6.85	99.94%
S04	57	4	0.00383	20.21	32.9	0.577	20.21	99.98%
S05	90	1	0.00038					
S05	90	2	0.00240					
S05	90	3	0.00193	25.90	155	1.722	25.90	99.99%

3.4 Outputs form the ACROPOLIS model.

The ACROPOLIS model combines dietary and non-dietary exposures using MCRA to estimate the daily intake of compounds of interest in the selected population, which in this case is the Italian male population. The non-dietary exposure is taken from the EUROPOEM database in this case to represent the exposure of operators applying PPP to grape vine. The MCRA estimates of daily intakes of tebuconazole are shown in Table 6.

Table 6. Output from MCRA for dietary intake of tebuconazole for Italian male population

	Compound name	Compound code	Contribution	Median (µg/kg bw/day)	Mean (µg/kg bw/day)	p25-p75 (µg/kg bw/day)
Total	Tebuconazole	RF-0403-001-PPP	97.50%	0.0403	0.335	(0 - 0.312)
Upper tail individuals > P97.5	Tebuconazole	RF-0403-001-PPP	95.10%	0.0702	2.24	(0 - 4.1)

The non-dietary data for exposure to tebuconazole are presented as µg a.s. /kg a.s. used which need to be converted into daily exposure values based on typical usage rates of tebuconazole in Italian grape vine. The ACROPOLIS model uses PPP usage data where available, and in the case of Italy the data from the EFSA project (Glass et al. 2012) have been used.

Mean	525.6522956
Median	275.2910707
P75	572.682807
P95	1858.854864

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