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

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ABSTRACT

The need for improved tools to estimate the cumulative and aggregate exposure to compounds such as plant protection products (PPPs) is recognised in the EU Regulation 1107/2009. A new model has been developed to estimate the exposure within a population to single compounds or compounds within a Cumulative Action Group, considering dietary and non-dietary sources and multiple exposure routes. To test the model a field study was carried out in Italy with operators applying tebuconazole fungicides, with measurements of dermal exposure collected. Whole urine samples were collected and analysed to provide values for the absorbed dose of tebuconazole, with duplicate diet samples collected and analysed as a measure of dietary exposures. The model provided predicted values of exposure for combined dietary and non-dietary routes of exposures which were compared to the measured absorbed dose values based on urinary analysis. The model outputs provided mean daily exposure values of 1.77 (±1.96) µg a.s./kg BW which are comparable to measured mean values from the biomonitoring field study of 1.73 (±1.31) µg a.s./kg BW. To supplement the limited measurement data available, comparisons against other models were also made and found to be comparable.

1. Introduction

The regulation of plant protection products (PPPs) in the European Union (EU) now requires the cumulative risk to be considered according to the regulation 1107/2009. Historically the risk assessments for human and environmental safety have been performed by considering the active substances within a single PPP, which is often a single active substance (a.s.). Rather than restricting the risk assessment on a single PPP, the combined exposure to compounds within a cumulative action group (CAG) from various sources and routes needs to be considered. The EU project ACROPOLIS (Aggregate and Cumulative Risk of Pesticides: an on-line integrated strategy) developed a general system for probabilistic modelling of cumulative and aggregate exposure within the EU (www.acropolis-eu.com). Cumulative exposure refers to the combined exposure from multiple compounds within the same CAG (USEPA, 2002). Aggregate exposure is defined as total exposure from dietary and non-dietary sources and is usually calculated with reference to a single compound (USEPA, 2001). The importance of aggregate and cumulative assessments is emphasised in EC (2005) Regulation 396/2005, Article 36, which calls for new methods to be used as soon as they are available. The ACROPOLIS aggregate model is

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described in Kennedy et al. (2014) which includes a range of case studies illustrating how some European non-dietary exposure models and databases can be incorporated. ACROPOLIS allows the user to take into account exposures from the use of several product types and use scenarios. The models are implemented within the web-based Monte Carlo Risk Assessment (MCRA) software version 8 (van der Voet et al., submitted for publication). Detailed case studies using MCRA for cumulative dietary exposure are presented in Boon et al. (2014).

The purpose of this paper is to describe a series of model/data comparisons used to test the ACROPOLIS model. As part of this, a biomonitoring field study was conducted with spray operators using tebuconazole products in vineyards in the Piedmont region of Italy. Biomonitoring techniques together with operator dermal dosimetry were used to measure the absorbed dose and dermal exposure of tebuconazole in the volunteers. In addition, duplicate diet sampling was used to provide data for external and absorbed dose. In this study the main comparisons involved aggregate but non-cumulative exposures for the single compound tebuconazole in order to make comparisons with the tebuconazole field measurements. Therefore, as a separate test of the aggregate and cumulative case, exposure to a CAG in the diet plus non-dietary exposure to tebuconazole was also estimated.

Previous studies have been reported which have measured urinary levels of pesticide parent compounds and metabolites, particularly for children of farmers and farm workers (Bradman et al., 2007; Egeghy et al., 2011). The early work to evaluate exposures to compounds within a CAG focussed on the organophosphate pesticides. Urinary levels of dialkyl phosphates (DAP) for the population have also been reported (Barr et al., 2011; Duggan et al., 2003; Heudorf and Angerer, 2001) to assess exposures from all routes. As an example of occupational exposures, Ueyama et al. (2012) reported that urinary DAP levels for a range of workers in Japan, including pesticide applicators, were similar to those reported previously. Exposure to pesticides as part of a total diet study has been performed by analysing community food samples (Gimou et al., 2008; Nougadère et al., 2012) and from duplicate diet samples (Melnyk et al., 1997, 2012, 2014).

For risk assessment it is important to be able to quantify the contribution of the different sources and routes of exposure. Spot urine samples provide limited information in terms of exposure, due to the variable time interval between exposure and sampling (Bradman et al., 2007). It is more informative to determine exposure in terms of the absorbed dose by conducting biomonitoring studies in which the whole urine sample is collected over the time period 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 (Hu et al., 2004). To derive an estimate of the contribution by the different routes of exposure, residues occurring in the diet during the exposure period under investigation need to be collected separately from other exposure sources. Therefore the biomonitoring field study in the ACROPOLIS project was designed to 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). The inhalation route of exposure was not measured, as this is considered to be a minor route of exposure for this application technique.

To carry out a test of the model all available data were used, and comparisons were made with as many intermediate results and alternative models as possible, to check for consistency. As described below, individual values for dermal and dietary exposures were compared to model estimates with MCRA, the model described in Lundehn et al. (1992) (henceforth referred to as the German Model) or the EUROPOEM (EUROpean Predictive Operator Exposure Model) database (EUROPOEM, 1996, 2002; van Hemmen, 2001), and ultimately with aggregate exposure predictions from the ACROPOLIS model as part of a testing exercise.

2. Materials and methods

The process of estimating aggregate exposure brings together various model and data components, each of which is described below. Various comparisons could potentially be made in order to assess the final estimate or intermediate calculations. Figure 1 shows how the estimation is constructed, and which comparisons are presented in this paper for model checking. Jointly considering the data for the dietary and non-dietary exposure routes provides an indication of the contribution of each of the routes to the total exposure, and how the measured "external" dose compares to the measured internal dose from the biomonitoring study.

There were 7 individual volunteers used in the study, of which 5, labelled S1–S5, provided both duplicate diet and urine samples. One of the participants provided measurements on 2 separate occasions, which were treated independently, effectively as separate individuals labelled S2 and S2B. Multiple working (spraying) days were included but due to practical difficulties, not all data components (duplicate diet, urine, dermal) could be collected from all study participants and days. Sample sizes are as follows: The 6 individuals labelled S1, S2, S2B, S3, S4, S5 provided duplicate diets on 3, 2, 2, 2, 4, and 3 consecutive days respectively (as seen in Table 4) giving 16 in total. Urine samples were available from these same individuals on 2, 1, 1, 1, 3, and 2 working days (Table 1). Only the first 9 of these urine measurements were actually used in our comparisons (as seen in Table 5) because one of the corresponding S5 duplicate diets was missing. For dermal exposure these individuals provided samples on 2, 2, 1, 0, 3, and 2 working days respectively. A further 2 study individuals provided dermal samples on 1 working day each, so there were 12 dermal samples in total.

2.1. Occupational exposure measurements

Field studies were performed in the Monferrato area, part of the Piedmont region of Italy with application of triazole fungicides to wine grape vineyards. As explained in Fustinoni et al. (2014), this area was selected based on high sales of tebuconazole from the previous season. Eligible vineyard estates were contacted with assistance from the local health authority and seven volunteers came forward following a meeting of individuals from these estates. The study was performed in accordance with Italian law 81/2008 for health and safety at the work place, under the supervision of an occupational health physician. Volunteers signed the informed consent form approved by the ethics committee of the University of Milan. Measurements were collected to determine the potential dermal exposure (PDE) and ADE, using whole body and patch dosimetry methods. The dermal dosimetry methodology followed a modified version of the OECD Guideline protocol (OECD, 1997). PDE and ADE were measured using outer cotton coveralls and inner cotton dosimeters respectively. Hand exposure was collected with a liquid hand wash poured on the subject's hands and captured in a basin. Head exposure was collected from a fabric head cover. Other clothing and equipment including respiratory equipment were as per normal practice for each individual. The ADE values reported below represent the total summed across body, head and hands. Full details of the field study including the dosimetry methods used are described in detail by Fustinoni et al. (2014). The analysis of the dermal samples is described in Mandic-Rajcevic, Maria Rubino, Vianello, Fugnoli, Polledri, Mercadante, Moretto, Fustinoni, Colosio (unpublished). Duplicate diets and urine samples were also collected on the days before, during and after the application period. Urine samples were collected for the 24 hour period prior to the start of the pesticide application and for periods between 24 and 48 hours following the end of the application. Two specific metabolites of tebuconazole had been identified for analysis in the urine samples, TEB-OH [(RS)-5-(4-chlorophenyl)-2,2-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-pentanediol] and TEB-COOH [(RS)-5-(4-chlorophenyl)-2,2-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)-3-ol-pentanoic acid] (Mercadante et al., 2014). Correlations were assessed between dermal exposures and urine-based exposures at different time periods. One of the results from Fustinoni et al. (2014) used to inform our model testing was that urine samples in the 24 hours immediately after the first application were generally the most highly correlated with dermal exposure. Urine measurements from this time period were therefore used in the comparisons. The total amount of excreted metabolites was converted to tebuconazole equivalent based on the molecular weights of the corresponding chemicals (Fustinoni et al., 2014). The accuracy in the determination of TEB metabolites in urine was estimated to range from 98 to 101%, based on the use of internal quality controls (Mercadante et al., 2014).

Table 1 summarises those data from the operator exposure field study for which urine measurements were available. Actual dermal exposure is labelled ADE (μg a.s.) and was measured on the inner garments for each individual. This value was then scaled by the respective amounts of a.s. used, to produce the final column in Table 1. Presenting exposure as a proportion of a.s. used is standard practice, and allows for a more consistent comparison of models, as for example in Table 2. For each individual, absorbed dose is expressed as the observed urine-based tebuconazole equivalent relative to bodyweight.