Integration of biological monitoring, environmental monitoring and computational modelling into the interpretation of pesticide exposure data: Introduction to a proposed approach

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A B S T R A C T

Open field, variability of climatic and working conditions, and the use of complex mixtures of pesticides makes biological and environmental monitoring in agriculture, and therefore risk assessment and management, very complicated. A need of pointing out alternative risk assessment approaches, not necessarily based on measures, but simple, user-friendly and reliable, feasible also in the less advanced situations and in particular in small size enterprises, arises. This aim can be reached through a combination of environmental monitoring, biological monitoring and computational modelling. We have used this combination of methods for the creation of “exposure and risk profiles” to be applied in specific exposure scenarios, and we have tested this approach on a sample of Italian rice and maize herbicide applicators. We have given specific “toxicity scores” to the different products used and we have identified, for each of the major working phases, that is mixing and loading, spraying, maintenance and cleaning of equipment, the main variables affecting exposure and inserted them into a simple algorithm, able to produce “exposure indices”. Based on the combination of toxicity indices and exposure indices it is possible to obtain semi-quantitative estimates of the risk levels experienced by the workers in the exposure scenarios considered. Results of operator exposure data collected under real-life conditions can be used to validate and refine the algorithms; moreover, the AOEL derived from pre-marketing studies can be combined to estimate tentative biological exposure limits for pesticides, useful to perform individual risk assessment based on technical surveys and on simple biological monitoring. A proof of principle example of this approach is the subject of this article.

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1. Introduction

The main tools currently available for the “in-the-field” pesticide risk assessment are biological and environmental monitoring, but in agriculture both tools show important limits. In particular, since working activities in agriculture are performed in an open environment, where the main route of absorption is via the skin, environmental airborne concentrations and related limits of exposure are of scarce utility. On the contrary, measurements of dermal dose involve very complicated and expensive procedures and cannot be carried out on a routine basis. Furthermore, there are no specific exposure limits. Even biological monitoring faces strong limitations, including lack of fully validated indicators and biological exposure limits. Additional difficulties are the instability of climatic and working conditions (Arbuckle et al., 1999; Harris and Solomon, 1992; Harris et al., 1992; Maibach et al., 1971; Moody et al., 1992), and the intermittent use of complex mixtures of pesticides, characterized by a variable composition (Hines et al., 2001), that deeply affect the possibility of carrying out accurate
risk assessment. It is, in fact, hard to collect data which are really representative of the average working conditions and not only of the specific and single situation being monitored.

It is thus apparent that in order to perform risk assessment in these conditions there is a need of simple, user-friendly and reliable approaches to estimate the levels of exposure (and of related occupational risk) experienced by the workers during typical, rather than actual, activities (Arbuckle et al., 2002). We refer to these typical conditions as scenarios. In order to build truly representative scenarios for agricultural activities, it is valuable to consider that some useful reference points exist and can be exploited. In particular, in the regulatory procedure performed in most industrialized countries leading to the authorization of the use of a specific compound – the so called “pre-marketing evaluation” – extensive information on physicochemical, toxicological and environmental characteristics is collected from controlled experimental and field studies. In particular, from the toxicological point of view, a nearly complete assessment of the toxicological profile, including in most cases skin absorption coefficients, toxicokinetic parameters of the parent compound and of the relevant metabolites is available. During the pre-marketing risk assessment process, a health based exposure limit of internal dose is established, that is the “Acceptable Operator Exposure Level” (AOEL), defined by the Directive 97/57/EC (establishing Annex VI to Directive 91/414/EEC) “… the maximum amount of active substance to which the operator may be exposed without any adverse health effects.” The AOEL is expressed as milligrams of the chemical per kilogram body weight of the operator. (CEC, 1991, 2001; EC, 1997).

As such, the AOEL is more suitable for risk assessment in the pre-marketing phase, where an estimate of the absorbed dose can be calculated by the used models, but it is not easily applicable in the “in field” risk assessment. In this case exposure is measured as airborne concentrations, as dermal dose (deposition) or as concentration of the compound under study or of its metabolites in body fluids. As a consequence, the relationship between exposure and biological monitoring data, and OEL can only be assessed by a thorough knowledge of ADME (absorption, distribution, metabolism and excretion) (Hakkert, 2001; Machera et al., 2003; Maroni et al., 1999).

We are currently developing an integrated user-friendly tool for risk assessment and management in agriculture, in which biological monitoring, environmental monitoring, and computational modelling are used. The main product of our effort is an exposure and risk profile, as a reliable way to forecast exposure levels of workers in typical scenarios from a minimum set of available information, aimed at performing a preliminary risk assessment even without the need of “in field” measurements. A proof of principle example of this approach is the object of this article.

2. Description of the approach

Our approach is essentially divided into three main tasks:

1. The setup of an evaluation grid for “in-the-field” risk assessment;
2. The setup of indices for exposure estimation;
3. Comparison between the risk class allocation provided by the “profile” (grid) and results of risk assessment performed on the basis of the results of biological and environmental monitoring.

2.1. Setup of an evaluation grid

The purpose of this effort is to create a “user friendly tool” adequate to evaluate the levels of occupational exposure and risk consequent to pesticide application, having in mind that it is possible to use even fairly toxic pesticides if the overall working conditions are such that farmer’s exposure is virtually negligible and that, on the contrary, even a relatively low toxicity product can pose an unacceptable risk if handled overlooking the most basic precautions.

To this aim we have chosen to develop a fairly simple $4 \times 4$ evaluation grid with four toxicity classes for the active ingredient, and four exposure classes resulting from the working conditions. Using an even number of classes (four, in our case) avoids the well-known risk of indecision, i.e., to drift to the center of the evaluation grid in case of unavailability or ambiguity of data. In the $4 \times 4$ evaluation grid shown in Table 1 there are 16 possible combination of toxicity and exposure classes, which are divided into four levels of risk: negligible (3 combinations), probably low (5 combinations), probably high (5 combinations), and unacceptably high risk (3 combinations), each identified by a different colour-coded area. To use the evaluation grid for “in-the-field” risk assessment, it is simply required to identify the toxicity class of the active principle used and to classify exposure into one of the four classes, as explained below. Once classifications of toxicity and exposure are reached, the position in the grid corresponds to one of the four levels of risk. If the outcome of the evaluation is not “negligible risk”, than specific action may be taken and their effects can easily be checked. These include the use of less toxic compounds, of more adequate personal protective devices, a better maintenance of equipment, the education of farmers, etc. The final procedure might become so simple and user friendly to allow “self-evaluation” by the farmer.

Of course, the first step is to assign appropriate values to the toxicity and exposure classes, as it will be specified below. The second step is to test the outcome of the first step with case studies. In order to do it, we have selected two cash crops, rice and maize, which are typical for Northern Italy and are characterized by a sufficiently well established and stable agricultural procedures which are, therefore relatively simple to model. The most beneficial characteristic for our initial proof-of-principle effort is that no re-entry activity is necessary in these crops after pesticide application.

2.2. Indices of exposure

2.2.1. Definition of toxicity indices

Classification, risk phrases and labels are defined during the toxicological evaluation of pesticides performed in the pre-marketing phase; from this information we ranked the pesticide

<table>
<thead>
<tr>
<th>Class</th>
<th>Exposure score</th>
<th>Toxicity score</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Low</td>
<td>NEGLIGIBLE</td>
</tr>
<tr>
<td>B</td>
<td>Probably low</td>
<td>NEGLIGIBLE</td>
</tr>
<tr>
<td>C</td>
<td>Probably high</td>
<td>LOW RISK</td>
</tr>
<tr>
<td>D</td>
<td>High</td>
<td>LOW RISK</td>
</tr>
</tbody>
</table>
formulations in four main toxicity groups, from the lowest levels of toxicity (group 1), to the highest level of toxicity (group 4).

Our ranking procedure considers each risk phrase in the frame of the agricultural occupational scenario, so that, as an example, absorption through ingestion is not crucial for farmers’ exposure, while long term and no-threshold effects are of a higher concern.

In the highest toxicity group, we have allocated the compounds with a higher acute toxicity (i.e., more risky in case of accidental overexposure) and those having particularly concerning risk phrases, such as carcinogenicity or teratogenicity (i.e., to account not only for the effects of continued use but also for the severe health consequences). Table 2 shows the toxicity scores attributed on the basis of the risk phrases allocated to the active ingredient. In case the farmer uses a mixture of pesticides, the pesticide with the highest ranking toxicity score is considered as the sole active ingredient of the whole mixture.

### 2.2.2. Definition of exposure indices

Through literature search and systematic observation of working activities in selected scenarios, we have identified the main variables affecting the levels of exposure to pesticides in the three main work phases in rice an maize crops (mixing and loading, application, cleaning and maintenance of machineries and personal protective devices) and the relations linking these variables. This approach is not novel, since it is that employed by different risk assessment algorithms (Lundehn et al., 1992). The identified variables have been divided in two main groups, i.e., those directly correlated with exposure levels and those whose increase or presence is associated with a reduction of the levels of exposure and risk.

A scoring system was established to assign numerical values to the various working conditions encountered in the field. Higher score numbers were assigned to conditions leading to use of a higher amount of pesticide (a larger treated area, a higher application dose, a higher concentration of active principle in the formulation) and to higher exposure in the different phases of work (less efficient equipment).

Based on the above consideration, the exposure index $I_{exp}$ is calculated as a time-averaged sum of those calculated for the three main working phases (mixing, MIX; application, APPL; in-field repair, REP), as described by Eq. (1):

$$I_{exp} = I_{MIX} + \%t_{MIX} + I_{APPL} + t_{APPL} + I_{REP} + \%t_{REP}$$  \hspace{1cm} (1)

$\%t_i$, being the percent fraction of the working time spent into the specific task $i$.

For each work phase (MIX, APPL, REP), the index ($I$) can be described by the following Eq. (2)

$$I = \text{dose} \ast I[PPD] \ast I[\text{operator skills}] \ast I[\text{machineries}]$$  \hspace{1cm} (2)

where dose is dependent on several parameters (see Tables 3 and 4), and PDD, operator skills and condition of machinery are modifying factors.

### Table 2

Toxicity scores based on the risk phrases allocated to the compound.

<table>
<thead>
<tr>
<th>Risk phrase</th>
<th>Score</th>
<th>Examples of products</th>
</tr>
</thead>
<tbody>
<tr>
<td>R22 DANGEROUS IF SWALLOWED</td>
<td>1</td>
<td>Terbutylazine – Propanil – Copper hydroxide – Ziram – Diquat – Benfuracarb</td>
</tr>
<tr>
<td>R36 EYE IRRITANT</td>
<td>1</td>
<td>Endosulfan – Dichlorvos</td>
</tr>
<tr>
<td>R20 DANGEROUS IF INHALED</td>
<td>2</td>
<td>Linuron – Methiocarb – Dichlorvos</td>
</tr>
<tr>
<td>R25 TOXIC IF SWALLOWED</td>
<td>2</td>
<td>Copper hydroxide – Methiocarb – Ziram – Benfuracarb</td>
</tr>
<tr>
<td>R23 TOXIC IF INHALED</td>
<td>3</td>
<td>2,4-D-Mancozeb – Methiocarb – Ziram – Dichlorvos</td>
</tr>
<tr>
<td>R43 SKIN SENSITIZER</td>
<td>3</td>
<td>Ziram – Diquat – Dichlorvos</td>
</tr>
<tr>
<td>R26 HIGHLY TOXIC IF INHALED</td>
<td>4</td>
<td>Linuron – Benfuracarb</td>
</tr>
<tr>
<td>R62 CAN REDUCE FERTILITY</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3

Scoring of main working conditions which determine or influence entity of pesticide applicator’s exposure during mixing/loading and application. Re-entry is not considered because it is not present in these activities, and the crop architecture was the same for all subjects – since they all worked on low crops.

<table>
<thead>
<tr>
<th>Phase of work</th>
<th>Variables influencing exposure</th>
<th>Less exposure</th>
<th>→</th>
<th>→</th>
<th>More exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mix/load</td>
<td>Number of loadings</td>
<td>1</td>
<td>2-5</td>
<td>&gt;5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Score</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Concentration of active principle (%)</td>
<td>&lt;50</td>
<td>50-90</td>
<td>&gt;90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Score</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Type of formulation</td>
<td>Soluble bags</td>
<td>Granules/liquid</td>
<td>Powder</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Duration of mixing and loading</td>
<td></td>
<td></td>
<td></td>
<td>Short</td>
</tr>
<tr>
<td></td>
<td>Time (% of total activities)</td>
<td></td>
<td></td>
<td></td>
<td>Long</td>
</tr>
<tr>
<td>Application</td>
<td>Use rate (kg/ha)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Score</td>
<td>&lt;0.1</td>
<td>0.1–2.5</td>
<td>&gt;2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Application pressure (bar)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Score</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treated area (ha)</td>
<td>&lt;10</td>
<td>10–20</td>
<td>&gt;20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Score</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Interventions on machines during application</td>
<td>None</td>
<td>1–2 times during the day</td>
<td>More than 2 times</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Score</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Condition of equipment</td>
<td>Good</td>
<td>Acceptable</td>
<td>Bad</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Score</td>
<td>0</td>
<td>4</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Duration of application</td>
<td></td>
<td></td>
<td></td>
<td>Short</td>
</tr>
<tr>
<td></td>
<td>Time (% of total activities)</td>
<td></td>
<td></td>
<td></td>
<td>Long</td>
</tr>
<tr>
<td>Maintenance</td>
<td>Maintenance of equipment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Score</td>
<td>Not done</td>
<td>Done</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Duration of maintenance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time (% of total activities)</td>
<td></td>
<td></td>
<td></td>
<td>Short</td>
</tr>
</tbody>
</table>

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that higher score numbers correspond to conditions leading to an increase of exposure (dose).

2.2.2.1. **Pesticide mixing and loading (MIX).** In this phase exposure occurs more likely as the consequence of episodic phenomena (contact with formulations in the state of powders, splashes from suspensions or foams) rather than from continuous contact. The variables to which a score was assigned are the following:

a) **Number of loadings/day:** This variable is related with tank capacity. Even though data are not univocal, it seems that having a large tank capacity reduces the levels of exposure because it reduces the number of mixing and loading events per unit of active ingredient (a.i.) applied (Arbuckle et al., 2002). The number of loading does not depend only on the tank’s size, but on other variables such as the need of using different formulations, or different concentrations.

b) **Concentration of active ingredient in the product:** The levels of exposure increase, in the same working conditions, with the increase of the concentration of the active ingredient in the product (Wester and Mailbich, 1985).

c) **Type of formulation:** It is well known and proved that the levels of exposure depend on the different types of formulation; in general, higher levels of exposure are observed with powders than with liquids. Levels are usually very low for granules, and negligible in case of use of soluble packages (Arnold and Beasley, 1989).

d) **Duration of mixing and loading** is a variable necessary for the calculation of the time averaged sum of indices of exposure.

2.2.2.2. **Pesticide application (APPL).** Literature data collected from “in-the-field studies” suggest that, despite a fairly large variability, application is the phase which most significantly contributes to the operator exposure (Arbuckle et al., 2002; Baldi et al., 2006). The variability of exposure data might be at least partially explained by the other variables of interest and in particular by the presence/absence of modifying factors, which will be described below. The variables to which a score was assigned are the following: use rate, treated surface, application pressure, interventions on machineries during application and condition of machineries. Exposure pattern is also strongly related to crop architecture, i.e., height of the plants and their density on the ground (Hughes et al., 2008). All available models assume that any increase of crop height is associated with an increase of the exposure. Higher distance between the rows of the crop allows operators to avoid contact with sprayed surfaces (Machera et al., 2003). In our pilot study, crop architecture was not taken into account due to fact that both the crops addressed belong to the “low” typology.

a) **Use rate:** the quantity (in weight) of product applied per surface unit (i.e., kg/ha) is considered a key variable (Arbuckle et al., 2002).

b) **Daily treated surface:** this parameter (hectares treated per day) enters, along with use rate (b, above) in the calculation of the amount of pesticide used per day.

c) **Duration of application:** (hours spent for the task). Duration is not related only to crop architecture and size of the treated areas, but also depends on the characteristics of the territory: for example, applying in a mountainous area needs more time than a similar kind of activity in a flat territory (Arbuckle et al., 2002; Coble et al., 2005).

d) **Application modalities and pressure:** This variable is at least partially related with crop height (for example, low crops are usually treated with boom application and high crops with sprayers). Available data consistently suggest that the highest levels of exposure are related with back pack application, followed by spray and then by booms (Garry et al., 2001; Nigg et al., 1990; Nuyttens et al., 2009b; Rutz and Krieger, 1992; van Hemmen, 1992). Other factors affecting operator exposure in this phase are the application pressure (Machera et al., 2003; Nuyttens et al., 2007), and the type and condition of the spraying devices (addressed later). In our proof of principle study, only boom application on low crops has been considered.

e) **Condition of the machineries and interventions on machineries during application:** If machineries are in good condition of maintenance, it is easily anticipated that there will be little if any need of interventions during application (Baldi et al., 2006). Similarly, the pesticide throw through the nozzles will be fluent, without a significant runoff or need of unanticipated maintenance in the field or at the farm. Exposure can be significantly reduced by the use of low pressure and anti-drift nozzles (Nuyttens et al., 2009b). As for boom, pressure is a key element in determining operator exposure, as well maintenance of the equipment (Machera et al., 2003; Nuyttens et al., 2009a). In particular, a well maintained apparatus avoids the need for the operator to exit the tractor to do non-scheduled maintenance activities. Heavy hand contamination occurs often due to poor general care of the workers and of resulting poor maintenance of the equipment (Machera et al., 2003). Also, if the equipment is well kept, the surfaces will be less contaminated, and contaminated surfaces are known to be a major source of exposure (Hines et al., 2001; Yoshida et al., 1990).

f) **Type of tractor used:** This variable will be specifically addressed in the paragraph on “modifying factors”.

2.2.2.3. **Cleaning and maintenance of machineries (REP).** Significant exposure of the worker may occur while performing these tasks. Field studies have shown that, in some cases, this task provides the highest contribution to worker’s exposure (Baldi et al., 2006; Coble et al., 2005). This working phase is hardly addressed by models, since it is difficult to estimate its contribution in quantitative terms. The easiest way to take this task into account is to evaluate the time spent on interventions (duration of each single intervention) and the frequency of interventions. As for the use of personal protective devices, the variable will be addressed in the next paragraph (“modifying factors”).

### Table 4

<table>
<thead>
<tr>
<th>Modifying factors</th>
<th>Less exposure</th>
<th>→</th>
<th>→</th>
<th>More exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of tractor Score</td>
<td>With cabin and carbon filter 0</td>
<td>With air-conditioned cabin 1</td>
<td>With cabin without air-conditioning 2</td>
<td>Open 3</td>
</tr>
<tr>
<td>Personal protective devices Score</td>
<td>Adequately used</td>
<td>Not used</td>
<td>0.7</td>
<td>1</td>
</tr>
<tr>
<td>Training/skill Score</td>
<td>Certificate or equivalent</td>
<td>None</td>
<td>0.5</td>
<td>1</td>
</tr>
</tbody>
</table>

Certificate of Letters
2.2.3. Modifying factors

Generally, these are factors that mitigate workers’ exposure with reference to situations where these do not operate. As highlighted by several studies, typical examples are the use of Personal Protective Devices (PPDs), of well-designed, efficient agricultural machinery and the level of operator’s skill (Arbuckle et al., 2002, 2005; Dosemeci et al., 2002). However, the opposite may occur (e.g.: not efficient machinery). Modifying factors have been assigned values from 0.5 to 1. The following factors have been considered:

a) Use of PPDs: PPDs provide effective protection only if they are adequate for the risk factor they are addressed to, in good condition of maintenance, and used in a proper way (Gomes et al., 1999; Libich et al., 1984). For example, some chemicals can easily permeate through gloves or boots made of certain polymers, thus not providing adequate protection against specific formulations (Brouwer et al., 2001). Gloves material is particularly important because in many studies the contribution to dermal exposure of hand deposition has been estimated to be 50% or more (Raldi et al., 2006; de Cock et al., 1995; Hines et al., 2001). If gloves are removed during work, and are worn again without having washed the hands, they might be significantly contaminated by the chemicals, and therefore become a source of exposure (Canning et al., 1998; Garrod et al., 2001; Guo et al., 2001; Machera et al., 2003; Sanderson et al., 1995).

b) Type of tractor used: The highest levels of exposure are observed during use of an open tractor. The exposure is significantly reduced, but not abolished, by using a closed tractor, and is negligible when an air-con tractor with filters is used (Arbuckle et al., 2002; Carman et al., 1982; Coble et al., 2005). Of course, in this case, doors and windows of the tractor’s cabin must remain closed while working, filters must be regularly changed, and people wearing contaminated clothes and gloves must not enter the tractor, in order not to contaminate internal cabin surfaces (Hines et al., 2001; Sanderson et al., 1995).

c) Operator’s skill: Operators’ awareness of the risks, and their skills in doing the job is the first and most important modifying factor to be considered in the evaluation (Gomes et al., 1999; Libich et al., 1984; London, 1994). Operators’ skills can be evaluated through a specific interview as well as by observing and ranking specific working procedures (e.g. awareness in the use of adequate PPD or the way the worker approaches application in windy days). It is important to remark that a well-trained agricultural worker is supposed to adopt good working practice in a broad definition, not only in term of use of PPDs. Therefore he avoids application in environmentally unsafe conditions, for example in very windy days, or unsafe working procedures, such as smoking during application or opening the windows of the air-con tractor.

2.3. Comparison of modelled risk assessment to that derived from field-measured exposure

The most reliable exposure limit for risk assessment of pesticide exposure in agriculture is represented by the AOEL. Being expressed as milligrams of the chemical per kilogram body weight of the operator, AOEL is not immediately comparable with external dose measures; however, pesticide dermal deposition can be used to estimate an internal dose, considering dermal absorption coefficients and worker’s body weight and then compared with the AOEL. Individual farmer’s exposure can be than expressed as % of the AOEL.

In a group of 24 maize and rice farmers who applied propanil or terbutylazine, we measured external exposure from which we estimated the internal dose. Urine metabolite excretion has been employed to confirm these internal dose estimates and a tentative excretion value corresponding to the absorption of active ingredient at the AOEL level of exposure, i.e., a tentative biological exposure limit (BEL) has been defined on the basis of the results of environmental and biological monitoring. The AOEL established for both propanil and terbutylazine is 0.02 mg/kg\textsubscript{bw} (CDGB, 2011; PPDB, 2011), and these compounds are classified with a toxicity score of 1 (Rubino et al., 2011). The estimated internal dose was then compared to the AOEL and we classified exposure as follows, taking into account that estimates necessarily show some degree of uncertainties, and that a conservative approach is needed.

Class A: exposure dose ≤33% of AOEL → negligible risk
Class B: exposure dose 34–66% of AOEL → low risk
Class C: exposure dose 67–90% of AOEL → high risk
Class D: exposure dose ≥100% of AOEL → unacceptable risk

This grid has been used to check the concordance with the risk class allocation obtained by the “risk profile” described above. This work has been performed in order to (i) evaluate the validity of the model’s estimates; (ii) fine tune some of the coefficients in the algorithm.

3. Results

3.1. Concordance of risk assessment performed by profile and by field measurements

Classification according to the algorithm of the 24 examined pesticide applicators (two of which have been examined twice in successive years, and two worked in shifts, so were considered twice) is shown in Fig. 1. All 28 exposure scenarios considered fell in the “negligible risk area” of our risk assessment grid (Table 1).

Based on the data of actual monitoring in the field (Rubino et al., 2011), in about half of the 28 examined workers the estimated internal dose was below 1% of AOEL. In only one case measured exposure resulted 80 times higher than the AOEL, as a consequence of accidental spilling of the compound during work activity and which of course could not be taken into account by the simplified “risk profile”.

3.2. Integration of environmental and biological monitoring to estimate a provisional biological exposure limit

We defined the biological exposure limit (BEL) as the quantity of a pesticide (or metabolite) excreted in the urine within 24h after cessation of a one-day application by an agricultural worker who has absorbed, through all possible routes of absorption, a dose equal to the AOEL established in the authorization process for the compound of concern.

Fig. 2 shows the results of the estimation of a provisional BEL for the pesticide propanil based on excretion of the main urinary metabolite, 3,4-dichloro-aniline. Full details of the monitoring campaign will be published elsewhere (Rubino et al., 2011). A statistically significant and fairly strong double logarithmic linear relationship is observed between pesticide metabolite excretion in 24-h post-application urine and the estimated internal dose, reported as ratio with AOEL. Extrapolation of the least-square regression line and of its 95% confidence limits to a ratio of 1 (i.e., exposure equal to 100% of the AOEL) yields the provisional BEL for propanil as the 95% upper limit of the calculated regression. On the basis of the reported results a total amount of 3,4-dichloro-aniline of less than 1000 µg in the 24-h post-application urine of a farmer who sprayed propanil shows that the worker is exposed at a dose less than the AOEL of 0.02 mg/kg\textsubscript{bw} per day. It is also apparent from the fairly high dispersion of the data that many more measurements are necessary to obtain fully reliable results. In particular, a
wider range of working conditions need to be explored, especially in the proximity of the AOEL.

The ‘protection factor’ used as the independent variable in the graph of Fig. 2 is in fact the reciprocal of the more commonly used ‘Hazard Quotient’ (HQ=exposure/AOEL). The use of the risk index (1/HQ) is justified by the need of providing to the workers a more understandable value such as a ‘school mark’, the value of which increases as the condition of safety improves.

![Graph showing risk assessment and ranking of 28 pesticide applicators](image1.png)

**Fig. 1.** Results of risk assessment and ranking of 28 pesticide applicators according to the proposed model.

![Graph showing relationship of urinary metabolite excretion to protection factor](image2.png)

**Fig. 2.** Relationship of urinary metabolite (3,4-dichloro-aniline, 3,4-DCA) excretion to protection factor (defines as AOEL/exposure) measured from biological monitoring and from under-clothes pads in 12 farmers who applied propanil. The pair of more external dotted lines is the 95% confidence limits of the double-logarithmic regression calculated on the whole set of results (regression line is the thick line; parameters are reported in the inset). A provisional BEL of approx. 1 mg of 3,4-DCA/L of 24-h urine is calculated as the upper 95% confidence limit of the regression at a value of the protection factor of 1 (e.g. with exposure at the AOEL).
4. Discussion and conclusions

Here we propose an approach to the assessment of pesticide users’ exposure that takes advantage of existing information on pesticide toxicity and is of relatively simple application. This information is integrated with observation of the local specific exposure scenario where the different variables that influence or determine exposure have been weighted and combined. This approach (“risk profile”) does not give accurate or consistent estimates of exposure, but rather a pragmatic information on the acceptability of the risk faced by the workers and, consequently, of the presence or the absence of the need to carry out preventive interventions.

We compared the outcome of this “risk profile” with the assessment of the exposure by measuring dermal deposition from which the internal dose was derived taking into account the dermal absorption rate of the compounds. When this internal dose was compared to the AOEL, a ranking of exposure was obtained and compared with the ranking of the “risk profile”. The two approaches gave consistent rankings, thus supporting the usefulness of the “risk profile” algorithm. However, it should be noted that all subjects, but one in the environmental monitoring approach fell in the “negligible risk” area. This limits somewhat the value of the comparison and will require assessment of higher exposure levels for further confirmation.

With regard to the “risk profile”, it should be also noted that, if it will turn out to be outside of the “negligible risk” area, it allows simulation of the outcome of selected changes (e.g.: change of a.i., improved PPDs, reduced treated surface per day). This will help in identifying specific preventive intervention, and give a judgement on the likelihood of improving, through the planned action(s), the working conditions up to levels of acceptability. These simple tools can be used also in small-size and family based enterprises, and by a risk assessor acting in the territory, without the need of high specialization in pesticide risk assessment. In addition, the “risk profile” approach can take into account some variables that strongly affect exposure levels and that are not explicitly considered by the most used deterministic model. Of course, neither approach can anticipate episodic overexposure due to accidental events. However, since such events are often the outcome of poor general conditions of occupational safety and hygiene in the farms and of a poor training of the workers, the possibility of occurrence is indirectly considered in our profile, in which the quality of machineries and the levels of training of the workers is taken into account.

Critical issues still exist in the use of check lists, which need to be filled in by expert agronomists or farmers’ supervisors, to avoid misleading answers, such as misreporting of the real use of adequate PPD or unreported contact with contaminated items, and with the fact that the approach does not take into account possible accidental overexposures.

Combining data of internal exposure as derived from dermal deposition/rate of absorption and biological monitoring data may allow the derivation of BEIs, i.e., urinary levels of the compound or of its metabolite(s) following exposure corresponding to the AOEL. For comparison The two currently used definitions of biological exposure limits are those referred to the BEI values proposed by the American Conference of Governmental Hygienists, interpreted as indicating the internal dose or dose surrogate that should arise from exposure via the airborne route alone at the TLV level, while the BAT value, proposed by the German Forschungsgemeinschaft, is defined as the maximal permissible quantity of chemical compound, its metabolites, or any deviation from the norm of biological parameters induced by these substances in exposed human beings (Morgan and Schaller, 1999).

Our preliminary data indicate that this is feasible. The provisional BEI that we identified for the propanil metabolite, 3,4-dichloro-aniline, may in principle be adequate to assist the interpretation of biological monitoring results. As a richer database of results is developed, estimates of the BEI can gradually improve and the quality of risk assessment is increased.

Certainly, measurement of urinary levels is less complex and cumbersome than measuring external exposure, and may become of help to assess the validity of the “risk profile” approach.

Finally, this approach may prove maximally effective when results from surveys in the field are shared on a cooperative regional, national or wider-scale basis.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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