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Exploring Novel Approaches to Pesticide Exposure and Risk Assessment

Exposure and Risk Profiles for a Safe Pesticide Use in Agriculture

STEFAN MANDIĆ-RAJČEVIĆ, MD

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Exposure and Risk Profiles for a Safe Pesticide Use in Agriculture

by

Stefan Mandić-Rajčević

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Tutor:	Prof. Claudio Colosio
Co-Tutor:	Prof. Federico Maria Rubino
Coordinator:	Prof. Giovanni Costa

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Università degli Studi di Milano a thesis entitled: **Exploring Novel Approaches to Pesticide Exposure and Risk Assessment,** in fulfillment of the requirements for the degree of Doctor of Philosophy (Occupational Medicine and Industrial Hygiene) of the Università degli Studi di Milano.

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Prof. Claudio Colosio

(Tutor)

Date: 4/2/2014

....

Prof. Federico Maria Rubino

(Co-Tutor)

Date: 4 Febbraio 2014

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I, **Stefan Mandić-Rajčević**, declare that this thesis is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

Signature ....

To my grandfather, Milorad. I wish you could be here to share this moment with us.
To my mother Dubravka, father Miomir, grandmother Elvira, and girlfriend Sanja. I would never have made it without your endless love and support.

Abstract

Introduction

Agrochemicals, short from agricultural chemicals, is a term used for various chemical products which are commonly used in agriculture. The most famous representative example of agrochemicals are pesticides, but it may also include fertilizers, hormones or similar chemical growth agents, as well as raw animal manure. Even as an active substance is authorized in European Union, and products containing this active substance are authorized and marketed, there is still a need for risk assessment to communicate and to manage risk with regard to the different risk groups, workers and the general population as a whole.

Overall Goal

The goal of this effort is the creation of Exposure and Risk Profiles, as a reliable, scientifically based way to forecast pesticide exposure and workers' risk 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.

Methodology

To reach our goal we have conducted a wide published literature search to define the process of pesticide application and the most common exposure determinants. Then we conducted two real-life field studies on exposure to pesticide in different use scenarios in the vineyards of the Region of Lombardy (one study in the framework of the ACROPOLIS project of the European Union, and another financed by INAIL). We collected field information in the form of a structured questionnaire, with a goal to record the variables previously identified as important modifiers of pesticide exposure. Also we collected exposure measurements, using two methodologies: skin pads and whole-body method, following in principle the OECD guidelines. Finally, we used the results from the field to develop a method that allows for a re-use of field data in risk assessment, by creating a Risk Assessment Scheme which can be used to assess risk in the field, without doing any measurements.

Results

We report the main phases of pesticide work and variables, together with their influence, as a result of our wide literature search. Also we report the results of two field studies, first on 7 workers applying Tebuconazole on 12 work-days, and second on 28 workers applying Mancozeb on 38 work-days. Finally, we show a proposed approach to using field measurements from our study in the Region of Lombardy to perform future risk-assessment in one defined scenario of closed and filtered tractors.

Discussion and Conclusions

Our work has tackled the problem of risk assessment for pesticide exposure in agriculture, which has been unfairly neglected in the past years. Through the use of literature data, field studies and computational modelling, we have managed to analyze and summarize the characteristics of pesticide application in agriculture, explore the real-life field conditions during pesticide application in vineyards in Italy, collect the field measurements necessary to do exposure and risk assessment, and to develop a method to use the data collected to produce a Risk Assessment Scheme. The study results and the above mentioned tool represent a step forward towards rapid, simple and scientifically based risk assessment in real-life conditions of pesticide application in agriculture.

Contents:

I. List of Abbreviations

A.I.	Active Ingredient
A.S.	Active Substance
ADI	Acceptable Daily Intake
AOEL	Acceptable Operator Exposure Level
APPL	Application phase
ARfD	Acute Reference Dose
BEI	Biological Exposure Index
BEI	Biological Exposure Index
DAR	Draft Assessment Report
DDT	Dichlorodiphenyltrichloroethane
EC	European Council
ED	Endocrine Disruptor
EFSA	European Food and Safety Authority
ETU	Ethylenethiourea
EU	European Union
FAO	Food and Agriculture Organization
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MIX	Mixing and Loading phase
MNTN	Maintenance phase
MRL	Maximum Residue Levels
PCB	Polychlorinated biphenyl
PPP	Plant Protection Product
RA	Risk Assessment
REP	Repair phase
RMS	Rapporteur Member State
STEF	Standardized Toxicity Efficacy Factor
TEB	Tebuconazole
TLV	Threshold Limit Value
USA	United States of America
WHO	World Health Organization

II. List of Tables

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IV. Executive Summary

Introduction

Agrochemicals, short from agricultural chemicals, is a term used for various chemical products which are commonly used in agriculture. The most famous representative example of agrochemicals are pesticides, but it may also include fertilizers, hormones or similar chemical growth agents, as well as raw animal manure. Pesticides are chemical compounds which are used to control pests, including insects, rodents, fungi and unwanted plants (weeds). They can be extracted from plants, or may be "synthetic".

Before an active substance can be authorized, it is necessary to perform the assessment of risk the active substance can pose to operators, workers, consumers, the environment and non-target plants and animals. Since our main interest is occupational health, we will be dealing with the risk assessment of operator exposure to pesticides. Pre-marketing risk assessment for occupational exposure to Plant Protection Products is a procedure aimed at demonstrating that the active substance, formulated as the commercial product(s) intended for marketing, is able to perform its task (*i.e.*, to suppress the target organism under field conditions) without causing inacceptable harm to the farm worker.

Even as an active substance is authorized in European Union, and products containing this active substance are authorized and marketed, there is still a need for risk assessment to communicate and to manage risk with regard to the different groups of stakeholders and to the general population as a whole (European Parliament, 2009).

Models are used in the pre-marketing risk assessment in European Union (see ***Section 1.6.***), and there have been attempts to use them as a risk assessment tool in field studies. The published literature mostly concludes that, since the models are based on exposure measures in experimental conditions, which are different from real-life field conditions in agriculture it is not adequate (fully reliable) to perform exposure and risk assessment in these conditions (Machera et al., 2009). It was demonstrated that the models underestimate the risk in low-use scenarios (when a small amount of active substance is used) and overestimate the exposure in high use scenarios (when a large amount of active substance is used), namely because the total exposure by these models is linearly dependent on the amount of active substance used (Protano et al., 2009; Rubino et al., 2012). In addition, the models do not take into account some specificities of real-life pesticide application conditions, such as the presence of a cabin with filters in a tractor, as well as the repetition of mixing and loading tasks, and many other situations of use and non-use of personal protective equipment.

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

The starting point for this activity is the definition of typical exposure and risk scenarios and the definition of the typical levels of exposure anticipated in these scenarios, necessary to extrapolate the data collected in the situation under study to other similar and comparable. In order to do it, it is necessary to study, in these scenarios, the relationships between selected variables affecting the levels of workers’ exposure in each of the above mentioned working phases.

Overall Goal

The goal of this effort is the creation of Exposure and Risk Profiles, as a reliable, scientifically based way to forecast pesticide exposure levels and risk 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.

Methodology

In order to reach the overall goal of this PhD project (see ***Section 1.9.***), we needed to address the objectives defined in the ***Section 1.9.1.***

Understanding the process of pesticide application was the first task in studying the process of pesticide preparation and application and defining the factors influencing exposure and risk. Many reference points already existed in the published literature, and in order to better understand the main phases of work with pesticides, a thorough literature search was done (see ***Section 2.1.***).

Even though literature data can be very useful in setting up and better describing the scenario(s), it is not enough to completely explore and define exposure and risk profiles, and offer a solution for in-field rapid risk assessment. Therefore, we organized two real-life field studies in the vineyards in North Italy.

ACROPOLIS is an EU-funded project with a goal of creating an On-Line Integrated Strategy for Aggregate and Cumulative Risk of Pesticides (Acropolis Project, 2013). As one part of the activities of the project field studies have been organized to assess the exposure to Tebuconazole (TEB). The study was conducted in Monferrato, which is a world-famous wine-producing area of Piedmont, Northern Italy, where the local cultivars are the source of commercially prized wine brands. TEB belongs to a large family of azole fungicides, several of which are used also in human therapy. Controlled use of these chemicals is considered safe for humans, although TEB causes malformations at high doses in animals both *ex vivo in vitro* and *in vivo* (EFSA, 2008; Giavini and Menegola, 2010).

In the same period of 2011 INAIL financially supported another real-life pesticide exposure and risk study (the “***Region of Lombardy***” study) conducted by our study team. The goal of this study was to assess the exposure and risk of workers using open tractors, as well as closed and filtered tractors while applying Mancozeb in vineyards of the Region. Mancozeb is another widely used agricultural fungicide. It is a manganese ethylenebis(dithiocarbamate) complex with zinc salt. Mancozeb formulations contain a percentage of ethylenethiourea (ETU), which is also a metabolic product of ethylenebisdithiocarbamates, which is known to have long-term effects characterized principally by antithyroid activity in experimental animals (Colosio et al., 2002).

In both studies, potential and actual dermal exposure was measured. *Potential* dermal exposure (in brief potential exposure) is defined as the amount of pesticide coming into contact with the working clothes and personal protective devices (Lesmes-Fabian et al., 2012b; Rajan-Sithamparanadarajah et al., 2004). *Actual* dermal exposure (in brief actual exposure) is defined as the amount of pesticide coming into contact with the workers' skin, available for absorption (Lesmes-Fabian et al., 2012b; Rajan-Sithamparanadarajah et al., 2004). Detailed methodology of both studies described in ***Sections 2.2.*** and ***2.3.***

Finally, we have developed methodologies to help us define exposure and risk determinants of pesticide applicators, as well as to extrapolate the risk from the measured active substance to a broader range of active substances. The above mentioned methodology is detailed in ***Sections 2.4.*** to ***2.8.***

Results and discussion

Through a systematic literature search (see ***Section 2.1.***) and field activities (see ***Sections 2.2.*** and ***2.3.***) we have identified main phases of work with pesticides, the exposure determinants in these work phases, and the relationships linking these variables.

A typical work-day with pesticides can be divided into Mixing and Loading (MIX), Application (APPL), Maintenance and Cleaning of machineries after work (MNTN). We have also explored the general "modifying factors" of pesticide exposure and risk (***Section 3.1.4.***).

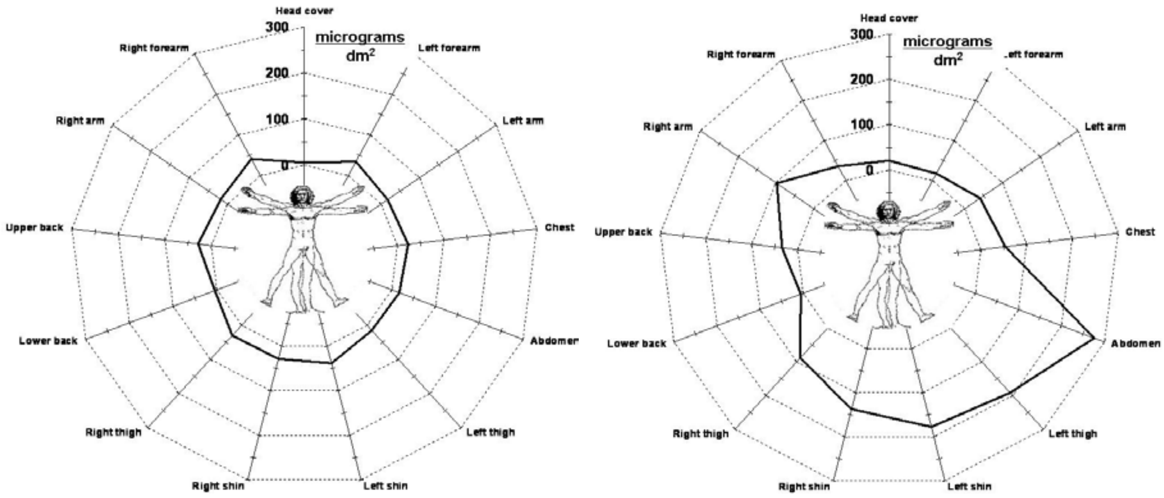


Figure 3.2.2. Spider plot of tebuconazole contamination on farmers' coveralls. (**A**) one who sprayed from a closed-cockpit tractor; (**B**) one who sprayed manually from a hose (passing by the left hip) hand-sprayer.

Small and middle-size enterprises that use pesticides are seldom subject to assessment of exposure and related health risks. In the ***ACROPOLIS*** study (***Section 3.2.***) we attempted to shed more light on the characteristics and determinants of exposure in actual working conditions during pesticide spraying in vineyards, and for the first time Tebuconazole exposure levels have been measured in field conditions.

A high variability in the general working conditions in study subjects was noticed (see ***Table 3.2.2.***). Work was carried out with hand-held equipment, open and closed tractors, and combinations of tractors and hand-held equipment even during the same work-day. The cause was most probably the characteristics of the terrain, as well as the different sizes of vineyards, which ranged from very small to larger ones. Finally this explains the differences in working hours recorded in our study.

Potential body exposure showed a high variability (see ***Table 3.2.4.***). This can mostly be explained by the different working modalities, sizes of estates, as well as the different length of exposure and amount applied. The potential and actual body exposure of our workers fall in the same range of those measured in open-field pesticide applicators with exposure to isoproturon (Lebailly et al., 2009), procymidone (Aprea, 2012) and terbutylazine (Vitali et al., 2009).

The cotton coverall used by the workers provided them with a high protection factor (98%). The protection provided in our study is higher than that reported by other authors for standard cotton garments (reportedly 73% to 88%) and in the range of the protection provided by Tyvek® coveralls (Aprea et al., 2005; Fenske et al., 1990; Vitali et al., 2009).

In the "***Region of Lombardy***" study (***Section 3.3.***) we tried to explore two work scenarios in more depth and with a higher number of study subjects (28 work-days with a closed and filtered tractor, and 9 work-days with an open tractor). As a standard for this kind of work, it was done only by men, also confirming the situation of the Acropolis study and literature data (Baldi et al., 2006; Lebailly et al., 2009; Vitali et al., 2009).

We noted an important difference in some characteristics of work-day between the workers using a closed and filtered tractor and those using an open tractor (see ***Table 3.3.2.***). For example, the amount of active substance per day, the area treated and the application time are all higher for a closed and filtered tractor. This can be explained by the fact that larger estates can afford better machineries, usually with larger tanks, in order to more efficiently do the work with pesticides.

Our study has shown that workers use most protection during the mixing and loading phase, since 97% of them used gloves and 81% of them used a mask in this phase (see ***Table 3.3.3.***). Application phase is not considered so dangerous, especially for workers using a closed and filtered tractor, judging by the use of personal protective equipment, while the use was higher in the maintenance and cleaning phase. Similar studies have shown that mixing and loading phase and the maintenance and cleaning phase might contribute the most to overall exposure and risk (Baldi et al., 2006; Coble et al., 2005). Our study has shown that gloves reduce hand exposure if used during the application phase, but only in the case of open tractors, while in the case of closed and filtered tractors, the difference in hand exposure between the workers who used gloves and those who did not was not notable (see ***Figure 3.3.2.***).

Risk assessment in field conditions is useful for several reasons. We can estimate the risk in different working conditions, we can suggest the modifications of working conditions to reduce the risk, and we can communicate the individual risk to workers so they would know how to improve or change their work habits. Nevertheless, doing risk assessment on individual workers in their normal working conditions is not easy. Time is needed to organize the study. Money is necessary for the costs of personnel, transport, sample collection and analysis. Moreover, often the individual risk assessment is valid only on the specific day, with the worker spraying a specific quantity of a pesticide, and just for the pesticide in question. It was our goal to explore the most used scenario in the Region of Lombardy study – the closed and filtered tractor, the most important factors that influenced the exposure of workers, and produce a tool that can be used for risk assessment in any situation when a closed and filtered tractor is used for pesticide application.

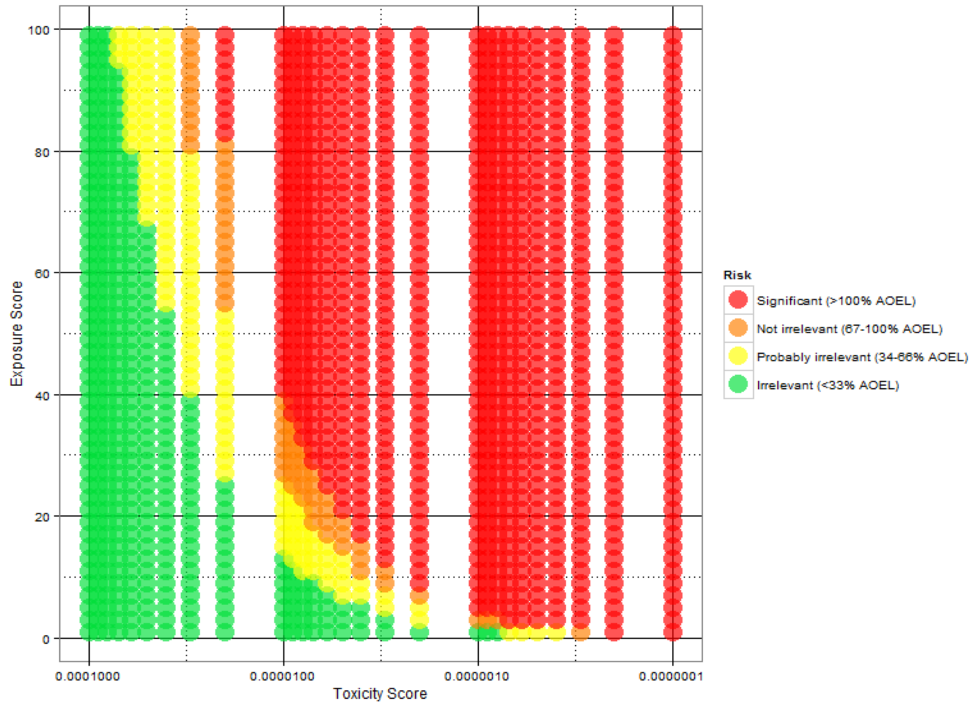


Figure 3.4.4. Risk Assessment Scheme for closed and filtered tractors for all toxicity scores and all exposure scores

Using the R programming language (R Core Team, 2012), a simulation is made to calculate the risk for each combination of the exposure score (from 1 to 100) and toxicity score values (54 values from 0.1 to 0.0000001). Both axes' values and the risk assessment done for each combination have been generated using the R code available as ***Supplementary material S.3.1***

The product is a table with 3 columns:

1. Exposure Score – or the "y" axis value
2. Toxicity Score – or the "x" axis value
3. Risk Assessment – the saturation of AOEL for that exposure and toxicity score combination

Using the ggplot2 package (Wickham, 2009) for R, it is possible to plot all of these values colouring the dots based on the level of AOEL saturation, as explained in the ***Section 2.8.4.***

Our methodology of using exposure and risk profiles and the risk assessment scheme should not be considered a replacement of the pre-marketing risk assessment tools. The models used in the pre-marketing cannot and do not consider all the characteristics of field activities. Our methodology and the resulting tool considers the work with pesticides as it is done in real-life conditions, and there are phases and variables not taken into account by the German model or the EUROPOEM. One example is the activity of cleaning and maintenance, not addressed by models in the pre-marketing (see ***Section 1.6.***), but it is an activity routinely done in real-life conditions and can bring about high exposure (see ***Section 3.1.3.***). There are also variables, such as the number of times Mixing and Loading is performed, which is also correlated also to the tank size, very important in real-life field conditions (see ***Section 3.1.1.***) but not taken into consideration by the existing models.

Conclusions

Our work has tackled the problem of risk assessment for pesticide exposure in agriculture, which has been unfairly neglected in the past years. Through the use of literature data, field studies and computational modelling, we have managed to analyze and summarize the characteristics of pesticide application in agriculture, explore the real-life field conditions during pesticide application in vineyards in Italy, collect the field measurements necessary to do exposure and risk assessment, and to develop a method to use the data collected to produce a Risk Assessment Scheme. The study results and the above mentioned tool represent a step forward towards rapid, simple and scientifically based risk assessment in real-life conditions of pesticide application in agriculture.

Future work

A lot of work remains to be done, especially in the field of collecting more measurements, improving the methods of exposure assessment, improving the methods of risk assessment, simplifying and streamlining the model creation by using new computational tools, and making the risk assessment tool available to as many users possible online. We plan to address the above mentioned areas of improvement, and, in contact with experts in the field try to implement their ideas for reaching safe pesticide use in agriculture.

1. Introduction

1.1. Definition and history of pesticides

Agrochemicals, short from agricultural chemicals, is a term used for various chemical products which are commonly used in agriculture. The most famous representative example of agrochemicals are pesticides, but it may also include fertilizers, hormones or similar chemical growth agents, as well as raw animal manure.

Pest is *a destructive living organism that attacks crops, food, livestock*. Pesticides are chemical compounds which are used to control pests, including insects, rodents, fungi and unwanted plants (weeds). They can be extracted from plants, or may be “synthetic”. Some pesticides are used both in agriculture, to kill pests that damage crops, as well as in public health to kill vectors of diseases, such as mosquitoes. Pesticides are chemical formulations which consist of one or more active ingredients (A.I.), also called active substances (A.S), and other ingredients, such as synergists, co-formulats, adjuvants, adesivants, and also solvents and compounds that improve absorption. In agriculture, horticulture, forestry and gardening, their role is the protection of crops, therefore they are also called Plant Protection Products (PPP).

It is believed that the use of inorganic chemicals to control insects could date back to classical Greece and Rome. Fumigant value of burning sulphur was mentioned by Homer, while insecticidal use of arsenic, and the use of soda and olive oil for the seed treatment of legumes was advocated by Pliny the Elder.

In the nineteenth century the fist systematic scientific studies into the use of chemicals for crop protection were starting. Work on arsenic compounds led to the production of “Paris green” in 1867, which was an impure form of copper arsenite. In the United States of America (USA) it was used to control the spread of the Colorado beetle, and by the 1900 it was so widespread that it led to the introduction of probably the first pesticide legislation in the world.

Between the First and the Second World War and during the Second World War, the number and complexity of chemicals for crop protection increased. Synthetic pyrethrum and pyrethroids were developed by a charitable-funded laboratory in England, the insecticidal potential of an already known substance, dichlorodiphenyltrichloroethane (DDT) was discovered in Switzerland and insecticidal organo-phosphoric compounds were developed in

Germany. The first soil-acting carbamate herbicides were discovered by industrial researchers in the United Kingdom and the organochlorine insecticide chlordane was introduced in the USA and in Germany (Hassall, 1982).

1.2. Characteristics and importance of pesticides

While they differ in many ways from other chemical substances produced by humans, especially for manufacturing and industrial uses, they share several similarities with pharmaceuticals. First, they are produced to control living species and therefore they are necessarily biologically active (toxic to target species); second, they are deliberately spread into the environment to reach their targets, therefore can be source of environmental pollution and human exposure (workers and consumers); third, they are produced to fight against pests, but the specificity of their toxicity for their targets is limited, therefore their use can endanger non target species, from useful insects such as bees to humans. For the reasons stated above, it is obvious that pesticides pose a risk for the health of humans, as well as the non-target organisms, but this risk needs to be evaluated in the context of the importance of pesticides for food production in the 21st century economy.

Crops can be affected by different pests, by the competition from weeds, as well as by several insects (and other arthropods), fungi, molluscs and bacteria. The result is a quantitative and qualitative loss.

The population of the World is predicted to increase to 9 billion people by 2050, and more importantly the world’s highest rates of population growth occur in areas highly dependent on the agriculture sector. There is capacity in the world to produce enough food to feed everyone adequately, but it has also been a challenge, since 870 million people still suffer from chronic hunger (see ***Figure 1.2.1.***). The increasing movement of people and goods, and the changes in production practices give rise to new threats from pests, diseases and invasive alien species.

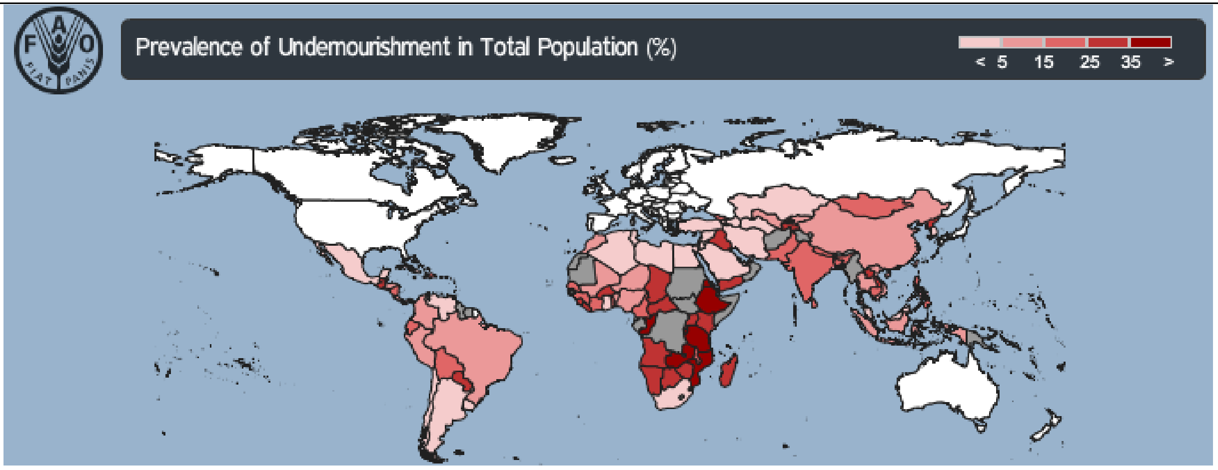


Figure 1.2.1. – Prevalence of Undernourishment in total population (source: FAO)

It is widely accepted that without the use of pesticides a significant proportion of the agricultural production goes lost to spoilage in the fields and to rotting and deterioration throughout the production and distribution process. Therefore, in particular in tropical countries, their use is unavoidable. In this perspective, the environmental and health risks related with their use need to be balanced by the benefit they yield to agricultural production and, in the fight to disease-bearing parasites, to the benefit to public health.

1.3. Pesticide use in the world

Since the first introduction of pesticides to the world of agriculture, new active substances have been continuously developed and "old" active substances have been losing their place in the market, also due to the onset of resistance in target organisms. In the last decade, the global sales of pesticides have been rising with a steady pace (see ***Figure 1.3.1.***), but at the same time the number of active substances have been decreasing.

A comprehensive renewal procedure was first laid down in 1991 (CEC, 1991), and in 1993 the European Commission launched the work program on the Community-wide review for all active substances used in the European Union. By that time, there were about 1,000 active substances and 10,000s of PPPs on the market. It was requested that each substance was re-evaluated to understand whether it could be still used safely with respect to human and environment health. To harmonize technical requirements and acceptance criteria, Directives have laid out comprehensive risk assessment and authorization procedures for active substances and products containing these substances. It is the responsibility of industry to provide the data showing that a substance can be used safely with respect to human health and the environment.

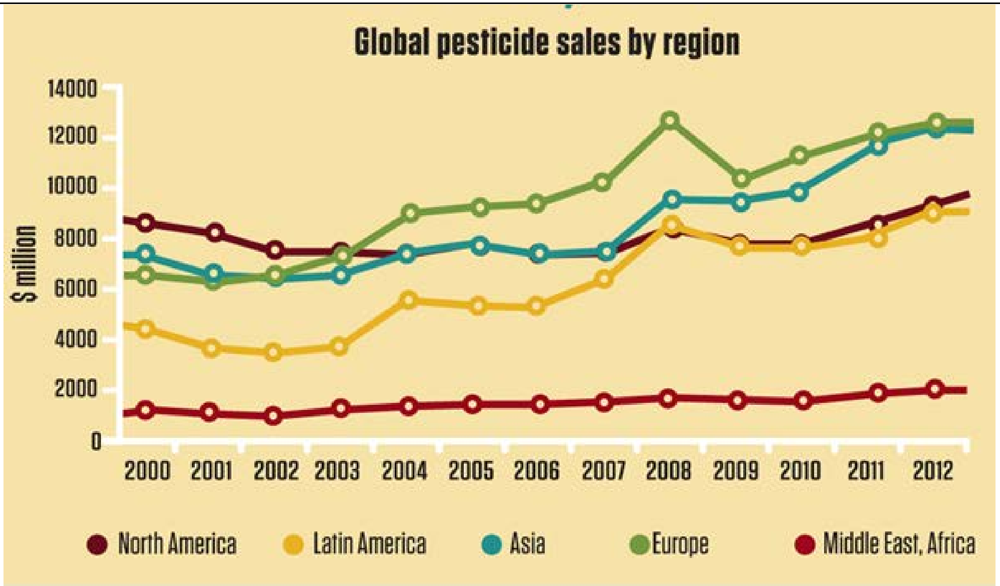


Figure 1.3.1. – Global pesticide sales by region (*source: Washington Post*)

The decisions only started to be taken in 2001, since and in March 2009 last decisions were taken. From around 1000 active substances on the market in at least one Member State before 1993, only 250 (26%) passed the harmonized EU safety assessment. For the majority of eliminated substances (67%) dossiers were either not submitted, were incomplete or the industry spontaneously withdrew them from the market. There is a possibility that many of these substances the request for re-authorization was not made because they were not commercial enough (low price, low use rate), and not because they posed a risk for humans or the environment.

Most of the substances in use were fairly safe, as demonstrated by the fact that only about 70 substances failed the review and were removed from the market, because the evaluation carried out did not show safe use with respect to human health and the environment.

1.4. Pesticide health risks

Having in mind that pesticides are intrinsically toxic substances, their effect can be harmful to non-target organisms, among which also humans. Harmful effect can be caused by a short-term high-level exposure, and they are considered acute, or they can be caused by a chronic low-level exposure, when they are considered long-term effects of pesticide exposure.

Acute pesticide poisonings are illnesses occurring within 48 hours from suspected or confirmed exposure to a pesticide (Thundiyil et al., 2008). Acute poisonings can be classified according to three main scenarios: intentional, accidental and occupational. Intentional poisonings result from an intention to cause harm, and they include self-harm (e.g. suicide). Accidental poisoning is unintentional, unexpected or not foreseen (e.g. human therapy overuse). Occupational poisonings occur during work, where a pesticide is being used in the context of the work process, including application, transportation, storage and disposal. Several estimates have been made regarding the number of poisonings and mortality (see ***Table 1.4.1.***), and the number could be around 250,000 to 500,000 poisonings with 3,000 to 30,000 deaths every year (Garcia, 1998; Jeyaratnam, 1985; Litchfield, 2005). Developing countries have a higher rate of occupational poisonings than developed, due to the climatic and socioeconomic conditions, although underreporting occurs (Litchfield, 2005). Developing countries also have a higher rate of intentional-suicidal poisonings, as pesticides are the most common method of suicide in the world (Bertolote et al., 2006). Asia is the continent where most suicides by pesticides occur (Buckley et al., 2004; Gunnell and Eddleston, 2003), followed by Africa.

Some health effects of chronic pesticide exposure have been well explored and documented, but among the emerging risks it is important to underline neurobehavioral effects, consequences of exposure to endocrine disrupting pesticides, and the need of further exploring the link between pesticide exposure and cancer.

Many studies have shown that acute pesticide poisoning has a serious effect on the neurobehavioral function of an individual, but now studies are showing that even low-level repeated exposure can have an effect on cognitive skills and behaviour. Low neuropsychological performance in tasks of integrative perception and visuo-constructional praxis were found in subjects chronically exposed to pesticides for more than 10 years (Roldan-Tapia et al., 2005).

Most studies of neurotoxicity have documented an increase in symptom prevalence and changes in neurobehavioral performance reflecting cognitive and psychomotor dysfunction, but many found little effect of pesticide exposure on sensory or motor function (Baldi et al., 2001; Farahat et al., 2003; Kamel and Hoppin, 2004; van Wendel de Joode et al., 2001).

But when all studies have been reviewed, there were no firm and consistent evidence that pesticides have neurobehavioral effects after long-term low-dose exposure, but the authors have stressed the possibility that one season exposure in not enough to yield measurable effects (Colosio et al., 2003). On the other hand, in a follow up of the PHYTONER study, results suggested long-term cognitive effects of exposure to pesticides were present, and the exposure rose the risk of evolution towards dementia (Baldi et al., 2011).

It is estimated that more than 870,000 people commit suicide every year. Pesticides have been used to commit suicide for decades, probably because of their availability (Eddleston, 2000), but now studies are appearing trying to link long term exposure to pesticides and mental health problems, possibly even as a predictor of suicide (London et al., 2005; Meyer et al., 2010), but these findings have been disputed by other studies (Pickett et al., 1998; van Wijngaarden, 2003). Studies have shown that workers exposed to pesticides often have suicidal ideas (Zhang et al., 2009), but further investigation is necessary.

Where	Cases	Intentional	Accidental	Occupational	Source
Worldwide	Estimate	2,000,000 (200,000 deaths)			(Jeyaratnam, 1990)
Worldwide	Estimate	873,000			(WHO, 2006)
Japan	346 cases of pesticide poisoning	70 %	8 %	16 %	(Nagami et al., 2005)
Taiwan, Taiwan's Poison Control Centers (PCCs)	4799 OP exposures July 1985 to December 2006	64.72 %			(Lin et al., 2008)
Taiwan Kaohsiung Medical University Hospital	75 patients admitted with OP acute poisoning between 1995 – 2005	61 (81.3 %)	14 (18.7 %)		(Tsai et al., 2007)
India, Civil Hospital of Ahmedabad	190 cases of OP acute poisoning	67.4 %	15.8 %	16.8 %	(Agarwal, 1993)
Turkey, Afyonkarahisar district	220 patients admitted to the local hospital 1995 – 2004; diagnosis of APP	75.9 %			(Yurumez et al., 2007)
Turkey	63 cases of pesticide poisonings	53 (84 %)	10 (16 %)		(Ozer et al., 2007)
Jordan	144 fatalities due to pesticides recorded in a 4-year survey	64.3%	24.3% (accidental + homicidal)		(Abdullat et al., 2006)
Ethiopia, Tikur Anbessa Hospital	50 cases of OP poisonings in 6 years	94 %			(Abebe, 1991)

Table 1.4.1. Pesticide poisoning reported in the literature (Satoh and Gupta, 2010)

Another important health effect is endocrine disruption, a term coined in 1993 (Colborn et al., 1993) to define effects caused by chemicals in an intact organism, due to changes in endocrine function. Several studies have shown that exposure to endocrine disruptors have an influence on a person's reproductive capability (Bonde et al., 2008; Garry, 2004; Hauser et al., 2003; Nicolopoulou-Stamati and Pitsos, 2001; Richthoff et al., 2003), thyroid function (Meeker, 2010) and risk for diabetes (Codru et al., 2007; Turyk et al., 2009). A link that requires more exploring is between exposure to EDs and different cancers (Garry, 2004). Several studies have shown a link between the concentration of known endocrine disruptors and testicular germ cell tumours (McGlynn et al., 2008, 2009; Purdue et al., 2009), but a case-control study of 876 adult men has shown no such link (Biggs et al., 2008). Finally, a few small studies have suggested an association between PCB exposure and prostate cancer (Hardell et al., 2006; Ritchie et al., 2005), whereas no association was reported in a recent Canadian study (Aronson et al., 2010).

Considering the importance of pesticides in agriculture, as well as public health, their use will continue. Although often inconclusive, epidemiologic studies suggest that human health effects occur at current exposure levels in occupational (and environmental) setting, and it is necessary to better understand the patterns of exposure and variability within the human population to better evaluate the risk to human health (Alavanja et al., 2004).

1.4.1. Risk assessment, risk management and risk communication

Although its principles were implicitly well-known since at least two centuries (the assessment of risk in long-distance trading of goods, which is the basis for setting insurance premium, is as old as Mesopotamic ages) Risk Assessment (RA) was formalized as a discipline in the USA towards the end of the 1970s. The necessity to frame the process arose as a consequence of two episodes which greatly stressed the public opinion and brought considerable debate into the scientific community: the consequences of the use of thalidomide as a drug (Brynner and Stephens, 2001) and of vinyl chloride in the synthesis of PVC (Markowitz and Rosner, 2002). In the case of thalidomide, a sedative drug against morning sickness of pregnant women was deemed as fully safe after the reassuring outcome of what was reputed at the time to be sufficiently extensive safety tests on animal models and was marketed all over the world. It was prescribed to and taken by millions of pregnant women and, as a consequence, an epidemics of teratogenic effects soon developed, which stroke millions of children throughout the world and their families, before the use of the drug was banned. In the case of vinyl chloride, a chemical commodity was supposed to be reasonably safe in normal conditions of use and was widely used both as a monomer in the production of polymers for manufacturing uses and as a propellant for spraying in consumer products. As manufacturing and use increased over a few decades, evidence of its carcinogenic properties accumulated, finally leading to a ban in its use and to an increased level of care in its production and use. These two crucial episodes highlighted the need of performing a full toxicological assessment *before* any chemical substance could be authorized for industrial, pharmaceutical or other uses, and in particular if their use was, as in the case of pesticides, intrinsically dissipative.

Pesticides are one of the best examples to follow the thread of the development of risk assessment of chemical substances. Since its first introduction into the market, in 1939, DDT showed very good insecticidal properties and its production and use grew, widening its application from public health (control of human external parasites, such as pediculosis and scabies), to environmental application in the eradication of Anopheles mosquito, the vector of malaria, from several endemic areas in temperate and tropical countries, to an efficient control of agricultural pests and even . Starting from the early sixties, with the publication of Rachel Carson’s *Silent Spring*, concern on the environmental consequences of its widespread use reached the public opinion and the scientific community over the world started to investigate the bioaccumulation and bio-magnification properties of DDT through the food chain and its possible link to possible effects on human health. As awareness strengthened in the seventies, limitations and bans were raised in the most developed Countries, leading to a significant reduction of DDT production and use and finally to a generalized international ban. The same fate was followed by a few other pesticides, which are all characterized by common chemical characteristics leading to very long persistence in the environment, to transmission through the human food chain and by potential long-term toxicity.

Public perception of failures of pesticide regulation leading to strong public health concern and severe and persistent environment contamination has been a strong driving force towards improvement and harmonization of the requirements for authorization of plant protection products. The approach which is currently adopted for regulation of pesticides is “reactive/preventive”, since it responds (‘reactive’) to damaging impacts for which there is convincing evidence of cause-effect relationship and takes regulatory action to ensure that similar impacts do not arise with new generation chemicals (‘preventive’). It is also “risk-based” since it relies on cost-benefit analysis as a basis for scientifically rational decision making (Tait, 2001).

1.5. Pesticide authorization process in the European Union

The role of regulation processes is to keep under constant control the consequences of the use of pesticides, with reference to a risk-benefit evaluation, and to prevent serious consequences to human health and to environment self-sustainability related to the use of these compounds. Since the Council Directive of 1991, the European Union recognizes that plant production is very important for agriculture and plant protection products are one of the most important ways of protecting plants and plant products against harmful organisms including weeds, and of improving agricultural production (CEC, 1991).

The regulation for the approval of active substances, of herbicide safeners and of synergists, enforces the precautionary principle as cited by Article 191.2 of the Consolidated Version of the Treaty on the Functioning of the European Union, which reads:

"*Union policy on the environment shall aim at a high level of protection taking into account the diversity of situations in the various regions of the Union. It shall be based on the precautionary principle and on the principles that preventive action should be taken, that environmental damage should as a priority be rectified at source and that the polluter should pay*"

The main legislation to regulate the registration procedure of pesticides in the EU was the Council Directive 91/414/EEC of July 1991 concerning the placing of plant protection products on the market (CEC, 1991). In 2009, in the light of experience gained from the application of the 1991 Directive and the "recent scientific and technical developments" that Directive was replaced by the Regulation No 1107/2009 of the European Parliament and of the Council (European Parliament, 2009), which is fully applicable as of 14 June 2011. *The Community shall act within the limits of the powers conferred upon it by this Treaty and of the objectives assigned to it therein. // In areas which do not fall within its exclusive competence, the Community shall take action, in accordance with the principle of subsidiarity, only if and in so far as the objectives of the proposed action cannot be sufficiently achieved by the Member States and can therefore, by reason of the scale or effects of the proposed action, be better achieved by the Community. // Any action by the Community shall not go beyond what is necessary to achieve the objectives of this Treaty.* (Art. 3b of the Consolidated Version of the Treaty on the Functioning of the European Union, Official Journal of the European Union).

The principle of Mutual Recognition of formulations (products) ensures the free movement of goods within the EU and helps avoid the un necessary duplication of work that was the standard procedure under the old legislation (CEC, 1991), where every product had to be authorized in each Member State separately.

1.5.1 Authorization of active substances

In the European Union (EU), no plant protection product can be used unless it has first been scientifically established that: (***a***) they have no harmful effects on consumers, farmers, local residents and passers-by; (***b***) they do not cause unacceptable effects on the environment; (***c***) they are sufficiently effective against target pests. As a direct consequence, the components of plant protection products placed on the market must not adversely affect human or animal health or the environment. The current regulation also allows the States members of the European Union to apply the precautionary principle where there is scientific uncertainty as to the risk with regard to human or animal health or the environment posed by the plant protection products.

The complex procedure leading to authorization of a new active substance as PPP is outlined in the scheme of ***Figure 1.5.1.***

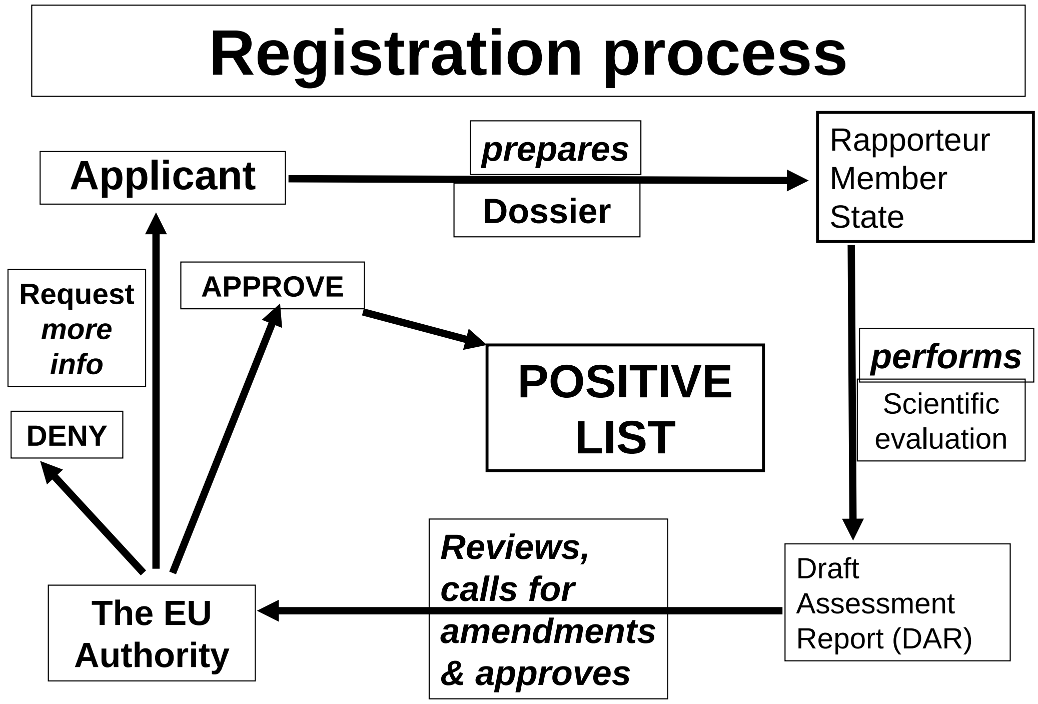


Figure 1.5.1. Procedure leading to authorization of a new active substance as PPP in the EU.

The Notifier or Applicant is (usually) the Company willing to have the active substance authorized for PPP use in the EU. The review of the information supplied in the Dossier is assigned to one Member State (Rapporteur), which is usually selected on the basis of the putative or requested use of the active substance and the expertise gained in the Country in reviewing products for that specific use (*e.g.*, grape in Southern European countries like France, Italy or Spain, potato in Central European countries like Germany or Ireland).

To apply for authorization of an active substance as PPP, the Applicant submits a dossier to the Member State which has been designated as the Rapporteur Member State (RMS). The dossier comprises all the study reports, data and information which are required by the Council Directive. The dossier is evaluated by the RMS and the results are summarized in a Draft Assessment Report (DAR). The DAR is then discussed by the Member States in a peer review process. The Member States, the EFSA and interested parties can comment on the DAR. Open matters to do with evaluation can be discussed in expert meetings (PRAPeR Expert Meetings) organized by the European Food Safety Agency (EFSA) for this purpose. Following the peer review, the EFSA sends a summary report, which includes its conclusions, to the European Commission. At the end of this process, the European Commission and the 27 Member States decide in a meeting with the Standing Committee on the Food Chain and Animal Health on the inclusion or non-inclusion of the active substance in Annex I of the Council Directive. The complete list of active substances submitted for EU approval is publicly accessible at the website of the Directorate General for Health and Consumers (SANCO, in the EU administrative jargon). For each active substance it is indicated the status and outcome of the authorization procedure. So far, nearly 500 active substances have been authorized.

This regulation, which is publicly available at the SANCO website, applies to active substances that are intended for one or more of following uses:

- Protecting plants or plant products against all harmful organisms […];
- Influencing the life processes of plants (e.g. growth, other than as a nutrient);
- Preserving plant products (excluding products subject to EU provisions on preservatives);
- Destroying undesired plants or parts of plants;
- Checking or preventing undesired growth of plants.

The EU regulation applies also to other categories of substances which are usually mixed with the active substance in the production process, or prior to application:

- *Safeners*: substances or preparations which are added to a plant protection product to eliminate or reduce phototoxic effects of the plant protection product on certain plants;
- *Synergists*: substances or preparations that can give enhanced activity to the active substance(s) in a plant protection product;
- *Co-formulants*: substances or preparations which are used in a plant protection product, but are neither active substances nor safeners or synergists;
- *Adjuvants*: substances or preparations consisting of one or more co-formulants, to be mixed by the user with a plant protection product to enhance its effectiveness.

Therefore the European Union will authorize only active substances that are sufficiently effective under reasonable conditions of use; that do not have immediate or delayed harmful effect on human health, including that of vulnerable groups and on animal health, directly or through drinking water, food, feed or air, or consequences in the workplace or through other indirect effects; that do not have any unacceptable effects on plants or plant products; that do not cause any unnecessary suffering and pain to vertebrates to be controlled; and finally that do not have any unacceptable effects on the environment.

1.5.2 Authorization of formulations

In accordance to the Regulation 1107/2009 of the European Commission (European Parliament, 2009), Plant Protection Products (PPPs) can be authorized in a Member State and can be placed on the market only if it complies with the requirement that:

- All substances in it; active substances, safeners and synergists have been approved and, if any of them is produced by a different source, their properties cannot deviate significantly from those included in the Regulation approving the substance;
- It does not contain co-formulants which are included in Annex III of the Regulation 1107/2009 (List of co-formulants which are not accepted for inclusion in plant protection products)
- It is formulated so that user exposure or other risks are limited as much as possible without compromising the efficacy of the product;

- It complies with all the safety requirements for active substances and PPPs
- Its physical and chemical properties have been determined and deemed acceptable for the use and storage of the product;
- The nature and quantity of all components and its residues in the environment and in crops and food can be determined by appropriate methods.

1.5.3. National authorization and mutual recognition

The regulation 1107/2009 has laid down harmonized rules for the approval of active substances and the placing on the market of plant protection products, including the rules on the mutual recognition of authorizations and on parallel trade. The goal is to increase free movement of such products and availability of these products in the Member states.

Under the new legislation, authorizations granted by one Member State should be accepted by other Member States where agricultural, plant health and environmental (including climatic) conditions are comparable. To facilitate such mutual recognition, the 27 Member States of the EU are assigned each to one of three zones with such comparable conditions. The zones of mutual recognition have been established as follows:

Zone A – North: Denmark, Estonia, Latvia, Lithuania, Finland, Sweden

Zone B – Centre: Belgium, Czech Republic, Germany, Ireland, Luxemburg, Hungary, Netherlands, Austria, Poland, Romania, Slovenia, Slovakia, United Kingdom

Zone C – South: Bulgaria, Greece, Spain, France, Italy, Cyprus, Malta, Portugal

The Member State may amend an authorization issued by another Member State, or refuse to authorize the plant protection product in their territory, where there are agricultural or environmental circumstances that require so, or where high level of protection of human and animal health, and the environment cannot be achieved.

1.5.4. Renewal and review of active substances

To have the same level of protection for all Member States, the decision to approve a PPP, to deny or to withdraw the approval is taken at Community (EU) level and authorization is subject to renewal to account for new information that may be emerging from field use. This procedure is analogous to farmacovigilance carried by EMEA and by the National authorities for pharmaceutical drugs.

By the new Regulation, 1107/2009, first approval of a product can be for a period not exceeding 10 years, after which all active substances approved have to be reviewed to be renewed. Renewal cannot be granted for a period longer than 15 years, and for some active substances (those covered by Article 4(7): substances that do not comply to all of the conditions required by this Regulation, but are necessary to combat a pest in an urgent matter) authorization cannot be renewed for a period longer than 5 years.

This effort now provides assurance that the substances currently on the EU market are acceptable for human health and for the environment.

1.5.5. Exposure limits in the authorization process

The use of PPPs necessarily entails the spread of active substances in the environment and the possibility that they contaminate workers, subjects of the general population and, through the general environment, also food and water sources. To protect humans from the possibly unavoidable contact with active substances in unnecessary and excessive amounts, limits need to be established during the regulatory process for the presence of PPP active substances at workplaces (mainly during pesticide application by farmers), as residues or contaminants in food and water and in the general environment. For each of these scenarios a different limit value has been developed.

1.5.5.1. Acceptable Operator Exposure Level (AOEL).

Protection of agricultural workers' health when using Plant Protection Products features several fundamental differences and further difficulty with respect to the much simpler case of workers in the manufacturing industry, mainly outdoor rather than indoor work, continuously changing job, time and exposure patterns rather than Tayloristic schemes, prevalent skin than respiratory absorption route of employed chemicals. These differences point at whole-body dosimetry rather than environmental monitoring as the most convenient strategy to allow quantitative risk assessment. To this purpose, an Acceptable Operator Exposure Level (AOEL) has to be established. The AOEL is a systemic dose, normalized as milligrams of active pesticide substance per kilogram of body weight ($mg * kg_{bw}^{-1}]_{day}$) which an agricultural worker can absorb through professional exposure in any one working day so that there will be no negative health consequences. The AOEL is determined on the basis of animal toxicology experiments which take into consideration as endpoint the biological effect (relevant to the human) which occurs at the lowest exposure level (the Lowes Observed (Adverse) Effect Level, LO(A)EL), then look for the (often extrapolated) exposure dose at which the effect is no longer observed in the most sensitive animal species (No Observed (Adverse) Effect Level, NO(A)EL). In turn, NO(A)EL values are extrapolated from animal studies (typically oral short-term toxicity studies; 90-day study, or occasionally 1-year dog study), performed in the pre-marketing development of a candidate active substance (EU SANCO, 2006).

NO(A)EL is translated into AOEL by further dividing the ‘safe’ dose assessed in the suitable animal model by empirical reduction factors which account for the uncertainties existing in the extrapolation from animal toxicity data to safeguard levels for the human population. The current hazard assessment for toxic endpoints for which the existence of a no-effect threshold dose is assumed employs a minimum (default) 100-fold uncertainty factor to extrapolate a ‘safe’ dose level for the general population based on animal toxicity data. The global 100-fold uncertainty factor is based on the assumption of a conservative 10-fold higher sensitivity of the human with respect to the most sensitive (or the default) animal species, and of a 10-fold difference of inter-individual variability in sensitivity between human subjects of the general population of different age, gender and general health status. Higher, but not (or seldom) lower values of the uncertainty factors can be applied when it is deemed that current unavoidable uncertainties recommend a more conservative approach to ensure that even the most sensitive human subject will go unharmed.

One source of debate is whether long-term and also chronic effects should be considered in determining the AOEL, rather than only those of short-term exposure studies: while it is commonplace that the use of agrochemicals by farmers is limited in time to the relatively short periods of application, whereas in the scenario of manufacturing industry exposure is considered to be continuous and appreciably constant throughout the working life of the person in the specific task, however it is also well-known that farmers’ working life spans a much wider period of their lives, even from late childhood to late post-retirement age. As a consequence, adverse health effects occurring late in age as the consequence of chronic exposure should also be taken into account.

To improve risk assessment, any information on human exposure derived from scientifically sound and ethically sustainable observations and studies can be used to confirm the validity of regulatory limit values derived from animal studies, but at the moment it is not allowed to perform *ad-hoc* studies in human subjects to derive information for regulatory purposes and, in particular, no data collected on humans can be used to lower the safety margins resulting from tests or studies on animals (European Parliament, 2009).

1.5.5.2. Acceptable Daily Intake (ADI).

This concept was first introduced in 1961 by the Council of Europe and later the Joint FAO/WHO Expert Committee on Food Additives (JECFA), a committee maintained the Food and Agriculture Organization (FAO) and the (WHO) World Health Organisation of the UN. The ADI takes into account the unavoidable presence of residues of PPPs in food and in drinking water which derive from the legitimate use of the formulated active substance, applied on crop cultures to protect them from pests, on crop products to prevent deterioration in their transport to food processing, to markets and to consumers, incorporated into meat and dairy from pasture and from silage, leaked into water reservoirs from use in the field.

An ADI value is established in the authorization process, based on the results of long-term studies on animals, by applying the same general criteria described above for the AOEL. Also the ADI is usually given in milligrams per kilogram of body weight (mg * $\text{kg}_{\text{bw}}^{-1}]_{\text{day}}$).

The ADI is considered a safe intake level for a healthy adult of normal weight when intake is appreciably constant in time. This may raise concern for substances with a tendency to show bio-accumulation in the organism or bio-magnification in the human food chain, as was the case with organo-chlorine pesticides, but currently the requirement for new active substances is that they are *per se* chemically labile in the environment, so that potential for build-up of levels of concern in the environment is now mostly negligible.

Increased safety factors for infants have been discussed, but are not needed, because elimination of chemicals is in fact often more rapid in children than in adults. The ADI does not take into account allergic reactions that are individual responses rather than dose-dependent phenomena.

1.5.5.3. Maximum Residue Levels (MRLs)

Conceptually closely related to the ADIs are the limit values referring to the maximum tolerable presence of residues in the several types of food which are produced and marketed downstream to the crops and in drinking water. Under the EU regulations, the Maximum Residue Levels (MRLs) are the upper legal levels of a concentration for pesticide residues in or on food or feed based on good agricultural practices and to ensure the lowest possible consumer exposure. The European Food Safety Authority (EFSA) is the administrative body of EU responsible for setting those limits. Regulation (EC) No 396/2005 establishes the MRLs of pesticides permitted in products of plant or animal origin intended for human or animal consumption.

MRLs are derived after a comprehensive assessment of the toxicological properties of the active substance (on the basis of which the ADI is established) and on the residue levels measured on or in crops treated according to the good agricultural practices defined for the product. Since consumer safety is the final aim for setting MRLs, values of the MRL are set at levels such that consumer intake of the active substance even in unbalanced diets based on food with the highest presence of residues does not exceed the ADI.

The maximum pesticide residue level in foodstuffs is 0.01 mg/kg for each active substance identified. This general limit is based on the expected sensitivity of available analytical methods and is applicable ‘by default’, *i.e.* in all cases where an MRL has not been specifically set for a product or product type. Some of the specific MRLs listed in Annex II are higher than the default limit, since there is evidence that the active substance is harmless to consumers’ health.

In some cases, provisional MRLs may be set and should then be listed in Annex III. Provisional MRLs should in particular be set in the some cases, among which are the occurrence of exceptional circumstances (*e.g.*, emerging local phenomena of food contamination), and in the course of harmonization procedures.

The Member States have to carry out official controls on pesticide residues in order to enforce compliance with Maximum Residue Levels. The results of the controls have to be reported to the Commission, to the other Member States and to EFSA, which publishes an Annual Report on Pesticide Residues in the EU based on the monitoring information.

Products which do not comply with the fixed limits cannot be marketed to consumers and may not be diluted with products with a lower level of residues in order to lower the mean level to below the limit. except in the case of certain processed and/or composite products listed by the Commission (Annex VI). In exceptional cases, products which do not comply with the limits set in Annexes I and II may be authorized by a Member State if the products do not represent an unacceptable risk. It should in fact be considered that agricultural products are produced at a substantial environmental, labour and economic cost and that unnecessary discard and destruction or diversion from their food use is ethically unjustified unless higher-rank interests, such as that to health protection need to be enforced.

1.5.5.4. Acute Reference Dose (ARfD).

The need to consider acute effects of pesticide residue intake has been acknowledged for many years, and the concept of the Acute Reference Dose (ARfD) was developed by the

Joint FAO/WHO Meeting on Pesticide Residues in 1994. Since then, there has been a progressive increase in the establishment of ARfDs for particular pesticides to address potential exposure to residues in food and drinking water at relatively higher doses for short-term periods, due to accidental or incidental events. JMPR has continuously updated its procedure on the setting of ARfDs.

The ARfD is defined as "an estimate of the amount a substance in food or drinking water, normally expressed on a body weight basis, that can be ingested in a period of 24 h or less without appreciable health risks to the consumer on the basis of all known facts at the time of the evaluation".

1.6. Pre-marketing pesticide exposure and risk assessment

Before an active substance can be authorized, it is necessary to perform the assessment of risk the active substance can pose to operators, workers, consumers, the environment and non-target plants and animals. Since our main interest is occupational health, we will be dealing with the risk assessment of operator exposure to pesticides.

Pre-marketing risk assessment for occupational exposure to Plant Protection Products is a procedure aimed at demonstrating that the active substance, formulated as the commercial product(s) intended for marketing, is able to perform its task (*i.e.*, to suppress the target organism under field conditions) without causing inacceptable harm to the farm worker.

As in any risk assessment, the risk is calculated as the ratio of actual internal dose to the regulatory limit: acceptable risk is exceeded if the ratio is >1, *i.e.*, if the internal dose is higher than that allowed by the regulatory limit. This task is accomplished by (***a***) evaluating the dose of active substance which reaches the farmer during agricultural activities, (***b***) estimating the resulting internal dose and (***c***) comparing with the maximum dose allowed by the toxicity characteristics of the active substance and established as part of the authorization process.

As anticipated, all calculations use the main parameters which are obligatorily part of the information collected in the evaluation Dossier or in the studies supplied with the active substance for which the application has been submitted: the Acceptable Operator Exposure Level (the health-based AOEL), the skin absorption factor (which is either experimentally measured or defaulted to 75% if its direct determination is not feasible), the concentration of active substance in the product, and the use rate (per hectare of surface).

The internal dose is calculated from the dose reaching the skin and the skin absorption coefficient, when this is available. In turn, the dose reaching the skin needs to be estimated from the amount of pesticide employed in a typical working day.

This quantity is often measured by performing studies in experimental farms, under standardized conditions, with workers doing spraying activities in different working scenarios, using different kinds of machineries, and different levels of protection. The resulting exposure is measured according to standardized methods, following the Guidelines of European Union (OECD, 1997).

The measured levels of exposure for each working scenario and level of protection are then generalized as milligrams of active substance deposited on farmers' clothes per kilogram of active substance used (exposure), and the levels of protection afforded by different types of Personal Protection Devices are expressed as the percent fraction of exposure that reaches the worker's skin.

1.6.1. The German model

To answer the requirements of (at that time) new European Union legislation 91/414/EEC (CEC, 1991) the "Uniform *Principles for Safeguarding the Health of Applicators of Plant Protection Products* (*Uniform Principles for Operator Protection*)" (Lundehn et al., 1992) described the characteristics of the German model. Specific exposures, namely inhalation exposure, hand exposure and remaining body exposure, later used for modelling, were determined on the basis of experimental studies conducted by the plant protection industry.

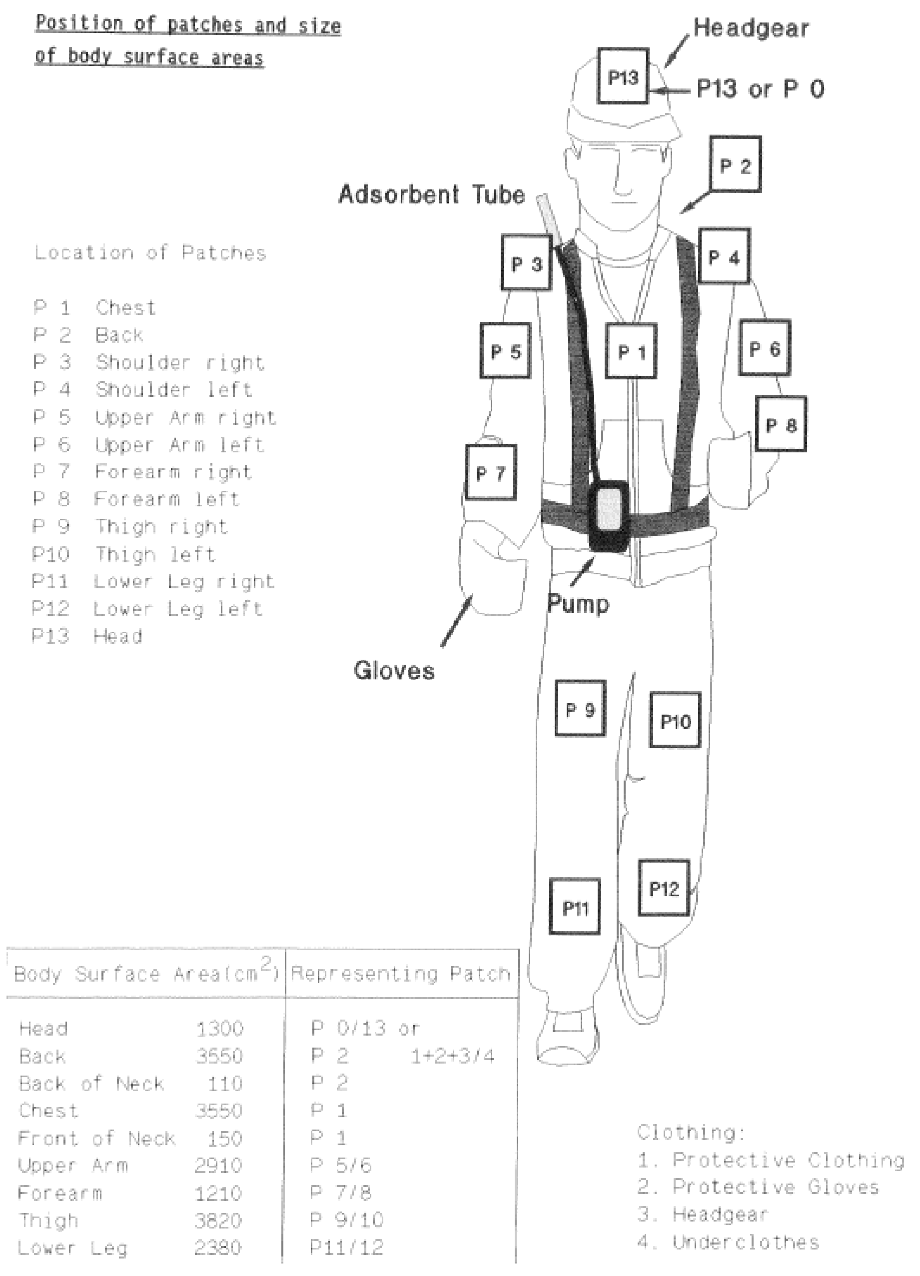
Inhalation exposure was determined using a tube, attached to the operator at mouth level, connected to a portable battery-powered pump, which sucked the same air an operator would breathe. The measured amount of active substance was standardized to the human respiration volume of 1.74 m^3/h.

The dermal exposure of the hands is measured by determination of the amount of the active substance in the rinsing fluid from the gloves or hands. For this purpose, the gloves or hands of a person were rinsed with a solvent after work. Phases "mixing and loading" and "application" were again sampled separately.

The exposure of the remaining body surface is measured by means of absorbent patches (made of cellulose) with a defined surface (e.g. 33 cm^2) that were attached to appropriate locations on the operator's clothing. Samples were only taken during the "application" phase of work. From the amount of active substance measured on the patches, the amount on each body region represented by a patch is calculated by extrapolating the surface of the patch to the surface of the region.

The “mixing and loading” and “application” phase were examined separately.

An illustration of the worker with patches’ location, taken from the original document describing the German Model approach, is available in ***Figure 1.6.1.***



Body Surface Area(cm^2)		Representing Patch
Head	1300	P 0/13 or
Back	3550	P 2 1+2+3/4
Back of Neck	110	P 2
Chest	3550	P 1
Front of Neck	150	P 1
Upper Arm	2910	P 5/6
Forearm	1210	P 7/8
Thigh	3820	P 9/10
Lower Leg	2380	P11/12

Figure 1.6.1. German Model methodology (from: Ludhen et al., 1992.)

Twenty three trials were made, for a total of over 100 exposure measurements, to determine the specific exposures in various use patterns and the protection offered by different kinds of Respiratory Protection Equipment and Personal Protection Equipment.

The protective gear and reduction coefficients are given in ***Table 1.6.2.***

Protective gear	Reduction coefficient	
	Dermal	Inhalation
Universal protective gloves (plant protection)	0.01	
Standard protective garment (plant protection) and sturdy footwear	0.05	
Protective clothing against chemicals; type 3	0	
Broad-brimmed headgear of sturdy fabric	0.5	
Hood and visor	0.05	
Particle filtering half-mask FF2-SL or half mask with particle filter P2	0.8	0.05
Half mask with combination filter A1P2	0.8	0.02

Table 1.6.1. – Elements of protective gear and reduction coefficients

The work-day characteristics taken into account by the German Model are:

- Application method
    - Tractor-mounted/trailed boom sprayer: hydraulic nozzles
    - Tractor-mounted/trailed broadcast air-assisted sprayer
    - Hand-held sprayer: hydraulic nozzles. Outdoor, high level target
    - Hand-held sprayer: hydraulic nozzles. Outdoor, low level target
- Formulation type
    - Wettable Granules (WG)
    - Wettable Powder (WP)
    - Liquid
- Active substance concentration (g/kg)
- Dermal absorption from product
- Dermal absorption from spray
- Respiratory Protection Equipment (RPE) during mixing and loading
    - None
    - FFP2SL or P2 mask
    - A1P1 mask
- Personal Protection Equipment (PPE) during mixing and loading
    - None
    - Gloves
- Respiratory Protection Equipment (RPE) during application

    - None
    - FFP2SL or P2 mask
    - A1P1 mask
- Personal Protection Equipment (PPE) during application
    - Head
        - None
        - Broad-brimmed headwear
        - Hood and visor
    - Hands
        - None
        - Gloves
    - Body
        - None
        - Coverall and sturdy footwear
- Dose (kg of product per ha)
- Work rate (ha per day)
- Operator weight

Based on the above listed work-day characteristics, the German model calculates the following exposures:

- Dermal exposure during mixing and loading
- Inhalation exposure during mixing and loading
- Dermal exposure during spray application
- Inhalation exposure during spray application
- Absorbed dose
- Predicted exposure

The predicted exposure is expressed as the absorbed dose divided by the operator weight (mg/kg of body weight per day), and this can be compared to the AOEL for the specific substance which is expressed in the same unit of measure.

1.6.2. The EURO-Poem

The European Commission has established the EUROPOEM expert group to develop a predictive operator exposure model on the basis of field studies (van Hemmen, 2001). Field study reports were requested from industry, European governments and academia, and were considered according to structured criteria. Studies were included in the EUROPOEM exposure database only if they were considered relevant for European agriculture. Several different use scenarios were defined, and relevant surrogate values were obtained for each use scenario for which sufficient data were available. The purpose of these values was then to be used in registration procedures for agricultural pesticides. This expert group developed a database of exposure data using only studies that were in agreement with the spirit of the OECD Guidance Document (OECD, 1997). The choice was made to use the 75th percentile for large databases, and not the geometric mean (van Hemmen, 2001).

EUROPOEM has proposed a tiered approach in the exposure assessment for risk assessment in authorization procedures. Three tiers are considered for operators:

- First tier: Most conservative estimate by using worst-case assumptions for all relevant variables. This estimate of exposure is compared with the appropriate AOEL, to estimate the risk ratio, which should be below 1 to pass the test.
- Second tier: If the risk ration is >1, the exposure-reducing effect of PPE may be considered in the second tier as well as relevant knowledge on dermal and inhalation absorption.
- Third tier: If the estimated exposure in the second tier is still above the AOEL, the only possible way to authorize the active substance is to show in a representative, well-designed study, that the level of exposure of the active substance and the use scenario under consideration are below the AOEL. Preferably this should be done with biological monitoring that can be interpreted on the basis of human pharmacokinetics. In such a case the ultimate answer to the test is given.

The work-day characteristics taken into account by the EURO-Poem are:

- Liquid concentrate formulation/Solid concentrate formulations
- Application method
    - Tractor-mounted/trailed boom sprayer: hydraulic nozzles
    - Tractor-mounted/trailed boom sprayer: rotary atomisers
    - Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha
    - Tractor-mounted/trailed broadcast air-assisted sprayer: 100 l/ha
    - Tractor-mounted/trailed broadcast air-assisted sprayer: 50 l/ha
    - Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target
    - Hand-held rotary atomiser equipment (2,5l tank). Outdoor, low level target
    - Hand-held rotary atomiser equipment (2,5l tank). Outdoor, high level target

  - home garden sprayer (5 litre tank). Outdoor, low level target
- Formulation type
  - Organic solvent-based
  - Water-based
  - Wettable Powder (WP) or Soluble Powder (SP)
  - Wettable Granules (WG) or Soluble Granules (SG)
  - Water soluble bags (SB)
- Active substance concentration (g/kg)
- Dermal absorption from product
- Dermal absorption from spray
- Container (capacity and closure)
- Personal Protection Equipment (PPE) during mixing and loading
  - None
  - Gloves
  - Gloves and FFP2 mask
  - Gloves and FFP3 mask
  - FFP2 mask
  - FFP3 mask
- Personal Protection Equipment (PPE) during application
  - None
  - Gloves
  - Gloves and impermeable coverall
- Dose (kg of product per ha)
- Work rate (ha per day)
- Application volume (l/ha)
- Duration of spraying (hours)
- Operator weight

Based on the above listed work-day characteristics, the EURO-Poem calculates the following exposures:

- Dermal exposure during mixing and loading
- Inhalation exposure during mixing and loading
- Dermal exposure during spray application
- Inhalation exposure during spray application

- Absorbed dose
- Predicted exposure
- Operator weight

The predicted exposure is expressed as the absorbed dose divided by the operator weight (mg/kg of body weight per day), and this can be compared to the AOEL for the specific substance which is expressed in the same unit of measure.

1.7. Post-marketing pesticide exposure and risk assessment

Even as an active substance is authorized in European Union, and products containing this active substance are authorized and marketed, there is still a need for risk assessment to communicate and to manage risk with regard to the different groups of stakeholders and to the general population as a whole (European Parliament, 2009).

Risk assessment of agricultural occupational exposure (as well as for other exposures) performed in the pre-marketing phase is aimed at ensuring that a formulated active substance, when applied in the field under the conditions established as Good Agricultural Practices, is safe for use and does not pose harm to farmers' health. In real-life working conditions, however, risk assessment is seldom, if any, performed since the task has many difficulties, mainly linked to economic cost, to the limited availability of trained personnel and logistics necessary to reach small, family based enterprises, which are often poorly covered by occupational health services, to the variability of working patterns, of climatic conditions and of the frequent use of mixtures of pesticides. The existence of epidemiological studies (Baldi et al., 2001) and of case reports which suggest that chronic low-level pesticide exposure can have long-term effects on the health of agricultural workers also suggest the necessity to perform risk assessment also in 'real-life', region specific field conditions.

In the field, exposure to pesticides comes from three main routes: dermal, inhalation and oral. During open-field farming (and pesticide spraying), the contribution of the oral route is considered negligible (unless accidental and non-predictable hand-to-mouth occurs) and inhalation has been demonstrated to contribute very little to the overall exposure, while exposure by absorption from the contaminated skin (the dermal route) accounts as that quantitatively most relevant. From the point of view of risk assessment, work with pesticides can be classified into three phases, each corresponding to specific modalities of farmer exposure: preparation of the product for application (mixing and loading), spraying (application) and finally maintenance of the agricultural equipment. In each of these phases, the worker can be exposed to the pesticides to a different extent, partly by direct contact with the mixture, and partly from contact with contaminated items.

1.7.1. Biological monitoring (biomonitoring)

Biomonitoring of exposure to pesticides involves the measurement of a pesticide, its metabolite(s) or biotransformation products in biological fluids such as urine or blood. Although it is widely used in many occupational and environmental health and exposure studies it is very important to understand the problems, implications and uncertainties involved in the biomonitoring process.

The advantage of biomonitoring is that the data are independent of the pathway of exposure. It measures integrated exposure from different routes and the amount found is some portion of what actually entered the body. For some active substances, such as azinphos-methyl, the analysis of urinary metabolites has shown greater sensitivity than dermal exposure monitoring (Franklin, 1984). However, it is critical to design the study appropriately, plan which biomarker to measure, in which fluid or tissue, as well as when and how many samples should be taken, and from which workers (Manno et al., 2010). Urine sample can be taken as a spot sample, or urine can be collected during a longer period of time. Collection of spot urine samples is sometimes considered to reduce participant burden and avoid potential confounding from additional chemical uses. In this case, the first morning void is often preferred because the urine is more concentrated, the sample represents a much longer window of accumulation (mostly 8 hours), and it is often correlated with total excretion over 24 hours. To evaluate other sources of variation samples can be taken days and/or weeks apart. But, in the common scenarios of pesticide use, the spot sample has some disadvantages. Since the exposure to pesticide is mostly intermittent, and the kinetic for most pesticides is not well known, it is difficult to estimate precisely where the peak of excretion will be. Therefore, some researchers consider the 24-hour sample to be more representative of the exposure (and excretion) during one work-day.

Another critical point of biological monitoring is the complexity of the toxicokinetic process. It can vary based on demographic variables (age, gender, genotypic and phenotypic variability, ethnicity), lifestyle (diet), co exposures, and certain medical conditions. This kind of variability in the toxicokinetic process makes interpretation of biomonitoring data complex. Multiple elimination routes and variable metabolism can complicate the measurement and interpretation of biomarkers analysed in urine samples.

There are many ways the interpretation of biological measurements can be confounded. In agriculture, the farmer may have been exposed to the pesticide(s) of interest in the days before the monitoring. Therefore, the biomarker level might not be at the baseline before sample collection in the study. In other cases, the farm worker might be exposed to the pesticide in the days following the monitoring, which would significantly interfere with the results of multiday post-application sample collection. For a successful interpretation of biological measurements it is necessary to collect important information from the farm worker regarding the activities resulting in pesticide exposure (start and end of important activities, tasks performed, equipment used) and most certainly the use of personal protective equipment.

For many pesticides the routes of metabolic biotransformation in humans are unknown, and the method for their detection need yet to be developed. Even for the pesticides with known metabolites, there is a lack of biological health-based limit values. Interpretation and risk assessment using biological monitoring data and is dependant of the existence of Biological Exposure Indices (BEIs). BEIs are guidance values for assessing biological monitoring results which represent the levels of determinants that are most likely to be observed in specimens collected from healthy workers who have been exposed to chemicals to the same extent as workers with inhalation exposure at the Threshold Limit Value (TLV). The BEI generally indicates a concentrations bellow which nearly all workers should not experience adverse health effects. These two facts, combined with a somewhat high cost of biological monitoring, both in money as well as the time and burden on the study participants and staff, makes biological monitoring difficult to use for post-marketing risk assessment.

1.7.2. Environmental monitoring

Environmental monitoring is a way to assess the exposure of workers which measures the exposure in the working environment. The exposure levels are estimated by measuring potential dermal exposure, actual dermal exposure and inhalation exposure (Maroni et al., 1999). Nevertheless, in open-field farming and application of pesticides, the inhalation exposure is considered negligible compared to dermal exposure (Dowling and Seiber, 2002; Flack et al., 2008; Wang et al., 2006), especially when respiratory protection is worn (Aprea et al., 1998). Therefore, we will be dealing with dermal exposure and risk assessment in the following sections.

Dermal exposure monitoring typically makes use of a set of dermal dosimeters for each individual participating in an exposure study, as well as hand-wash or wipe sampling to measure the hands exposure (Brouwer et al., 2000; OECD, 1997). The measurements are collected during the whole work-day or during a set of work activities. Collection of dermal doses (using dermal dosimeters) has the advantage of providing information about specific routes of exposure and also provides exposure information on a specific activity being monitored.

The method gives us information on the potential exposure (contamination found on the workers' clothes and personal protective devices) and actual exposure (contamination found on the workers' skin, ready for absorption). Therefore, a number of assumptions and empirical parameters regarding the transport and distribution of the chemical on and through the skin and lungs are required to be able to accurately estimate the internal dose.

Both exposure measurement approaches have been used in farm work exposure and risk assessment, and each approach has advantages and disadvantages based on information provided, uncertainty, participant burden and resource requirements. Biological monitoring provide better evidence of the occurrence of exposure and absorption, which is a more toxicologically relevant measure of internal dose. The biomarkers account for all exposure(s) and routes, while using dermal dosimetry it is possible to compare different routes of exposure, as well as estimate how different field conditions influence the said exposure routes. Drawbacks of dermal exposure monitoring are the complexity and burden of sample collection, the cost, and the need for extrapolation of contamination found on the dosimeters (pads, clothes cuts) to the whole body, which can increase the uncertainty in the exposure and risk assessment.

Factors to be considered when deciding between these two methods are collected in ***Table 1.7.1.***

Factor	**Biological monitoring**	**Environmental monitoring**
Internal dose assessment	+++	+
Availability of limits	+	+++
Burden on farmers	+++	+
Application cost	+++	+
Accuracy	+++	++
Analysis of field conditions and PPE	+	+++

Table 1.7.1.: Comparison of characteristics of biological and environmental monitoring

1.7.3. Algorithms and models (surrogates of exposure)

Having in mind the limitations of biological and environmental monitoring (see ***Sections 1.7.1.*** and ***1.7.2.***), alternative methods for exposure and risk assessment have been developed. They differ in their complexity and reliability, and vary from the use of expert opinion (Harris et al., 2005; Marquart et al., 2003), pre-marketing models (Lundehn et al., 1992; van Hemmen, 2001), to the use of combination of literature data, measurements and expert opinion (Colosio et al., 2012; Dick et al., 2010; Dosemeci et al., 2002). In the following sections these approaches will be described in more detail.

1.7.3.1. The use of pre-marketing models

Models are used as pre-marketing risk assessment tools in most European Union countries (see ***Section 1.6.***), and there have been attempts to use it as a risk assessment tool in field studies. The published literature mostly concludes that, since the models are based on exposure measures in experimental conditions, which are different from real-life field conditions in agriculture it is not adequate (fully reliable) to perform exposure and risk assessment in these conditions (Machera et al., 2009). It was demonstrated that the models underestimate the risk in low-use scenarios (when a small amount of active substance is used) and overestimate the exposure in high use scenarios (when a large amount of active substance is used), namely because the total exposure by these models is linearly dependent on the amount of active substance used (Protano et al., 2009; Rubino et al., 2012). In addition, the models do not take into account some specificities of real-life pesticide application conditions, such as the presence of a cabin with filters in a tractor, as well as the repetition of mixing and loading tasks, and many other situations of use and non-use of personal protective equipment.

1.7.3.2. Agricultural Health Study quantitative method

The National Cancer Institute (NCI), the National Institute of Environmental Health Sciences (NIEHS) and the United States Environmental Protection Agency (EPA) have conducted a prospective cohort study (the Agricultural Health Study, AHS) of more than 90,000 farmers, farmers' spouses and commercial applicators in Iowa and North Carolina (USA) to evaluate cancer and other disease risk associated with pesticides, other agricultural exposures and lifestyle factors (Dosemeci et al., 2002).

To answer the problem of assessment of exposure to agricultural pesticides, which has been limited to the use of surrogates of exposure (type of farm, chemicals used, job title) in chronic disease research, the group of authors have described a quantitative approach developed for the Agricultural Health Study to estimate applicator exposure to more than 50 individual pesticides, using questionnaire responses and pesticide information published in the literature.

At the enrolment into the study, the pesticide applicators completed a questionnaire consisting of time (number of exposed years, average annual number of days used, phases of handling) and intensity (frequency of mixing, method of application, use of personal protective equipment) related pesticide exposure questions. Applicators who completed the enrolment questionnaire were also given a take-home questionnaire to obtain additional information on the pesticide handling, use of an enclosed mixing system, type of tractor, procedures to clean pesticide application equipment, personal hygiene, the practice of changing clothes after a spill, and frequency of replacing old gloves.

The questionnaire responses were used to develop chemical-specific exposure scenarios. The general algorithm is presented below:

$$\boldsymbol{Intensity\ Level} = (\boldsymbol{Mix} + \boldsymbol{Appl} + \boldsymbol{Repair}) \times \ \boldsymbol{PPE}$$

Where:

- Mix (mixing status)
    - Never (score 0)
    - <50% of time mixed (score 3)
    - 50%+ of time mixed (score 9)
- Appl (application method)
    - Does not apply (score 0)
    - Application methods for different groups of pesticides
        - Herbicides (from aerial-aircraft to hand spray, score 1-9)
        - Insecticides (from aerial-aircraft to mist blower, score 1-9)
        - Animal insecticides (from ear tags to powder duster, score 1-9)
        - Fungicides (from seed treatment to mist blower, score 1-9)
        - Fumigants (from gas canister to pour fumigant, score 2-9)
- Repair (repair status)
    - Does not repair (score 0)
    - Repair (score 2)
- PPE (Personal Protective Equipment use)

  - PPE-0 (0% protection)
  - PPE-1 (20% protection)
    - Face shields or goggles
    - Fabric/leather gloves
    - Other protective clothing, such as boots
  - PPE-2 (30% protection)
    - Cartridge respirator or gas mask
    - Disposable outer clothing
  - PPE-3 (40% protection
    - Chemically resistant rubber gloves
  - Scores for each PPE type are
    - PPE-0 = 1.0
    - PPE-1 = 0.8
    - PPE-2 = 0.7
    - PPE-3 = 0.6
    - PPE-1 & PPE-2 = 0.5
    - PPE-1 & PPE-3 = 0.4
    - PPE-2 & PPE-3 = 0.3
    - PPE-1 & PPE-2 & PPE-3 = 0.1

Example of the score for a situation.

Pesticide used: 2,4-D

Mixing status: Personally mixes pesticides more than 50% of time (score 9)

Application method: Backpack spray (score 8)

Repair status: personally repairs application equipment (score 2)

PPE status: Wears rubber gloves and boots (PPE-1 & PPE-3, score 0.4)

$$Intensity\ level = (Mix + Appl + Repair) \times\ PPE = (9 + 8 + 2)\ \times 0.4 = 7.6$$

The main sources of assigned exposure weights were the monitoring data in published scientific literature. Results of various monitoring data has been compared, between individual exposure variables (mixing versus applying) as well as within a selected variable (e.g. in application: ground boom versus backpack application).

This approach, despite some limitations, represented a step forward in the estimation of pesticide exposure in epidemiological studies, and the method has been modified and improved several times in the years after.

Unfortunately, this method has not been designed for real-life field risk assessment, and being based on published literature and generic databases (PHED, 1992), the exposure estimates in different scenarios cannot be considered representative for agricultural work in Europe, or in different crop (vineyards).

1.7.3.3. Task-Exposure Matrix (TEM) method

Over a number of years efforts have been made to improve pesticide exposure estimates in epidemiological studies. The most actual approaches have been the collection of work histories (job titles), job-exposure matrices (JEMs), expert assessment of work histories, and self-reports of exposure. The approach of using job titles as exposure surrogates has some important limitations when applied to farming. The problem is that job titles such as “farmer” encompass such a huge group of tasks in which pesticide exposure varies significantly. There have been reports on farm-related job titles being poor surrogates for pesticide exposure, with over three-quarters of farm jobs being assessed as having no likelihood of pesticide exposure when considered by an occupational hygienist (Dick et al., 2010).

Job-exposure matrices have at least two axes, one covering a range of jobs and the other axis being the agents of interest. Some matrices have also a third axis, which covers the time in order to allow for changes in work practices or agents over the study period. The cells of the matrix are populated with exposure estimates that may indicate exposure (exposed/unexposed), exposure ranking (low/medium/high), or the probability of exposure. Assessment of pesticide exposure by experts is generally considered the best approach to exposure estimation where reliable biomonitoring data are not available (de Cock et al., 1996; Garcia et al., 2000).

Although TEMs can be considered an important step forward for epidemiological studies on health effects of pesticide exposure, they are not suitable for field risk assessment, due to the fact that they are based on specific country’s pesticide use in the past, and the fact that they give a semi-quantitative exposure assessment, but no precise risk assessment of pesticide use in agriculture.

1.7.3.4. First step towards Exposure and Risk Profiles

After considering all the advantages and limitations of the above mentioned approaches to exposure and risk assessment of pesticide exposure in agriculture, we have done a study in rice and corn pesticide applicators (Rubino et al., 2012) and explore the possibility of using the field measurements to create a "user friendly tool" adequate to evaluate the levels of occupational exposure and risk consequent to pesticide application (Colosio et al., 2012), 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.

		Toxicity score			
Class	**Exposure score**	**1**	**2**	**3**	**4**
A	**Low**	**NEGLIGIBLE**	**NEGLIGIBLE**	**LOW RISK**	**HIGH RISK**
B	**Probably low**	**NEGLIGIBLE**	**LOW RISK**	**HIGH RISK**	**HIGH RISK**
C	**Probably high**	**LOW RISK**	**LOW RISK**	**HIGH RISK**	**UNACCEPTABLE RISK**
D	**High**	**LOW RISK**	**HIGH RISK**	**UNACCEPTABLE RISK**	**UNACCEPTABLE RISK**

Table 1.7.2. Semi-quantitative scheme for the evaluation of pesticide-related health risk for farmers, based on an estimate of increasing exposure levels

To this aim a fairly simple 4 x 4 evaluation grid with four toxicity classes for the active ingredient was chosen, 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 centre of the evaluation grid in case of unavailability or ambiguity of data. In the 4 x 4 evaluation grid shown in ***Table 1.7.2.*** there were 16 possible combination of toxicity and exposure classes, which were 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 was 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 was to assign appropriate values to the toxicity and exposure classes. The second step is to test the outcome of the first step with case studies. In order to do it, two cash crops, rice and maize, which are typical for Northern Italy were selected.

Definition of toxicity indices

Classification, risk phrases and labels were defined during the toxicological evaluation of pesticides performed in the pre-marketing phase; from this information the pesticide formulations were ranked in four main toxicity groups, from the lowest levels of toxicity (group 1), to the highest level of toxicity (group 4).

The ranking procedure considered each risk phrase in the frame of the agricultural occupational scenario, so that, as an example, absorption through ingestion was not crucial for farmers’ exposure, whilst long term and no-threshold effects were of a higher concern.

In the highest toxicity group, 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) have been allocated. ***Table 1.7.3.*** 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 was considered as the sole active ingredient of the whole mixture.

	Risk phrase	Score	Examples of products
R22	**DANGEROUS IF SWALLOWED**	**1**	**Terbutylazine – Propanil – Copper hydroxide – Ziram – Diquat - Benfuracarb**
R36	**EYE IRRITANT**	**1**	**Endosulfan - Dichlorvos**
R20	**DANGEROUS IF INHALATED**	**2**	**Endosulfan**
R25	**TOXIC IF SWALLOWED**	**2**	**Linuron – Methiocarb - Dichlorvos**
R23	**TOXIC IF INHALATED**	**3**	**Copper hydroxide – Methiocarb – Ziram - Benfuracarb**
R43	**SKIN SENSITIZER**	**3**	**2,4 D – Mancozeb – Methiocarb – Ziram - Dichlorvos**
R26	**HIGHLY TOXIC IF INHALATED**	**4**	**Ziram – Diquat - Dichlorvos**
R62	**CAN REDUCE FERTILITY**	**4**	**Linuron - Benfuracarb**

Table 1.7.3. Toxicity scores based on the risk phrases allocated to the compound.

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 was 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 $\mathbf{I_{exp}}$ was 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 Equation (1):

$$\boldsymbol{I_{exp} = I_{MIX} \times \%t_{MIX} + I_{APPL} \times \%t_{APPL} + I_{REP} \times \%t_{REP}}$$

$\boldsymbol{\%t_i}$ 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 equation (2)

$$\boldsymbol{I_{PHASE} = Dose \times I[PPD] \times I[Operator\ Skills] \times I[Machineries]}$$

where DOSE is dependent on several parameters (see below and in ***Table 1.7.4.*** and ***Table 1.7.5.***), and PDD, Operator Skills and condition of Machinery are modifying factors (see below).

Tables 3 and **4** report examples of the scoring system assigned to the various working conditions encountered in the field so that higher score numbers correspond to conditions leading to an increase of exposure (DOSE).

Phase of work	Variables influencing exposure	Less exposure	→	→	More exposure
Mix/Load	**Number of loadings**	1	2-5	>5	
	Score	0.5	1	2	
	Concentration of active principle (%)	<50	50-90	>90	
	Score	0.5	1	2	
	Type of formulation	Soluble bags	Granules/liquid	Powder	
	Score	0	1	2	
	Duration of mixing and loading **Time (% of total activities)**	Short			Long
Application	**Use rate (kg/ha)**	<0.1	0.1-2.5	>2.5	
	Score	1	2	3	
	Application pressure (bar)	<3	3-5	5-10	>10
	Score	1	2	3	4
	Treated area (ha)	<10	10-20	>20	
	Score	1	2	3	
	Interventions on machines during application	None	1-2 times during the day	More than 2 times	
	Score	0	1	2	
	Condition of equipment	Good	Acceptable	Bad	
	Score	0	4	8	
	Duration of application **Time (% of total activities)**	Short			Long
Maintenance	**Maintenance of equipment**	Not done	Done		
	Score	0	30		
	Duration of maintenance **Time (% of total activities)**	Short			Long

Table 1.7.4. 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.

Modifying factors	**Less exposure**	→	→	**More exposure**
Type of tractor	With cabin and carbon filter	With air-conditioned cabin	With cabin without air-conditioning	Open
Score	0	1	2	3
Personal Protective Devices	Adequately used	Not used		
Score	0.7	1		
Training/skill	Certificate or equivalent	None		
Score	0.5	1		

Table 1.7.5. Scoring of modifying factors.

1.8. New tools for pesticide exposure and risk assessment in agriculture

The main tools currently available for the “in-the-field”, namely biological and environmental monitoring, show important limits in agriculture. 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. Moreover, real-life exposure measurement is very expensive due to the necessity to perform non-standard chemical measurements (Hoppin et al., 2006).

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).

New tools adequate to perform pesticide risk assessment even in absence of field measurement are therefore missing. In principle, this task is based on the knowledge of the relationships between different variables affecting the levels of exposure in the four typical working phase of pesticide application in agriculture: mixing and loading of products, application on the crops, re-entry in the treated field and maintenance and cleaning of equipment and personal protective devices (PPDs). The starting point for this activity is the definition of typical exposure and risk scenarios (Machera et al., 2003; Machera et al., 2002; Maroni et al., 2000) and the definition of the typical levels of exposure anticipated in these scenarios, necessary to extrapolate the data collected in the situation under study to other similar and comparable. In order to do it, it is necessary to study, in these scenarios, the relationships between selected variables affecting the levels of workers’ exposure in each of the above mentioned working phases.

1.9. Goals and objectives

The goal of this effort is the creation of Exposure and Risk Profiles, as a reliable, scientifically based way to forecast pesticide exposure levels and risk 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.

To reach this goal we have defined objectives and sub-objectives.

1.9.1. Objectives:

1. Define the main phases of pesticide work and known factors which influence exposure
    - Search available published literature
    - Identify determinants and modifiers of exposure
    - Explore and compare their contribution to exposure
    - Set-up a base for collecting field information
2. Collect information and measurements in real-life field conditions
    - Based on the literature search, define the variables of interest and a method for their collection
    - Organize real-life field studies to collect the measurements of exposure
3. Analyze the data from field studies
    - Perform exposure and risk assessment of workers participating in real-life field studies
    - Develop methods for accurate exposure assessment
    - Develop methods for accurate risk assessment
    - Define the variables influencing exposure (and risk) in field conditions
4. Develop methodology to use the field data for risk assessment
    - Develop methods for generalizing results of field studies to a wide group of pesticides (or all pesticides)
    - Develop a methods for the creation of a Risk Assessment Scheme (Exposure and Risk Profile)
5. Create an Exposure and Risk Profile for the most frequent pesticide application method
    - Test the method of doing risk assessment without measurements

2. Materials and Methods

In order to reach the overall goal of this PhD project (see ***Section 1.9.***), we needed to address the objectives defined in the previous sections (see ***Section 1.9.1.***).

Understanding the process of pesticide application was the first task in studying the process of pesticide preparation and application and defining the factors influencing exposure and risk. Many reference points already existed in the published literature, and in order to better understand the main phases of work with pesticides, a thorough literature search was done (see ***Section 2.1.***).

Even though literature data can be very useful in setting up and describing better the scenario(s), it is not enough to completely explore and define exposure and risk profiles, and offer a solution for in-field rapid risk assessment. Therefore, we organized two real-life field studies in the vineyards North Italy.

ACROPOLIS is an EU-funded project with a goal of creating an On-Line Integrated Strategy for Aggregate and Cumulative Risk of Pesticides (Acropolis Project, 2013). As one part of the activities of the project field studies have been organized to assess the exposure to Tebuconazole (TEB). The study was conducted in Monferrato, which is a world-famous wine-producing area of Piedmont, Northern Italy, where the local cultivars are the source of commercially prized wine brands. Due to the nature of the hilly landscape, small vineyards, ranging from 200 m^2 to 6,000 m^2 are most common and their uphill laying and irregular size command the use of small, mainly open-cockpit tractors for towing small-volume spraying tanks and for manual spraying of smaller or physically unattainable garden vineyards. Among many active ingredients used in vineyards, tebuconazole is often applied to fight the uncontrolled growth of wine-spoiling moulds which greatly deteriorate the quality of the product. TEB belongs to a large family of azole fungicides, several of which are used also in human therapy. Controlled use of these chemicals is considered safe for humans, although TEB causes malformations at high doses in animals both *ex vivo in vitro* and *in vivo* (EFSA, 2008; Giavini and Menegola, 2010).

In the same period of 2011 INAIL financially supported another real-life pesticide exposure and risk study conducted by our study team. The goal of this study was to assess the exposure and risk of workers using open tractors, as well as closed and filtered tractors while applying Mancozeb in vineyards of the Region. Mancozeb is another widely used agricultural fungicide. It is a manganese ethylenebis(dithiocarbamate) complex with zinc salt. Mancozeb formulations contain a percentage of ethylenethiourea (ETU), which is also a metabolic product of ethylenebisdithiocarbamates, which is known to have long-term effects characterized principally by antithyroid activity in experimental animals (Colosio et al., 2002).

In both studies, potential and actual dermal exposure was measured. *Potential* dermal exposure (in brief potential exposure) is defined as the amount of pesticide coming into contact with the working clothes and personal protective devices (Lesmes-Fabian et al., 2012b; Rajan-Sithamparanadarajah et al., 2004). *Actual* dermal exposure (in brief actual exposure) is defined as the amount of pesticide coming into contact with the workers' skin, available for absorption (Lesmes-Fabian et al., 2012b; Rajan-Sithamparanadarajah et al., 2004). Detailed methodology of both studies described in ***Sections 2.2.*** and ***2.3.***

Finally, we have developed methodologies to help us define the factors influencing exposure and risk of pesticide applicators, as well as extrapolate the risk from the measured active substance to a whole range of active substance. The above mentioned methodology is detailed in ***Sections 2.4.*** to ***2.8.***

2.1. Literature search

Searching is part of conducting a review on a topic, and this process is extremely important, as mistakes can result in biased or incomplete evidence base. It is necessary to precisely define the question we are trying to answer, and have in mind all the important concepts we are gathering knowledge about.

The interest of this search were articles published in the last 25 years portraying the exposure to pesticide, division of work in phases, most important variables affecting exposure to pesticides in the field. Additionally, we concentrated on the articles of authors that have tried to estimate exposure to pesticide using work variables such as number of mixing and loadings, duration of activities, area treated and personal protection devices (PPDs) used during all the phases of work with pesticide.

2.1.1. Keywords and combinations of keywords

Here we list the basic concepts of interest for our work, and the keywords (combinations of keywords) used to retrieve the published articles on these topics.

1. General knowledge on pesticide exposure and risk assessment in agriculture (Keywords: *pesticide, exposure, risk, agriculture*)

2. Studies in the field/vineyards on pesticide exposure
   (Keywords: *field/vineyard, pesticide exposure, pesticide risk*)
3. Variables/Determinants influencing pesticide exposure
   (Keywords: *variable(s), determinant(s), pesticide exposure, agriculture, pesticide risk, predictor(s)*)
4. Methods used for pesticide exposure and risk assessment
   (Keywords: *assessment, exposure, risk, dosimetry, environmental monitoring, biological monitoring, estimate*)
5. Activities influencing the reduction of exposure and risk
   (Keywords: *pesticides, agriculture, practice(s), training, safety, knowledge, attitudes*)

2.1.2. Articles retrieved and used in the project.

Based on the initial keyword search, more than 60 articles were found, covering the topics of pesticide exposure and risk assessment, environmental and biological monitoring during pesticide application, determinants of exposure, and interventions on reducing the exposure and risk of agricultural workers. Using the references from the identified articles, it was possible to select a group of more than 150 articles of interest dealing with pesticide exposure assessment, risk assessment, and the determinant and modifiers of pesticide exposure and their contribution to the overall exposure and risk.

This literature search has resulted in a definition and systematization of current knowledge on pesticide importance in the modern world, exposure and risk assessment in the pre- and post-marketing phase, and needs for improvement of existing risk assessment techniques (see ***Introduction.***). It has also given us a base of methods to use in our field studies, as well as guidelines to develop new methods where necessary to achieve the objectives of our work (see ***Methodology***).

2.2. Acropolis study

2.2.1. Study overview

The study was run from May to July 2011 in the area of Monferrato, Piedmont, Northern Italy (***Figure 2.2.1.***), and involved farmers using different modes of pesticide application (***Figure 2.2.2.***).

Figure 2.2.1. Monferrato vineyards

Seven farmers were invited to participate based on their use of TEB in the vineyards. Five study subjects were independent farmers, one was an employee and one was an independent specialized hired professional. The workers were required to avoid changing their normal work-day routine, and were offered feedback on personal exposure and risk assessment and suggestion for improvement of their work routine. All individuals read and signed the informed consent form approved by the Ethical Committee of the University of Milan.

Figure 2.2.2. Application of fungicides by the participating farmers: from an open-drive tractor (up) and by manual spraying (down).

2.2.2 Study protocol

The study protocol is defined in two coordinated levels of data collection:

1) Data collection sheet consisting of questions regarding the characteristics of the farmer, the farm, and the work-day;

2) Assessment of potential and actual dermal exposure, i.e. monitoring head, body, and hands exposure during all phases of work with pesticides.

2.2.3. Data collection sheet

A data collection sheet to explore determinants and modifiers of exposure was developed and filled in by trained members of the research team during the investigated work-days. It was divided into several parts including: information about the enterprise, the worker, each working phase (mixing and loading, application, cleaning of machineries), as well as the use of personal protective devices during each phase of the work. A version in English of the data collection sheet used in this study is reported as ***Supplementary material S1.***

2.2.4. Personal dermal exposure monitoring

On each application day the farmer wore a working attire consisting of underwear (a cotton t-shirt and cotton boxers), a cotton coverall as the working suit and a hospital-type non-woven fabric head cover (see ***Figure 2.2.3.***). Dermal exposure assessment was performed along the Organization for Economic Co-operation and Development guidelines (OECD, 1997).

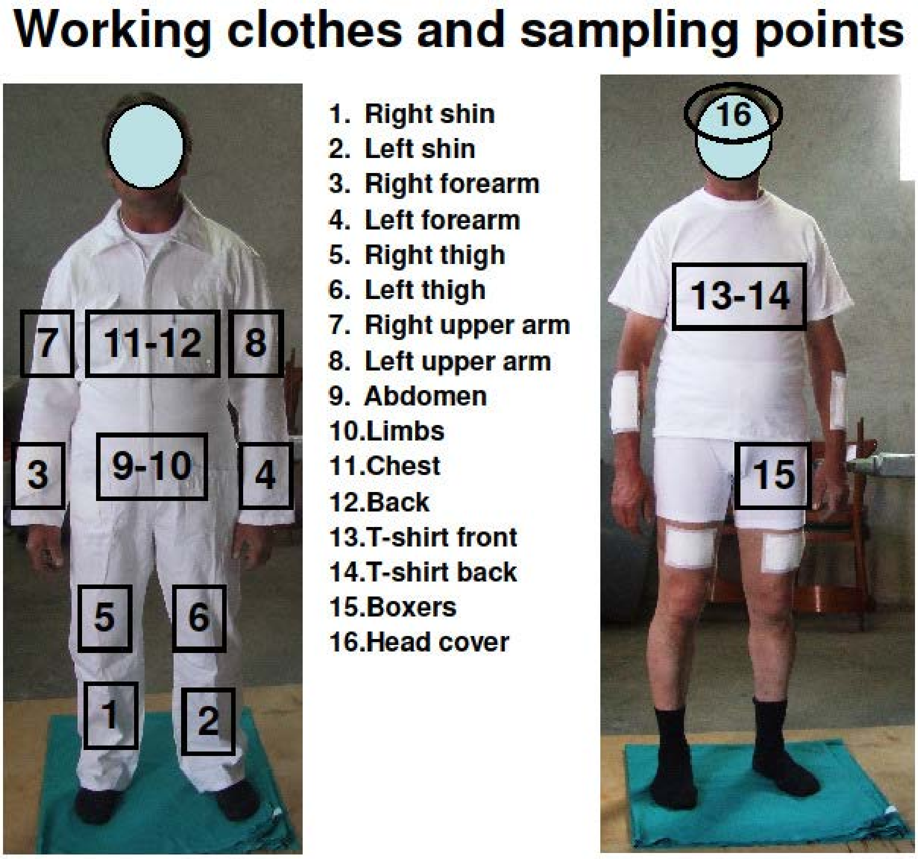


Figure 2.2.3. A typical investigated subject with normalized clothing used for field sampling: coverall (left) and underwear (right).

To collect samples for hand exposure assessment, farmers were required to notify a study team member when they wish to wash their hands, so that the sampling procedure could be performed before. Their hands were washed with a total volume of 100 mL of a 20% v/v mixture of isopropyl alcohol in water, which was poured in four to five aliquots on the subject's hands and collected in an underneath basin. The handwash was also performed at the end of work activities, just before the worker disrobed.

The gloves were farmers' own, therefore their contamination (potential hand exposure) was not taken into consideration since their possible prior contamination with pesticide residues or leakiness could not be assessed in quantitative terms.

At the end of the application activity, the farmer disrobed the work attire, which was cut on-site by the field investigators. The coverall was cut in 12 sections and the underwear (t-shirt and boxers) was cut in 3 sections, as detailed in ***Figure 2.2.3.*** An additional sample was obtained from the head cover.

For each work-day 12 coverall cuts, 3 underwear sections, one head cover, one to five hand wash samples were collected and analyzed, for a total number exceeding 230 samples for the entire study. All samples were kept in a cool and dark place before shipping to the laboratory, where they were processed and frozen until the analysis.

2.2.5. Sample preparation and measurement

Sample preparation and analysis were performed in the blind, after sample coding. TEB on dermal samplers was determined after desorption by an aqueous/alcoholic solution in the presence of tebuconazole-D6 as internal standard, by liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS).

Coverall and underwear. The sections of the coverall/underwear or the head cover were individually stored into food-grade polyethylene bags at the moment of cutting. Individual bags were weighted to obtain the net weight of canvas. A desorption solution of aqueous methanol (25% v/v) containing tebuconazole-D6 (Dr. Ehrenstorfer, LabService, Anzola Emila, Italy) at the concentration of 100 µg/L was prepared. For every 20 g of fabric a 100 ml volume of desorption solution was added and the desorption was operated shaking the samples for 2 h at room temperature. The recovery of the procedure, estimated spiking 10 and 100 µg of TEB to each sample, ranged from 82 to 111% (CV% 6.9).

Handwash. The handwash liquid (about 100 mL) was spiked with tebuconazole-D6 to a final concentration of 100 µg/L.

Analysis. A sub-sample of each solution was filtered and analyzed by a high performance liquid chromatography system (Surveyor, Thermo Scientific, Rodano, Italy) equipped with a Betasil C18 column (150 mm length, 2.1 mm internal diameter and 5 µm particle size; Thermo Scientific, Rodano, Italy) kept at room temperature, using a isocratic mixture of aqueous formic acid (0.5%) and methanol (30:70) at 0.25 ml/min as eluent. The liquid chromatograph was interfaced with a LC-MS/MS (TSQ Quantum Access, Thermo Scientific, Rodano, Italy) equipped with a heated-electro spray ionization source. The ionization source parameters were: spray voltage 4500 V, ion transfer tube temperature 350°C, vaporization temperature 300°C, nitrogen as sheath gas and auxiliary gas operating at the pressure of 50 and 5 units (arbitrary scale), tube lens offset 76 V. Collision-inducted dissociation was performed using Ar as the collision gas at a pressure of 1.5 mTorr. TEB and tebuconazole-D6 were detected in the positive ion mode and quantification was based on multiple reaction monitoring (MRM) following the transition m/z 308 → 70 + 308 →125 +308 →151 for TEB and m/z 314 → 72 + 314 → 125 + 314 → 154 for TEB-D6. Retention times were 10.32 min and 10.17 min, respectively, for TEB and tebuconazole-D6. The method had a precision of less than 10%, evaluated as the coefficient of variation, with accuracy between 95 and 103%. The limits of quantification was 0.6 µg/L for TEB in the coverall, underwear or head cover solutions and 1.1 µg/L for TEB in hand-wash solutions.

2.2.6. Data management and statistical analysis

From concentration of TEB (mg/L) in the individual samples, the absolute amount in the original field sample was calculated as TEB (mg). The potential body exposure was calculated as the sum of regional exposures which were measured from the cuts (cut ID from 1 to 12) of the coverall (see ***Figure 2.2.3.***) according to the formula:

$$Potential\ exposure_{body}(mg) = \sum_{coverall\ cut\ =1}^{12} TEB_{i\ coverall\ cut}\ \ (mg)$$

The actual body exposure was calculated from the amount of TEB measured in the t-shirts (cuts ID 13 and 14) and boxers (cut ID 15), plus the extrapolation from the underwear to the surface not covered by underwear (see formula below), which was calculated using the Mosteller formula (Mosteller, 1987), considering the proportions of a normal healthy male.

$$
\begin{aligned}
&Actual\ exposure_{body}(mg) \\
&\quad = \sum_{underwear\ cut\ =13}^{15} TEB_{i\ underwear\ cut}\ (mg) \\
&\quad + \frac{TEB_{boxer}\ (mg)}{boxer\ area\ (dm^2)}\ X\ uncovered\ leg\ area\ (dm^2\ ) \\
&\quad + \frac{TEB_{t-shirt}\ (mg)}{t-shirt\ area\ (dm^2)}\ X\ uncovered\ arm\ area\ (dm^2\ )
\end{aligned}
$$

The actual total exposure was calculated summing actual body exposure with the amount of TEB on hand and head according to the formula:

$$
\begin{aligned}
&Actual\ exposure_{total}(mg) \\
&\quad = Actual\ exposure_{body}\ (mg) + TEB_{hand}\ (mg) + TEB\ _{head}(mg)
\end{aligned}
$$

Data management and statistical analyses were performed in custom Microsoft Excel® Worksheets and in the R Language and Environment for Statistical Computing (R Core Team, 2012; Wickham, 2009).

Since the sample was small, and the continuous variables were not normally distributed, medians, minimum and maximum values, as well as non-parametric statistical tests (Mann-Whitney-Wilcoxon test) were used in the description of results and in the statistical analyses. Protection factor is the fraction of pesticide retained by the barrier of the work clothing layer (Lima et al., 2011), and was calculated as:

$$
Protection\ Factor = \frac{Potential\ Body\ Exposure}{Potential\ Body\ Exposure + Actual\ Body\ Exposure}
$$

expressed in percentages.

2.3. Region of Lombardy study

2.3.1. Study overview

This study was organized from April to July 2011 in Mantova and Pavia (***Figure 2.3.1.***) regions of Lombardy. Meetings were organized with local unions to present our study and study protocol, and companies which spray Mancozeb were invited to participate in our study.

All individuals participating in this study read and signed the informed consent form approved by the Ethical Committee of the University of Milan.

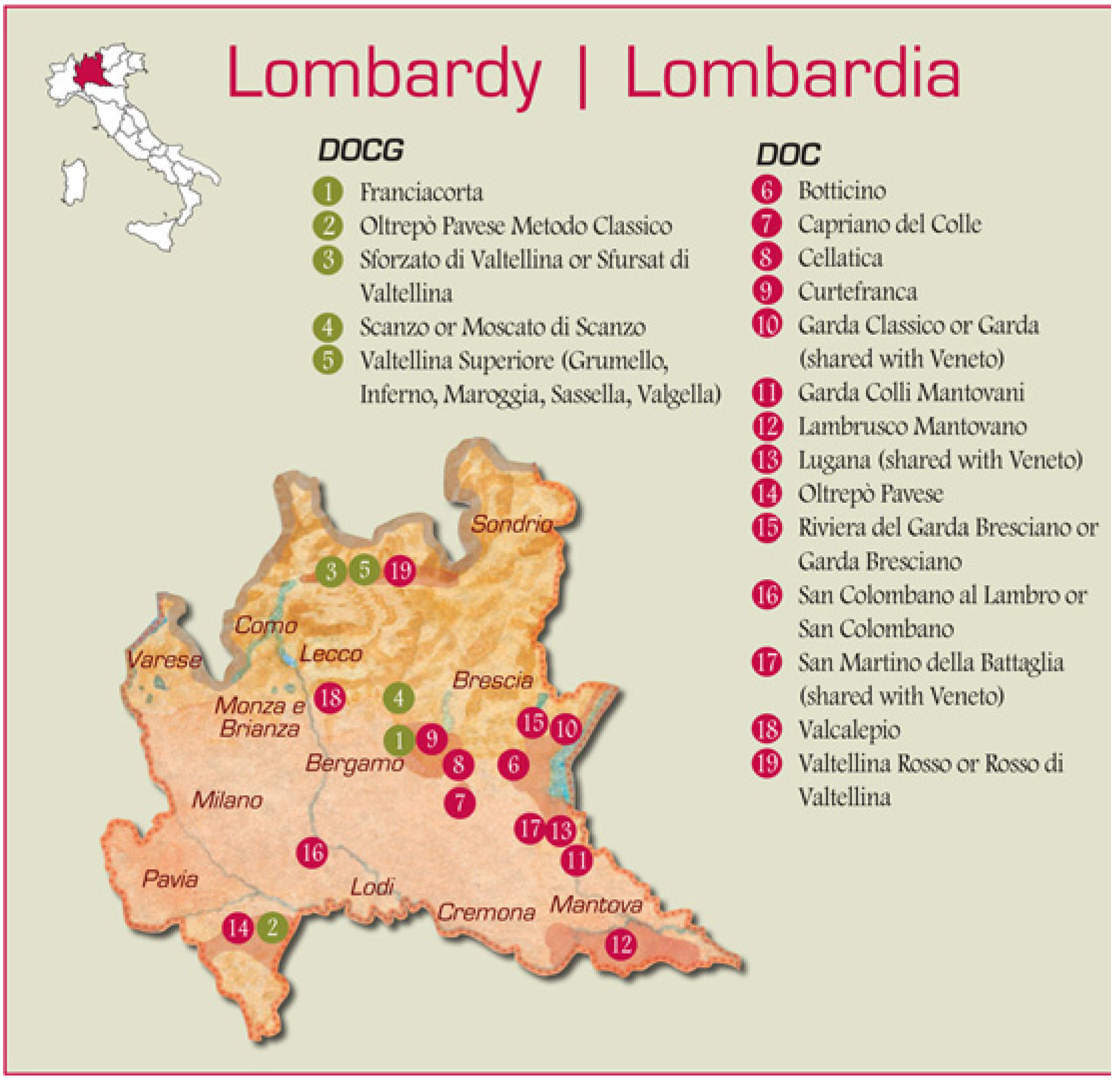


Figure 2.3.1. Lombardy region, and protected wine types of Pavia and Mantova

Twenty three companies expressed their interest to participate in our study, and their contact information was collected in the first meeting. For these companies, a second meeting was held where the study protocol was explained in more detail. All companies were instructed to contact our researchers 3-5 days before their intended pesticide treatment with Mancozeb.

2.3.2. Study protocol

The study protocol defined three levels of data collection:

1) Data collection sheet consisting of questions regarding the characteristics of the farmer, the farm, and the work-day;

2) Assessment of potential and actual dermal exposure

2.3.3. Data collection sheet

This study utilized the same Data Collection Sheet as the Acropolis study. It is available as ***Supplementary Material S1***.

2.3.4. Personal dermal exposure monitoring

Skin exposure was assessed according to Organization for Economic Co-operation and Development (OECD) guidelines (OECD, 1997) with the use of square 0.01 m^2 pads made of Whatman n°1 filter paper (Prodotti Gianni, Milan).Ten pads were placed on the clothes used during application (4 pads), under the clothes on the skin (5 pads) and on the collar, above clothes (1 pad). Pads on the clothes estimate the potential dermal dose, that is the amount of applied active ingredient which reaches the subject; those under the clothes, on the skin, estimate the actual dermal dose, that is the amount of compound able to reach the uncovered skin, available for absorption. For details see ***Figure 2.3.2.*** and ***Table 2.3.1.***

Pad No		Position	Proportion of body surface (%)
1	clothes	Chest	17%
2	clothes	Right glove	3%
3	clothes	Right thigh	9%
4	clothes	Collar	3%
		Total	31%
5	skin	Chest	17%
6	skin	Right forearm	3%
7	skin	Left forearm	3%
8	skin	Right thigh	9%
9	skin	Left thigh	9%
10	skin	Back	17%
		Total	58%

Table 2.3.1. Pads, their location and % of body surface they represent

Hand skin exposure was assessed by collecting the hand-wash liquid. Workers were asked to notify the study team each time they would usually wash their hands during the work-day, and they were asked to wash their hands with 200 mL of iso-propanol first. At least one hand-wash was collected, at the end of the work-day, but the workers were not asked to change their daily routine (e.g. to wash their hands more often) to allow us to collect more samples.

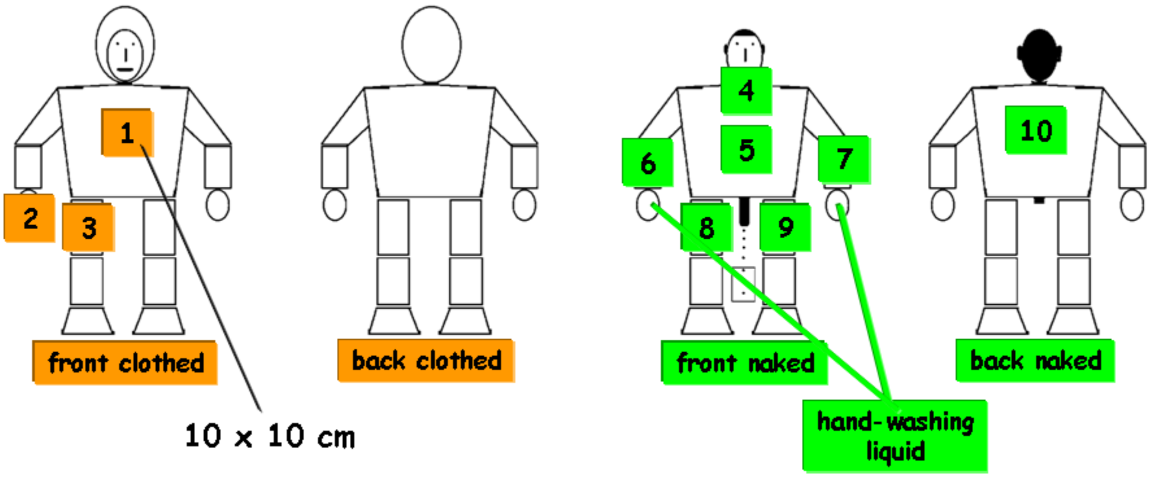


Figure 2.3.2. Placement of pads on farmers' bodies: over the garments (pads 1-3) and under the garments (pads 4-9).

Levels of respiratory exposure to applied pesticides were not monitored. Published literature on field studies suggests that dermal exposure accounts for the most significant fraction (93-99.9%) of pesticide exposure in open-field farming, while respiratory exposure does not provide a significant contribution to the overall exposure (Aprea et al., 2005; Flack et al., 2008; Vitali et al., 2009). In addition, the burden of the workers was significantly reduced with this decision, with an increase of their compliance to the already rather burdensome study protocol.

2.3.5. Sample preparation and measurement

The determination of ETU in different kind of samples (pad, hand wash and urine) was obtained by liquid chromatography-mass spectrometry, namely with Acquity UPLC system (Waters, Milford, MA, USA) coupled with a triple quadrupole Waters TQD mass spectrometer.

For quantitative analysis the TQD detector was used with an ESI interface in positive ion mode (ESI+). The MRM acquisition used to quantify ETU was: m/z 103 → 44 (CV 36, CE 16) ; for internal standard ETU D4 quantification was obtained in SIR: m/z 107 (CV35).

UPLC separation was performed on a Waters UPLC HSS T3 1.8 µm (2.1 x 100mm) column kept at 28°C, by gradient elution with a mixture containing variable proportion of water and methanol, delivered at a flow rate of 0.4 ml/min. The retention time of ETU and its internal standard was 1.3 min.

Briefly, urine samples (2ml) were diluted with water (1ml), spiked with ETU D4 and purified using diatomaceous earth column (ChemElut® 3ml unbuffered, Varian, Poole, UK). In particular, after loading, analyte was eluted with dichloromethane (6 ml * 5), with an interval of 10 min between different aliquots; the eluate was evaporated to dryness under a gentle stream of nitrogen and reconstituted with 0.1% formic acid (2ml) and finally injected onto the chromatographic system (3 µl). The calibration curve (constructed with a pool of urine of no-smoking subjects) was linear in the range 2.5-100 µg/l.

External and internal Pads samples (8x12.5cm) were spiked with ETU D4, inserted in a polypropylene tube and desorbed with 8 ml of water, vortexed for 10 minutes, centrifuged and an aliquot was injected onto UPLC after a suitable dilution factor with 0.1% formic acid. The calibration curve was linear in the range 1-50µg for external pads and 5-500ng for internal pads.

Hand wash samples were centrifuged, diluted 1:20 in 0.1% formic acid (1ml), spiked with ETU D4 and finally injected in UPLC (3 µl). The calibration curve for these samples was linear in the range 0.2-4 mg. For all the type of samples the mean recovery (at least > 80%) and the absence of matrix effect were verified. Further details of the method will be described elsewhere.

2.3.6. Data management and statistical analysis

From concentrations of Mancozeb (mg/L) in the individual samples, the absolute amount in the original field sample was calculated in mg (mg of Mancozeb). The potential body exposure was calculated as the sum of regional exposures which were measured from the pads (pads from 1 to 4, see Figure XX), takin into the account the surface of the pad and the body region represented by each pad, according to the formula:

$$Potential\ exposure_{body}(mg) = \sum_{pad=1}^{4} Mancozeb_{pad}(mg/dm^2) \times Body\ area\ represented_{pad}\ (dm^2)$$

The actual body exposure was calculated as the sum of regional exposures calculated from the amount of Mancozeb measured in the skin pads (pads from 5 to 10) multiplied by the surface of each region represented by the pads.

$$Actual\ exposure_{body}(mg) = \sum_{pad=5}^{10} Mancozeb_{pad}(mg/dm^2) \times Body\ area\ represented_{pad}\ (dm^2)$$

Data management and statistical analyses were performed in custom Microsoft Excel® Worksheets and IBM SPSS version 20.

Medians, minimum and maximum values, as well as non-parametric statistical tests (Mann-Whitney-Wilcoxon test) were used in the description of results and in the statistical analyses. Protection factor is the fraction of pesticide retained by the barrier of the work clothing layer (Lima et al., 2011), and was calculated as:

$$Protection\ Factor = \frac{Potential\ Body\ Exposure}{Potential\ Body\ Exposure + Actual\ Body\ Exposure}$$

expressed in percentages.

2.4. Risk assessment

Risk from pesticide exposure is calculated from the exposure assessed using the method described above, and taking into account the Acceptable Operator Exposure Level (see ***Section 1.5.5.1.***) and dermal absorption coefficient of each pesticide, as defined in the authorisation process.

AOEL is expressed as milligrams of absorbed active substance per kilogram of body weight of the worker, per day (mg/kg bw/day). All these information are available online in the authorisation document of each active substance.

The absorbed level of active substance is calculated by using the dermal exposure coefficient:

$$Absorbed = Exposure\ \times Dermal\ Absorption$$

Where ***Absorbed*** is the absorbed amount of a pesticide, ***Exposure*** is the calculated (assessed) exposure from the field study, and ***Dermal Absorption*** is the coefficient of dermal absorption stabilised in the authorisation process.

The absorbed amount of pesticide is then divided by the body weight of each worker to calculate the absorbed amount in the same measure units as the AOEL:

$$Absorbed_{kg} = \frac{Absorbed}{Body\ Weight}$$

Finally, risk is calculated as the saturation of the AOEL, expressed in percentages, as:

$$Risk = \frac{Absorbed_{kg}}{AOEL}$$

2.5. Hand exposure and risk assessment

The agricultural workers participating in our studies washed their hands from 1 to 6 times during the work day. The initial analysis of results of body and hand exposure monitoring showed that hand exposure accounts for 99% of total dermal exposure. Having in mind that the goal of this study was to assess risk of workers applying pesticides in the open field, and use this data to develop tools for rapid in-field risk assessment, we found it necessary to develop a method for more accurate assessment of the risk coming from hands exposure.

Observing the behaviour of workers in the field, we have seen that they wash their hands a number of times during the work-day. Since the method for hand exposure assessment assumes that the hand-wash using iso-propanol washes off the most of pesticide exposure (as would washing with soap and water), we are obliged to consider that after the hand-washing has been done, the worker is no longer exposed to the “washed-off” amount of the pesticide. In other words, the worker has been exposed to the measured amount of pesticide (in one hand-wash sample) for a specific period of time, namely the time that passes between two washing of hands. It is also necessary to consider that the dermal absorption coefficient stated in the authorisation process, expressed in percentages, assumes an exposure lasting the whole work day (8 hours). Therefore, time is an important factor in determining the absorbed amount of the active substance, which is in turn used for the risk assessment (Cherrie and Robertson, 1995; Frasch et al., 2014).

Therefore, the risk assessment from hand exposure to pesticides needs to be corrected for the fact that the duration of exposure can be much shorter than the 8 hour work day. This can be done by using the formula for the half-life of First order Reactions (First order Kinetics):

$$Exposure = Exposure_0 \times \ e^{-k\Delta t}$$

Where:

Exposure is the exposure measured by the hand-wash method.

Exposure$_0$ is the exposure at the beginning of absorption (beginning of the time interval is considered the most conservative method to avoid underestimating risk).

$\boldsymbol{k}$ is the constant characteristical for each active substance, calculated using the dermal absorption coefficient from the absorption process (and having in mind that the duration of exposure $\boldsymbol{\Delta t}$ is 8 hours) like in this example for Mancozeb (dermal absorption coefficient 0.24%):

$$Exposure \quad = \ Exposure_0 \times e^{-k\Delta t}$$

$$0.0024 \times Exposure_0 = \ Exposure_0 \times e^{-k\Delta t}$$

$$0.0024 = \ e^{-k\Delta t}$$

$$-k\Delta t = -6.011$$

$$-k \ \times 8 = -6.011$$

$$k = 0.751$$

Knowing the value of the constant $\boldsymbol{k}$, we are able to calculate the absorbed dose (***Absorbed***) of the pesticide based on the amount we measured in the hand-wash liquid, and having in mind the duration of exposure ($\boldsymbol{\Delta t}$).

$$Absorbed = Exposure_0 - Exposure$$

$$Absorbed = \frac{Exposure}{e^{-k\Delta t}} - Exposure$$

From the absorbed dose calculated in this way, the risk is assessed more accurately, and it allows us to take into account also the number of hand washes done during the work day.

2.6. Analysis of exposure determinants

The goal of this research project was to develop a tool for rapid pesticide risk assessment in the field of agriculture, using the lowest necessary number of variables. These variables were identified first using the wide published literature search, and afterwards field studies.

First, a descriptive evaluation of the results of field studies was done. Since exposure measurements are not normally distributed, medians, minimums and maximums were used.

Second, a visual comparison of effects of different variables to the total exposure and risk was done using box plots and scatter plots.

Third, where the first two methods were not indicative enough to decide whether to include a variable in the risk assessment process, non-parametric statistical methods, such as Man-Whitney and Kruskall Wallis tests were used.

2.7. Generalization to a group of pesticides

Studies aiming at pesticide exposure and risk assessment have a drawback of describing only risk from a specific active substance. It is as if the study showed only specific points on a map, but never the whole map.

2.7.1. Tracer substances

Tracer substances have been used in the field of pesticide studies for some time. It is possible to use them in the field of pesticide exposure assessment, as well as the analysis of drift during pesticide application (Garcia-Santos et al., 2011; Lesmes-Fabian and Binder, 2013; Lesmes-Fabian et al., 2012a). The method considers adding a substance with specific characteristics, namely the low limit of detection and quantification, rapid quantification, solubility in spray mixtures, minimum physical effect on droplet evaporation, distinctive property differentiating it from background or natural-occurring substances, stability, moderate cost and nontoxicity. A tracer substance is put into the mixture of pesticides in a specific proportion to the active substance of interest. Based on that proportion, the quantity of the active substance of interest is calculated from the quantity of the tracer substance.

Agricultural workers use many different active substances during the year, and even mixtures of active substances. Analyzing exposure samples for every active substance used would be impossible due to the cost and time necessary to do so (see ***Section 1.7.1.*** and ***1.7.2.***). The methodology of tracer substances allows us to analyse the exposure to only one of the active substances, and then based on the proportion of the substances in the mixture, calculate the exposure to all of the active substances in the mixture.

2.7.2. Using data from the authorization process

As detailed in the ***Introduction*** (see ***Section 1.5.***), each active substance to be used in the European Union needs to be approved and registered. In this process, information is collected about the active substance, and one accepted, this data is available freely online for each and every pesticide.

Some of this values have been mentioned before, such as the Acceptable Operator Exposure Level and the Dermal Absorption Coefficient. They are necessary for the risk assessment, and are used in our studies.

Another information available is the quantity of active substance to be used per hectare of plant culture (e.g. quantity of active substance per hectare of vineyards). This information allows us to calculate the proportion of the active substances used in our studies (e.g. Mancozeb) to "ALL" the other pesticides that are registered to be used in the European Union.

The logic behind this is that of tracer substances (see ***Section 2.7.1.***). Once we have measured the exposure to a specific substance, we can calculate what the exposure to another substance would be, in the same conditions, considering the proportion of the use rates of the substance we measured and the one we want to extrapolate to, as seen in ***Figure 2.7.1.***

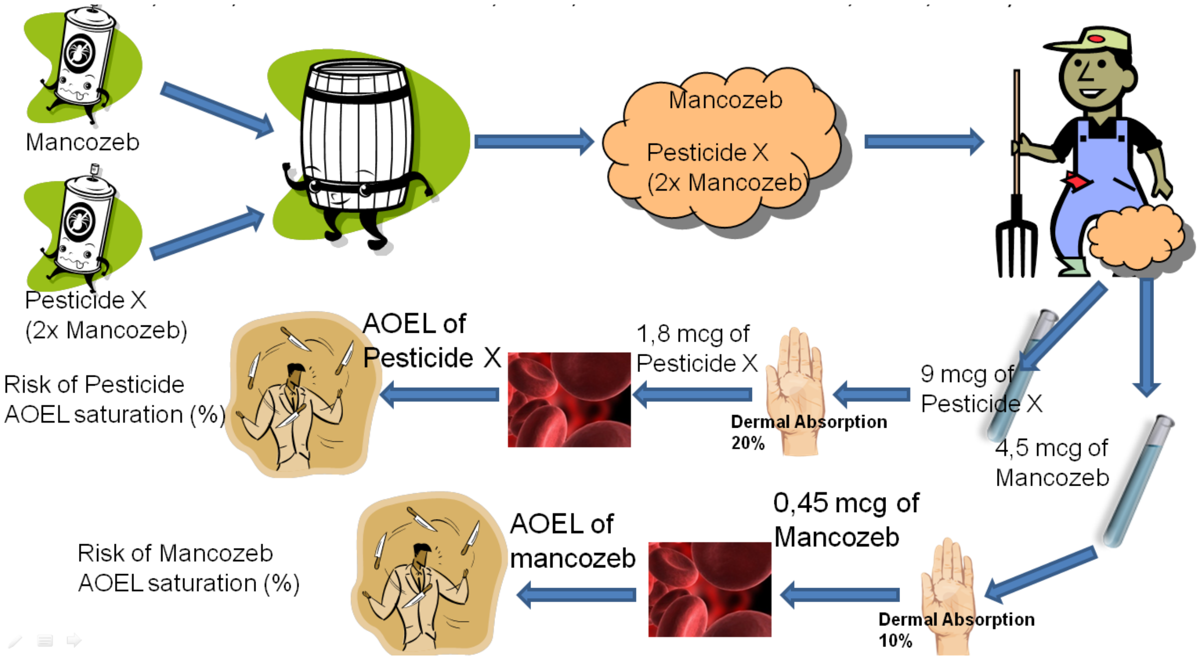


Figure 2.7.1. Applying the logic of tracer substances to theoretical mixtures of pesticides.

Therefore, also the risk of a substance can be calculated using the information from the authorisation process (AOEL, dermal absorption, use rate).

The following equations explain the ***Figure 2.7.1.*** mathematically:

$$\mathrm{Risk}_{\mathrm{Mancozeb}} = \frac{\mathrm{Exposure}_{\mathrm{Mancozeb}} \times \mathrm{Dermal\ Absorption}_{\mathrm{Mancozeb}}}{\mathrm{Weight}\ \times \mathrm{AOEL}_{\mathrm{Mancozeb}}}$$

$$\mathrm{Risk}_{\mathrm{Pesticide\ X}} = \frac{\mathrm{Exposure}_{\mathrm{Pesticide\ X}} \times \mathrm{Dermal\ Absorption}_{\mathrm{Pesticide\ X}}}{\mathrm{Weight}\ \times \mathrm{AOEL}_{\mathrm{Pesticide\ X}}}$$

And if we express the Risk of pesticide X as a function of the Risk of Mancozeb:

$$\mathrm{Risk}_{\mathrm{Pesticide\ X}} = \frac{\mathrm{Risk}_{\mathrm{Mancozeb}} \times \mathrm{AOEL}_{\mathrm{Mancozeb}} \times \mathrm{Exposure}_{\mathrm{Pesticide\ X}} \times \mathrm{Dermal\ Absorption}_{\mathrm{Pesticide\ X}} \times \mathrm{Weight}_{\mathrm{Subject}}}{\mathrm{AOEL}_{\mathrm{Pesticide\ X}} \times \mathrm{Exposure}_{\mathrm{Mancozeb}} \times \mathrm{Dermal\ Absorption}_{\mathrm{Mancozeb}} \times \mathrm{Weight}_{\mathrm{Subject}}}$$

The Exposure of pesticides can be expressed as the fraction of use rate:

$$\mathrm{Exposure}_{\mathrm{Pesticide\ X}} = \mathrm{Fraction}\ \times \mathrm{Use\ Rate}_{\mathrm{Pesticide\ X}}$$

$$\mathrm{Exposure}_{\mathrm{Mancozeb}} = \mathrm{Fraction}\ \times \mathrm{Use\ Rate}_{\mathrm{Mancozeb}}$$

Therefore, the equation becomes:

$$\mathrm{Risk}_{\mathrm{Pesticide\ X}} = \frac{\mathrm{Risk}_{\mathrm{Mancozeb}} \times \mathrm{AOEL}_{\mathrm{Mancozeb}} \times \mathrm{Fraction} \times \mathrm{Use\ Rate}_{\mathrm{Pesticide\ X}} \times \mathrm{Dermal\ Absorption}_{\mathrm{Pesticide\ X}} \times \mathrm{Weight}_{\mathrm{Subject}}}{\mathrm{AOEL}_{\mathrm{Pesticide\ X}} \times \mathrm{Fraction} \times \mathrm{Use\ Rate}_{\mathrm{Mancozeb}} \times \mathrm{Dermal\ Absorption}_{\mathrm{Mancozeb}} \times \mathrm{Weight}_{\mathrm{Subject}}}$$

And simplified:

$$Risk_{Pesticide\ X} = \frac{Risk_{Mancozeb} \times AOEL_{Mancozeb} \times Use\ Rate_{Pesticide\ X} \times Dermal\ Absorption_{Pesticide\ X}}{AOEL_{Pesticide\ X} \times\ Use\ Rate_{Mancozeb} \times Dermal\ Absorption_{Mancozeb}}$$

2.7.3. Standardized Toxicity Efficacy Factor

Coefficient of risk from pesticide X and from Mancozeb can be expressed as:

$$\frac{Risk_{Pesticide\ X}}{Risk_{Mancozeb}} = \frac{AOEL_{Mancozeb} \times Use\ Rate_{Pesticide\ X} \times Dermal\ Absorption_{Pesticide\ X}}{AOEL_{Pesticide\ X} \times\ Use\ Rate_{Mancozeb} \times Dermal\ Absorption_{Mancozeb}}$$

Both in the numerator and the denominator in the equation above we see the same elements: *AOEL*, *Dermal Absorption* and the *Use Rate*.

Therefore, if these three elements are expressed as one number, the Standardized Toxicity Efficacy Factor (STEF), which takes into account the Toxicity of an active substance (through the AOEL and the Use Rate) and the Efficacy of the substance (through the Use Rate) can be expressed as:

$$STEF_{Pesticide} = \frac{AOEL_{Pesticide}}{Dermal\ Absorption_{Pesticide} \times Use\ Rate_{Pesticide}}$$

And the ratio of risks as:

$$\frac{Risk_{Pesticide\ X}}{Risk_{Mancozeb}} = \frac{STEF_{Mancozeb}}{STEF_{Pesticide\ X}}$$

Therefore, risk of a pesticide X can be expressed as the risk of Mancozeb, multiplied by the ratio of the STEF of Mancozeb and the STEF of pesticide X:

$$Risk_{Pesticide\ X} = Risk_{Mancozeb} \times \frac{STEF_{Mancozeb}}{STEF_{Pesticide\ X}}$$

2.8. Creation of an Exposure and Risk Profile for Closed and filtered tractors

To create an exposure and risk profile for the scenario “Closed and filtered tractor”, simulation of exposure and risk is done. Using the analysis of the field exposure and risk data from the Region of Lombardy study on the exposure to Mancozeb, a realistic “worst case scenario” is defined. The worst case scenario is defined using the variables that influence exposure and risk, and considering their value when the exposure (and risk) is the highest. For example, in the case of a closed and filtered tractor, a worker that performs more than one mixing and loading action during the day, washes his hands only once (at the end of the workday) and uses no personal protective equipment.

2.8.1. Simulation of exposure scores

The worst case scenario exposure is given a score of 100, representing the 100% of the maximum (worst case) exposure. All other combinations of variables influencing exposure must have a score (percentage) lower than the worst case. This is the exposure score, and it takes values from 100 to (theoretical) 1, or in percentages from 100% to 1% of the worst case scenario.

2.8.2. Simulation of toxicity scores

Toxicity score of a pesticide is the value of the Standardizes Toxicity Efficacy Factor (STEF) of that particular pesticide. This values are calculated using the information available in the authorization document for every active substance, and using the formula from the ***Section 2.7.3.*** In order to acquire the range of toxicity scores for authorized active substances in the European Union, a list of fungicides, with their AOELs, dermal absorption factors and use rates (for vineyards or general) was created. Then STEF values were calculated for each active substance, and the minimum and maximum values were taken as reference for the simulation of exposure and risk.

2.8.3. Risk assessment

Risk assessment for all of the active substances registered in the European Union is performed using the exposure and risk data for Mancozeb from the Region of Lombardy study. For each point of the Exposure score, and each value of the Toxicity score (values chosen to represent all registered active substances in the European Union), risk is calculated using the following formula (detailed in ***Section 2.7.***):

$$Risk_{Pesticide\ X} = Risk_{Mancozeb} \times \frac{STEF_{Mancozeb}}{STEF_{Pesticide\ X}}$$

Risk is expressed as the saturation of AOEL in percentages.

2.8.4. Risk Assessment Scheme construction

Risk Assessment Scheme is a tool that can be used for rapid risk assessment in the field, without using exposure measurements, relying on the study of Mancozeb exposure (Region of Lombardy study). It represents a simulation of possible exposure scores, toxicity scores (as detailed in ***Sections 2.8.1.*** and ***2.8.2.***) and the risk assessment, expressed as the saturation of AOEL (detailed in ***Section 2.8.3.***).

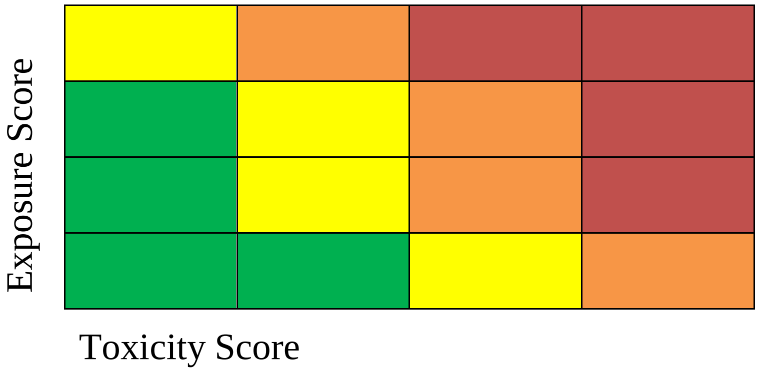


Figure 2.8.1. Example of a risk assessment grid/scheme

The combinations of Exposure and Toxicity scores are plotted on, and the marks are coloured by the Risk for each combination of Exposure and Toxicity score (see ***Figure 2.8.1.***). The colours are:

- ***Green*** – for risks between 0 and 33% of AOEL saturation
- ***Yellow*** – for risks between 34 and 66% of AOEL saturation

- ***Orange*** **–** for risks between 67 and 100% of AOEL saturation
- ***Red*** **–** for risks higher than 100% of AOEL saturation

The risk phrases associated to the above defined risk levels are: irrelevant risk (green), probably irrelevant risk (yellow), not irrelevant risk (orange), and significant risk (red).

The simulation of Exposure and Toxicity scores is performed using the R Language and Environment for Statistical Computing (R Core Team, 2012), while the construction of the Risk Assessment Scheme is done using the ggplot2 package for R (Wickham, 2009).

3. Results

3.1. Factors influencing pesticide exposure in field application

Through a systematic literature search (see ***Section 2.1.***) and field activities (see ***Sections 2.2.*** and ***2.3.***) we have identified main phases of work with pesticides, the variables which affect the levels of exposure to pesticides in these work phases, and the relationships linking these variables.

A typical work-day with pesticides can be divided into Mixing and Loading (MIX), Application (APPL), Maintenance and Cleaning of machineries after work (MNTN). We have also explored the general "modifying factors" of pesticide exposure and risk (***Section 3.1.4.***). The following sections describe these phases and the exposure determinants and modifiers.

3.1.1. 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 Maibach, 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.

3.1.2. Pesticide application (APPL).

Literature data collected from "in-the-field studies" suggest that, despite a fairly high 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".

3.1.3. Cleaning and maintenance of machineries (MNTN)

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").

3.1.4. Modifying factors.

Generally, these are factors that modify 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; Arbuckle et al., 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). In some studies, boots were found protective only if combined with a coverall (Ohayo-Mitoko et al., 1999). 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 (Baldi 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.

3.2. Acropolis study result (exposure and risk assessment for 12 work-days)

3.2.1. Study subjects

The main relevant characteristics of the subjects are shown in ***Table 3.2.1.*** A total of 7 healthy male workers were followed during their normal working activities, which include the preparation of the mixture and filling the tank of the tractor-mounted or hand-held sprayer (mixing and loading), spraying the pesticide (application) and in some cases routine after-work cleaning of the equipment (cleaning). Three workers worked for 1 day each, three worked for 2 days (two workers for two consecutive days and the other for two non-consecutive days, with a break of three weeks), and one worked for 3 consecutive days. All personal exposure monitoring measures were considered as independent, and are reported per work-day. There were a total of 12 work-days, which are chronologically coded from A to L in the Tables and in text.

Estate size and position were disclosed by the vineyard owners, who supplied real estate maps and authorized photo- and video recording. The brand of TEB fungicide used, its composition and applied amounts were disclosed by the farmers to the investigators in the field on the basis of the official records kept at the estate.

Table 3.2.1. Main personal characteristics of the participating farmers

Worker ID	**Work-day Code**	**Age years**	**Height cm**	**Weight kg**	**Body Surface [a] dm^2**	**Hand**
1	A, E	49	180	100	250	Right
2	B	50	180	95	238	Right
3	C, D	51	178	91	225	Right
5	F, G, H	40	168	57	133	Right
6	I, J	41	185	90	231	Right
7	K	47	170	78	184	Right
8	L	36	180	90	225	Right
Minimum	-	36	168	57	133	-
Median	-	47	180	90	225	-
Maximum	-	51	185	100	250	-

[a] Calculated according to Mosteller (Mosteller, 1987)

3.2.2. Characteristics of work-days

The work conditions during the examined work-days are reported in ***Table 3.2.2***. In all work-days when vineyard treatment was performed, meteorological conditions were deemed adequate by the farmers, with little if any wind or rain and at external temperature and humidity within seasonal variability.

The first phase of every work-day was mixing and loading. This phase was often repeated during the day, depending on the size of the vineyard, the size of the tank, and the application modality. The median number of mixing and loadings was 4 (from 2 to 5). In the majority of cases (11 workdays) the product was in the form of hydro-soluble or wettable powder, while only on 1 occasion the worker used wettable granules. All commercial products contained at the same concentration of 4.5% w/w of TEB. Dispersible sulphur (different brands) was also added to the sprayed mixture.

Table 3.2.2. Synopsis of application conditions in the examined work-days

		Mixing and Loading		**Application and Cleaning**						**General working conditions**	
Worker ID	**Work-day Code**	**Type of formulation** (1)	**Mixing (n)**	**Application Mode**	**Treated Area (ha)**	**Amount of TEB used (g)**	**Tank Capacity (L)**	**Interventions**	**Cleaning**	**Conditions of machineries**	**Total work time (h)**
1	A	WP	4	Open tractor	5.0	198.0	400	Yes	Yes	Clean	5
2	B	WP	3	Open tractor	6.0	594.0	600	No	No	Clean	6
3	C	WP	2	Open tractor	2.0	99.0	300	No	No	Clean	5
3	D	WP	3	Hand-held hose	4.0	67.5	300	No	No	Clean	6
1	E	WP	5	Open tractor	6.0	594.0	300	Yes	No	Clean	8
5	F	WP	4	Closed tractor	5.0	148.5	400	No	No	Dirty	10
5	G	WP	3	Closed and open tractor	4.0	148.5	400	No	No	Dirty	9
5	H	WP	1 + 4 (2)	Open tractor and back-pack	2.5	148.5	400 + 16[b]	No	No	Dirty	3
6	I	WP	4	Open tractor	17.0	1,530.0	1400	Yes	Yes	Clean	10
6	J	WP	2	Open tractor	10.0	900.0	1400	Yes	Yes	Clean	10
7	K	WP	4	Open tractor	1.8	117.0	300	No	Yes	Clean	7
8	L	GN	4	Closed tractor	3.0	1260.0	800	No	Yes	Clean	8
% positive	-	-	-	-	-	-	-	33%	42%	-	-
Minimum	-	-	2	-	1.8	67.50	300	-	-	-	3
Median	-	-	4	-	4.5	173.25	400	-	-	-	6
Maximum	-	-	5	-	17.0	1,530.00	1400	-	-	-	10

(1) WP = wettable powder; GN = wettable granules

(2) Backpack sprayer

The second phase of work was application. On 7 work-days the workers applied using an open tractor, on 2 work-days a closed tractor, on 1 work-day a hand-held sprayer, on 1 work-day a combination of an open tractor and a hand-held sprayer, and on 1 work-day a combination of an open and a closed tractor. Daily treated area ranged from 1.8 to 17 hectares per day (the usual unit for acreage in Italy; 1 hectare = 0.01 square kilometre or approximately 0.25 acres), with a median of 4.5 hectares, depending on the application modality and the nature of the estate's landscape, which ultimately dictates application speed. The workers used a median of 3.85 kg of formulation (range 1.5 - 34 kg). Taking into account the concentration of TEB in the product, the median amount of TEB sprayed during a typical work-day was 173 (range 67-1530) g. The median tank capacity was 400 L (from 16 L for the back-pack sprayer, to 1400 L for a big tractor-mounted sprayer in a larger estate).

Cleaning was performed in five work-days and consisted in washing the equipment and the interior of the tank with a water hose. In general the work place and the equipment were considered to be in clean conditions on 9 work-days out of 12. Total work time varied between 3 and 10 h.

3.2.3. Personal Protective Devices

Information regarding the use of personal protective devices is shown in ***Table 3.2.3***. All workers wore cotton coveralls, and underneath white cotton t-shirt and boxers, which were specifically supplied for the investigation (see also ***Figure 2.2.3.*** in the Methodology section). The other personal protective equipment such as working boots, gloves and masks, were not supplied by the study team, and large differences were noticed among farmers regarding their availability and use. Only 2 workers (5 work-days) wore protective shoes, while 4 workers (5 work-days) wore generic shoes, and one worker wore open shoes (slippers) on 2 work-days. Most of workers (6 out of 7, and 10 work-days out of 12) had

		Characteristics of Personal Protection Devices (PPDs)					**Use of personal protection devices in different phases of work**					
							Mixing and Loading		**Application**		**Cleaning of machines**	
Worker ID	**Work-day Code**	**Feet protection**	**Gloves available**	**Material of gloves**	**Quality of gloves**	**Mask available**	**Gloves**	**Mask**	**Gloves**	**Mask**	**Gloves**	**Mask**
1	A	Protection shoes	Yes	Neoprene	Used	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	B	Generic closed shoes	Yes	Neoprene	Used	Yes	Yes	Yes	No	No	-	-
3	C	Generic closed shoes	Yes	Neoprene	New	Yes	Yes	Yes	No	Yes	-	-
3	D	Generic closed shoes	Yes	Rubber	Used	Yes	Yes	Yes	No	No	-	-
1	E	Protection shoes	Yes	Neoprene	New	Yes	Yes	Yes	Yes	Yes	-	-
5	F	Protection shoes	Yes	Neoprene	New	Yes	Yes	Yes	No	No	-	-
5	G	Protection shoes	Yes	Neoprene	Used	Yes	Yes	Yes	No	No	-	-
5	H	Protection shoes	Yes	Neoprene	Used	Yes	No	Yes	Yes	Yes	-	-
6	I	Generic open shoes	No	-	-	Yes	No	Yes	No	Yes	No	Yes
6	J	Generic open shoes	No	-	-	Yes	No	Yes	No	Yes	No	Yes
7	K	Generic closed shoes	Yes	Neoprene	New	Yes	Yes	Yes	Yes	Yes	Yes	No
8	L	Generic closed shoes	Yes	Neoprene	New	No	Yes	No	No	No	Yes	No
% positive	-	-	83%	-	-	92%	75%	92%	33%	58%	60%	60%

Table 3.2.3. Personal protection devices used during the work-days

work gloves available, but the gloves' material and condition varied. Five workers (5 work-days) wore new professional gloves (neoprene), while there was a worker (2 work-days) that wore no gloves. Six workers had a face mask with a filter available (11 out of 12 workdays).

3.2.4. Total contamination and the distribution of contamination

Table 3.2.4. summarizes the potential and actual exposure for each work-day.

The median potential body exposure was 6.18 (range 1.68 - 21.50) mg while the median actual body exposure was 0.20 (range 0.01 – 0.80) mg. Cotton coverall has provided the workers with a median protection factor of 98% (from 90% to 99%).

Table 3.2.4. TEB potential and actual dermal exposures.

		TEB potential exposure	**TEB actual exposure**				
		Body (1)	**Body (1)**	**Head**	**Hands**	**Total**	**Normalized total (2)**
Worker ID	**Work-day Code**	**mg**	**mg (% of total)**	**mg (% of total)**	**mg (% of total)**	**mg (% of total)**	**mg/kg of a.s.**
1	A	1.68	0.05 (19)	0.02 (8)	0.18 (73)	0.25 (100)	1.25
2	B	6.51	0.42 (16)	0.13 (5)	2.02 (79)	2.57 (100)	4.33
3	C	12.56	0.33 (9)	1.67 (45)	1.68 (46)	3.68 (100)	37.19
3	D	21.50	0.42 (30)	0.52 (37)	0.47 (33)	1.41 (100)	20.92
1	E	5.58	0.61 (71)	0.10 (11)	0.15 (18)	0.86 (100)	1.45
5	F	6.20	0.04 (10)	0.10 (23)	0.28 (67)	0.42 (100)	2.83
5	G	4.32	0.07 (21)	0.07 (22)	0.18 (57)	0.32 (100)	2.13
5	H	5.52	0.06 (5)	0.10 (9)	1.01 (86)	1.17 (100)	7.87
6	I	10.84	0.80 (31)	0.09 (3)	1.66 (66)	2.54 (100)	1.66
6	J	14.15	0.68 (44)	0.04 (3)	0.82 (53)	1.54 (100)	1.71
7	K	6.15	0.01 (2)	0.45 (80)	0.11 (18)	0.56 (100)	4.80
8	L	3.43	0.01 (7)	0.03 (20)	0.11 (73)	0.16 (100)	0.12
Minimum	-	1.68	0.01 (2)	0.02 (3)	0.11 (17)	0.16	0.12
Median	-	6.18	0.20 (18)	0.10 (16)	0.38 (61)	1.02	2.48
Maximum	-	21.50	0.80 (71)	1.67 (80)	2.02 (86)	3.68	37.19

(1) Body: torso + limbs (without hand and head exposure)

(2) Normalized total: TEB total actual exposure (mg) per kilogram of active substance applied during the work-day

Median head exposure was 0.10 (range 0.02 – 1.67) mg. The workers washed their hands from 1 to 5 times during the work-day. In handwash a median level of 0.38 (range 0.11 – 2.02) mg was found. Median total actual exposure was 1.02 (range 0.16 – 3.68) mg. Body exposure contributed to the total actual exposure with a median value of 18%, while the head contributed with 16%, and the hands with 61%. When taking into account the amount of active substance used during the work day, the median total actual exposure was 2.48 (range 0.12 – 37.19) mg per kg of active substance applied.

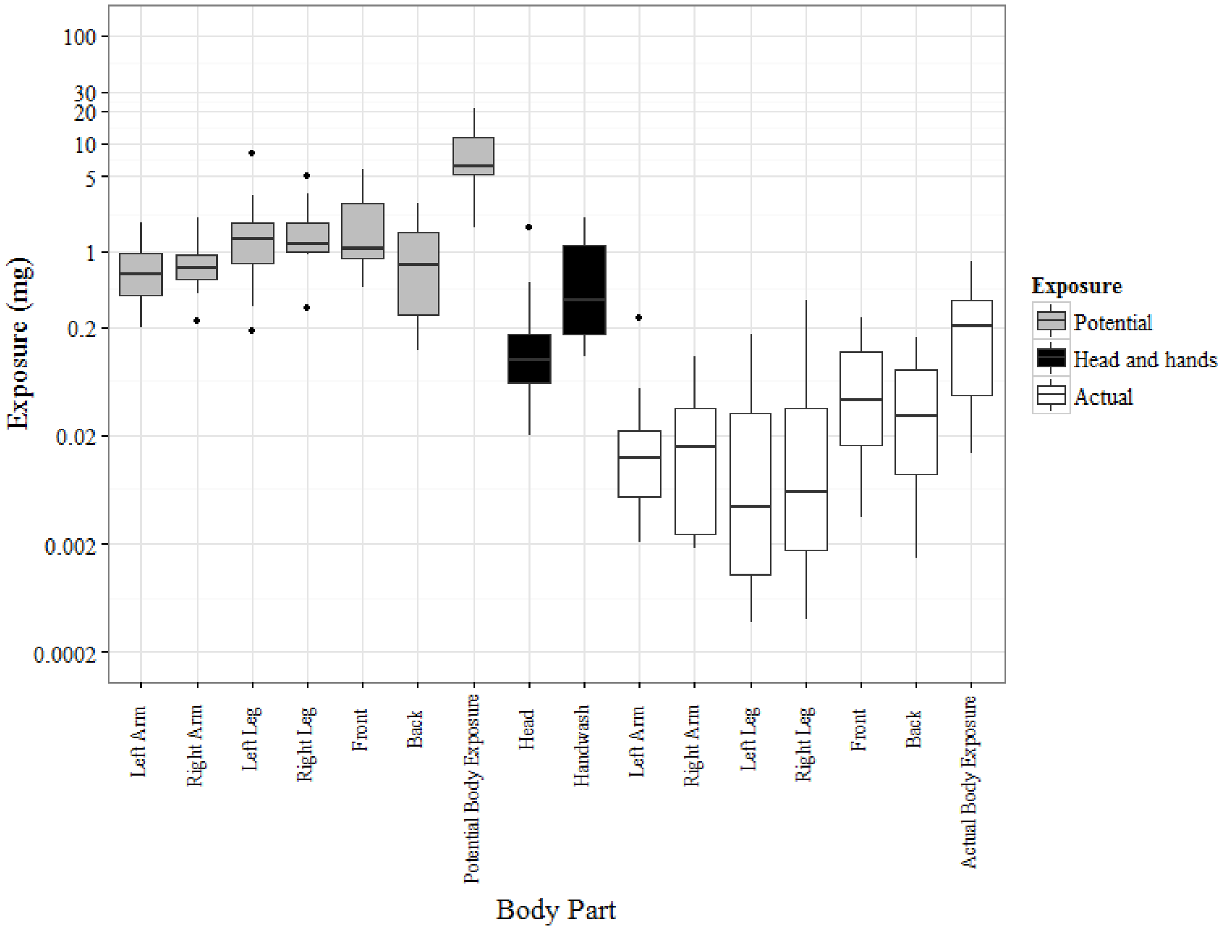


Figure 3.2.1. TEB potential and actual exposure by regions of the body
Arm (left and right): upper arm + forearm;
Leg (left and right): thigh + shin;
Front chest + abdomen;
Back upper back + lower back

Potential and actual exposure of different body parts is shown in ***Figure 3.2.1.*** The potential exposure box plots suggest that the most exposed regions of the body are the legs, followed by the front, back and arms (contamination measured on the clothes). Left and right parts of the body are almost equally exposed, while the front is slightly more exposed than the back. The actual exposure box plots paint a different image, with front and back exposure being the highest, followed by legs' and arms' exposure (contamination measured on the skin).

Table 3.2.5. Contamination (mcg) measured on coverall and underwear cuts per area (cm^2)

	Area (1)	TEB contamination per area		
Cut/Location	**Median (cm^2)**	**Minimum (mcg/cm^2)**	**Median (mcg/cm^2)**	**Maximum (mcg/cm^2)**
Chest	1560	0.027	0.485	1.726
Abdomen	1730	0.173	0.361	2.763
Upper and lower back	1960	0.083	0.366	1.394
Left upper arm	980	0.038	0.204	0.979
Right upper arm	960	0.024	0.226	0.976
Left forearm	1180	0.092	0.360	0.919
Right forearm	1320	0.146	0.338	1.075
Limbs	3090	0.007	0.073	0.528
Left thigh	1660	0.091	0.307	1.919
Right thigh	1960	0.078	0.309	0.923
Left shin	2720	0.036	0.324	1.778
Right shin	2480	0.008	0.250	1.372
Head cover (2)	1000	0.010	0.049	0.837
T-shirt front	3940	0.004	0.014	0.058
T-shirt back	4200	0.002	0.007	0.031
Boxers	3640	0.001	0.026	0.043

(1) Coverall and underwear size varied from the international standard S to XXL and was appropriate to workers stature

(2) The head cover was of a standard size for all workers

To explore the differences in the contamination of different body regions independently of their surface, we have standardized the exposure of each cut by its surface area (***Table 3.2.5.***). On the coverall, the most contaminated regions are the chest, back and the abdomen, with the median contamination of 0.485 mcg/cm2, 0.366 mcg/cm2 and 0.361 mcg/cm2 respectively. They are followed by the forearms, thighs and shins. The least exposed regions are the upper arms and the limbs. The most contaminated underwear cut are the boxers, followed by the front of the t-shirt and the back of the t-shirt, with contaminations of 0.026, 0.014 and 0.007 mcg/cm2 respectively.

The spider-plots of ***Figure 3.2.2.*** compare the distribution of pesticide deposition on the coverall of a worker who sprayed from an open tractor (left), and of a worker who used a hand-held sprayer (right). The two plots not only show a much higher median contamination of the worker working with hand-held sprayer, but also a different distribution of exposure.

The use of gloves in different phases of the work was explored to assess how it influenced the exposure of hands. ***Figure 3.2.3.*** shows the levels of hand contamination depending on the use of gloves during the two phases known to give the major contribution to the total daily exposure, namely mixing and loading, and application. Although not statistically significant (Man-Whitney U test, U = 21, p = 0.2091), probably due to the small size of the examined group, the use of gloves, especially during the mixing and loading phase, may lower the exposure of hands by more than 50%.

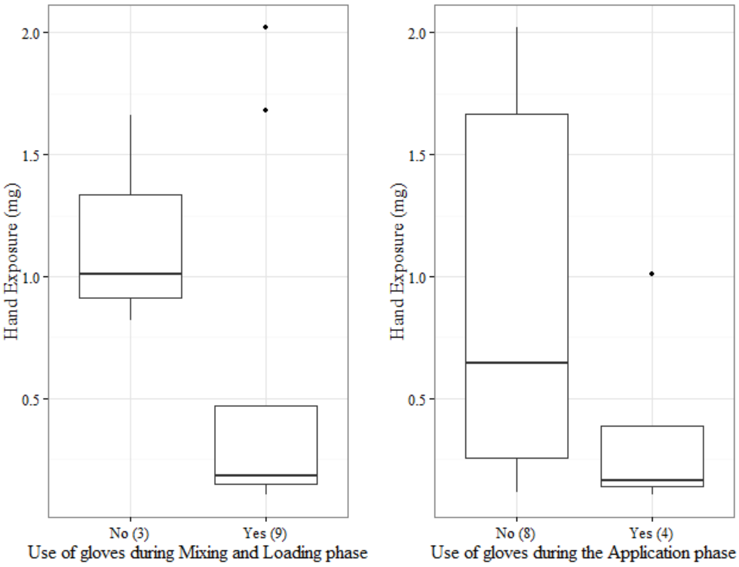


Figure 3.2.3. Hand exposure depending on the use of gloves during mixing and loading and during application

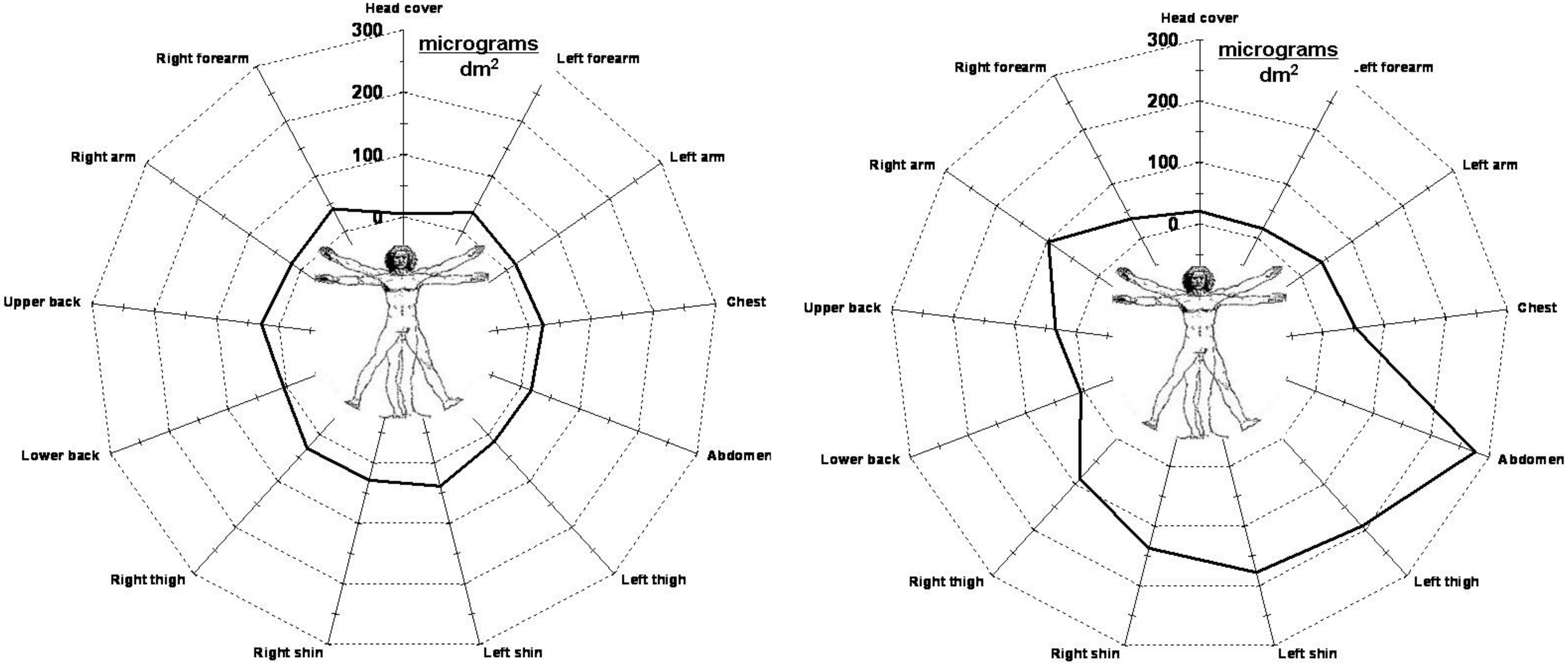


Figure 3.2.2. Spider plot of tebuconazole contamination on farmers' coveralls. (**A**) one who sprayed from a closed-cockpit tractor; (**B**) one who sprayed manually from a hose (passing by the left hip) hand-sprayer.

Table 3.2.6. contains the risk assessment information for tebuconazole for each work-day, using the field measures as well as using the German model (Lundehn et al., 1992) with different settings, as explained in the Methodology. The median AOEL saturation calculated from the field measures was 4.73% (range 0.76 – 17.38%). Hands had the highest contribution to the overall risk, with a median risk of 2.19%, and they were followed by the body (median 1.20%, range 0.07 – 3.74%) and the head (median 0.57%, range 0.09 – 7.97%).

The median risk calculated using the German model was 6.94% (range 1.12 – 77.42%), when using the dermal absorption specified for tebuconazole in the authorisation documents (EFSA, 2008). When using the default values for concentrated product (25% dermal absorption) and diluted product (75% dermal absorption) the median risk increases to 31.35% (range 6.15 – 226.54%), and when using the default value of 75% dermal absorption for both mixing and loading and application, the median risk increases to 37.27% (range 6.32 – 445.11%).

Table 7. summarizes the risk assessment done for a group of conazole fungicides, considering the ratio between the use rate of tebuconazole in vineyards, and each of the other conazoles. For penconazole, triticonazole and ciproconazole, the median AOEL saturations were 0.35, 0,51 and 2,99% respectively. For bromuconazole, the median AOEL saturation was 21,76%, and for epoxiconazole the median AOEL saturation was 85,01%. It is worth noting that for all the conazole, except epoxiconazole, the risk was lower than the limit of 100% AOEL in all work scenarios. For epoxiconazole, the limit was exceeded on 6 out of 12 work-days, out of which on 4 occasions an open tractor was used, in one occasion a hand-held pressure hose was used, and in one occasion a combination of an open tractor and a back-pack method.

Table 3.2.6. Risk assessment for each work-day for TEB using the field measures and the German Model Risk is expressed as AOEL saturation (Exposure/AOEL).

Wid	Wd	Weight (kg)	Risk Body (AOEL Saturation)	Risk Head (AOEL Saturation)	Risk Hands (AOEL Saturation)	Risk Tebuconazole (AOEL Saturation)	German model 13% (AOEL Saturation)	German Model 25%-75% (AOEL Saturation)	German Model 75% (AOEL Saturation)
1	A	100	0,22%	0,09%	0,78%	1,09%	1,89%	10,42%	10,70%
2	B	95	3,74%	0,59%	9,21%	13,55%	9,58%	51,52%	52,37%
3	C	91	1,41%	7,97%	8,00%	17,38%	1,37%	7,66%	7,80%
3	D	91	1,45%	2,48%	2,24%	6,17%	8,20%	41,63%	42,44%
1	E	100	1,34%	0,43%	0,65%	2,42%	5,67%	31,25%	32,09%
5	F	57	0,38%	0,75%	2,13%	3,26%	2,39%	12,84%	13,05%
5	G	57	1,06%	0,54%	1,37%	2,97%	2,39%	12,84%	13,05%
5	H	57	0,29%	0,78%	7,68%	8,75%	10,66%	31,45%	73,48%
6	I	90	2,81%	0,42%	7,99%	11,22%	77,42%	226,54%	445,11%
6	J	90	3,09%	0,21%	3,94%	7,24%	45,54%	133,26%	261,83%
7	K	78	0,10%	2,48%	0,59%	3,17%	1,12%	6,15%	6,32%
8	L	90	0,07%	0,15%	0,55%	0,76%	24,31%	108,96%	109,56%
Min		57	0,07%	0,09%	0,55%	0,76%	1,12%	6,15%	6,32%
Median		90	1,20%	0,57%	2,19%	4,72%	6,94%	31,35%	37,27%
Max		100	3,74%	7,97%	9,21%	17,38%	77,42%	226,54%	445,11%

Table 3.2.7. Risk assessment for each work-day for TEB and characteristic conazoles registered in the European Union

Wid	Wd		Tebuconazole (AOEL Saturation)	Penconazole (AOEL Saturation)	Triticonazole (AOEL Saturation)	Ciproconazole (AOEL Saturation)	Bromuconazole (AOEL Saturation)	Epoxiconazole (AOEL Saturation)
1	A	Open tractor	1,09%	0,08%	0,12%	0,69%	5,03%	19,65%
2	B	Open tractor	13,55%	1,02%	1,48%	8,60%	62,54%	244,29%
3	C	Open tractor	17,38%	1,30%	1,89%	11,03%	80,22%	313,34%
3	D	Hand-held hose	6,17%	0,46%	0,67%	3,92%	28,48%	111,24%
1	E	Open tractor	2,42%	0,18%	0,26%	1,54%	11,17%	43,63%
5	F	Closed tractor	3,26%	0,24%	0,36%	2,07%	15,05%	58,77%
5	G	Closed and open tractor	2,97%	0,22%	0,32%	1,88%	13,71%	53,55%
5	H	Open tractor and back-pack	8,75%	0,66%	0,95%	5,55%	40,38%	157,75%
6	I	Open tractor	11,22%	0,84%	1,22%	7,12%	51,78%	202,28%
6	J	Open tractor	7,24%	0,54%	0,79%	4,59%	33,42%	130,53%
7	K	Open tractor	3,17%	0,24%	0,35%	2,01%	14,63%	57,15%
8	L	Closed tractor	0,76%	0,06%	0,08%	0,48%	3,51%	13,70%
MIN	-	-	0,76%	0,06%	0,08%	0,48%	3,51%	13,70%
MEDIAN	-	-	4,72%	0,35%	0,51%	2,99%	21,76%	85,01%
MAX	-	-	17,38%	1,30%	1,89%	11,03%	80,22%	313,34%

3.3. Region of Lombardy study

3.3.1. Study subjects

	Type of tractor								
	Total			Closed and Filtered tractor			Open tractor		
	Minimum	Median	Maximum	Minimum	Median	Maximum	Minimum	Median	Maximum
Age of participants (years)	**32**	**45**	**63**	**32**	**46**	**63**	**36**	**43**	**54**
Height of participant (cm)	**162**	**175**	**190**	**162**	**174**	**190**	**162**	**175**	**184**
Weight of participant (Kg)	**60**	**80**	**120**	**60**	**78**	**100**	**62**	**90**	**120**
Body Surface (dm^2)	**167**	**194**	**248**	**167**	**194**	**230**	**167**	**209**	**248**

Table 3.3.1. Summary information on workers depending on the type of tractor used

The main characteristics of study subjects are summarized in Table 3.1.1. A total of 29 male workers were followed during their normal working activities, which comprise of preparation for work, mixing and loading of the active substance into the tank of the tractor, spraying of the pesticide (application phase) and in some cases cleaning of the equipment and washing of the tank (cleaning). Individual characteristics of the workers are reported in the ***Supplementary Table S.2.1.*** There were 37 work-days in total.

As in the Acropolis study, the size of the vineyard and the estate were disclosed by the owner. The farmers informed us on the brand, composition and amount of the pesticide (Mancozeb) used, and the official record is kept at the estate.

3.3.2. Characteristics of work-days

Summary of work conditions is reported in ***Table 3.3.2.***, while the individual information on each work-day is reported in ***Supplementary Table S.3.2***.

In order for a work-day to start the meteorological conditions were necessarily deemed adequate by the farmers or their employers, with no strong wind and temperature within seasonal variability.

The first phase of work, is Mixing and Loading. This phase can be repeated several times during the day, depending on the area to be treated and the capacity of the tank of the tractor. The median number of Mixing and Loadings was 2, with a minimum of 1 and maximum of 7. In 86% of examined work-days the workers used Mancozeb in the form of granules, and in 14% in the form of wettable powder. The median amount of active substance used was 7.5 (range: 0.5 – 30) kg. The median tank capacity was 1000 litres, with a minimum of 200 and a maximum of 3000 litres. As reported in ***Table 3.3.2.***, the median, minimal and maximal tank capacity for Closed and Filtered tractor and Open tractors differed substantially. The median tank capacity for Open tractors was more than 3 times smaller than that of the Closed and filtered tractors (300 compared to 1000 litres). This is reasonable considering that the median area treated for Closed and filtered tractor was 6 hectares, while for the Open tractor it was 3 hectares. Cleaning was done in 78% of cases in total, but more often on work-days where workers used a closed tractor (89%) and less when workers used and open tractor (44%).

Median work day lasted 3.5 hours, ranging from just over 1 hour to more than 11 hours.

		Type of tractor											
		Total				Closed and Filtered tractor				Open tractor			
		Column N %	Minimum	Median	Maximum	Column N %	Minimum	Median	Maximum	Column N %	Minimum	Median	Maximum
Form of Product	Granules	**86%**				**89%**				**78%**			
	Wettable Powder	**14%**				**11%**				**22%**			
Total Amount of AS per Day (kg)			**.5**	**7.5**	**30.0**		**.5**	**7.5**	**30.0**		**1.5**	**4.7**	**30.0**
Number of Mixing and Loading			**1**	**2**	**7**		**1**	**2**	**6**		**1**	**2**	**7**
Tank capacity (l)			**200**	**1000**	**3000**		**300**	**1000**	**3000**		**200**	**300**	**1500**
Area treated (ha)			**1.0**	**6.0**	**20.0**		**1.5**	**7.0**	**20.0**		**1.0**	**3.0**	**17.0**
Cleaning done	No	**22%**				**11%**				**56%**			
	Yes	**78%**				**89%**				**44%**			
Total Application Time (min)			**70**	**210**	**687**		**85**	**223**	**687**		**70**	**180**	**611**

Table 3.3.2. Summary of work-day characteristics depending on the type of tractor used

3.3.3. Personal Protective Devices

Information regarding the availability and use of Personal Protective devices is summarized in ***Table 3.3.3***., and the extensive individual information is available in ***Supplementary Table S.3.3.***

Contrary to the Acropolis study, workers' clothes were not dictated or influenced by our study design. Participants were allowed to decide what the work clothes and personal protective equipment they would use. In most cases (73%) the workers used a mono-use coverall, in 16% of cases a multy-use coverall, and in 11% of cases no coverall, meaning they worked in regular clothes. The workers using an open tractor never worked without a coverall. The coverall state was deemed adequate (new) in all cases of mono-use coveralls. In most other cases it was clean, and only in 5% of cases dirty.

Gloves were available to workers in 97% of cases (96% for closed and filtered tractor, and 100% for open tractor). In 5% of cases the gloves were made of latex, in 62% of cases plain rubber, and in 30% of cases they were professional chemical gloves. In half of the cases the gloves were new, and in the other half they were used.

Inhalatory protection was not available in 13% of work-days, while on 73% of cases the workers used a mask with a filter, and on 14% of cases they used a plain paper mask.

As in the Acropolis study, there was a high variability in the use of PPEs. During Mixing and loading 97% of workers used gloves, and 81% of workers used a mask. During the Application phase, only 32% of workers used gloves, and 35% of workers used a mask. In the work-days with a closed tractor, this number was much lower (18% for gloves and 21% for a mask), while in the work-days with an open tractor the workers used gloves and a mask in 78% of occasions. Most workers (78%) used gloves during the maintenance and cleaning of machineries after work, and 24% used a mask.

				Type of tractor	
			Total	Closed and Filtered tractor	Open tractor
Coverall Type	None		11%	14%	0%
	Multy		16%	14%	22%
	Mono-use		73%	71%	78%
Coverall State	No coverall		11%	14%	0%
	Dirty		5%	0%	22%
	Clean		11%	14%	0%
	New		73%	71%	78%
Gloves Available	No		3%	4%	0%
	Yes		97%	96%	100%
Gloves' Material	No gloves		3%	4%	0%
	Latex		5%	0%	22%
	Rubber		62%	64%	56%
	Professional		30%	32%	22%
Gloves' State	No gloves		3%	4%	0%
	Used		48%	46%	56%
	New		49%	50%	44%
Inhalatory Protection	None		13%	15%	11%
	Paper		14%	14%	11%
	Filter		73%	71%	78%
Gloves MIX	No gloves		3%	4%	0%
	Yes		97%	96%	100%
Inhalatory MIX	No	No mask	3%	0%	11%
		Not used	16%	14%	22%
	Yes		81%	86%	67%
Gloves APPL	No		68%	82%	22%
	Yes		32%	18%	78%
Inhalatory APPL	No	No mask	3%	0%	11%
		Not used	62%	79%	11%
	Yes		35%	21%	78%
Gloves MNTN	No	No gloves	11%	11%	11%
		Not used	11%	11%	11%
	Yes		78%	79%	78%
Inhalatory MNTN	No	No mask	14%	11%	22%
		Not used	62%	64%	56%
	Yes		24%	25%	22%

Table 3.3.3. Availability and use of Personal Protective Devices

3.3.4. Total contamination and the distribution of contamination

		Type of tractor		
		Total	Closed and Filtered tractor	Open tractor
External Body Exposure (mg)	Minimum	.0137	.0137	.1236
	Median	.2865	.1477	4.6689
	Maximum	13357.7720	3096.1366	13357.7720
Glove Exposure (mg)	Minimum	.0008	.0008	.0010
	Median	.0577	.0242	.2018
	Maximum	5.8958	5.6779	5.8958
Body Skin Exposure Total (mg)	Minimum	.0001	.0001	.0007
	Median	.0016	.0011	.0052
	Maximum	.5228	.5228	.0971
Hand Exposure (mg)	Minimum	.0196	.0196	.0501
	Median	.1367	.0681	.2406
	Maximum	4.7243	4.0233	4.7243
Total Skin Exposure (mg)	Minimum	.0003	.0003	.0553
	Median	.1120	.0644	.2900
	Maximum	4.7774	4.0755	4.7774
Body Risk (%)	Minimum	0.0164%	0.0164%	0.0381%
	Median	0.1145%	0.1036%	0.2670%
	Maximum	44.8116%	44.8116%	5.4232%
Hands Risk (%)	Minimum	0.0002%	0.0002%	0.0010%
	Median	0.0024%	0.0018%	0.0043%
	Maximum	0.1655%	0.0935%	0.1655%
Total Risk (%)	Minimum	0.0167%	0.0167%	0.0418%
	Median	0.1150%	0.1052%	0.2756%
	Maximum	44.8184%	44.8184%	5.4246%

Table 3.3.4. Potential exposure, actual exposure and risk assessment summary

Table 3.3.4. summarizes the potential and actual exposure for each application method (Open and Closed and filtered tractor), as well as the risk from body and hands exposure, and the total risk. Additional detailed information for each work-day considered is available in ***Supplementary Table S.3.3.***

The median Potential body exposure was 0.2865(range 0.0137 – 13,357) mg, while the median glove exposure was 0.0577 (range 0.008 – 5.8958) mg. Exploring the distribution Potential body exposure and glove exposure levels between Closed and Open tractor shows a statistically significant difference in the case of Potential body exposure (Mann-Whitney U Test, $p < 0.001$) and no statistically significant difference in the case of glove exposure.

The median actual body exposure was 0.0016 (range 0.0001 – 0.5228) mg, while the median actual hand exposure was 0.1367 (range 0.0196 – 4.7213). The median total actual exposure was 0.1120 (range 0.0003 – 4.7774) mg. There was no statistically significant difference in the distribution of hand exposure between the Closed and Open tractor. On the other hand, there was a statistically significant difference in the distribution of actual body exposure (Mann-Whitney U Test, $p < 0.05$) and total actual exposure (Mann-Whitney U test, $p < 0.05$) between the Closed and Open tractor.

The median risk calculated from body exposure was 0.1145% AOEL saturation (range 0.0164 – 44.8116%), while the median risk calculated from the exposure of the hands, considering the number of times the workers washed their hands and time of exposure, was 0.0024 (range 0.0002 – 0.1655) %. The median total risk was 0.1150 (range 0.0167 – 44.8184) %. There was no statistically significant difference between the distribution of risk between the two groups (Mann-Whitney U Test, $p = 0.373$, $p = 0.080$, $p = 0.336$ respectively).

The median protection factor of work clothes used by the worker was 99.137% (blocking 99.137% of exposure). Exploring the protection factor by types of coverall shows that normal clothes have a median protection factor of 72.371%, a multy-use coverall 98.642% and a mono-use coverall 99.481% (see ***Table 3.3.5.***)

		Coverall Type			
		Total	None	Multy	One
Protection Factor Body (%)	Minimum	43.550	43.550	95.983	93.469
	Median	99.137	72.371	98.642	99.481
	Maximum	99.999	99.969	99.918	99.999

Table 3.3.5. Protection factor provided by the work clothes

Potential and actual exposure of different body regions are shown in ***Figure 3.3.1***. The potential exposure box plots suggest the most externally exposed regions are the legs of a worker, followed by the chest and the back, and finally the arms. Actual exposure box plots suggest the most exposed regions are the arms, followed by the legs, and finally chest and back.

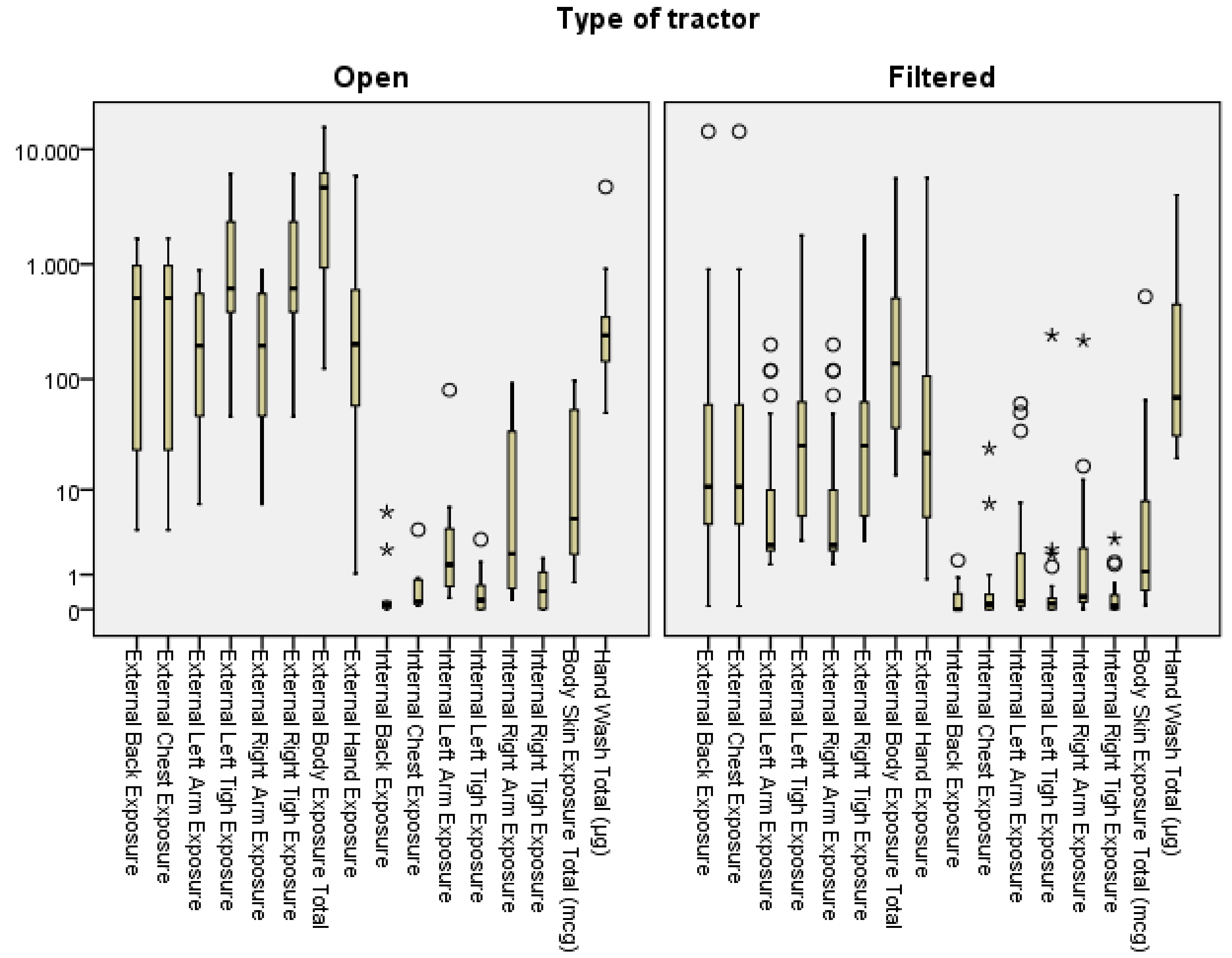


Figure 3.3.1. Potential and actual exposure of body regions. Comparison between the Open and Filtered tractor.

Workers which used and Open tractor are generally more exposed than workers using a Closed and filtered tractor.

We explored how the use of gloves in the phase of application influences hand exposure in ***Figure 3.3.1.***

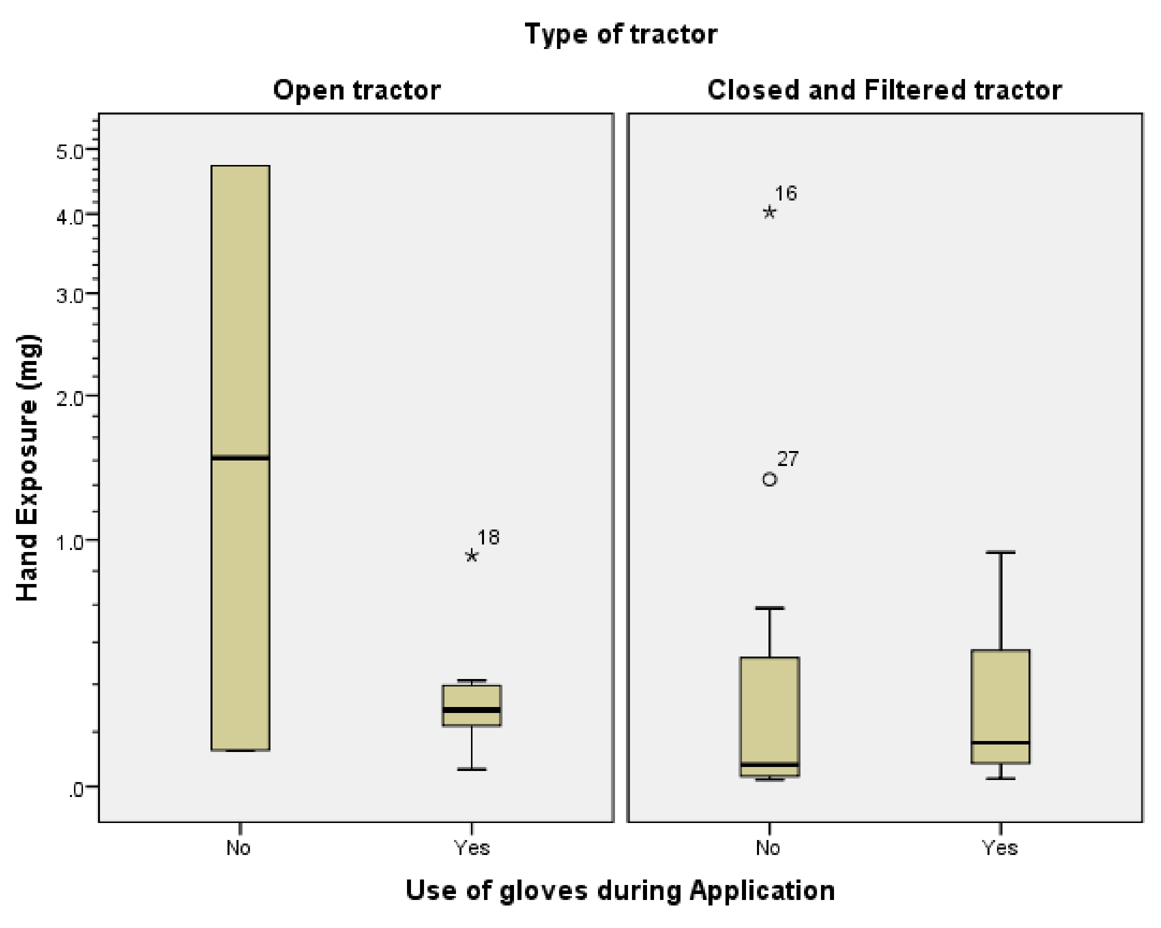


***Figure** 3.3.2*. Hand exposure depending on the use of gloves during Application and the type of tractor

In the case of Open tractor application, the use of gloves reduces hand exposure, while in the case of Closed and filtered tractor there appears to be no difference in hand exposure whether the worker uses the gloves or not.

3.4. Risk assessment profile and the Risk Assessment Scheme

Risk assessment in field conditions is useful for several reasons. We can estimate the risk in different working conditions, we can suggest the modifications of working conditions to reduce the risk, and we can communicate the individual risk to workers so they would know how to improve or change their work habits. Nevertheless, doing risk assessment on individual workers in their normal working conditions is not easy. Time is needed to organize the study. Money is necessary for the costs of personnel, transport, sample collection and analysis. Moreover, often the individual risk assessment is valid only on the specific day, with the worker spraying a specific quantity of a pesticide, and just for the pesticide in question. In this chapter, it is our goal to explore the most used scenario in the Region of Lombardy study – the closed and filtered tractor, the most important factors that influenced the exposure of workers, and produce a tool that can be used for risk assessment in any situation when a closed and filtered tractor is used for pesticide application.

3.4.1. Scenario definition

As stated above, our initial scenario is defined as the use of a closed and filtered tractor. The exposure can be that of the body, of the hands and respiratory exposure. Each of these exposures depends on field conditions and the Personal Protective Devices used. Since the workers' treated area differed (as seen in ***Section 3.3.***) the work-day total risk was standardized for 20 hectares.

Therefore factors influencing standardized total risk the most are:

1. Number of Mixing and Loadings during the work-day (one or more than one)
2. Number of hand washing during the work-day (one at the end of the work-day, or more than one)

3. Type of body protection and the phases in which it is used (no coverall, multy-use coverall or mono-use coverall)

4. Type of hands' protection and the phases in which it is used (no gloves used, or any kind of gloves used)

5. Type of respiratory protection and the phases in which it is used (no protection used, or a mask with a filter used)

Having defined the factors influencing the risk, it is possible to define the "worst-case scenario", which would be the scenario, always having in mind that the worker is using a closed and filtered tractor for a standardized work-day of 20 hectares, where the median risk is the highest.

In our case, it is the scenario where:

- The worker mixes and loads the pesticide more than once during the work-day
- The worker washes his hands only once, at the end of the work-day
- The worker does not use any kind of body protection (no coverall)
- The worker does not use any kind of hands' protection (no gloves)
- The worker does not use any kind of respiratory protection (no mask)

The median risk, considering this "worst case scenario" and the exposure to Mancozeb is 4.245% AOEL saturation.

3.4.2. Exposure and exposure score

Having defined the base risk of 4.245% AOEL saturation for Mancozeb in the "worst case scenario", we can give this risk (and exposure) a score of 100% or 100 points. Any other work situation, e.g. using gloves, or washing hands more often, will reduce the risk by a number of points, since it can only improve on the worst case scenario.

The “best case scenario” can be defined as the one where:

- The worker mixes and loads the pesticide only once during the work-day
- The worker washes his hands more than once during the work day
- The worker uses a mono-use coverall in all phases of work
- The worker uses gloves in all phases of work
- The worker uses a mask with a filter in the mixing and loading phase and the cleaning phase (not significant in the Application phase because of the specific scenario in which the worker is protected in a cabin of a closed and filtered tractor)

In this “best case scenario” the worker’s risk is just 0.04% AOEL saturation of Mancozeb, or 1% of the “worst case scenario”, and therefore the “best case scenario” exposure is given a score of 1.

Consequently, the exposure score can have values from 100 (the “worst case scenario”) to 1 (the “best case scenario”) depending on the number of mixing and loadings, the hand washes and the PPDs used. A linear scale can be used to represent these scores.

3.4.3. Toxicity score

Since the exposure and risk assessment has been done for Mancozeb, and the workers use many different registered active substances to protect their vineyards, it was necessary to calculate the exposure and risk for a wide range of active substances. Using the methodology described in ***Sections 2.7.3.*** and ***2.8.2.*** of this text STEF values have been calculated for a large sample of active substances (fungicides registered in the European Union).

Active Principle	STEF
FLUDIOXONIL	0,0907692
FOSETIL ALLUMINIO	0,0025000
AZOXYSTROBIN	0,0020000
KRESOXIM METHYL	0,0010827
FLUTOLANIL	0,0009051
FENAMIDONE	0,0008513
DIFENOCONAZOLO	0,0008205
TOLCLOFOS METILE	0,0006478
IPRODIONE	0,0004000
ZOXAMIDE	0,0003636
PENCONAZOLO	0,0003077
PROPINEB	0,0003061
DIETOFENCARB	0,0002985
FLUOPICOLIDE	0,0002861
TIABENDAZOLO	0,0002597
PROPICONAZOLO	0,0002474
QUINOXIFEN	0,0002244
TRITICONAZOLO	0,0002114
TRIADIMENOL	0,0001914
PYRACLOSTROBIN	0,0001500
MANCOZEB	0,0001333
IPROVALICARB	0,0001261
TRIFLOXYSTROBIN	0,0001185
FUBERIDAZOLO	0,0001140
CARBOSSINA	0,0001004
PICOXISTROBIN	0,0000860
METALAXIL-M	0,0000750
PROQUINAZID	0,0000635
FLUTRIAFOL	0,0000615
DODINA	0,0000570
MANEB	0,0000556
FENHEXAMID	0,0000480
PROCLORAZ	0,0000471
TRIFLUMIZOLO	0,0000466
DIMETOMORF	0,0000417
TETRACONAZOLO	0,0000381
CIPROCONAZOLO	0,0000364
PENCICURON	0,0000337
FENBUCONAZOLO	0,0000314
BENALAXIL	0,0000300
CYAZOFAMID	0,0000300
MEPANIPIRIM	0,0000260
HIMEXAZOL	0,0000235
TEBUCONAZOLO	0,0000231
CYPRODINIL	0,0000189
DITIANON	0,0000143
BUPIRIMATE	0,0000143
FOLPET	0,0000133
IMAZALIL	0,0000122
PROTIOCONAZOLO	0,0000107
TIOFANATO METILE	0,0000087
FENPROPIDIN	0,0000080
BITERTANOLO	0,0000077
FAMOXADONE	0,0000060
CAPTANO	0,0000056
BROMUCONAZOLO	0,0000050
FLUAZINAM	0,0000047
METCONAZOLO	0,0000032
CIMOXANIL	0,0000015
DODEMORF	0,0000015
EPOXICONAZOLO	0,0000013
FENPROPIMORF	0,0000009
TIRAM	0,0000006
SPIROSSAMMINA	0,0000005
ZIRAM	0,0000004
ETRIDIAZOLO	0,0000002

Table 3.4.1. Sample of fungicides registered in the European Union and their STEF values.

Table 3.4.1. shows the range of fungicides registered in the European union and their STEF scores, which have values from 0.1 to 0.0000001. Therefore, to cover the risk assessment of the authorized active substances in the European Union, a group of values from 0.1 to 0.0000001 can be selected to represent all of the toxicity scores. A logarithmic scale needs to be used to represent these scores, and 54 values have been selected to cover the range from 0.1 to 0.0000001.

3.4.4. The Risk Assessment Scheme

Using the R programming language (R Core Team, 2012), a simulation is made to calculate the risk for each combination of the exposure score (from 1 to 100) and toxicity score values (54 values from 0.1 to 0.0000001). Both axes' values and the risk assessment done for each combination have been generated using the R code available as ***Supplementary material S.3.1***

The product is a table with 3 columns:

1. Exposure Score – or the "y" axis value
2. Toxicity Score – or the "x" axis value
3. Risk Assessment – the saturation of AOEL for that exposure and toxicity score combination

Using the ggplot2 package (Wickham, 2009) for R, it is possible to plot all of these values colouring the dots based on the level of AOEL saturation, as explained in the ***Section 2.8.4.***

The code necessary for plotting the values for exposure and toxicity score, and colouring them depending on the risk is available in ***Supplementary material S.3.2.***

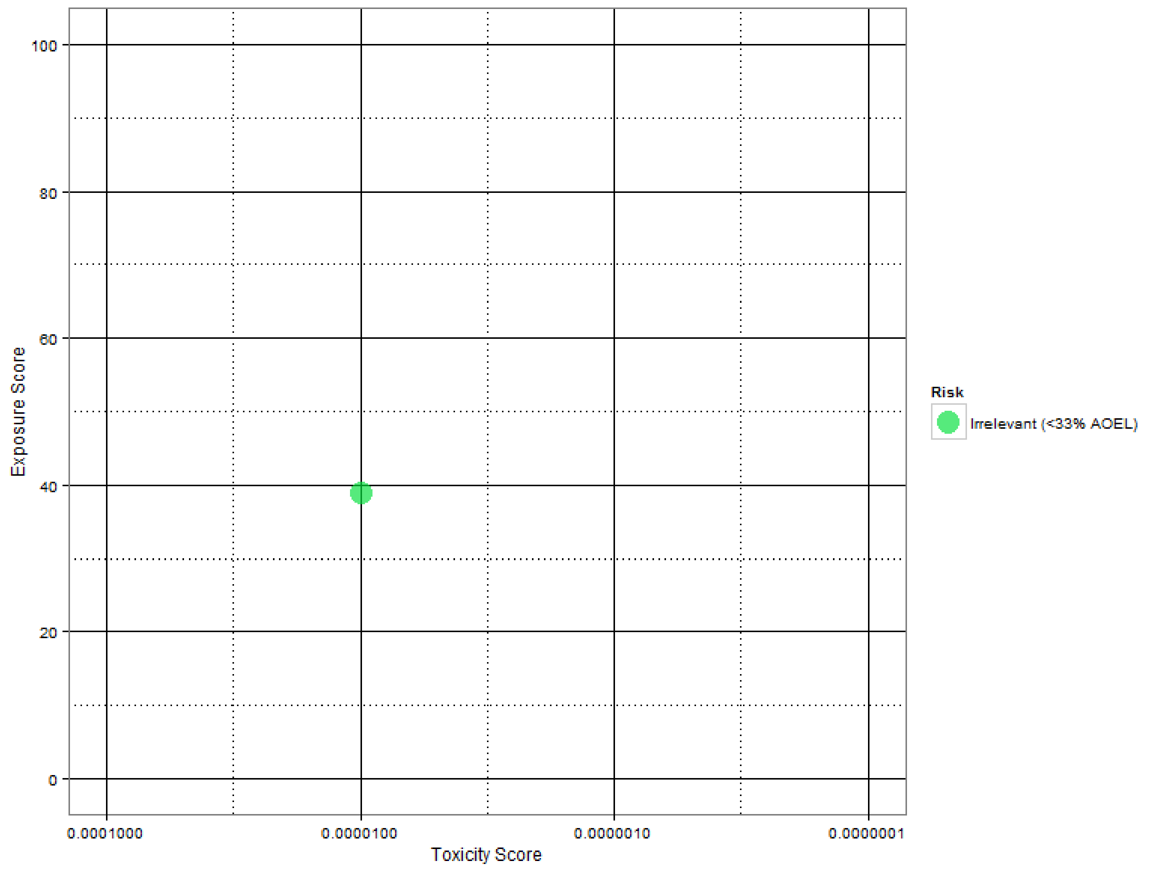


Figure 3.4.1. Risk assessment for one exposure and toxicity score combination

The process of plotting all possible risks for all possible exposure and toxicity scores starts with calculating each individual risk for each individual exposure and substance (toxicity). ***Figure 3.4.1.*** shows an exposure of 40 points (40% of the worst case scenario) for a substance that has the Standard Toxicity Efficacy Factor of 0.00001. The risk assessment for this combination has a value less than 33% of AOEL saturation, therefore it is collared green. This is exactly as having a risk assessment done for a specific work situation in the field, for a worker applying a specific pesticide.

Since our interest is to generalize the results of risk assessment results from our field studies, we might ask ourselves what would all the risks from that specific substance be, considering that from the worst to the best case scenarios we have 100 theoretical possibilities for the exposure score.

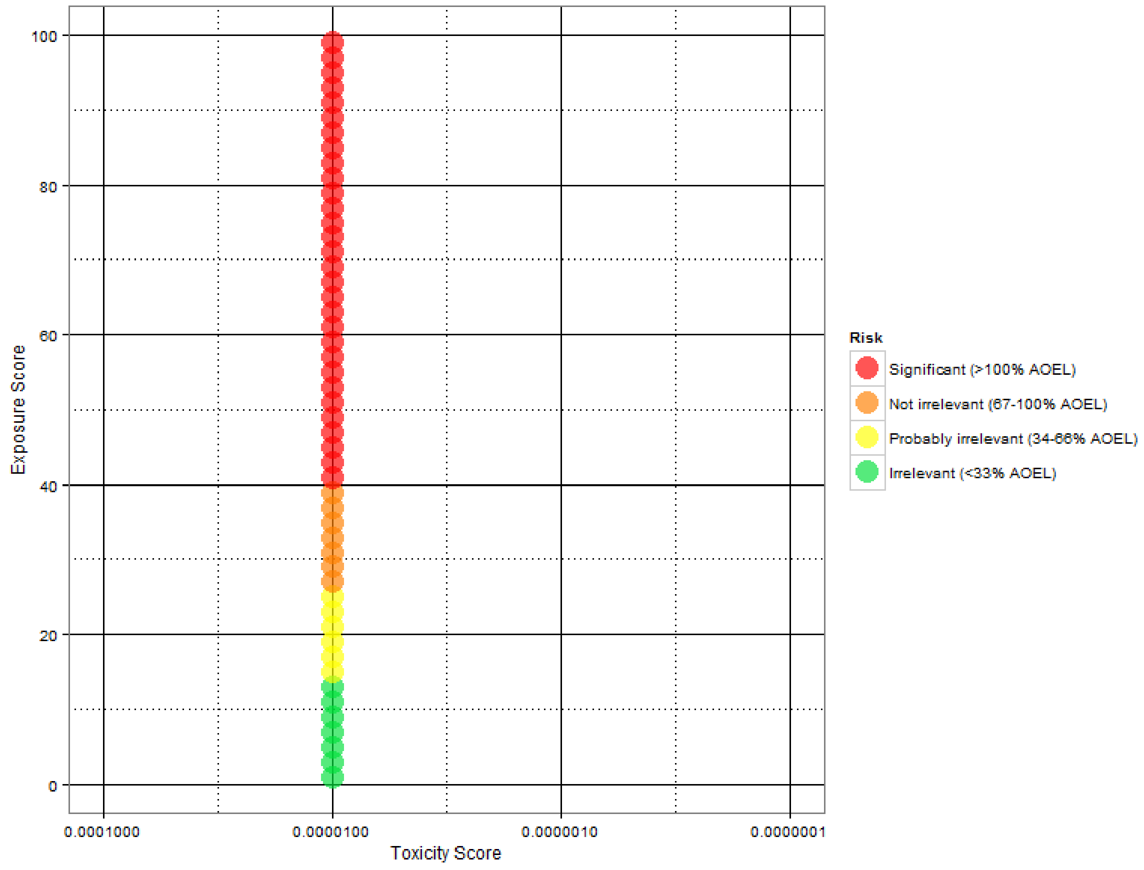


Figure 3.4.2. Risk assessment for one toxicity score value and all possible exposure score values

Figure 3.4.2. shows all possible exposure scores in the case of a pesticide with a toxicity score of 0.00001. The dots are coloured by the saturation of AOEL, and they take all four possible categories, from less than 33% AOEL saturation (Irrelevant risk) for exposure scores of less than 14 points, between 34 and 66% AOEL saturation (Probably irrelevant risk) for exposure scores between 15 and 26, between 67 and 100% AOEL saturation (Not irrelevant risk) for exposure score between 27 and 40, and finally arriving to risks over 100% AOEL saturation for exposures superating 40 points.

Our interest, of course, lies in being able to use our measurements for the risk assessment not only in many exposure situations (denoted by the exposure score), but also in the situation when different active substances are used (denoted by the toxicity score of a substance).

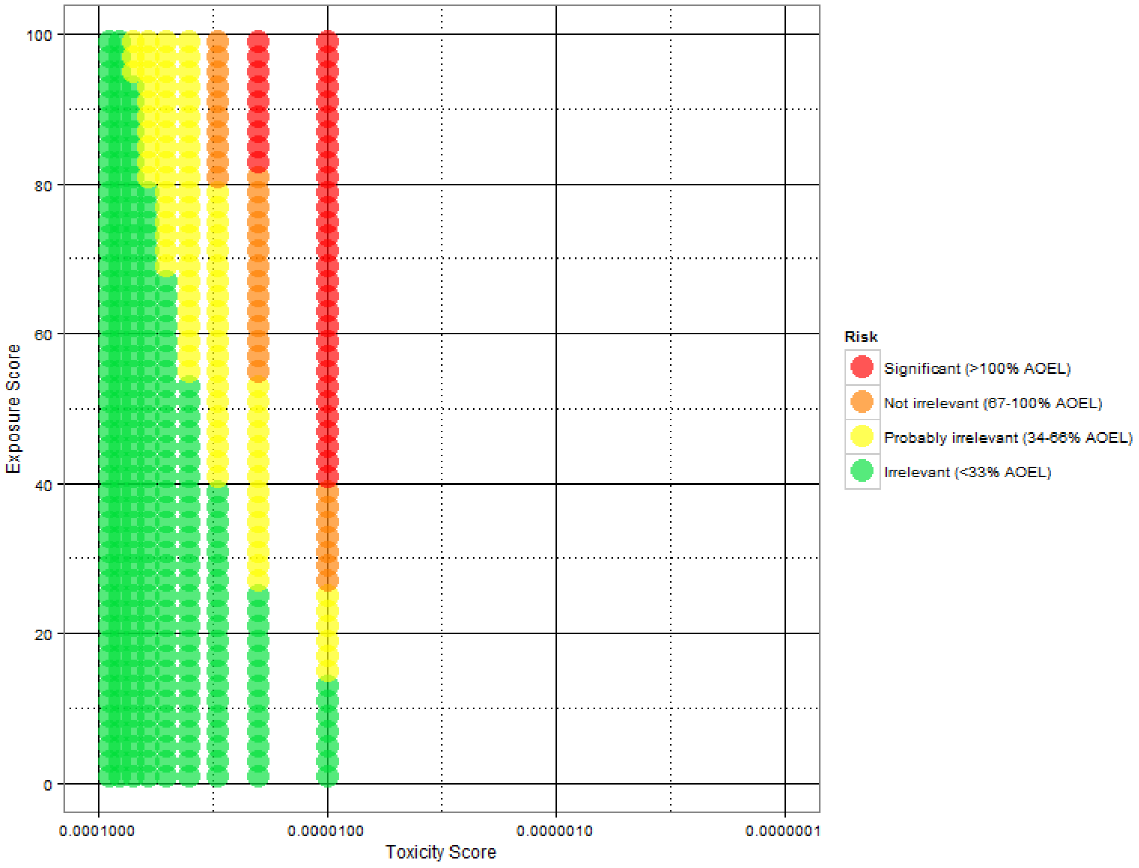


Figure 3.4.3. Risk assessment for all exposure score values and several toxicity score values

A risk assessment considering all exposure score values and several toxicity score values is shown in ***Figure 3.4.3.*** For all the substances having a toxicity score higher than 0.0001, even the worst case scenario using the closed and filtered tractor has a risk of less than 33% AOEL saturation (green colour, irrelevant), and therefore these values are not shown in the figure. From the value of 0.0001 of the toxicity score to the value 0.00001 it is possible to see how the risk values change depending on the exposure score, and the risk gets higher even with lower exposures.

Finally, to cover the whole spectrum of possibilities for both exposure score and toxicity score, we have produced Figure 3.4.4. as the Risk Assessment Scheme for the scenario of closed and filtered tractor application of pesticides.

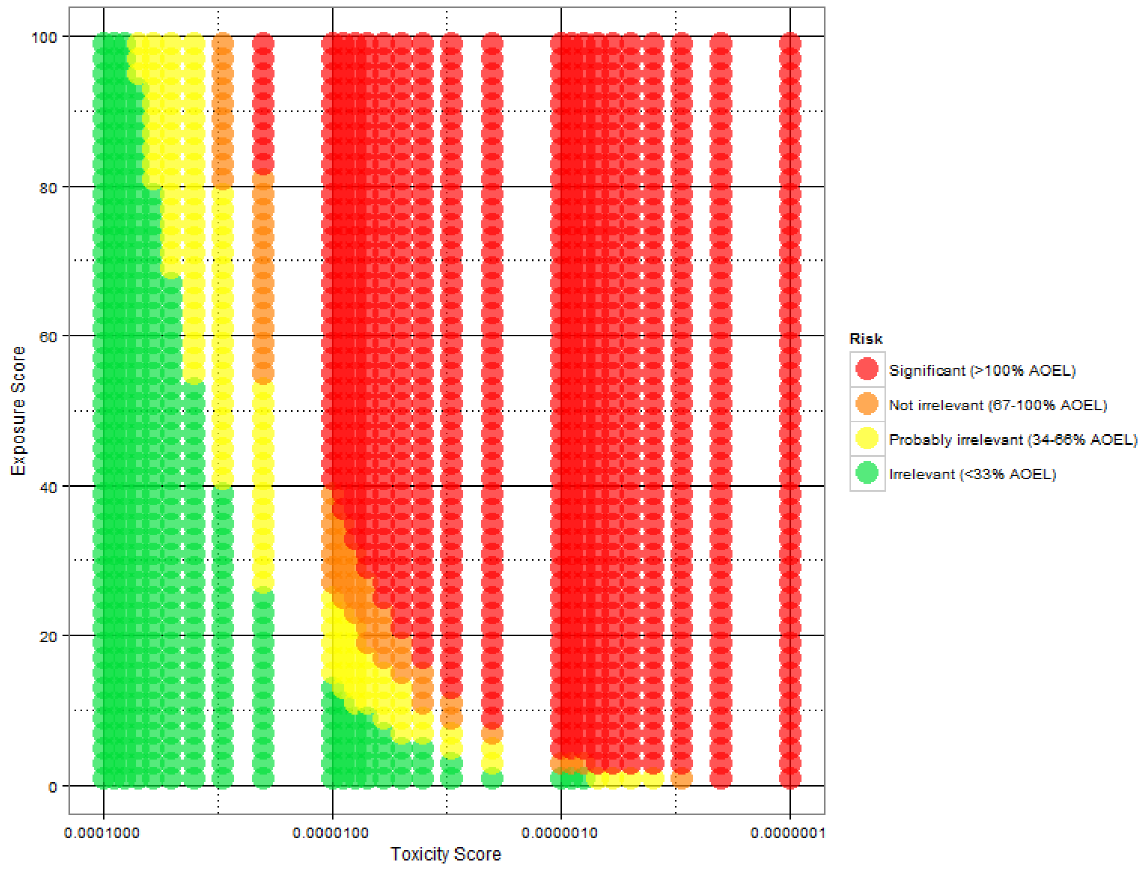


Figure 3.4.4. Risk Assessment Scheme for closed and filtered tractors for all toxicity scores and all exposure scores

The substances with toxicity scores higher than 0.0001 have a risk lower than 33% AOEL saturation in any exposure scenario, having in mind always the use of a closed and filtered tractor. As we follow the x axis the value of toxicity score gets lower, and from the point of 0.0000001 and less all the risk assessments for any exposure score will give a value higher than 100% AOEL saturation (Significant risk, coloured red). Therefore it is not necessary to show the values of toxicity score higher than 0.0001 (irrelevant risk at all exposure levels) and lower than 0.0000001 (significant risk at all exposure levels).

3.4.5. Risk assessment example

To test the idea of doing risk assessment without measurements done in the field, we have organized a mission to one of the vineyards.

Figure 3.4.5. shows the worker during the mixing and loading phase. He is using a mono-use coverall, professional chemically resistant gloves and a mask with a filter. He performs mixing and loading one time during the work-day.

Figure 3.4.5. Worker during the mixing and loading phase

After finishing with the preparation of the active substance Maneb, the worker proceeded to the Application of the pesticide in the vineyard (see ***Figure 3.4.6.***).

Figure 3.4.6. Worker applying Maneb using a closed and filtered tractor

During the work-day, the worker covered 8 hectares of vineyards using the closed and filtered tractor. He used no body protection, no gloves and no mask during this phase of work, and washed his hands only once at the end of the work-day (see ***Figure 3.4.7.***).

Finally, at the end of the work-day the worker performed maintenance and cleaning. In this phase, he used no body protection and no mask, but he used the professional chemically resistant gloves (see ***Figure 3.4.8.***).

Work conditions useful for our risk assessment using the Risk Assessment Scheme:

- 1 mixing and loading

- 1 handwash at the end of the work-day
- Mono-use coverall (mixing and loading phase)
- Professional gloves (mixing and loading, and maintenance phases)
- Mask with a filter (mixing and loading phase)
- Active substance used: Maneb (STEF: 0.00006)
- Area treated: 8 hectares

Figure 3.4.7.

Worker's personal protective devices during the Application phase (no body protection, no gloves, no mask)

Figure 3.4.8.

Worker's personal protective devices during the cleaning and maintenance phase (no body protection, no mask, professional gloves)

Using the work conditions described above, and the proposed table of points based on the results of the Region of Lombardy study (***Supplementary table S.4.1.***) it is possible to calculate the Exposure score, starting from the worst case scenario of 100 points for a closed and filtered tractor:

- Base score: 100 points
- 1 mixing and loading (-65 points)

- 1 handwash (-11 points)
- Body protection – mono-use coverall during mixing and loading (-10 points)
- Hands protection – professional gloves during mixing and maintenance (-2 points)
- Respiratory protection – mask with a filter during mixing and loading (+1 point)
- Area treated compared to standard 20 hectares (2.5 times less)
- **Total points: 13 / 2.5 = 5.2 points**

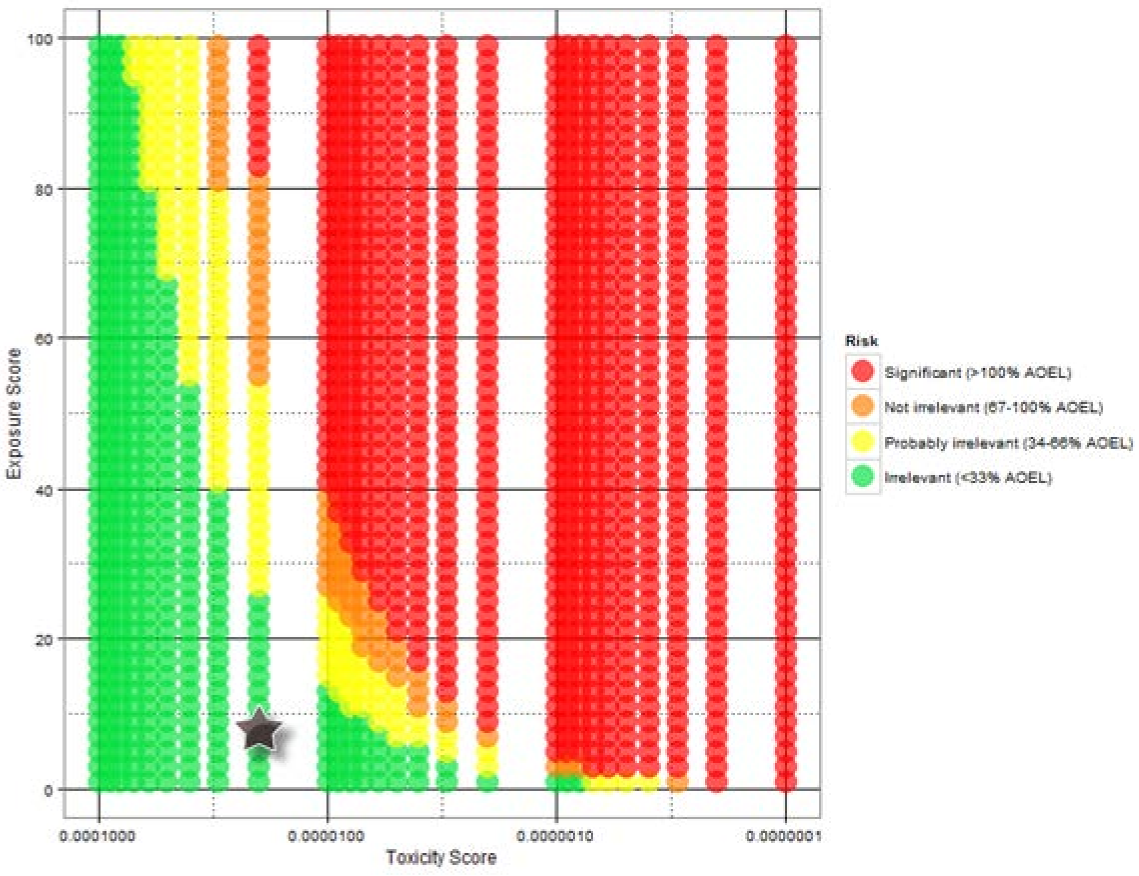


Figure 3.4.9. Risk assessment using the Risk Assessment Scheme with worker's position denoted by a star

Using the calculated exposure score, and the toxicity score of Maneb, it is possible for us to find the risk assessment for the above described work-day. As shown in Figure 3.4.9., the worker is in the green zone, meaning his risk is estimated to be less than 33% AOEL saturation for Maneb.

4. Discussion

4.1. Acropolis Study

Small and middle-size enterprises that use pesticides are rarely subject to assessment of exposure and related health risks. In this study we attempted to shed more light on the characteristics and determinants of exposure in actual working conditions during pesticide spraying in vineyards, and for the first time TEB exposure levels have been measured in field conditions.

Work with pesticides in the field is traditionally carried out mostly by men, due to the high physical strain on the body and the potential risks, which was the case in our study (see ***Table 3.2.1.***). Our experience is that in family enterprises sometimes the women help the men, and in professional pesticide application their job is mostly to organize the work and support the male workers.

A high variability in the general working conditions in study subjects was noticed (see ***Table 3.2.2.***). Work was carried out with hand-held equipment, open and closed tractors, and combinations of tractors and hand-held equipment even during the same work-day. The cause was most probably the characteristics of the terrain, as well as the different sizes of vineyards, which ranged from very small to larger ones. Finally this explains the differences in working hours recorded in our study.

Personal protective equipment is one of the most important determinants of exposure in open field farming. Our study showed a high variability in the access to the basic personal protective equipment (shoes, gloves and masks), and an even larger variability in their use in the different work phases (see ***Table 3.2.3.***). It is worth noting that one of our workers (work-days I and J) used slippers during his two working days. Most workers did not have new and adequate (chemically protective neoprene) gloves. The minimum set of personal protective devices that the worker must wear when handling pesticides and the interpretation of the label's instructions is most commonly left to the workers and/or their employers.

Potential body exposure showed a high variability (see ***Table 3.2.4.***). This can mostly be explained by the different working modalities, sizes of estates, as well as the different length of exposure and amount applied. The potential and actual body exposure of our workers fall in the same range of those measured in open-field pesticide applicators with exposure to isoproturon (Lebailly et al., 2009), procymidone (Aprea, 2012) and terbutylazine (Vitali et al., 2009).

The cotton coverall used by the workers provided them with a high protection factor (98%). The protection provided in our study is higher than that reported by other authors for standard cotton garments (reportedly 73% to 88%) and in the range of the protection provided by Tyvek® coveralls (Aprea et al., 2005; Fenske et al., 1990; Vitali et al., 2009). Several studies have identified hand exposure as the main contributor to the total skin exposure (Aprea et al., 2005; Baldi et al., 2006; Lebailly et al., 2009), and our study has confirmed this finding by showing a median contribution of 61% to total actual exposure. In particular workers who use gloves during mixing and loading and application phases have much lower hand exposure (***Figure 3.2.3.***). However it should be noted that re-use of gloves is known to increase the exposure of the hands, because of damages to the gloves or their internal contamination, because workers often do not wash their hands after removing the gloves and wearing them again (Canning et al., 1998; Garrod et al., 2001; Guo et al., 2001; Machera et al., 2003).

Head exposure contribution is rarely mentioned in studies of pesticide exposure in real-life field conditions. In our study head exposure gave a median contribution of 16% to total actual exposure (see ***Table 3.2.4.***). The head cover is the least contaminated part among the external dosimeters (see ***Table 3.2.5.***), but, since the workers did not wear any personal protection on the head, it can also be considered a skin dosimeter. In this light, it would be the most contaminated part of the skin after hands. This can present a significant addition to the absorbed dose, having in mind the relatively high dermal absorption in the head region, although the physical barrier represented by the hair to penetration through the scalp should be taken into account (Poet and McDougal, 2002).

Looking at the difference of hand contamination between the workers who did or did not use gloves in the mixing and loading and the application phases, and the contribution of head exposure to the total actual exposure, it can be concluded that hand protection, preferably with new neoprene gloves, and head protection, with a disposable cover, should always be worn while working with pesticides.

It should be noted (see ***Figure 3.2.1.***) that the highest contribution to the potential exposure was that of the legs, while the highest contribution to actual exposure was that of the front and back. Assuming that the cotton coverall worn by the workers provide the same level of protection at any body region, these data may be explained by the closest contact of clothes with skin in the trunk zone, which is not the case with the clothes over arms and legs. To our knowledge this is the first report underlying this difference.

Different working conditions, especially in the application modalities, are known to entail different levels of exposure to workers, being highest for hand-held and lowest for the use of air-conditioned tractor with carbon filters (Garry et al., 2001; Nuyttens et al., 2007). Our data, although obtained from a small sample population, confirm this difference in both absolute values and the distribution of exposure. The use of hand-held equipment led to a much higher contamination of lower parts of the body (abdomen, back, legs), and especially a peak on the left side of our right-handed worker. This was likely due to the fact that the worker had the hose of the sprayer passing and in close contact with the left part of his body. The spider graphs (see ***Figure 3.2.2.***) and the box-plots showing how exposure depends on the body region (***Figure 3.2.1.***) may be a good risk communication method when giving feedback to the workers regarding their work practices.

Using the German model for Risk Assessment of our workers, in real-life working conditions, has confirmed the tendency of pre-authorization models to underestimate the exposure when small amounts of active substance are used (the case of small, family based farms) and overestimate exposure when large amounts of pesticides are used (the case of large farms, usually with better tractors and PPDs) (Protano et al., 2009; Rubino et al., 2012).

In this study we used the information from the authorization process of Penconazole, Triticonazole, Ciproconazole, Bromuconazole and Epoxiconazole, to perform theoretical risk assessment, treating Tebuconazole as a tracer in the mixture of these conazoles. Only for Epoxiconazole, the median and maximal risk were high (85% and 313% AOEL saturation), and in 6 out of 12 work days the risk would have been unacceptable. This information could be explained by the quite low AOEL of this active substance (0.008 mg/kg bw/day) and high dermal absorption (50%) (PPDB, 2013), compared to Tebuconazole (EFSA, 2008).

This field study has nevertheless some limitations, most of which were intrinsic to the unavoidable compromise between the extent of information to be gained and the intervention on the farmers’ daily work. Although we tried to minimize the interference with normal working conditions of pesticide applicators, it is possible that the workers may have somewhat modified their practices because of the presence of external observers. For example, unattended farmers most likely would have worn shirts and jeans, or a Tyvek® coverall over their normal clothes, as we observed in previous studies (Rubino et al., 2012; Vitali et al., 2009). In conclusion, these facts could have resulted in an exposure lower than that normally occurring.

Information on the workers' height and weight, which is used to calculate the individual body surfaces, and for the extrapolation of exposures measured by the t-shirt and boxers, was collected by interview from the workers, and there is no guarantee that this information is truly accurate; however it was estimated that there is likely no more than 5% uncertainty on the exposure estimate. Furthermore the surface of different body regions depends also on the distribution of fat and muscles, so that more obese people have a larger trunk and smaller limb relative surface. Although our sample consisted of healthy, working men, some differences in the percentages represented by different body regions are possible, but at the moment this contribution is impossible to account for, but it is likely to be low.

Another limitation of the present study is the relatively small number of participants, but this is a reality for the real-life field studies of pesticide exposure, especially in small-size enterprises, where the standard is having 2 to 10 participants working on several work-days (Aprea, 2012; Rubino et al., 2012; Vitali et al., 2009).

In the ACROPOLIS study a potential exposure to TEB in the range of milligrams per person per work day was found, however the use of personal protective equipment was very efficient in preventing the fungicide from reaching the skin. Educational and preventive actions to raise farmers' awareness on health risks following pesticide exposure should be focused on a better use of personal protective equipment and especially new gloves resistant to chemicals, suitable coveralls, and head protection, because even small improvements in the use of these devices could greatly increase the protection of workers (Keifer, 2000).

4.2. Region of Lombardy Study

In this study we tried to explore two work scenarios in more depth and with a higher number of study subjects (28 work-days with a closed and filtered tractor, and 9 work-days with an open tractor). As a standard for this kind of work, it was done only by men, also confirming the situation of the Acropolis study and literature data (Baldi et al., 2006; Lebailly et al., 2009; Vitali et al., 2009).

We noted an important difference in some characteristics of work-day between the workers using a closed and filtered tractor and those using an open tractor (see ***Table 3.3.2.***). For example, the amount of active substance per day, the area treated and the application time are all higher for a closed and filtered tractor. This can be explained by the fact that larger estates can afford better machineries, usually with larger tanks, in order to more efficiently do the work with pesticides.

Personal protective equipment is known to be one of the most important determinants of exposure during the day (Gomes et al., 1999; Libich et al., 1984). In this study, a opposed to the Acropolis study (see ***Section 3.2.***) the workers had the freedom to choose the personal protective equipment they used during the work-day, and even the phases of work in which they would use them. Therefore, our results show also the real availability of personal protective equipment and use in different phase (see ***Table 3.3.3.***). Only 11% of workers had no body protection, meaning they worked in normal clothes, and none of them using an open tractor. Most of the workers had gloves available when analyzing all work-day together, and again all of the workers using an open tractor had gloves. This speaks to workers understanding of the higher risk of exposure when using this kind of machinery, as opposed to a closed and filtered tractor. The analysis of glove exposure was made difficult by the fact that around half of the workers in total did not use new gloves, but those already used for work with pesticides.

Our study has shown that workers use most protection during the mixing and loading phase, since 97% of them used gloves and 81% of them used a mask in this phase (see ***Table 3.3.3.***). Application phase is not considered so dangerous, especially for workers using a closed and filtered tractor, judging by the use of personal protective equipment, while the use was higher in the maintenance and cleaning phase. Similar studies have shown that mixing and loading phase and the maintenance and cleaning phase might contribute the most to overall exposure and risk (Baldi et al., 2006; Coble et al., 2005). Our study has shown that gloves reduce hand exposure if used during the application phase, but only in the case of open tractors, while in the case of closed and filtered tractors, the difference in hand exposure between the workers who used gloves and those who did not was not notable (see ***Figure 3.3.2.***).

Workers using an open tractor had 30 times higher external body exposure than those working using a closed and filtered tractor. When analyzing glove exposure, the much lower use of gloves in the application phase has to be considered a factor, as well as the fact that cotton pads were used as the dosimeter. Body skin exposure was only 5 time higher in open tractor operators (see ***Table 3.3.4.***), which can be explained by high efficiency of personal protective equipment. The protections of 98 and 99% for cotton and mono-use coveralls are in the range of the Acropolis study results, and somewhat higher than literature data report.

Hand exposure represented more than 95% of total skin exposure, but when taking into account the number of hand washes during the day and the time of exposure, it represented less than 5% of the total risk. Literature has identified hand exposure as the most important in pesticide application (de Cock et al., 1995; Hines et al., 2001; Machera et al., 2003), but it is important to consider that all of the contamination found on the hands does not necessarily translate into risk. Many factors influencing dermal absorption need to be considered, such as the duration of exposure (before the contamination is washed away), loading effect, evaporation, continued absorption (Frasch et al., 2014).

Risk assessment performed for our workers has shown that all of them are several times (up to several hundred times) below the AOEL for Mancozeb, with median risk of workers using open tractors being double that of workers using closed and filtered tractors (see ***Table 3.3.4.***).

This study had some limitations, as does any field study. The data regarding the area treated, amounts of pesticides used, as well as some work times were acquired by interviewing the workers, therefore relying on their word. In most cases this problem was avoided by comparing the information collected from the workers with the field notes of the investigators and photographs taken during each work-day.

Dermal exposure assessment in the field of pesticides, as well as risk assessment, present an activity with many decisions to be made. The OECD Guidelines (OECD, 1997) offer the possibility to use pads or whole body dosimetry for exposure assessment of workers. In these study we used the pads methodology in order to observe the real working conditions in the field, and to be able to use the data collected for future risk assessment. Another point of discussion might be represented by the reference values from the authorization process. Dermal absorption coefficients used to calculate the absorbed dose might not be the best and most accurate way of calculating how much of the active substance has actually been absorbed, since they do not take into account the time of exposure and the loading effect (Frasch et al., 2014). Nevertheless, most state of the art, and commonly used methods for the calculation of exposure and risk have been used, and until new methods are developed there is no way to remove these limitations.

This study has analyzed field conditions, personal protective devices, exposure and risk of two groups of workers, one using closed and filtered tractors, and another using open tractors, which applied pesticides in vineyards. Although the use of open tractors submitted workers to higher exposure and risk, the personal protective devices were very efficient to reduce this exposure, resulting in total risk lower than the limit values in all cases.

4.3. Risk Assessment Scheme

Exposure assessment in the field, and risk assessment using field measurements is a time consuming and costly activity, also burdensome for the study subjects, which is seldom performed in agriculture (see ***Section 1.7.***). Many factors reduce the possibility of evaluating risk on regular basis, among which the unstable environmental conditions, location of farms which are usually spread over a large surface, far away from research centres and high cost of laboratory analyses. Moreover, the exposure and risk assessment done once, in specific work conditions, may not be representative of another situation, even if the worker uses the same machinery.

With the introduction of the idea of Exposure and Risk profile and the Risk Assessment Scheme, we made an attempt to develop a methodology which allows for generalization and re-use of our field data for simple, but scientifically based, risk assessment in real life field conditions. The idea of using field measurements to create a tool for exposure and risk assessment is not new (see ***Section 1.7.2.***). It has been done by the creators of the German model (Lundehn et al., 1992), as well as the EUROPOEM (van Hemmen, 2001). Contrary to these two models, the Risk Assessment Scheme is based on real-life field measurements, acquired during the application of pesticides in vineyards in Italy. The German model and the EUROPOEM are based on generic databases which, although larger than our study group, are collections of filed measurements done in somewhat experimental conditions, which makes their output not necessarily representative of the activities in real-life conditions. Comparing the risk assessment done in the Acropolis study with the output of the German model, showed that the latter overestimates the exposure when larger quantities of pesticides are used in the field, while for smaller quantities (common in small and family-based enterprises) the risk might be underestimated (see ***Section 3.2.***).

Other authors have attempted to develop methods for risk assessment without using field measurements. An example is the Agricultural Health Study (Dosemeci et al., 2002), where the authors developed a questionnaire and an algorithm in order to be able to assess pesticide applicators' exposure and correctly define the exposed and non-exposed subjects in epidemiological studies. Our approach was similar, since we used a literature search (see ***Section 2.1.***) to define the variables influencing exposure and to rank them based on the literature data, but we have gone beyond using just literature data. Throughout our studies we tried to quantify the influence of different field variables and assign them real-life values for exposure and risk, combining the approaches used in all of the above mentioned methods (see ***Sections 2.2.*** and ***2.3.***). We have also introduced the use of the R Programming Language for Statistical Computing (R Core Team, 2012) to enhance risk assessment and perform simulations of exposure and risk in different working conditions.

The German model and the EUROPOEM are the official tools used for pesticide risk assessment in the authorization process. They are based on many studies, standardized and joined together to create an exposure assessment tool. Our goal was to create a method that would be simple and easy to apply, but also cheap, which would allow for risk assessment in real-life field conditions. Therefore it may be possible to use the measurements collected in the studies included in the German model and EUROPOEM, together with the methodology developed by our group, to create Risk Assessment Schemes based on these two models. This would allow for a tool that is already officially accepted to be available to risk assessors, farm owners and pesticide applicators, but in a form which is easily utilized in field conditions.

An added value of the method developed for the Exposure and Risk profiles and the proposed Risk Assessment Scheme is that they can also be used as a risk communication and risk management tools, since they shows clearly to the workers in what "zone" of risk they are, and more importantly why they found themselves in that zone and what changes they could make to reduce the risk. In our risk assessment example (see ***Section 3.4.5.***) we have tested the use of our Risk Assessment Scheme in a real-life scenario. Had the worker been in any zone with higher risk, it would have been simple explaining to him what changes in the work conditions he needs to make in order to lower his risk. The options are to use more personal protective devices, or use them in all the phases of work, or to apply to a smaller surface, use a bigger tank (thus reducing the number of mixing and loadings), and washing hands more often during the work day.

One of the most obvious limitations of using field measurements to develop a tool for risk assessment is the representability of our sample. The work-days monitored may or may not be representative of the work conditions in another place, or on another day. It is encouraging to know that the existing models (the German model and EUROPOEM) are based on a higher but similar number of work-days per study. For example, 12 trials were done for tractor mounted equipment applying on high crops, then another 3 trials with the same setting, 3 trials for tractor mounted equipment on low crops, 15 trials for hand-held equipment on high crops, etc…(Lundehn et al., 1992). In the creation of the Risk Assessment Scheme, 28 trials with closed and filtered tractors applying on high crops (vineyards) were used (see ***Section 3.3.***). As new field studies are done, it is possible to unite the results and create a more representative model, also increasing the number of variables used in this model.

Finally, our methodology of using exposure and risk profiles and the risk assessment scheme should not be considered a replacement of the pre-marketing risk assessment tools. The models used in the pre-marketing cannot and do not consider all the characteristics of field activities. Our methodology and the resulting tool consider the work with pesticides as it is done in real-life conditions, and there are phases and variables not taken into account by the German model or the EUROPOEM. One example is the activity of cleaning and maintenance, not addressed by models in the pre-marketing (see ***Section 1.6.***), but it is an activity routinely done in real-life conditions and can bring about high exposure (see ***Section 3.1.3.***). There are also variables, such as the number of times Mixing and Loading is performed, which is also correlated also to the tank size, very important in real-life field conditions (see ***Section 3.1.1.***) but not taken into consideration by the existing models.

Future work on this approach should be directed at:

- Increasing the number of field measurements
- Increasing the number of variables taken into account
- Generalization and validation of the model for other cultures
- Improving the methods for field exposure and risk assessment
- Development of Risk Assessment Schemes for open tractors, as well as hand-held application
- Development of an electronic version of the Risk Assessment Scheme, available on-line and/or for hand-held devices to allow higher accessibility

5. Conclusions

Our work has tackled the problem of risk assessment for pesticide exposure in agriculture, which has been unfairly neglected in the past years. Through the use of literature data, field studies and computational modelling, we have managed to analyze and summarize the characteristics of pesticide application in agriculture, explore the real-life field conditions during pesticide application in vineyards in Italy, collect the field measurements necessary to do exposure and risk assessment, and to develop a method to use the data collected to produce a Risk Assessment Scheme. The study results and the above mentioned tool represent a step forward towards rapid, simple and scientifically based risk assessment in real-life conditions of pesticide application in agriculture. A lot of work remains to be done, especially in the field of collecting more measurements, improving the methods of exposure assessment, improving the methods of risk assessment, simplifying and streamlining the model creation by using new computational tools, and making the risk assessment tool available to as many users possible online.

Our future work will address the above mentioned areas of improvement, and, in contact with experts in the field try to implement their ideas for reaching safe pesticide use in agriculture.

6. Acknowledgements

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My work and stay in Italy would have been impossible without the scholarship of the ***Italian Ministry of Education*** and the kind hospitality of the ***International Centre for Rural Health of the San Paolo Hospital*** and all the colleagues of the ***Department of Health Sciences of the University of Milan***.

7. References

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8. Supplementary material

Supplementary Material S1 - Data collection sheet used for the recording of field conditions during the study of exposure to TEB. Version in English language.

Company Information Company ID:______________

Company stamp

Name of the company: ____________________________________

Address: ____

Town: ____

Province: ____

Region: __________________________________

Name of the responsible person in the company:
____

Contact phone: __________________________________ or
____

Total surface under fields: __________ (ha) Surface of vineyards: _________ (ha)

Number of workers engaged in spraying pesticides: ______________

Comments:
____

____
____

____
____

Worker information

Worker ID: ______________

Last name: ________________________________

First name: ________________________________

Date of birth: _ _ / _ _ / _ _ _ _

Telephone number: _________________________

Mobile phone number: _______________________

Has the worker signed the consent form?

1) YES **2) NO**

Province of birth (Country if foreigner): ___________

Province of living: ____________________

Region of living: _______________________

Height: ______ (cm) Weight: _______ (kg)

Sex: ________ Age: ___________

Primary hand: 1) Right 2) Left

Years of education (school): ________________

Knowledge of Italian (only foreigners):
1) Bad 2) Medium 3) Good

Does he consume alcohol regularly:
1) Yes 2) No

Smoking status:
1) Non smoker 2) Smoker 3) Ex-smoker

Does he have any dermatological problems:
1) Yes 2) No

Does he suffer from any chronic disease:
1) Yes 2) No

Local or systemic?
1) Local 2) Systemic

Does he use any dermatological medications:
1) Yes 2) No

Which medication? __________________________

When is the last time he sprayed pesticides?
Date: _ _ / _ _ / _ _ _ _
How many days ago? ______________

Does he spray pesticides in some other company?
1) Yes 2) No

Working day information

Working day ID: ______________

Date of work: _ _ / _ _ / _ _ _ _

Name of the product:
____

Active substance:
____

Concentration of the active substance in the product:

_______________________________ (%)

Size of the tank: ___________ (litres or hectolitres)

Amount of product per hectare: _________ (g/ha)

Wind:
1) No wind 2) Light wind 3) Strong wind

Job title of the worker (usual job):

____

Study name: ___________________________

Formulation:
1) Powder
2) Granules
3) Bags
4) Liquid

Amount of the product per ONE tank:
________ (kg)

Amount of mixture per hectare: __________ (l/ha)

Phases of work that he does (circle all):
1) Mixing and loading
2) Spraying (Application)
3) Cleaning and maintenance
4) Re-entry (After how many days? ___________)

Personal protective devices

Body protection:
1) None (normal clothes)
2) Mono-use coverall
3) Multy-use coverall

What is he wearing under the coverall:
1) Normal clothes 2) Underwear

Material of the gloves:
1) Latex 2) Rubber 3) Neoprene (profess.)

What does he wear on his feet:
1) Normal shoes 2) Boots 3) Protective shoes (antiinfor.)

Head protection:

Coverall material:
1) Cotton
2) Tyvek
3) ____________________

Does he have gloves:
1) Yes 2) No

Condition of the gloves:
1) Used 2) New

Inhalatory protection (mask):
1) None 2) Paper 3) Filter

1) None 2) Hat 3) Hood of the coverall

Personal protective devices in different phases of work (check):

Phase of work	Coverall	Gloves	Mask	Head prot.
Mix and load				
Application				
Maintenance				

Comments:
____
_____
____
_____
____
_____
____
_____
____
_____
____
_____
____
_____
____
_____

Mixing and loading

Where is mixing done?
1) Pre-mixture container 2) Directly in the tank

Average time for a mixing and loading: _______(min)

Did any incidents happen during mixing and loading (e.g. splash or spill)?
1) Yes 2) No

Number of mixing and loading for that working day:
____

Comments:
____
___
____
___
____
___
____
___
____
___

Application

Application mode:
1) Hand-sprayer 2) Tractor sprayer

How old is the sprayer equipment? _______ (years)

How old is the tractor (if any)? _______ (years)

Culture type:
1) Herb 2)Tree

Sprayer type:
1) Atomisator
2) Nebulisator
3) Sprayer withut air assistance
4) Sprayer with air assistance
5) Backpack pump

Distance between rows: ______________ (m)

Liters of mixture per hectare: ____________

Area treated during the day:
_________________ (ha)

Number of applications during the day?
____

Total duration of application during the day?
___ (h)

Working pressure: ___________ (bar)

Did the worker spray on himself or the tractor?
1) Yes 2) No

How many times? _______________

How long (average) did it last?
_____________(min)

Incidents during application?
1) Yes 2) No

(l/ha)

Average duration of one application:
_________(min)

Did the worker exit the tractor during application?
1) Yes 2) No

Did the tractor have problems during the spraying?
1) Yes 2) No

Comments:
____
____
____
____
____
____
____
____
____
____

Maintenance

Does the worker wash the tank after the treatment?
1) Yes 2) No

How much time? ______________ (min)

Does the worker wash the tractor after the treatment?
1) Yes 2) No

How much time? ______________ (min)

Where does the water go?
1) Ground 2) Container

Incidents during this phase?
1) Yes 2) No

Comments:
____
____
____
____
____
____
____
____
____
____

Reduction factors

Type of tractor:
1) Open 2) Closed 3) Closed with filters

Is the tractor and the sprayer maintained regularly?
1) Yes 2) No

What kind of education (diploma) does he have?
____
_

Does he have the licence to spray pesticides?
1) Yes 2) No

How would he rate his skill
(1 = bad; 10 = great)? _______

How would he rate the toxicity of the substance
(1-10)? _______

How would he rate his exposure of the day
(1-10)? _______

Are filters changed regularly (every 2000 hours)?
1) Yes 2) No

How many years of experience does the worker have?
__________________ (years)

Does he have any kind of agricultural education?
1) Yes 2) No

Comments:
____
_____
____
_____
____
_____
____
_____
____
_____
____
_____

Supplementary material S2 – Detailed individual characteristics of study subjects and work-days in the Region of Lombardy study

Table S.2.1. Individual personal characteristics of study subjects

Subject ID	Work-day ID	Age (years)	Height (cm)	Weight (kg)	Body Surface (dm^2)
1	1	57	178	93	214
2	2	32	190	100	230
3	3	47	175	78	195
5	4	56	185	60	176
6	5	63	180	82	202
7	6	59	172	76	191
8	8	44	177	76	193
9	9	53	165	80	191
10	10	42	170	89	205
11	11	41	184	78	200
12	12	45	186	90	216
13	13	36	184	120	248
14	14	60	178	95	217
15	15	55	171	80	195
16	16	42	168	95	211
17	17	41	163	81	192
17	18	41	163	81	192
18	19	53	175	90	209
19	20	43	175	75	191
21	21	54	162	62	167
21	22	54	162	62	167
21	23	54	162	62	167
22	24	44	165	78	189
22	25	44	165	78	189
22	26	44	165	78	189
23	27	53	172	75	189
23	28	53	172	75	189
23	29	53	172	75	189
24	30	41	175	60	171
25	31	45	183	90	214
26	32	41	173	100	219
27	33	47	178	102	225
28	34	48	182	100	225

Subject ID	Work-day ID	Age (years)	Height (cm)	Weight (kg)	Body Surface (dm^2)
30	36	34	178	76	194
30	37	34	178	76	194
31	38	33	190	90	218
31	39	33	190	90	218
Minimum	-	32	162	60	167
Median	-	45	175	80	194
Maximum	-	63	190	120	248

Table S.2.2. Individual work conditions in the Region of Lombardy Study

Subject ID	Work-day ID	Product Form	Amount of A.S used (kg)	Number of MIX	Tank Capacity (l)	Type of tractor	Area treated (ha)	Cleaning	Total Work Time (minutes)
1	1	GN	2,2	2	1500	Filtered	5,2	Yes	240
2	2	GN	1,5	1	1000	Filtered	7,0	Yes	160
3	3	GN	4,8	2	1500	Filtered	3,5	Yes	150
5	4	GN	1,9	1	1000	Filtered	3,5	Yes	190
6	5	GN	2,9	2	1000	Filtered	3,0	Yes	140
7	6	GN	7,7	2	1250	Filtered	4,1	Yes	210
8	8	GN	9,0	2	3000	Filtered	6,0	Yes	260
9	9	GN	4,5	1	2000	Filtered	4,0	Yes	155
10	10	PD	5,7	3	500	Filtered	7,0	Yes	210
11	11	GN	1,5	4	200	Open	5,0	Yes	220
12	12	GN	1,9	1	1000	Filtered	4,0	Yes	90
13	13	GN	4,7	2	1500	Open	3,0	No	180
14	14	GN	9,7	2	1500	Filtered	7,0	No	270
15	15	GN	5,4	2	500	Filtered	3,0	Yes	235
16	16	GN	11,5	4	800	Filtered	17,0	Yes	687
17	17	PD	21,0	6	500	Filtered	15,0	Yes	660
17	18	PD	1,7	1	300	Open	1,2	No	70
18	19	GN	11,5	3	800	Open	17,0	Yes	420
19	20	GN	3,6	2	250	Open	2,0	Yes	150
21	21	GN	22,5	3	800	Filtered	12,0	Yes	450
21	22	GN	30,0	4	800	Open	16,0	No	611
21	23	GN	7,5	1	800	Filtered	4,0	Yes	135
22	24	GN	22,5	3	1000	Filtered	15,0	Yes	450
22	25	GN	30,0	4	1000	Filtered	20,0	Yes	520
22	26	GN	15,0	2	1000	Filtered	10,0	Yes	270

Subject ID	Work-day ID	Product Form	Amount of A.S used (kg)	Number of MIX	Tank Capacity (l)	Type of tractor	Area treated (ha)	Cleaning	Total Work Time (minutes)
23	27	GN	22,5	3	1000	Filtered	15,0	Yes	465
23	28	GN	30,0	4	1000	Filtered	20,0	Yes	560
23	29	GN	7,5	1	1000	Filtered	5,0	Yes	135
24	30	GN	7,1	1	800	Filtered	7,0	Yes	135
25	31	GN	1,5	1	200	Open	1,0	Yes	70
26	32	PD	14,0	7	300	Open	10,0	No	490
27	33	GN	6,5	1	1000	Open	1,0	No	80
28	34	PD	0,5	1	300	Filtered	1,5	Yes	85
30	36	GN	28,1	4	1000	Filtered	20,0	Yes	340
30	37	GN	4,2	1	1000	Filtered	3,0	No	100
31	38	GN	18,8	5	1000	Filtered	20,0	Yes	350
31	39	GN	7,5	2	1000	Filtered	8,0	No	200
Minimum	-	-	0,5	1	200	-	1,0	-	70
Median	-	-	7,5	2	1000	-	6,0	-	210
Maximum	-	-	30,0	7	3000	-	20,0	-	687

Table S.2.3. Personal protective devices in the Region of Lombardy Study

		Available Personal Protective Equipment						Mixing and Loading		Application		Cleaning	
Subject ID	Work-day ID	Coverall Type	Coverall State	Gloves available	Gloves material	Gloves state	Inhalatory protection	Gloves	Inhalatory	Gloves	Inhalatory	Gloves	Inhalatory
1	1	Multy	Clean	Yes	Rubber	New	Filter	Yes	Yes	No	No	Yes	No
2	2	One	New	Yes	Rubber	Used	Filter	Yes	Yes	No	No	Yes	No
3	3	Multy	Clean	Yes	Rubber	Used	Paper	Yes	Yes	No	No	Yes	Yes
5	4	One	New	Yes	Rubber	Used	Filter	Yes	Yes	No	No	Yes	No
6	5	Multy	Clean	Yes	Rubber	New	Filter	Yes	Yes	Yes	Yes	Yes	Yes
7	6	None		Yes	Rubber	Used	No	Yes	No	No	No	Yes	No
8	8	Multy	Clean	Yes	Rubber	New	Filter	Yes	Yes	No	Yes	Yes	Yes
9	9	One	New	Yes	Rubber	Used	No	Yes	No	No	No	No	No
10	10	One	New	Yes	Rubber	Used	Filter	Yes	Yes	No	No	Yes	No
11	11	One	New	Yes	Rubber	Used	Filter	Yes	Yes	Yes	Yes	Yes	No
12	12	One	New	Yes	Rubber	Used	Filter	Yes	Yes	No	Yes	Yes	No
13	13	One	New	Yes	Latex	New	Filter	Yes	No	Yes	Yes	Yes	No
14	14	None		Yes	Rubber	New	Filter	Yes	Yes	No	No	Yes	Yes
15	15	None		No			No	No	No	No	No	No	No
16	16	One	New	Yes	Rubber	New	Filter	Yes	Yes	No	No	Yes	No
17	17	One	New	Yes	Rubber	New	Paper	Yes	Yes	No	No	Yes	No
17	18	One	New	Yes	Rubber	New	Filter	Yes	Yes	Yes	No	Yes	No
18	19	One	New	Yes	Rubber	New	Filter	Yes	Yes	Yes	Yes	Yes	No
19	20	Multy	Dirty	Yes	Rubber	Used	Filter	Yes	Yes	No	Yes	No	No
21	21	One	New	Yes	Neoprene	New	Filter	Yes	Yes	Yes	Yes	Yes	Yes
21	22	One	New	Yes	Neoprene	Used	Filter	Yes	Yes	Yes	Yes	Yes	Yes
21	23	One	New	Yes	Neoprene	Used	Filter	Yes	Yes	No	No	Yes	No
22	24	One	New	Yes	Neoprene	New	Filter	Yes	Yes	No	No	Yes	No

		Available Personal Protective Equipment						Mixing and Loading		Application		Cleaning	
22	25	One	New	Yes	Neoprene	Used	Filter	Yes	Yes	No	No	Yes	No
22	26	One	New	Yes	Neoprene	Used	Filter	Yes	Yes	No	No	Yes	No
23	27	One	New	Yes	Neoprene	New	Filter	Yes	Yes	Yes	Yes	Yes	Yes
23	28	One	New	Yes	Neoprene	Used	Filter	Yes	Yes	No	No	Yes	Yes
23	29	One	New	Yes	Neoprene	Used	Filter	Yes	Yes	No	Yes	No	No
24	30	One	New	Yes	Neoprene	New	Filter	Yes	Yes	No	No	No	No
25	31	One	New	Yes	Rubber	Used	None	Yes	No	Yes	No	Yes	No
26	32	One	New	Yes	Neoprene	Used	Filter	Yes	Yes	No	Yes	Yes	Yes
27	33	Multy	Dirty	Yes	Latex	New	Paper	Yes	Yes	Yes	Yes		
28	34	None		Yes	Rubber	Used	No	Yes	No	No	No	Yes	No
30	36	One	New	Yes	Rubber	New	Filter	Yes	Yes	Yes	No	Yes	No
30	37	One	New	Yes	Rubber	New	Paper	Yes	Yes	No	No		
31	38	One	New	Yes	Rubber	New	Filter	Yes	Yes	Yes	No	Yes	No
31	39	One	New	Yes	Rubber	New	Paper	Yes	Yes	No	No		

Table S.2.4. Exposure and risk assessment for all work-days in the Region of Lombardy Study

Subject ID	Work-day ID	External Body Exposure (mg)	Glove Exposure (mg)	Skin Exposure (mg)	Handwash (mg)	Total Skin Exposure (mg)	Body Risk (% AOEL sat.)	Hands Risk (% AOEL sat.)*	Total Risk (% AOEL sat.)
1	1	0,04	0,19	0,0011	missing	0,0011	0,0832%	0,0044%	0,0875%
2	2	0,04	0,11	0,0005	0,0615	0,0620	0,0371%	0,0005%	0,0376%
3	3	0,29	0,02	0,0011	0,0867	0,0878	0,1030%	0,0031%	0,1061%
5	4	0,91	0,56	0,0007	missing	0,0007	0,0633%	0,0024%	0,0656%
6	5	0,14	0,01	0,0025	0,0224	0,0249	0,2194%	0,0006%	0,2200%
7	6	1,45	0,01	0,0005	0,0312	0,0317	0,1145%	0,0005%	0,1150%
8	8	0,47	0,02	0,0044	0,1725	0,1769	0,4338%	0,0033%	0,4371%
9	9	0,05	0,01	0,0003	0,0267	0,0270	0,0316%	0,0004%	0,0320%
10	10	3096,14	0,52	0,0313	0,6509	0,6821	0,6947%	0,0277%	0,7225%
11	11	15,58	0,63	0,0007	0,2321	0,2328	0,0713%	0,0025%	0,0738%
12	12	0,16	0,37	0,0003	missing	0,0003	0,0337%	0,0006%	0,0344%
13	13	13357,77	0,00	0,0948	0,1430	0,2378	5,4232%	0,0014%	5,4246%
14	14	0,03	0,00	0,0055	0,0440	0,0495	0,4006%	0,0018%	0,4025%
15	15	0,40	1,19	0,5228	0,4464	0,9692	44,8116%	0,0068%	44,8184%
16	16	0,20	0,03	0,0139	0,5809	0,5948	0,9702%	0,0102%	0,9804%
17	17	5,61	0,17	0,0522	4,0233	4,0755	4,4215%	0,0935%	4,5150%
17	18	0,91	0,06	0,0020	0,3471	0,3491	0,1711%	0,0043%	0,1754%
18	19	6,24	0,60	0,0971	0,9150	1,0121	0,6345%	0,0647%	0,6993%
19	20	4,67	0,48	0,0038	0,1082	0,1120	0,0381%	0,0037%	0,0418%
21	21	0,71	5,68	0,0010	0,4656	0,4667	0,1287%	0,0091%	0,1378%
21	22	1,49	5,90	0,0494	0,2406	0,2900	0,2670%	0,0086%	0,2756%
21	23	0,16	0,37	0,0016	0,0653	0,0669	0,0517%	0,0011%	0,0528%
22	24	0,02	0,04	0,0001	0,0196	0,0197	0,0164%	0,0003%	0,0167%

Subject ID	Work-day ID	External Body Exposure (mg)	Glove Exposure (mg)	Skin Exposure (mg)	Handwash (mg)	Total Skin Exposure (mg)	Body Risk (% AOEL sat.)	Hands Risk (% AOEL sat.)*	Total Risk (% AOEL sat.)
22	25	0,50	0,00	0,0005	0,0393	0,0398	0,0457%	0,0009%	0,0466%
22	26	0,13	0,00	0,0004	0,0368	0,0372	0,0396%	0,0006%	0,0403%
23	27	0,87	0,09	0,0077	0,9314	0,9391	0,7165%	0,0186%	0,7352%
23	28	29,57	0,27	0,0644	1,3715	1,4360	3,2456%	0,0329%	3,2785%
23	29	0,12	0,00	0,0006	0,4250	0,4256	0,0630%	0,0059%	0,0689%
24	30	0,03	0,09	0,0001	0,0225	0,0226	0,0198%	0,0004%	0,0201%
25	31	0,93	0,07	0,0010	0,3107	0,3116	0,0799%	0,0052%	0,0851%
26	32	6,10	0,20	0,0532	4,7243	4,7774	1,5871%	0,1655%	1,7526%
27	33	0,12	0,02	0,0052	0,0501	0,0553	0,3483%	0,0010%	0,3493%
28	34	0,03	0,01	0,0169	0,1422	0,1591	0,5414%	0,0019%	0,5432%
30	36	0,02	0,01	0,0009	0,0681	0,0690	0,0809%	0,0011%	0,0819%
30	37	0,01	0,00	0,0007	0,0242	0,0249	0,1041%	0,0002%	0,1043%
31	38	0,11	0,00	0,0070	0,1313	0,1382	0,5340%	0,0019%	0,5359%
31	39	0,08	0,01	0,0007	0,0285	0,0292	0,1002%	0,0003%	0,1005%
Minimum	-	0,01	0,00	0,0001	0,0196	0,0003	0,0164%	0,0002%	0,0167%
Median	-	0,29	0,06	0,0016	0,1367	0,1120	0,1145%	0,0024%	0,1150%
Maximum	-	13357,77	5,90	0,5228	4,7243	4,7774	44,8116%	0,1655%	44,8184%

Supplementary Material S3 – R programming language code for simulating exposures and toxicity scores, and generating the Risk Assessment Scheme

Supplementary Material S.3.1. – Code for generating exposure and toxicity scores

```
#### Simulate data ####
# Base risk: 0.04245 (4.245 % AOEL)

# X axis (STEF values)
x <- numeric()
for (i in 0.1^seq(1, 7)) {
  x <- c(x, seq(i, 2 * (i/10), -i/10))
}

# Y axis (EXPOSURE points)
y <- seq(1, 100, 2)

# Make data frame
vecx <- numeric()
vecy <- numeric()

for (i in x) {
  for (j in y) {
    vecx <- c(vecx, i)
    vecy <- c(vecy, j)
  }
}

dfxy <- data.frame(vecx, vecy)

# Change the names of the data frame
names(dfxy) <- c("ToxScore", "ExpoScore")

# Calculate the STEF coeficient
dfxy$StefCoef <- 0.000133333 / dfxy$ToxScore

# Calculate risk for each ExpoScore and each ToxScore
dfxy$Risk <- with(dfxy, 4.245 * ExpoScore/ 100 * StefCoef)
```

Supplementary Material S.3.2. – Code for generating the Risk Assessment scheme

```
# ENGLISH - Closed and filtered tractor risk assessment

closedRiskPlotPHD <- ggplot(dfxy, aes(x = ToxScore, y = ExpoScore,
color = RiskRecF)) +
  geom_point(size = 7, alpha = 2/3) +
  scale_x_continuous(limits = c(0.0001, 0.0000001),
                     name = "Toxicity Score",
                     trans = reverselog_trans(10),
                     breaks = c(0.001, 0.0001, 0.00001, 0.000001,
0.0000001)) +
  scale_y_continuous(name = "Exposure Score",
                     minor_breaks = c(10, 30, 50, 70, 90),
                     breaks = c(0, 20, 40, 60, 80, 100)) +
  scale_color_manual(values = c("#01DF3A", "#FFFF00", "#FF8000",
"#FF0000"),
                     name = "Risk",
                     labels = c("Irrelevant (<33% AOEL)", "Probably
irrelevant (34-66% AOEL)", "Not irrelevant (67-100% AOEL)",
"Significant (>100% AOEL)"),
                     guide = guide_legend(reverse = TRUE)) +
  theme_bw() +
  theme(panel.grid.major = element_line(colour = "black", size =
0.7),
        panel.grid.minor = element_line(colour = "black", linetype =
"dotted"))
```

Supplementary Material S4 – Proposed point reductions based on the results of the Region of Lombardy study

<table>
<tr><td>Tractor Type</td><td colspan="9"><u>Closed and filtered</u></td></tr>
<tr><td>START SCORE</td><td colspan="9">100</td></tr>
<tr><td>Number of MIX</td><td colspan="5"><u>1</u></td><td colspan="4"><u>More than 1</u></td></tr>
<tr><td></td><td colspan="5">-65 pts</td><td colspan="4">-0 pts</td></tr>
<tr><td>Number of HW</td><td colspan="2"><u>1-2</u></td><td colspan="3"><u>More than 2</u></td><td colspan="2"><u>1-2</u></td><td colspan="2"><u>More than 2</u></td></tr>
<tr><td></td><td colspan="2">-11 pts</td><td colspan="3">-12 pts</td><td colspan="2">-0 pts</td><td colspan="2">-10 pts</td></tr>
<tr><td>Body protection</td><td><u>None</u></td><td colspan="3"><u>Multy</u></td><td><u>Mono</u></td><td><u>None</u></td><td colspan="2"><u>Multy</u></td><td><u>Mono</u></td></tr>
<tr><td>Total</td><td>-0 pts</td><td colspan="3">-15 pts</td><td>-20 pts</td><td>-0 pts</td><td colspan="2">-65 pts</td><td>-80 pts</td></tr>
<tr><td>Mix/Load</td><td></td><td colspan="3">7</td><td>10</td><td></td><td colspan="2">35</td><td>40</td></tr>
<tr><td>Application</td><td></td><td colspan="3">4</td><td>5</td><td></td><td colspan="2">15</td><td>20</td></tr>
<tr><td>Maintenance</td><td></td><td colspan="3">4</td><td>5</td><td></td><td colspan="2">15</td><td>20</td></tr>
<tr><td>Hand protection</td><td colspan="3"><u>No gloves</u></td><td colspan="2"><u>Gloves</u></td><td colspan="2"><u>No gloves</u></td><td colspan="2"><u>Gloves</u></td></tr>
<tr><td>Total</td><td colspan="3">-0 pts</td><td colspan="2">-2 pts</td><td colspan="2">-12 pts</td><td colspan="2">-13 pts</td></tr>
<tr><td>Mix/Load</td><td colspan="3"></td><td colspan="2">1</td><td colspan="2"></td><td colspan="2"></td></tr>
<tr><td>Application</td><td colspan="3"></td><td colspan="2"></td><td colspan="2"></td><td colspan="2"></td></tr>
<tr><td>Maintenance</td><td colspan="3"></td><td colspan="2">1</td><td colspan="2"></td><td colspan="2"></td></tr>
<tr><td>Respiratory protection</td><td colspan="3"><u>No protection</u></td><td colspan="2"><u>Mask</u></td><td colspan="2"><u>No protection</u></td><td colspan="2"><u>Mask</u></td></tr>
<tr><td>Total</td><td colspan="3">+3 pts</td><td colspan="2">-0 pts</td><td colspan="2">+5 pts</td><td colspan="2">-0</td></tr>
<tr><td>Mix/Load</td><td colspan="3">2</td><td colspan="2"></td><td colspan="2">3</td><td colspan="2"></td></tr>
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<tr><td>Maintenance</td><td colspan="3">1</td><td colspan="2"></td><td colspan="2">2</td><td colspan="2"></td></tr>
<tr><td>FINAL SCORE</td><td colspan="9"></td></tr>
</table>

Supplementary Table S.4.1. Proposed point values for risk assessment using the Risk Assessment Scheme

9. Personal Gratitude

I do not believe it is possible to mention and thank all the people who have been there for me during this 3-year PhD period, and have forever changed my life. Bellow is an attempt to do so...

I would like to express special gratitude to my tutor, ***Professor Claudio Colosio***, for finding a perfect research match for my interests, encouraging me to produce results, and for helping me grow as a research scientist, as a colleague, and even as a diplomat! Your endless motivation, strong will, and the power to overcome obstacles have been an inspiration in these three years.

My work would not exist without ***Professor Federico Maria Rubino***. Scientific discussions (not to say "fights") with you have been the source of the best breakthroughs I had. Thank you for destructing and reconstructing my ideas with just a few sentences and a good reference, for the respect you gave me, diplomacy, and all the work you selflessly did and do for others, including me.

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I could always count on ***Professor Gabri Brambilla***. Thank you for your spirit, tolerance, strong support, advice, and the sense of security you gave me in difficult moments. You have managed to improve my experience in Italy, and for this I will be forever grateful.

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I was lucky to spend 3 years working with incredible colleagues at the ***International Centre for Rural Health of the San Paolo Hospital***. Thanks to ***RaminTabibi*** and ***Ezra Mrema***, who helped me with their experience as foreigners in Milan, saved me from the civil servants at the Agenzia dell'Entrate, ASL, Poste Italiane and Questura, supported me the whole time as researchers, as (senior) PhD students, as friends. To ***Chiara Somaruga***, ***Giulia Rabozzi***, ***Maria Grazia Martinazolli*** for welcoming me into the family, for all you taught me, for the first aperitivos, and for helping me get to know Milan. To ***Francesca Vellere*** and ***Maryam Sokooty***, not only for being great colleagues but also acting as my mother and grandmother, making sure I eat enough, but not too much. To ***Lorenzo Fugnoli*** and ***Giorgio Vianello***, for living in the field with me, listening and responding to all my requests and complaints, even hosting me in Pavia. Thanks also to ***Iseballa Cucchi***, for being part of the family at least for a short while.

A special thank you to the boys who brought new life into our Centre, ***Massimiliano Mazzi*** and ***Patrick Cadeddu Martin***. Both of you have been incredible friends to me, at work and outside of work. Thank you for putting up with me, for the energy you brought to work every day, the jokes, the laughs. Another special thank you to our girls, ***Federica Masci*** and ***Lucia Zanini***. You made the work feel like fun, and improved our health (laughing and stretching is the best way).

Finally, it has been a great pleasure working with students of the School of Medicine, ***Ruggero Rizzo***, ***Gaia Varischi*** and ***Emanuela Bossi***, as well as students of the School of Prevention Techniques, ***Marco Zuffada***, ***Francesca Gatto***, ***Mauro Colosio***, ***Iolanda Melone***, ***Valeria*** and ***Giusy Di Lorenzo*** and ***Pietro Grillo Ruggieri***. I also have to mention the staff of the Occupational Health Unit of the San Paolo Hospital, ***Rosamaria Bentoglio***, ***Samantha Astro***, ***Teresa Costantiello***, ***Marcella Pedranzini***, and ***Giuseppina Rota***, as well as my colleagues from the Security and Prevention Service of the San Paolo Hospital, ***Simona Fortunato*** and ***Camilo Crespoli***.

My life in Milan would not exist without the friends who helped me start liking the city (at least a little bit) and miss Belgrade a little bit less. If I had to give special recognition to one person, it would be ***Roberto Felace***. After 3 years that I have known you, I still consider the day we met as my luckiest day in Milan. You have constantly been a great friend, and are probably the person with the largest heart I have ever met in my life. I would have to write another thesis to describe you, so I will just stop here. The only problem I have now is to figure out how to make you move to Belgrade. Of course, I can never forget the people I met thanks to you, that have accepted me like you did, as their brother. Thank you, ***Andrea Amato***, ***Luca Santoro***, ***Davide Samueli***, and ***Antonio Amato***!

When I arrived to the Ripamonti Residence it was a strange place, and I could not believe I will be able to spend 3 years there. The people I met made it became my home, and made me feel as part of something great. I am grateful to ***Domenico Losquadro*** (***Lo Squalo***) for being one of my first friends in Ripamonti. He introduced me to a guy that seemed his brother, ***Andrea Tomasi***, who turned out to become my brother, and one of my best friends in Italy. We cooked together, he taught me to make home-made pasta, came to Belgrade to taste the sweet life, and brought me to his little village to meet his family. Both of you have been the source of my energy while you were in Ripamonti. First year in Ripamonti would not be complete without the positivity of ***Daniela Perrone***, strange buttery cakes of ***Daria*** (***Dasha***) ***Solomakha***, a still unexplained pair of friends ***Aurelia Brogno*** and ***Mariafrancesca Colonnese***, so different but inseparable, another person with love for the whole world ***Dimitra Sota***. ***Diletta Pellegrini*** and ***Vanessa Cesari***, and of course my French roomate ***Romain Buclon***.

After some time I met ***Sonia Mendes***, my dietician (unsuccessful), ***Jonathan Heywood***, the wizard of economy and political sciences, and ***Claudio Argiolas***, who claims to study something called “musicology”, and ***Andrea Gennari***, the oil searching football player. The four of you have been real friends to me, in good and in bad, and have made me happy more times than anyone could remember. I can never thank you enough.

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10. About the Author

Personal Info	
Name and last name	Stefan Mandic-Rajcevic
Date and place of birth	1st May 1985, Belgrade, Serbia
Address	Via Muzio Attendolo Detto Sforza 6, 20141 Milan, Italy
Address (2)	Obilicev venac 21, 11000 Belgrade, Serbia
Phone numbers	+39 339 6670449 (Italy) +381 61 2299112 (Serbia)
E-mail	rajcevic_stefan@yahoo.co.uk stefan.mandic-rajcevic@unimi.it
ResearchGate profile	https://www.researchgate.net/profile/Stefan_Mandic-Rajcevic/
PubMed Publications	http://www.ncbi.nlm.nih.gov/pubmed/?term="Mandic-Rajcevic"[Author]

Education	
• Period	January 2011 – February 2014 (expected)
	PhD in Occupational Medicine and Industrial Hygiene at the University of Milan (Italy) Thesis title: "Exploring Novel Approaches to Pesticide Exposure and Risk Assessment – Exposure and Risk Profiles for a Safe Pesticide Use in Agriculture"
• Period	October 2004 – July 2010
	Medical School at the University of Belgrade
• Period	September 2000 – June 2004
	First Belgrade's High school – Science and mathematics group