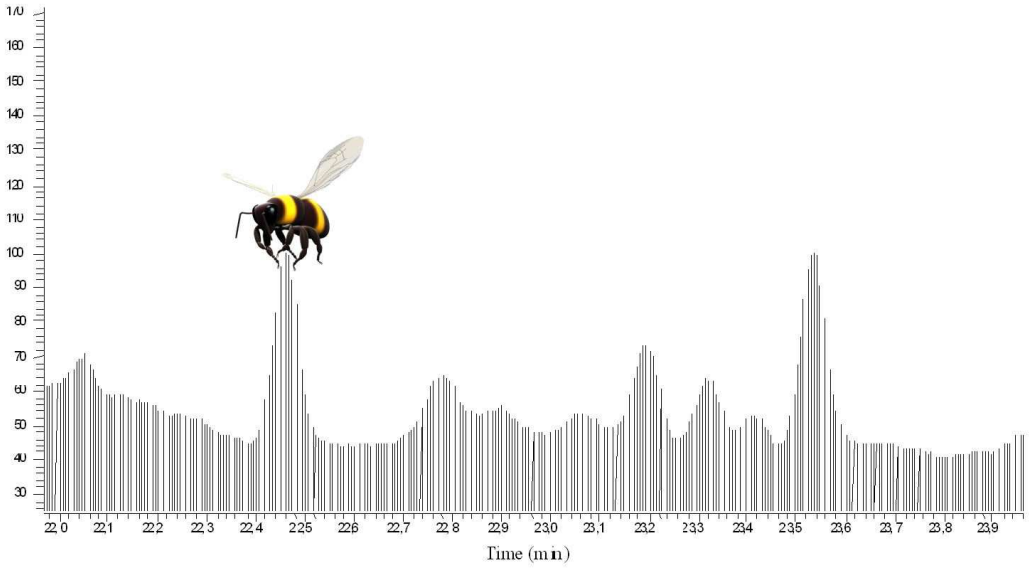
Plant protection product risk assessment:

Distribution and experimental validation in terrestrial ecosystems



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Plant protection product risk assessment: Distribution and experimental validation in terrestrial ecosystems

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CHAPTER I

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1.1 AGROCHEMICALS RISK ASSESSMENT IN THE EU

Risk assessment tries to estimate the probability of adverse effects to occur (Tarazona & Vega, 2002). Risk assessment process is complex because of the need a multidisciplinary approach. For this reason, for regulatory purposes, simplified approaches were developed. In EU, official procedures for environmental risk assessment are described in the Technical Guidance Document (TGD) in support of Commission Directive 93/67/EEC, Commission Regulation (EC) No 1488/94 and Directive 98/8/EC of the European Parliament and of the Council. Risk assessment guidelines for plant protection products are reported in the Annex VI of the Directive 91/414 EC, which contain the "Uniform Principles"; the harmonised criteria for evaluating products at a national level. All these risk assessment methods are based on a step-wise tiered procedures comprising: the effect assessment, the exposure assessment and the risk characterisation.

Effect assessment. The effects assessment comprises the following steps:

- hazard identification or identification of the effects of concern
- dose (concentration)/response (effect) assessment

The effect assessment is carried out through the extrapolation for each considered compartment (water, terrestrial and air) of the PNEC (Predicted No Effect Concentration), intended as the concentration below which an unacceptable effect will most likely not occur. The PNEC is determined dividing the lowest short-term $L(E)C_{50}$ or long-term NOEC (No Effect Concentration) by an appropriate assessment factor. The assessment factor reflects the degree of uncertainty in extrapolating from toxicity test data for a limited number of species to the “real” environment.

The adequacy and the completeness of toxicity data considered should be evaluated during the assessment. In some case (e.g. to predict the toxicity of chemicals with a non specific mode of action), specific methods for estimating properties of a chemical from its molecular structure, QSAR (Quantitative Structure-Activity Relationships), could be used to assist the evaluation of data.

Exposure assessment. In view of uncertainty in the assessment of exposure of the environment, exposure levels should be derived on the basis of both *measured data*, if available, and *model calculations*.
In case of measured data different criteria are available to determine their accuracy and reliability. The evaluation follows a stepwise procedure:

- evaluation of the sampling and analytical methods employed and the geographic and time scales of the measurement campaigns
- local or regional scenarios assignment of data taking into account the sources of exposure and the environmental fate of the substance
- comparison between measured data and calculated PEC.

In case of model calculation, the assessment is based on standardized scenarios at different scales (local, regional and continental). All potential emission sources need to be analysed, and the releases and receiving environmental compartments identified. Also, the fate of the substance once released to the environment needs to be considered taking into account biotic and abiotic transformation processes. The quantification of distribution and degradation of the substance (as a function of time and space) leads to an estimate of PEC at local and regional scale.
The FOCUS (FOrum for the Co-ordination of pesticide fate models and their Use) simulation models and scenarios for groundwater and surface water are examples of standardized models used in exposure assessment according to Council Directive 91/414/EEC.

Risk characterisation. For risk assessment purposes it is common to use quotients which combine exposure and effect in order to characterise risk. In the TGD quantitative risk characterization is calculated by comparing the PEC with the PNEC. Depending on the PEC/PNEC ratios, it is possible to:

- determine whether further information/testing may lead to a revision of these ratios;
- ask for further information/testing when appropriate;
- refine the PEC/PNEC ratio.

In **Figure 1.1** a general risk assessment outline is reported.

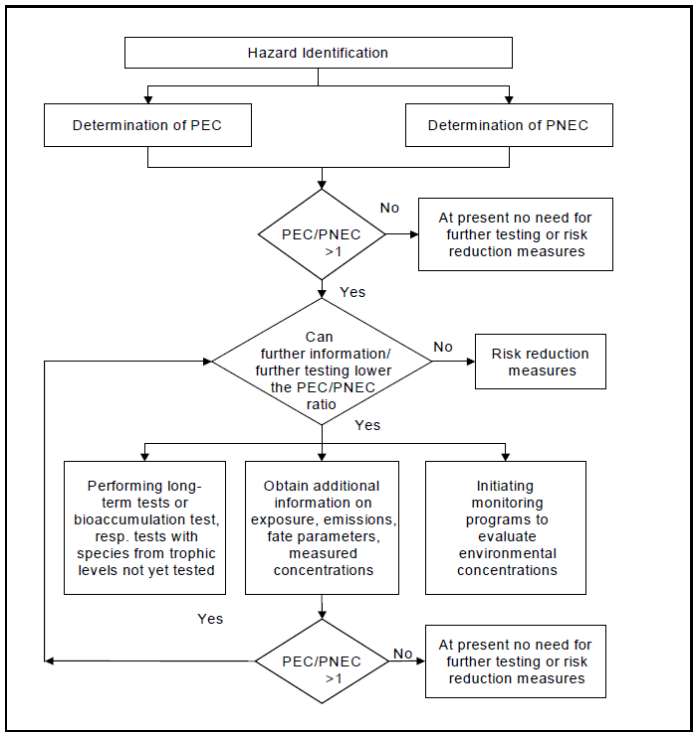

Figure 1.1: General procedure for environmental risk assessment (European Commission, 2003).

Within the framework of Directive 91/414/EEC risk characterisation approach is not uniform, currently, it uses TER (Toxicity Exposure Ratio) values for terrestrial vertebrates, earthworms and aquatic organisms along with HQ values for bees and beneficial arthropods and ETR (Exposure Toxicity Ratio) for terrestrial plants.

1.2 CRITICAL ISSUES

In official European procedures, specifically adressed to fulfil the requirements of chemical regulations, risk assessment is, generally, performed on more or less standardised scenarios, where the territory, at different scale levels is described without taking into account the spatial variability of parameters (Sala & Vighi, 2008).
These approaches represent a powerful tool to characterize potential risk and to rank chemicals in use, anyway results obtained with these approaches are no means truly representative of actual site-specific conditions and so are difficult to relate to the risk posed to real ecosystems (Vaj et al., 2009).

A site-specific approach, specifically addressed to aquatic ecosystems is proposed in the European Water Framework Directive (Directive 2000/60/EC).

For terrestrial ecosystems, as described in the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC and in explicit stepwise schemes of the European and Mediterranean Plant Protection Organization (EPPO), the risk assessment approach is the *targeted risk assessment*: the risk is individually characterized for each compartment and a simple process compare the PEC with the toxicity data for the species considered relevant for this particular compartment (Tarazona & Vega, 2002). Site specific risk assessment in this case is hampered by the complexity of terrestrial ecosystems.

In particular, the most critical step is exposure evaluation. While assessing exposure in these ecosystems specific behavioural and biological features of target organisms should be taken into account. The behaviour of organisms is quite different in epigeous and hypogeous ecosystems. Hypogeous organisms are exposed mainly to pesticides that reach the soil, epigeous organisms may be exposed directly or indirectly to pesticide and the matrices involved depend on their diet and behaviour. The scale of the assessment is, therefore, target-dependent: for hypogeous organisms the geographic unit for the assessment is the field whilst for epigeous organisms geographic unit could vary and depend on the forage area of the organism. Depending on the target *taxa* the most suitables exposure models should be selected. The difficulties in determining exposure in terrestrial organism is reflected in the not uniform risk characterisation approaches of EU guidelines.

In case of target organism with a relevant forage area, exposure assessment is impossible due to the variability of concentration in terrestrial environment from the treated field to the outside area. This is the case of pollinators, which feeeding area may reach some kilometers. In this case official procedure available (EPPO/OEPP, 2003) are based on the Hazard Quotient (the ratio between the application rate and an ecotoxicological endpoint), and not on actual exposure estimates. An Hazard Quotient approach based on data published in Candolfi et al. (2000) is suggested also of non target arthropods. In this case the HQ is calculated by dividing the crop-specific application rates (in-field exposure scenario) or drift rates (off-field exposure scenario) by the median lethal rate (LR50). In the HQ, the application rate represents a rough indicator of exposure. A realistic quantitative assessment of exposure is not performed.

1.3 STRUCTURE OF THE RESEARCH

The aim of this research was to analyses the main critical issues of agrochemicals risk assessment in terrestrial ecosystems. Particular attention was paid in exposure assessment at different scale levels. Starting from the field scale, different exposure models were applied in order to evaluate the pesticide mass balance in the specific case of vineyard. Official procedures were taken into account considering the FOCUS (2003) scenario prediction and the Ganzelmeier et al., 1995 studies. The foliage interception fractions reported in FOCUS (2003) were critically analysed with the support of experimental data.
From drift percents reported in Ganzelmeier et al., (1995) exposure in not target compartments (soil, vegetation) was evaluated. Predictive efficiency of the approach at a field scale was estimated starting from the specific cases of a vineyard-hedgerows and a vineyard-herbaceous strip systems
The adopted approach was up scaled in order to produce an exposure index for larger scales. The developed index was preliminarily validated and a sensitivity analysis was carried out. The exposure index was, then, integrated in a specific method developed to assess risk for pollinators. The developed procedure was applied in 13 field sites of the European ALARM (Assessing LArge scale environmental Risks for biodiversity with tested Methods) project. The validation of the entire procedure is now ongoing in collaboration with a research group of Reading University.
Predictive approaches application and validation were supported by agrochemical analytical methods development and validation in collaboration with a research group of the CSIC (IDAEA) of Barcelona.

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CHAPTER II

GC-MS determination of 10 insecticides (pyrethroids, organophosphates, organochlorines) in non crop leaves.

Abstract
The role of hedgerows in pesticide risk mitigation for ecosystems is underlined in different studies. Anyway the possibility of exposure to pesticide of hedgerows' organisms should be considered. An integrated approach based on sound analytical methodologies coupled with model predictions should be adopted to assess exposure for non target organisms. The aim of this work was to develop an analytical method based on pressurized liquid extraction (PLE), solid phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS) analysis to quantify ten commonly used insecticides in non crop leaves. Instrumental LODs obtained were comprised between 0.1 and 4 pg. The precision intra and inter-day was always below 10%. The methodology was applied to the determination of the selected insecticides in non crop leaves samples collected in two sites in North East Italy. In the natural area all the compounds searched where below the LOD whilst in the intensive area only chlorpyrifos was detectable. The levels found ranged between 0.030-0.171 µg/g Dry Weight (DW).

Keywords: leaves, non-crop, insecticides, GCB PLE, GC-MS

Barmaz, S., Diaz-Cruz, M.S., Vighi, M., Barceló, D., 2009. GC-MS determination of 10 insecticides (pyrethroids, organophosphates, organochlorines) in non crop leaves. Submitted to *J. of Agricultural and Food Chemistry*.

2.1 INTRODUCTION

Vegetated patches between fields may be exposed to pesticides because of direct drift. Direct drift is defined as the amount product that comes directly from the nozzles and is deflected out of the treated area by the action of air flow during the application process (Combellack et al., 1982; Hilbert, 1992; Carlsen et al., 2006). The amount of spray drift is independent from molecular properties of the active ingredients and depends rather on meteo-climatic conditions (e.g. wind speed, turbulence, temperature and humidity), application factors (e.g. sprayer type, nozzles type, release height and driving speed) and formulation (Carlsen et al., 2006). Exposure to pesticide residues, mostly insecticides, on plant parts may affect different taxa of beneficial arthropods such as bees. The official risk assessment procedures (OEPP/EPPO, 2003) to assess risk for pollinators are based, at least in the preliminary phases, on the calculation of the Hazard Quotient (the ratio between application rate and LD50 for *Apis mellifera*); any attempt to quantify actual exposure is not made, furthermore exposure on non crop vegetation is considered negligible. These issues underline the need of sound analytical methods to measure the amount of insecticide that reach vegetated patches between fields in order to define the role of these structures in exposure to pesticides.

During the productive season 2007 data on pesticide application were collected in the field sites of the Field Site Network (FSN) of ALARM project (Settele et al., 2005). Starting from data on pesticides load the most used insecticides were selected and included in the analytical method development. Insecticides used in studied agricultural situations belong to different chemicals classes (**Table 2.1**): organophosphorus (dimethoate, malathion, fenitrothion, chlorpyrifos and chlorpyrifos methyl), organochlorines (α e β endosulfan), pyrethroids (α cypermethrin, λ cyhalothrin and deltamethrin) and benzoylureas (flufenoxuron).

Up to the author's knowledge few are the studies on pesticide concentration on non-crop leaves in agro-ecosystems. Foliage, and in particular pine needles, have been extensively used as biomonitors for organochlorine contamination (Reishl et al., 1987; Calamari et al., 1994; Villa et al., 2003; Xu et al., 2004). In Barriada-Pereira et al., (2004) an analytical method to quantify 21 organochlorines pesticides in tree leaves was developed, optimizing different SPE clean up phases, and applied in tree species (*Castanea sativa, Corilus avellana, Juglans regia* and *Quercus robur*). A method to assess concentration of organophosphates (diazinon, chlorpyrifos, methidathion and their oxon) in pine needle compartments was proposed in Aston et al., (1996). Recently many papers were produced on analytical methods to determine concentration of different pesticides on leaves of different crop species, such as tobacco (Lee et al., 2008), Bengal gram

(Chowdhury et al., 2007), cranberry (Putnam et al., 2003), plum and cashew (Marco et al., 2006).
Methods for pesticide analysis in plant tissues were developed in the field of food control; for instance in (Tanaka et al., 2007) a simple one step extraction and clean up by PLE different class of pesticides in green leafy vegetables was proposed. The most critical step in method optimization in most of these works was the clean up step: in case of complex matrices, such as plants materials, the presence of interferences may obscure the analytical signal of studied compounds and commonly used phases seems not always adequate for vegetal materials (Barriada-Pereira et al., 2004).

The objective of the present work is to develop a method based on pressurized liquid extraction (PLE) as extraction technique, on solid phase extraction (SPE) for clean up, and gas chromatography-mass spectrometry (GC-MS) analysis for the determination of commonly used insecticides, selected starting from application data collected in the FSN of ALARM project as reported before, in leaves of different non crop species. The developed method was successfully applied to the determination of investigated active ingredients in non crop leaves collected in a natural area and an intensive agricultural area in North East Italy.

Table 2.1: Compounds under study and their physico-chemical properties (Tomlin, 2003).

Compound	MW g/mol	Molecular formula	CAS number	Vapor pressure mPa 20°C[1]	Henry's constant Pa/ m^3 mol	Solubility in water Mg/ l	Log K_{ow}
dimethoate	229.3	$C_5H_{12}NO_3PS_2$	60-51-5	0.25	1.42×10^{-6}	23.3×10^{3}	0.704
malathion	330.4	$C_{10}H_{19}O_6PS_2$	121-75-5	5.30	1.21×10^{-2}	145	2.75
fenitrothion	277.2	$C_9H_{12}NO_5PS$	122-14-5	$18.00^{(1)}$	9.42×10^{-2}	14	3.43
chlorpyrifos	350.6	$C_9H_{11}Cl_3NO_3PS$	2921-88-2	2.70	6.76×10^{-1}	1.4	4.70
chlorpyrifos-M	322.5	$C_7H_7Cl_3NO_3PS$	5598-13-0	3.00	3.72×10^{-1}	2.6	4.24
α endosulfan	406.9	$C_9H_6Cl_6O_3S$	959-98-8	0.83	1.48	0.32	4.74
β endosulfan	406.9	$C_9H_6Cl_6O_3S$	33213-65-9	0.83	0.07	0.33	4.79
α cypermethrin	416.3	$C_{22}H_{19}Cl_2NO_3$	97955-44-7	2.3×10^{-2}	6.9×10^{-2}	3.97×10^{-3}	6.94
λ cyhalothrin	449.9	$C_{23}H_{19}ClF_3NO_3$	91465-08-6	2×10^{-4}	2×10^{-2}	0.005	7.0
deltamethrin	505.2	$C_{22}H_{19}Br_2NO_3$	52918-63-5	1.24×10^{-5}	3.13×10^{-2}	$< 0.2\times10^{-3}$	4.6
flufenoxuron	488.8	$C_{21}H_{11}ClF_6N_2O_3$	101463-69-8	6.52×10^{-9}	7.46×10^{-6}	0.00152	4.0

[1] **At 25°C, for malathion at 30°C and for flufenoxuron and at 20°C for α cypermethrin , always in mPa**

2.2 MATERIALS AND METHODS

Chemicals and materials. High purity standards (purity > 95%) of all the target compounds and the Internal Standard (IS, PCB 30, 2.4.4'-trichlorobiphenyl. CAS no.38444-73-4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Three Surrogate Recovery Standards (SRS, dimethoate-D6, trans-cypermethrin-D6 and fenitrothion-D6) were selected between those available on the market (Dr. Ehrenstorfer, Augsburg, Germany) as the most representative of target compounds starting from the chemicals properties of each compound. Standard working solutions were prepared in n-hexane pesticide grade and stored at –20°C. The SRS were added before the extraction to each sample, the IS was added just before the analysis and used for the quantitative analysis. The organic solvents (n-hexane, acetone, acetonitrile, toluene, dichloromethane) used were pesticide grade. Glass-fiber filters used for the extraction in the stainless steel PLE extraction cells (33 ml) were from Dionex (Sunnyvale, CA, USA). The Hydromatrix, daily activated for 30 minutes by ultrasonication in acetone and dichloromethane, was supplied by Varian (Palo Alto, CA, USA). The neutral aluminum oxide (Alumina) used for the on-line clean up (daily activated at 150°C over night) was from Merck (Darmstadt, Germany).
As the on-line clean up was inefficient to remove the co-extracted interferences different phases and elution solvents for off-line solid phase extraction (SPE) were tested: Florisil SPE cartridges, 2 g (Isolute, International Sorbent Technology, UK); C18 (LiChrolut RP-18, 500 mg, Merck, Darmstadt, Germany) and Graphitized Carbon Black (ENVI Carb cartridges, 500 mg, Supelco, Bellefonte, PA, USA). High quality gases were used in drying and concentration steps (nitrogen) and gas chromatographic analysis (helium).

Sample preparation. For the method development, leaves coming from uncontaminated areas in North-Eastern Italy were used. Each foliage sample was collected during the productive season 2007, packaged in aluminum foils and preserved at –20°C upon arrival until analysis. After a bibliographic research freeze-drying was selected as drying technique: this technique permit the elimination of water in different kind of matrix with limited losses for relative non volatile compounds. Reported time for foliage lyophilisation found in literature was comprised between 8 h (Columé et al., 2001) and 96 h (Smirle et al., 2004). An intermediate lyophilisation time in our case was sufficient: frozen samples were freeze-dried for 48 h at –40°C at a pressure of 10^{-2} mBar.
Freeze-dried samples were then grounded in a commercial blender accurately cleaned with acetone; it is critical to enhance solvent extraction efficiency that the sample is reduced in fine powder. The samples selected for recovery studies were foliage of *Corylus avellana* and *Salix alba*, the

percent of water of these plant species was determined and ranged between 37% and 40%.

Extraction. Extraction was carried out using an ASE 2000 (DIONEX, USA) PLE system. The optimized extraction parameters were: 100° C, 1500 psi, 3 min of static time, 120 s of nitrogen purge and 60% flush volume. Different extraction conditions were tested in order to determine the best solvent and the number of extraction cycles. In a first series of recovery 3 g of freeze-dried sample were extracted with acetone in 33 ml stainless steel PLE extraction cells, filled with 5 g of Alumina to perform the on-line cleanup.
The upper empty space was filled with Hydromatrix. The obtained extracts resulted dark green coloured and very cloudy also after ultra-centrifugation (20 min, 12000 rpm). For this reason other recovery studies were carried out reducing the amount of extracted sample (1.5 g Dry Weight, DW), consequently the concentration of co-extracts, and testing as extraction solvent the mixture acetone: dichloromethane (50:50). For each extraction condition three samples spiked with 0.3 μg/g of target compounds and 0.1 μg/g of SRS were processed. The absence of contamination of the selected samples and of the system was confirmed with blank tests. To determine the optimal number of cycles of extraction, for each recovery series, the extract were collected in two phases: first two cycles and second the third cycle.

Clean up. One of the most critical steps in pesticide determination in complex matrices like biological ones seems to be the clean up procedure. In case of plant materials the presence of interferences like pigments, lipids and waxes may obscure the analytical signal of target compounds (Barriada-Pereira et al., 2004). Chlorophyll and carotenoids are typical co-extractants in vegetables matrices; these compounds are of low volatility and are not apparent interferences in GC-MS determination, but they may accumulate in the liner of the system causing problems in the transfer of analytes in the column and on peak shape (Mol et al., 2007). The need of an efficient clean up step is, therefore, crucial.
Different extracting materials and elution solvents for clean up were checked in order to evaluate which combination provided the better removal of co-extracts and higher recoveries:

- Florisil SPE cartridges, 2 g, conditioned with 20 ml of acetone: dichloromethane (80:20) and eluted with 20 ml acetone: dichloromethane (80:20)
- Florisil SPE cartridges, 2 g conditioned with 20 ml of acetone: hexane (80:20) and eluted with acetone: hexane (80:20)
- C18 conditioned with 10 ml of acetone and eluted with 10 ml acetone: dichloromethane (80:20)
- ENVI Carb (In this case 1 g of sample, extracted with acetone and processed as reported before, was used to test the clean up

methodology. The absence of contamination of the sample used was confirmed with blank tests) conditioned with 10 ml of acetonitrile and eluted with 10 ml of acetonitrile and 10 ml of acetonitrile: toluene (95:5), (modified from Amvrazi et al., 2006*)*;

- ENVI Carb conditioned with 10 ml of acetone and eluted with 10 ml of acetone and 10 ml of acetone: toluene (80:20)
- ENVI Carb conditioned with 10 ml of acetonitrile and eluted with 10 ml of acetonitrile and 10 ml of acetonitrile: toluene (80:20)

For each combination one trial was done directly loading into the cartridge a standard solution with a concentration of 0.5 μg/ml of each analyte.
The cleanup method with best recovery was selected for the purification of the PLE extracts. After the purification step all samples were taken to dryness, added with the IS (final concentration 1 μg/ml) and reconstituted in 1 ml of hexane.

Instrumental analysis. GC-MS method development was done starting from (Hildebrandt et al., 2007). In this work a multi-residues methodology to assess 30 widely used pesticides in groundwater and soil based either on SPE or PLE extraction procedures was developed. A good linearity was obtained over a concentration range of 0.005 – 0.750 μg / ml for nearly all the compounds. Instrumental limit of detection ranged from 0.5 and 5.7 pg.
The compounds investigated in (Hildebrandt et al., 2007) comprise a large amount of active ingredients and some of the target compounds of the present work (dimethoate, fenitrothion, malathion, chlorpyrifos) but any pyretroids compound was included. In the present study, the GC-MS method was optimized in order to include also three pyretroids (α cypermethrin, λ cyhalothrin and deltamethrin) commonly used in the control of a wide range of crop pests in relevant crops such as cereals, hops, potatoes, vegetables, oil seed rape, grapes, citrus and soybean (Tomlin, 2003). The GCMS analysis was carried out with a Finnigan Trace 2000 gas chromatograph coupled with a Trace 2000 MS system with an Electron Impact (EI) ionization mode and equipped with an AS 2000 auto sampler. The detector voltage was settled to 500 V. Compounds separation was achieved with a capillary column HP-5MS of 30 m×0.25-mm i.d. and a film thickness of 0.25 μm from J&W Scientific (Folsom, CA USA) with the following temperature gradients: initial temperature 60° C (holding 1 min) to 175°C at 12°C / min to 235°C at 3°C/min and finally to 310°C at 8°C / min (holding 10 min). Helium was used as a carrier gas with a flow rate of 1 ml/min and 5 min of solvent delay. The injection volume was 2 μl and the injection was achieved in a splitless mode with a splitless time of 0.8 min, the injector temperature was 280°C. A standard mixture of target compounds, SRS and IS was acquired in SCAN mode to selected the characteristic fragment ions of each compound using the NIST/EPA/NIH mass spectral library (NIST 98) to confirm them.

Samples acquisition was performed in the Selected Ion Monitoring (SIM) mode for improved sensitivity. Internal standard quantification was done automatically with Xcalibur software. In **Figure 2.1** a chromatogram of a standard solution acquired in SIM mode is reported. Between the selected fragment ions, one was used for quantification and three for identification purpose.

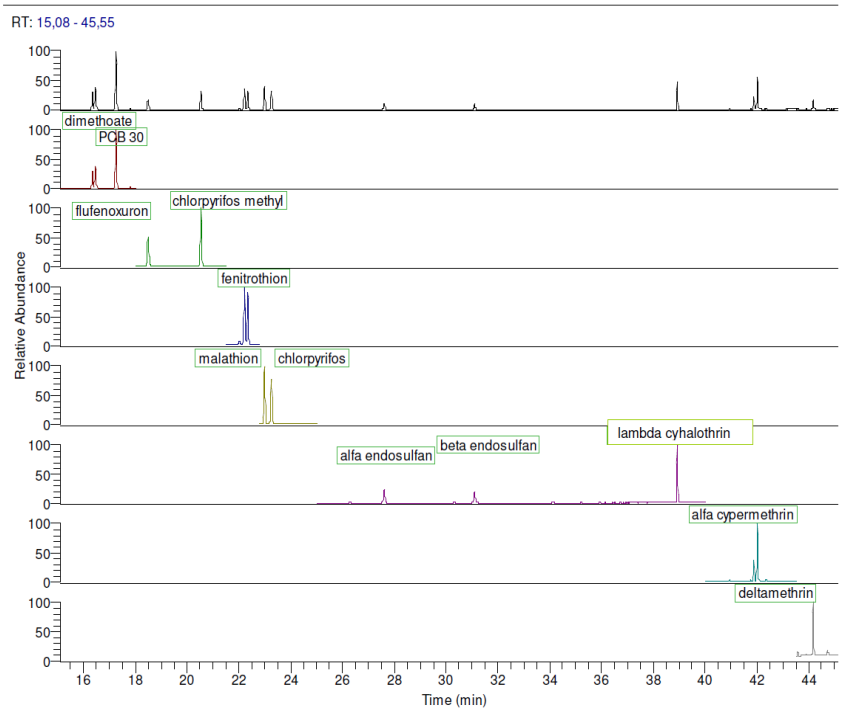

Figure 2.1: Chromatogram of a standard mixture solution (5 µg/ml) of the target compounds, IS and SRS acquired in SIM mode.

For the IS three characteristic ions were selected: one was used for quantification and two for identification purpose (**Table 2.2**)

Table 2.2: Selected fragment ions of studied compounds used for quantification (m/z_q) and identification (m/z_i) purposes.

Time window (min)	Retention time (min)	Compound	m/z_q	m/z_i	m/z_i	m/z_i
8.00-18. 00	16.35	dimethoateD6	99	87	131	235
	16.44	dimethoate	93	87	125	229
18.00-21.50	17.36	PCB 30	256	186	258	/
	18.59	flufenoxuron	331	268	333	488
21.50-22.80	20.63	chlorpyrifos methyl	286	125	197	321
	22.32	fenitrothionD6	131	115	266	283
22.80-25.00	22.44	fenitrothion	125	109	277	260
	23.09	malathion	173	125	127	330
	23.36	chlorpyrifos	314	197	199	351
25.00-40.00	27.73	α endosulfan	195	207	241	406
	31.22	β endosulfan	241	207	195	406
	38.99	λ cyhalothrin	181	197	141	449
40.00-43.50	41.93	transcypermethrinD6	169	133	181	421
	42.06	α cypermethrin	163	127	181	415
43.50-47.00	44.22	deltamethrin	253	181	209	505

Quantification. Quantification was carried out with the internal standard calibration method adding a known amount of IS (final concentration of 1 μg/ml) both to reference standard solutions and to the samples. PCB 30 was selected as IS because of its absence in the commercial mixtures of PCBs. SRS was added to control the losses of target compounds during extraction and clean up steps. The calibration curves for each analytes were constructed starting from eight standard solutions in n-hexane with progressive concentration: 0.01, 0.05, 0.1, 0.2, 0.5, 1, 2 and 5 μg/ml and the calibration range was evaluated on at least 6 points calculating the correlation coefficient of the curves.

Instrumental limit of detection and quantification of each compound was calculated on the basis of the 3:1 and 10:1 signal-to-noise ratio, analyzing the standard solutions at the lowest concentration levels. The limit of detection and quantification of the method was determined starting from matrix-matched standards at lowest concentrations levels taking into account the amount of sample extracted, the pre-concentration factor and the injection volume (2 μl). The instrumental precision was evaluated analysing one standard solution (0.5 μg/ml) several times during a day (intra-day precision) and in different days (inter-day precision) and evaluated by the calculation of the relative standard deviation (RSD).

2.3. RESULTS AND DISCUSSION

2.3.1 Clean up efficiency

C18 and Florisil SPE demonstrated in some case percent of recovery lower than 50% (chlorpyrifos, chlorpyrifos methyl, α endosulfan) and recoveries higher than 150% for pyrethroids, β endosulfan, flufenoxuron (**Table 2.3**). ENVI Carb between all the SPE phases tested, appeared to be the most efficient one for foliage clean up in terms of percent of recovery and of co-extractives removal. Different combinations of conditioning/eluting solvents were evaluated. In **Table 2.4** results of these trials are reported.
The use of toluene in the elution step is critical because GCB is well known to adsorb planar molecules, including chlorophyll and other pigments but also pesticides with planar functionality; toluene (typically 25%) is often added to the eluent to desorb these pesticides from the SPE column *(20)*.
As described previously three different clean up methods with Envi-Carb cartridges were tested:
1. conditioning with 10 ml of acetonitrile and elution with 10 ml of acetonitrile and acetonitrile: toluene (95:5);
2. conditioning with 10 ml of acetone and elution with 10 ml of acetone and acetone: toluene (80:20);
3. Conditioning with 10 ml of acetonitrile and elution with 10 ml of acetonitrile and acetonitrile: toluene (80:20).
Method 1 was tested on samples extracted with acetone and processed as reported before. The methods 2 and 3 refer to the percent of recovery obtained by loading a standard solution directly on the cartridges. It could be observed that flufenoxuron showed percents of recovery of 49-53 % when loaded directly on the cartridge but it was not detectable in the purified extract. These results demonstrate a loss of this compound during the extraction process. Methods 2 and 3 showed recoveries acceptable for most of the compounds (60-140%).

Table 2.3: Percent of recovery (%R) under different eluting (e)/conditioning (c) condition with different SPE phases. FL: FLORISIL®. LiC18: LiChrolut RP-1

Compound	SPE phase	Conditioning (c) solvent	Elution (e) solvent	V (c) ml	V (e) ml	% R
	FL	ace:hex 80:20	ace:hex 80:20	20	20	70
dimethoate	FL	ace:dcm 80:20	ace:dcm 80:20	20	20	75
	LiC18	ace:dcm 80:20	ace:dcm 80:20	10	10	68
	FL	ace:hex 80:20	ace:hex 80:20	20	20	nd
flufenoxuron	FL	ace:dcm 80:20	ace:dcm 80:20	20	20	69
	LiC18	ace:dcm 80:20	ace:dcm 80:20	10	10	>150
chlorpyrifos methyl	FL	ace:hex 80:20	ace:hex 80:20	20	20	40
	FL	ace:dcm 80:20	ace:dcm 80:20	20	20	41
	LiC18	ace:dcm 80:20	ace:dcm 80:20	10	10	nd
	FL	ace:hex 80:20	ace:hex 80:20	20	20	60
fenitrothion	FL	ace:dcm 80:20	ace:dcm 80:20	20	20	67
	LiC18	ace:dcm 80:20	ace:dcm 80:20	10	10	58
	FL	ace:hex 80:20	ace:hex 80:20	20	20	71
malathion	FL	ace:dcm 80:20	ace:dcm 80:20	20	20	104
	LiC18	ace:dcm 80:20	ace:dcm 80:20	10	10	50
	FL	ace:hex 80:20	ace:hex 80:20	20	20	38
chlorpyrifos	FL	ace:dcm 80:20	ace:dcm 80:20	20	20	48
	LiC18	ace:dcm 80:20	ace:dcm 80:20	10	10	nd
	FL	ace:hex 80:20	ace:hex 80:20	20	20	32
α endosulfan	FL	ace:dcm 80:20	ace:dcm 80:20	20	20	49
	LiC18	ace:dcm 80:20	ace:dcm 80:20	10	10	nd
	FL	ace:hex 80:20	ace:hex 80:20	20	20	143
β endosulfan	FL	ace:dcm 80:20	ace:dcm 80:20	20	20	>150
	LiC18	ace:dcm 80:20	ace:dcm 80:20	10	10	72
	FL	ace:hex 80:20	ace:hex 80:20	20	20	>150
λ cyhalothrin	FL	ace:dcm 80:20	ace:dcm 80:20	20	20	>150
	LiC18	ace:dcm 80:20	ace:dcm 80:20	10	10	133
α	FL	ace:hex 80:20	ace:hex 80:20	20	20	>150
cypermethrin	FL	ace:dcm 80:20	ace:dcm 80:20	20	20	>150
	LiC18	ace:dcm 80:20	ace:dcm 80:20	10	10	143
	FL	ace:hex 80:20	ace:hex 80:20	20	20	>150
deltamethrin	FL	ace:dcm 80:20	ace:dcm 80:20	20	20	>150
	LiC18	ace:dcm 80:20	ace:dcm 80:20	10	10	139

Table 2.4: Percent of recovery obtained with ENVI Carb: method 1. Conditioned with 10 ml of acetonitrile and eluted with 10 ml of acetonitrile and acetonitrile: toluene (95:5); method 2. Conditioned with 10 ml of acetone and eluted with 10 ml of acetone and acetone: toluene (80:20); method 3. Conditioned with 10 ml of acetonitrile and eluted with 10 ml of acetonitrile and acetonitrile: toluene (80:20). nd: not detected.

Compound	Method 1	Method 2	Method 3
dimethoate	107	60	62
flufenoxuron	nd	49	53
chlorpyrifos M	39	81	63
fenitrothion	45	62	67
malathion	61	84	92
chlorpyrifos	35	77	75
α endosulfan	24	103	76
β endosulfan	25	93	70
λ cyhalothrin	53	123	86
α cypermethrin	49	80	60
deltamethrin	54	104	76

2.3.2 Method validation.

PLE extraction and SPE clean up. In **Table 2.5** results of two different recovery studies are reported. In the first recovery trial, samples were fortified with 0.3 μg/g DW of standard solution; 3 samples were extracted with acetone 100% and 2 with a mixture of acetone and dichloromethane (50:50). In the second trial, samples were extracted with acetone 100% and two levels of concentration were tested (0.3 and 0.7 μg/g DW). In the first recovery series, the clean up was carried up with method 2 (elution with acetonitrile: toluene, 80:20) because it demonstrated percents of recovery sensibly higher than method 3 (elution with acetone: toluene, 80:20) anyway the obtained eluate was not sufficiently clear. After a second clean up step with method 2 a clearer eluate was obtained. For this reason the second recovery extracts were cleaned up directly with method 3.
According to the EU guideline (SANCO/10232/2006), the recovery percent should be comprised within 70-110%. The percent of recovery obtained in sample extracted with acetone were in some cases below 70% but the RSD was below the 15% in most of the cases. Furthermore, the recovery percents obtained for the two different levels of concentration tested were similar. The mixture acetone: dichloromethane demonstrated to be in most of the cases less efficient: recoveries below the 60% were obtained for α cypermethrin, deltamethrin, chlorpyrifos methyl and malathion. Endosulfan (α and β) could not be quantified because these compounds were present in

the samples used for the recoveries, probably because of a contamination of the selected samples.
In the first recovery series, different fractions of the sample (2 PLE-cycles + 1 PLE-cycle) were extracted and processed separately. In the last fraction, all the compounds were below the limit of detection, this fact underlined that two PLE cycles in this case were sufficient.
The second recovery study confirmed the results obtained in the first series with the exception of pyrethroids (α cypermethrin, λ cyhalothrin and deltamethrin); these compounds shown a drastic decrease of recovery percent (50%). This fact could be justified by a degradation of these compounds during the sample preparation and underlines the importance of using a SRS for correct pyrethroids quantification; trans-cypermethrin D6 appeared to be a good SRS for the pyrethroids in study as it shows the same behaviour of target compounds. The relative standard deviation was for most of the target compounds below the 15 %; only dimethoate and deltamethrin showed higher RSD in the second series of recoveries.

GC-MS analysis. The GC-MS method developed allowed a good chromatographic separation of the compounds in study. The SIM method permitted to search a maximum of 10 fragment ions in each chromatographic time window (**Table 2.2**).

Table 2.5: Recovery percent (R%) and in parenthesis the % Relative Standard Deviation (RSD) at different level of concentration (*l* = level: level 1 correspond to 0.3 µg/g DW, level 2 correspond to 0.6 µg/g DW) and with two different extraction condition (*s* = solvent: *a* is for acetone and *a:d* for acetone and dichloromethane 50:50). *n* is the number of trials.

Compound	Recovery 1				Recovery 2				Recovery 3				Recovery 4			
	n	s	l	R% (RSD)	n	s	l	R% (RSD)	n[1]	s	l	R% (RSD)	n	s	l	R% (RSD)
dimethoateD6	3	a	1	79(9)	2	a:d	1	84-86	2	a	1	84-89	3	a	2	85(25)
dimethoate	3	a	1	69(5)	2	a:d	1	61-64	2	a	1	68-68	3	a	2	64(16)
flufenoxuron	3	a	1	nd	2	a:d	1	nd	2	a	1	nd	3	a	2	nd
chlorpyrifos methyl	3	a	1	91(9)	2	a:d	1	53-57	2	a	1	84-89	3	a	2	80(4)
fenitrothionD6	3	a	1	141(7)	2	a:d	1	124-126	2	a	1	81-89	3	a	2	82(9)
fenitrothion	3	a	1	98(6)	2	a:d	1	78-86	2	a	1	62-62	3	a	2	67(5)
malathion	3	a	1	82(7)	2	a:d	1	50-57	2	a	1	69-70	3	a	2	66(5)
chlorpyrifos	3	a	1	84(10)	2	a:d	1	69-70	2	a	1	81-90	3	a	2	80(4)
α endosulfan	3	a	1	-	2	a:d	1	-	2	a	1	82-88	3	a	2	89(14)
β endosulfan	3	a	1	-	2	a:d	1	-	2	a	1	59-64	3	a	2	64(8)

[1] **In this case 3 replicates of the recovery trial were processed but all the compounds presented lower values for one of the trial, it was decided to considered only two trial since the recoveries percents at this level were yet tested in the first series**

Table 2.5: (continued)

Compound	Recovery 1				Recovery 2				Recovery 3				Recovery 4			
	n	**s**	**l**	**R% (RSD)**	**n**	**s**	**l**	**R% (RSD)**	**n[1]**	**s**	**l**	**R% (RSD)**	**n**	**s**	**l**	**R% (RSD)**
λ cyhalothrin	3	a	1	85(15)	2	a:d	1	66-67	2	a	1	39-48	3	a	2	44(8)
*trans*cypermethrinD6	3	a	1	84(6)	2	a:d	1	93-96	2	a	1	57-68	3	a	2	53(23)
α cypermethrin	3	a	1	63(4)	2	a:d	1	54-55	2	a	1	35-40	3	a	2	29(18)
deltamethrin	3	a	1	47(7)	2	a:d	1	47-52	2	a	1	23-26	3	a	2	18(16)

[1] In this case 3 replicates of the recovery trial were processed but all the compounds presented lower values for one of the trial, it was decided to considered only two trial since the recoveries percents at this level were yet tested in the first series

In **Table 2.6** quality parameters of the whole method are reported. For all the compounds the interday precision was evaluated injecting a standard solution of 0.5 μg/ml in 5 different days and evaluated with the relative standard deviation, for all studied compound the value of this parameter was below 10%. Intraday precision was evaluated by measuring a standard solution (0.5 μg/ml) 9 times during the same day, also in this case the relative standard deviation was very low (< 5%). The correlation coefficients of the calibration curves were comprised between 0.9955 and 0.9998.
The instrumental limit of detection achieved was comprised between 0.1 and 4 pg and similar or lower than those achieved by Hildebrandt et al. (2007) (for the same active ingredients between 2.1 and 3 pg). The limits of detection obtained for the whole method was compared with those obtained with other methodologies for multiresidues analysis in comparable matrices. In Obana et al., (2001) an analytical method for multi-residue analysis in fruit and vegetables had been developed using two different matrices (orange and spinach). The extraction techniques was based on homogenization of 20 g of samples with ethyl-acetate and clean up with two-layer column (graphitized carbon and water absorbent polymer). The quantification was carried out with a different detector (GC-NCI-MS and GC-FDP) depending on the target compound. The LOD of the method obtained were, with respect to that of the present work, lower for some compounds (α cypermethrin: 3 ng/g; deltamethrin: 2 ng/g and endosulfan α and β: 0.5 ng/g), comparables for dimethoate (20 ng/g) and higher for the other organophosphates (chlorpyrifos: 10 ng/g; chlorpyrifos-methyl: 10 ng/g; fenitrothion: 15 ng/g and malathion: 20 ng/g) and λ cyhalothrin (2 ng/g). In Tanaka et al., (2007) a method to determine six insecticides, a fungicide and an herbicide green leafy vegetables was developed. The LODs obtained for chlorpyrifos, chlorpyrifos methyl and malathion were of 3 ng/g. In Mol et al., (2007) the LOD for different pesticide in lettuce (similar to leaves) was lower for some compounds (deltamethrin: 14 ng/g; fenitrothion 3 ng/g and dimethoate 17 ng/g), comparable for α cypermethrin (8 ng/g) and α endosulfan (10 ng/g) and higher for the other insecticides (chlorpyrifos: 2 ng/g; β endosulfan: 20 ng/g and malathion: 5 ng/g).

Table 2.6: Quality parameters for the GC-MS based developed method

Compound	Precision (RSD%)		Sensitivity				Linearity	
	Intraday n=9	Interday n=5	LOD_i pg	LOQ_i pg	LOD_m ng/g FW	LOQ_m ng/g FW	C.Range (µg/ml)	(r^2)
dimethoate	2	3	2	7	26	88	0.05-1	0.9955
flufenoxuron	1	8	0.1	0.4	-	-	0.05-5	0.9998
chlorpyrifos M	1	3	0.1	0.5	0.1	0.2	0.01-2	0.9993
fenitrothion	1	5	1	2	6	20	0.01-2	0.9974
malathion	1	3	1	3	0.5	2	0.01-2	0.9987
chlorpyrifos	1	4	0.3	1	0.1	0.5	0.01-2	0.9981
α endosulfan	2	1	1	3	14	48	0.01-5	0.9987
β endosulfan	2	1	1	3	1	2	0.01-5	0.9986
λ cyhalothrin	2	2	0.5	2	0.5	2	0.01-2	0.9980
α cypermethrin	1	1	4	13	11	37	0.01-2	0.9980
deltamethrin	3	3	2	6	23	78	0.01-2	0.9980

[1] i = instrumental, m = method. The sensitivity was calculated for the method with best recovery percent for all the compounds.

2.3.3 Application of the method

Area of study. The developed method was applied to the determination of the selected pesticides in foliage samples coming from an intensive agricultural area (Meolo Basin) and a natural area (Upper Livenza Basin) of North East Italy. In particular, foliage of three different non crop species were collected (e.g. *Corylus avellana, Acer campestre, Edera elix*) from hedges at two different height (1 and 2 m) in three different points identified along the diagonal of selected areas of 4x4 km square. The sampling periods were established in function of the main insecticide application period. The sampling points were maintained in each sampling date. In **Figure 2.2** the position of the sampling areas Meolo field site and Livenza field sites is reported. In **Figure 2.3** the position of sampling point and vineyards (data collected in 2004 and 2007) is reported.

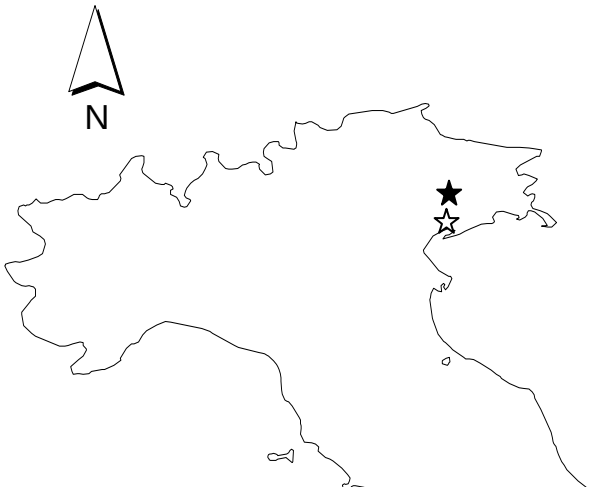

Figure 2.2 the position of the sampling areas Meolo field site (white star) and Livenza (Dark star) field sites.

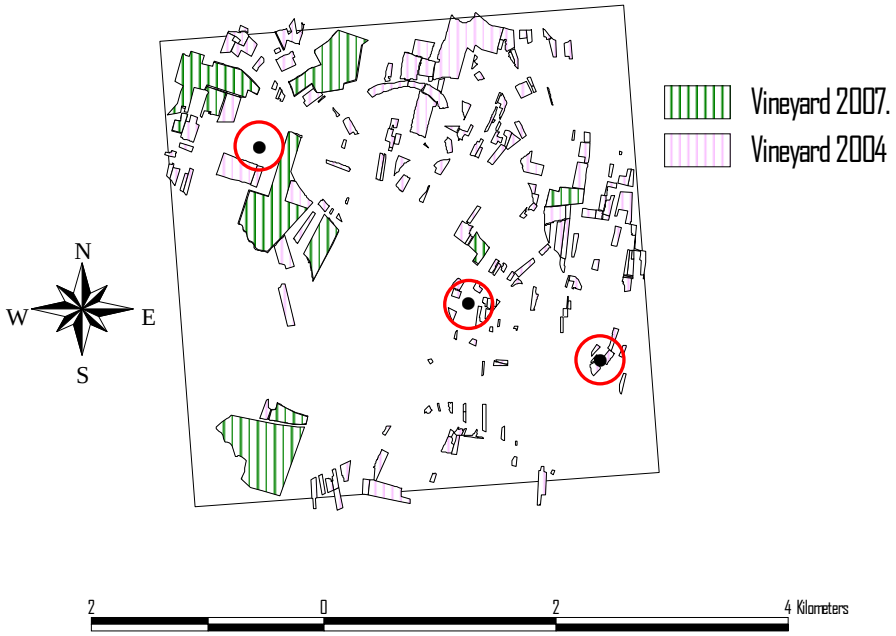

Figure 2.3: Meolo field site: position of sampling point and of vineyards (data collected in 2004 and 2007).

From the data on land use collected from the local consortium, the most used insecticide in the intensive site resulted to be chlorpyrifos. The unique crop applied with a relevant amount of insecticides was the vineyard. The surface area applied with chlorpyrifos was the 65 % of the total vineyard area (365 ha). The reference field site is located in natural area occupied mostly by wood and private fields at 50 km from the intensive site. Pesticide application in this area is negligible.
In **Table 2.7** the sampling period and position of collected samples in Meolo field and in Livenza field site are listed.

Table 2.7: Sampling periods and collected species, height (h) and coordinates of sampling points.

Field site	Data	Coordinates	h	Species	code
Meolo (agricultural)	16/04/2007	45°39'33.58" 12°26'10.00"	2	*Corylus avellana*	1.2
		45°39'51.99" 12°25'11.91"	2	*Acer campestre*	2.2
		45°40'36.98" 12°23'57.30"	2	*Edera elix*	3.2
	14/06/2007	45°39'33.58" 12°26'10.00"	1	*Corylus avellana*	1.1
			2		1.2
		45°39'51.99" 12°25'11.91"	1	*Acer campestre*	2.1
			2		2.2
		45°40'36.98" 12°23'57.30"	1	*Edera elix*	3.1
			2		3.2
	20/07/2007	45°39'33.58" 12°26'10.00''	1	*Corylus avellana*	1.1
			2		1.2
		45°39'51.99" 12°25'11.91"	1	*Acer campestre*	2.1
			2		2.2
		45°40'36.98" 12°23'57.30	1	*Edera elix*	3.1
			2		3.2
	09/07/2007	45°40'36.98" 12°23'57.30	1	*Edera elix*	3.1
			2		3.2
Livenza (reference site)	14/06/2007	46°01'13.76" 12°29'46.49"	2	*Corylus avellana*	1.2
		46°01'32.29" 12°29'13.06"	2		2.2
		46°01'20.68" 12°29'35.25"	2		3.2

Experimental part. The developed method was applied first on part of collected samples. for each sample two sub samples were processed and analyzed, and in some cases measured twice. As supported in (Anastassiades et al., 2003) "pesticides residues analysis using gas chromatography the quantification of certain pesticides is affected by the chromatographic response enhancement effect, (…) an improvement of the peak shape and intensity of affected compounds when they are injected in the presence of a complex matrix (…) not using matrix-matched standards is well established to provide erroneously high results ". The easiest way to avoid problems in quantification linked to matrix enhancement is to construct calibration curves with matrix-matched standards.

For this reason a series of blank extracts were prepared and fortified with target compounds in order to construct calibration curves for each compound. In this case too, the calibration range was evaluated using at least 6 points from eight standard solutions with progressive concentration: 0.01, 0.05, 0.1, 0.2, 0.5, 1, 2, and 5 μg/ml. In **Table 2.8** the parameters of the calibration curves obtained are reported.

Table 2.8: Calibration range (cr; μg/ml) and correlation coefficient of the calibration curves constructed with matrix-matched standards.

	dimethoate	**chlorpyrifos methyl**	**fenitrothion**	**malathion**	**chlorpyrifos**	**α endosulfan**	**β endosulfan**	**λ cyhalothrin**	**α cypermethrin**	**deltamethrin**
cr	0.01-5	0.01-5	0.01-2	0.01-5	0.01-5	0.01-5	0.01-5	0.01-5	0.05-5	0.01-2
r^2	0.9910	0.9998	0.9969	0.9993	0.9998	0.9995	0.9994	0.9983	0.9968	0.9931

During sample analyses a loss in sensitivity took place; the area of the internal standard and of the SRS dropped down. This fact caused some difficult in the active ingredients quantification. The use of matrix-matched standards demonstrated to be in this case conflictive; the calibration curves obtained with matrix-matched standards showed a very good linearity but the repeated injection of samples and matrix-matched standards caused probably an accumulation of matrix components in the liner of the gas chromatograph causing problems in the transfer of analytes in the column and on peak shape and a loss of sensitivity in the MS analyzer. The further analyses and internal standard based quantification procedures were done, therefore, using standard solutions prepared in n-hexane.
To obtain reliable results a third replicate of all the analyzed samples was processed and valuated. Furthermore, for a more complete picture, new samples were analyzed. In **Table 2.9** results of these analyses are listed. Reported concentrations are corrected on the basis of the percent of recovery obtained for the SRS; in this case fenitrothion-D6 appeared to reflect better than dimethoate-D6 and trans-cypermethrin D6 the behaviour of chlorpyrifos. All the pesticides included in the method were investigated at both sites.

In the natural field site all chemicals were below the detection limit confirming the reliability of this area as a reference site. In the intensive site, as expected, all the active ingredients searched were below the limit of detection with the exception of chlorpyrifos which concentration ranged between 0.030-0.171 µg/g DW. In **Table 2.9** only data for chlorpyrifos are reported

Table 2.9: Concentration of chlorpyrifos in analysed foliage samples. nd, not detected.

Field site	Period	Sample	Species sampled	µg/g DW
Meolo (agricultural)	16/04/2007	1.2	*Corylus avellana*	nd
		2.2	*Acer campestre*	nd
		3.2	*Edera elix*	0.030
	14/06/2007	1.1	*Corylus avellana*	0.094
		1.2	*Corylus avellana*	0.034
		2.1	*Acer campestre*	0.058
		2.2	*Acer campestre*	0.038
		3.1	*Edera elix*	0.064
		3.2	*Edera elix*	0.052
	02/07/2007	1.1	*Corylus avellana*	0.099
		1.2	*Corylus avellana*	0.061
		2.1	*Acer campestre*	0.044
		2.2	*Acer campestre*	0.032
		3.1	*Edera elix*	0.171
		3.2	*Edera elix*	0.075
	09/07/2007	3.1	*Edera elix*	0.042
		3.2	*Edera elix*	0.043
Livenza (reference site)	14/06/2007	1.2	*Corylus avellana*	nd
		2.2	*Corylus avellana*	nd
		3.2	*Corylus avellana*	nd

In **Figure 2.4** a chromatogram of a selected sample is reported.

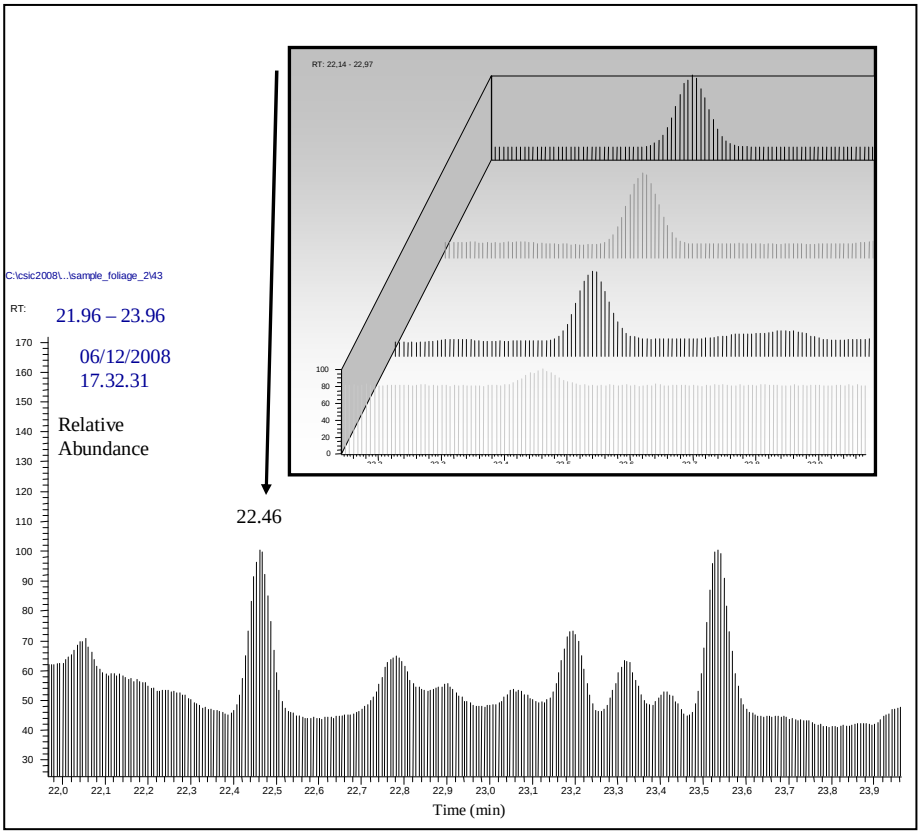

Figure 2.4: Enhanced figure of the chromatogram of a selected sample and fragments ions (m/z: 314, 197, 199 and 351) of chlorpyrifos in the same sample.

The concentration of chlorpyrifos was generally higher at 1 m of height than at 2 m. The distribution of pesticide in non target vegetation generally showed a vertical distribution trend linked to the application pattern. In vineyard-hedgerows systems at a height between 1 and 2 m above ground the concentration is nearly double of the mean (Otto et al., 2009); this means that just after the application the maximum concentration is reached at this height. Data on application were available only for biggest fields of Meolo Field site. In these fields the main application dates are concentrated in the period comprised between the 14th of May until the 27th of July.
The most relevant applications dates (considering the hectares applied) were the beginning of June (6th-8th June) and in the period comprised between the 16th and the 25 of July. Some application occurs later in the season. This may explain the absence of contamination in the samples of *Corylus*

avellana and *Acer campestre* collected in April and the following trend. The contamination of *Edera elix* in the first sampling date is probably linked to an exceptional application of chlorpyrifos in the area near to the sampling point; generally application of chlorpyrifos in vineyard occurs later in the season. The trend of the concentration did not show relevant differences in June and July sampling dates for both *Corylus avellana,* with a sensible increase from June to July and *Acer campestre* samples for which concentration in June and July are comparable. In the case of *Edera elix* a peak (the maximum concentration observed) the 2nd of July with a concentration about 6 times higher than in the first sampling date was observed at 1 m of height, a similar trend is observed at 2 m, after a week the concentration decreased to a level comparable to those observed in the first sample.

Since one of the goals of this study was to determine the background concentration of the main applied active ingredients in an intensive site, the sampling points are selected randomly and were independent from the field position. As expected, the results obtained are relatively homogeneous.

In conclusion, the developed method demonstrated to be efficient for the analysis of a wide range of insecticides in such a complex matrix like plant tissues even if some problems, actually common in complex matrix analysis, affected the analytical determination. The LOD reached were low and comparable to those reported in previous studies and the level of precision reached was high (inter and intraday precision calculated as % RSD always below 10%). The analysis of natural samples coming from an agricultural area permitted to obtain preliminary data on concentration of pesticides on foliage of non crop vegetation; this data will contribute, together with the analyses of samples coming form other sites to evaluate the role of non crop vegetation in exposure to insecticides for beneficial arthropods.

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CHAPTER III

Plant protection product exposure assessment at a small scale: Pesticide mass balance in vineyard

Exposure to pesticides for non-target arthropods may take place in the field (e.g. on crop or soil) or off-field (e.g. soil or non crop species). The accumulation of pesticides on field edge vegetation (vegetated strips or hedgerows) may determine risk for beneficial arthropods. The objective of this work is to describe the behaviour of insecticides in terrestrial ecosystems modelling the distribution on crops, soil, herbaceous species and hedgerows. Starting from Ganzelmeier drift predictions and FOCUS Surface Water Scenario, the fate of sprayed insecticides in and off-field was modelled considering experimental vineyards selected in an intensive agricultural area in North-East Italy. The predictions were compared with experimental data. A good agreement was find out for predictions off field even if some variability affects experimental data. In case of exposure in the field the foliage interception values estimated from experimental data (0.4-0.6) were generally lower than foliage interception reported in FOCUS Surface Water Scenario (0.7) for vineyard during full canopy stage.

Keywords: Drift, model, vineyard, exposure, foliage interception

3.1 INTRODUCTION

The complexity of the terrestrial environment requires that differences in behaviour and biology of target organisms, as well as different emission routes and environmental fate of pesticides have to be taken into account to assess exposure (Barmaz et al., 2008). The behaviour of organisms is quite different in epigeous and hypogeous ecosystems. Hypogeous organisms are exposed mainly to pesticides that reach the soil, epigeous organisms may be exposed directly or indirectly to pesticide and the matrices involved depend on their diet and behaviour. Then if exposure in soil organisms is, in most of the cases linked, to the amount of pesticide that reach the soil and could be predicted at a field scale, in epigeous ecosystems exposure evaluation is complicated by the fact that organisms move from natural to agricultural patches covering a larger scale (Barmaz et al., 2009).
An integrated approach based on sound models is needed in order to predict the distribution of pesticides from the field to the off- crops areas, as a starting point to assess exposure in terrestrial ecosystems at a larger scale and in more complex landscape scenarios. In **Figure 3.1** an outline of pesticide mass balance and references of methods to predict concentration in different compartments are reported.

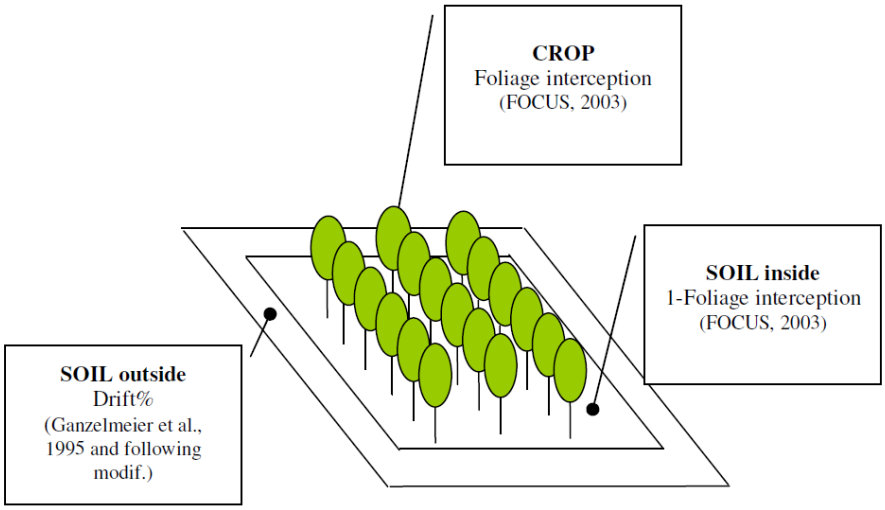

Figure 3.1: Scheme of the distribution of an insecticide in the vineyard.

The amount of pesticide that reaches the target is linked to the crop species, the phenological stage and the way of application; the amount of pesticide

intercepted by crop plants depends on the specific plant interception values. Interception is defined as the fraction of retained spray with respect to the delivered dose (Koch and Weisser, 2001). In the EU-procedure, under the EU-directive 91/414/EEC, it is stated that the concentration in environmental compartments should be predicted starting from models validated on Community level, in case of foliar interception the approach is based on data from Becker et al. 1999 and Van de Zande et al. 1999 (Linders et al., 2000). Harmonized plant interception values for different crop species are reported in the FOCUS Surface Water Scenario which aim is to "(…) assist in establishing relevant Predicted Environmental Concentrations (PECs) in surface water bodies which, in combination with the appropriate end points from ecotoxicology testing, can be used to assess whether there are safe uses for a given substance" (FOCUS, 2003).

Exposure in terrestrial ecosystems depends upon the concentration in different compartments; if potential exposure in the field for epigeous organisms depends on foliar interception, exposure outside the field depends on drift percent. Direct drift, defined as the movement of pesticide thought the air during application, is one of the main mechanism by which pesticides may reach off-crop areas. Spray drift was studied intensively in a series of studies by Ganzelmeier (1995) and Rautmann (2001). The results of this studies are currently in use in pesticide registration procedure in the EU (Wang and Rautman, 2008).

Exposure on non-crop vegetation is not considered in risk assessment schemes for some organism like honeybees (OEPP/EPPO, 2003), even if its role in exposure may be relevant. Recently models to evaluate the fraction of pesticide leaving the field by wind drift (Birkved et al., 2006) and to predict the fraction of pesticide intercepted by hedgerows (Lazzaro et al., 2007) were proposed.

In this work, starting from Ganzelmeier et al., (1995) and FOCUS (2003), the distribution of insecticides in different compartments was modelled using data collected in experimental vineyards. The predictions were compared with empirical data obtained from the analysis of specific tracers (chlorpyrifos and endosulfan) in leaves and soil samples collected during two productive seasons (2006 and 2008).

3.2 MATERIALS AND METHODS

3.2.1 Area of study

In order to evaluate exposure in and off field, two experimental vineyards were considered. During the productive season 2006 foliage samples were collected from two different hedgerows adjacent to a vineyard in North East Italy. Foliage samples (about 200 g fresh weight) were collected at both the sides of the hedgerow on two different transects at 3m of height (**Figure 3.2**). Collected samples were packaged in aluminium foils and stored at -20°C.

Samples were collected in different periods:

- before application (10th May 2006)
- after the main application dates (25th June and 18th July 2006).

The main application dates were:

- 15th May, 3rd June, 21st June 2006 (endosulfan)
- 11th July 2006 (chlorpyrifos).

Data on pesticide application (**Table 3.1 a** and **b**) and hedgerows distance from the field and width were collected directly in the field.

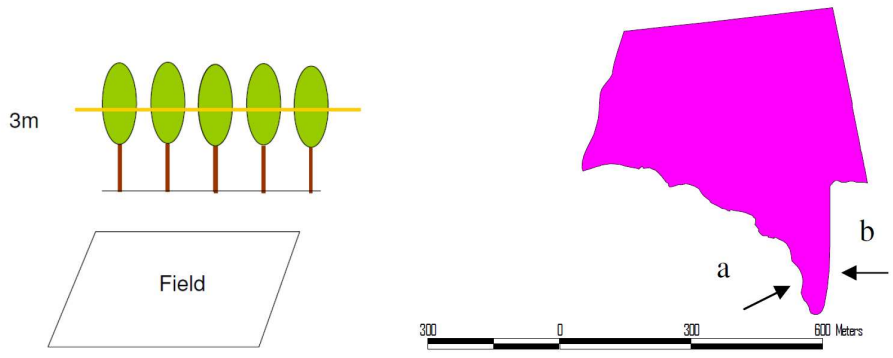

Figure 3.2: Position of the sampled hedgerows and sampling height .

Table 3.1: a) Distance from the field and width of the considered hedgerows, b) applied active ingredients and relative application rates.

a)

	Distance (m)	Thickness (m)
Transect A	7	6
Transect B	5	4

b)

	Application rate (g/ha)
chlorpyrifos	450
endosulfan	700

The distribution of pesticides inside the field was modelled starting from data collected in another experimental vineyard cultivated with two cultivars (*Pinot Grigio* and *Prosecco*). Foliages of *Vitis vinifera* were collected during the productive season 2008 just after a chlorpyrifos application (15th July) along two transects for each field from the last four rows. In **Figure 3.3** a map with the position of the vineyard and the transects is reported. The field owner provided an unique application rate (450 g/ha) for both the fields. Anyway the two cultivars were quite different in term of height and shape. The application rate was so re-calculated considering the row length (m) and the row height (m). The estimated application rates were 630 g/ha for *Pinot Grigio* and 253 g/ha for *Prosecco.*

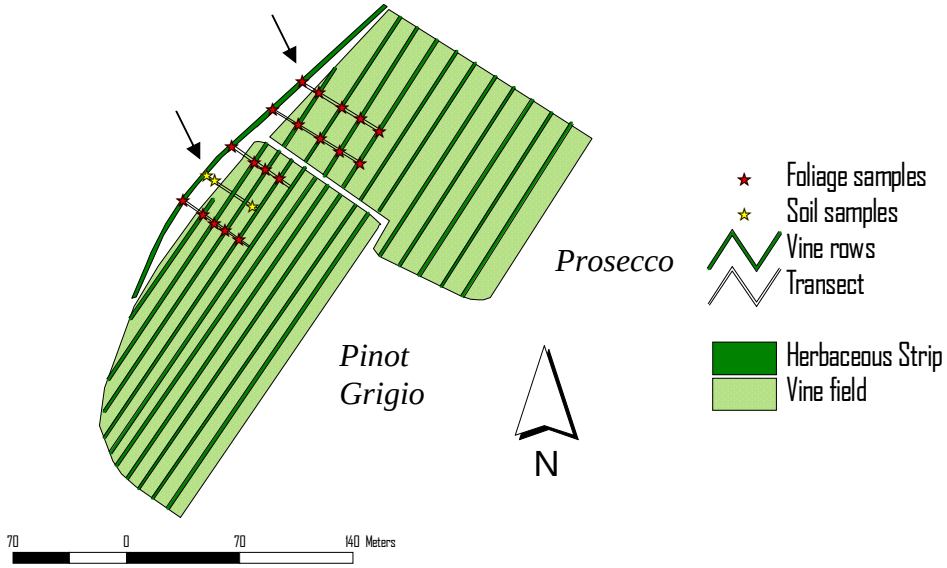

Figure 3.3: Experimental vineyard (productive season 2008). Red stars foliage samples (herbaceous and crop leaves), yellow stars soil samples (within vine rows, 4m from the field margin, 10m from the field margin), the arrows indicate the position of the herbaceous strip.

Different species of herbaceous plants were collected at the end of each transect from a vegetated strip along a ditch. In **Table 3.2** the main characteristics of the herbaceous strips are reported.

3.2: Characteristic of the herbaceous strips

	Distance (m)	Width (m)	Sampled species
Transect 1	9.0	1	*Phragmites communis*
Transect 2	10.5	1	*Urtica dioica* *Phragmites communis* *Fallopia convolvulus*
Transect 3	8.0	1	*Urtica dioica* *Phragmites communis*
Transect 4	8.7	1	*Phragmites communis* *Fallopia convolvulus*

In *Pinot Grigio* field also soil samples were collected. The sampling depth was comprised between 5-10 cm, samples were collected between the vine rows, at 4m from the field margin and at 10 m from the field margin. For each sampling point a pool of 3 sub-samples was prepared.

3.2.2 Exposure prediction

Exposure in the field. Exposure in the field may arise from contact with the soil or with the applied crop. The LAI or the soil cover determines to some extent the amount of substance intercepted by the crop and, therefore, the amount of pesticide that reach the soil is corrected for crop interception. In FOCUS (2003) for each crop, four interception classes are defined depending on the crop stage (**Table 3.3, Figure 3.4**). Starting from these values the fraction that reaches the soil is calculated as:

$$f_{soil} = 1 - f_{\text{int}} \qquad (\mathbf{3.1})$$

where f_{soil} is the fraction of the application rate that reach the soil and f_{int} the intercepted fraction (0.70 for vineyard in the considered sampling period, see **Table 3.3**) . From **Equation 3.2** the concentration on soil is calculated as follow:

$$C_{soil} = \frac{(AR * (1 - f_{\text{int}})) * 1000}{p * d} \qquad (\mathbf{3.2})$$

where C_{soil} is the soil concentration (μg/kg), *AR* is the application rate (mg/m^2), *p* is the depth reached by the pesticide (in this case the thickness of the sampling layer was between 5 and 10 cm), *d* the soil density (1500 kg/m^3, Finizio et al., 2001), 1000 conversion factor (mg to μg). The total amount that reaches the crop leaves could be obtained subtracting the total amount that reaches the soil (f_{soil}*AR*hectares applied) from the total amount applied.

$$a_{fol} = T - (f_{soil} * AR * S) \quad \textbf{(3.3)}$$

where a_{fol} is the total amount of pesticide that reaches the crop (g), *T* is the total amount of pesticide applied (surface area of the field-ha)**AR*-g/ha), and *S* is the surface area of the field (ha).

Table 3.3: Foliage interception fraction for vineyard (from FOCUS, 2003)

	no interception	**minimal crop cover**	**intermediate crop cover**	**full canopy**
BBCH-code [1]	00 – 09	10 – 19	20 – 39	40 – 89
Vines	0.4	0.5	0.6	0.7

[1] Indicative, adapted coding, the BBCH-codes mentioned do not exactly match (BBCH, 1994).

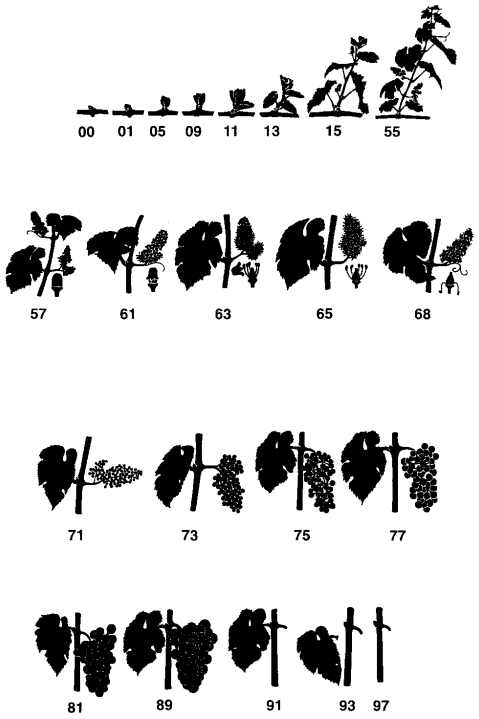

Figure 3.4: BBCH growth stages for grapevine (BBCH, 1994). 00-08 Stage 0: Sprouting/Bud development, 11-19 Stage 1: Leaf development, 53-57, Stage 2: Inflorescence emergence, 60-69 Stage 6: Flowering, 71-79, Stage 7: Development of the fruits, 81-89 Stage 8: Ripening of berries and 91-99 Stage 9: Senescence.

Exposure off field. The main mechanism by which pesticide may reach non-target areas outside the field is direct drift. The drift percent could be derived from Ganzelmeier et al. (1995) and Rautmann et al. (2001), studies, which are largely used in EU-pesticide registration procedures. This studies allow determining the concentration of pesticide in function of the distance of the field, the treated crop and its phenological stadium. The output of the Ganzelmeier predictions is a percent of drift (with respect to the application rate; **Table 3.4**).

Table 3.4: Drift % in function of the distance from the field margin (Ganzelmeier et al., 1995)

Distance (m)	Drift %[1]
1	-
2	-
3	7.5
4	-
5	5.2
7.5	2.6
10	1.7
15	0.8
20	0.4
30	0.2

[1] in % relative to the application rate in l/ha or kg/ha, for grapevine fields in late growth stage

These percents were interpolated with a curve in the form:

$$y = ax^{(-b)} \quad \textbf{(3.4)}$$

where y is the amount drifted off as a function of the distance from the field (x) and *a* and *b* are coefficient of the curve. The curve obtained for the vineyard is reported in **Figure 3.5.**

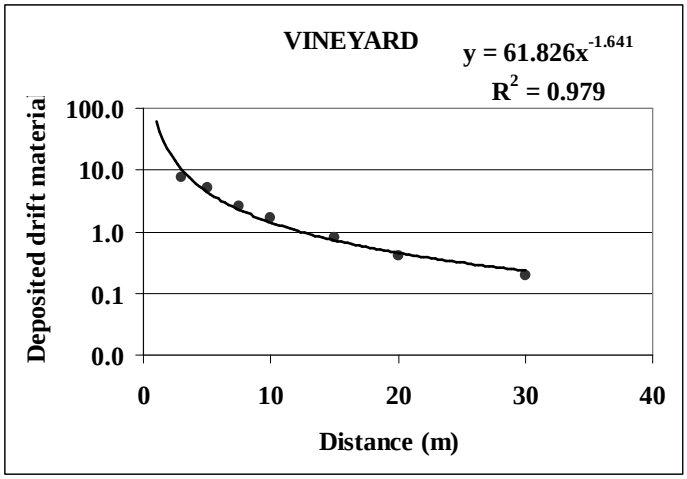

Figure 3.5: % of drift in function of the distance from the field margin derived Ganzelmeier et al. 1995.

The obtained equation was forced to have the maximum drift percent (100) when the distance from the field converges to zero:

$$y = \frac{a}{x^b + \frac{a}{100}} \quad \textbf{(3.5)}$$

For the vineyard, late growth stage, the following equation was obtained (**Figure 3.6**):

$$y = 12.409x^{(-0.897)} \quad \textbf{(3.6)}$$

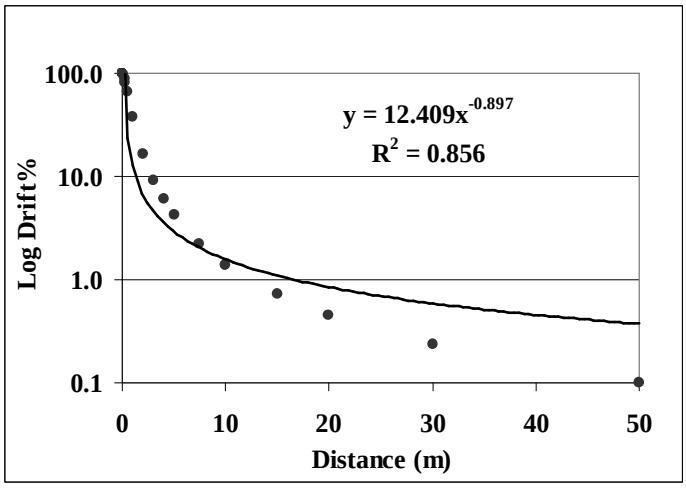

Figure 3.6: % of drift in function of the distance from the field margin, the curve is forced to 100 when the distance converges to 0.

The amount of pesticide that reached non-crop vegetation was calculated determining the amount of pesticide that reached the strip comprised between the side of the vegetation nearer to the field and the opposite side as represented in **Figure 3.7.**

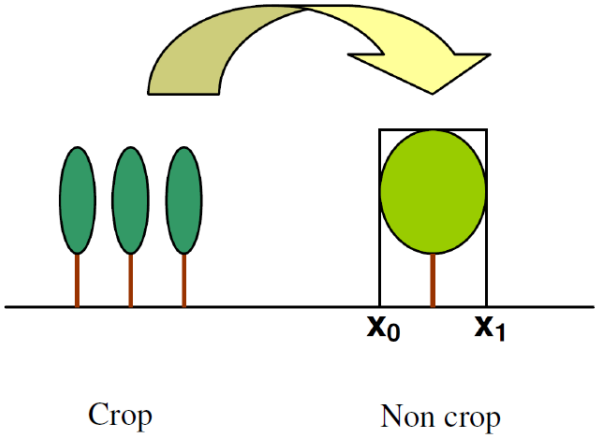

Figure 3.7: Schematic representation of the PEC determination in off field crop.

In particular, the total amount of pesticide that reaches a vegetated patch near a field is calculated integrating **Equation 3.6** from x_0 to x_1 **(**see **Figure 3.7).** The concentration on plant parts is calculated dividing the total amount of pesticide obtained by the foliage surface area calculated starting from the Leaf Area Index (m^2/m^2). The concentration was converted in a weight/weight concentration using the SLW (Surface Leaf Weight, the weight of a surface unit of foliage) In **Table 3.5** the reference for determining the LAI and SLW are reported.

Table 3.5: LAI (m^2/m^2) value and SLW (g/m^2, fresh weight) for herbaceous species and vineyard.

	LAI	SLW
vineyard	6[1]	203[4]
hedgerows	6[2]	208[5]
herbaceous species	1[2]-4[3]	173[6]

[1] Maximum LAI for Piacenza Scenario (FOCUS, 2001)
[2] Otto et al., 2009
[3] Otto unpublished data
[4] Fanizza et al., 1991
[5] Mean values obtained from literature
[6] Vile et al., 2005

In case of hedgerows foliage the sampling dates were not the same day of the application date. The decay of pesticide on leaves was, then, modelled using the empirical models to predict temporal decay of actives ingredients on plants parts reported in Leistra (2005).
The following mechanisms were considered (see chapter V for more details):

- The rate of **volatilisation**, described as a function of vapour pressure (Smit et al. 1998)

$$\log CV = 1.528 + 0.466 \log VP \quad (\mathbf{3.7})$$

where *CV* is the cumulative volatilization (% of initial mass on plant tissues) and *VP* the vapor pressure (mPa)

- **Photodegradation**: photodegradation rate (*Kph*, day^{-1}) may be calculated for DT_{50} taken from literature to predict the mass remaining after a specific time according to the first order kinetics:

$$m_2 = m_{1*} e^{(-kph*t)} \quad (\mathbf{3.8})$$

where m_1 is the initial mass on the plant and m_2 is the mass on plant (after photodegradation, mg).

- The wash-off by rain shower, described by the rule (FOCUS, 2003):

$$m_3 = m_2 * e^{-(W*T)} \quad (\mathbf{3.9})$$

where W is the foliar wash off coefficient (mm^{-1}), T the rain fall (mm), m_2 is the initial mass on plant and m_3 is the final mass on

plant. The foliage wash off coefficient is calculated as (FOCUS 2003):

$$W = 0.016 * Ws^{(0.3832)} \qquad (\mathbf{3.10})$$

where *Ws is the* water solubility (mg/l).

3.2.3 Exposure validation

Chemicals and materials. High purity standards (purity > 95%) of all the target compounds and the Internal Standard (IS, PCB 30, 2.4.4'-trichlorobiphenyl. CAS no.38444-73-4) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Dr. Ehrenstorfer (Augsburg, Germany). Standard working solutions were prepared in n-hexane pesticide grade and stored at –20°C. The organic solvents (n-hexane and ethyl acetate) used were pesticide grade. For the clean up Graphitized Carbon Black SPE (ENVI Carb cartridges, 500 mg, Supelco, Bellefonte, PA, USA) were selected. High quality gases were used in drying and concentration steps (nitrogen) and gas chromatographic analysis (helium).

Foliage samples

Sample preparation. In case of hedgerows, foliage samples, stored at -20°C from the sampling date till the analysis, were cutted finely and added with sodium sulphate anhydrous (granular, 12-60 Mesh J.T. Baker activated overnight at 100°C) before the extraction. In case of herbaceous and *Vitis vinifera*, samples were lyophilized for 48h before the extraction.

Extraction. Chopped samples were extracted with n-hexane for 45 minutes divided in three cycles (15 min each). In case of hedgerows samples 1 g of fresh sample was extracted, in the other cases about 2 g of fresh sample (0.5 g dry weight) were extracted. Before the extraction to monitor losses during extraction and clean up phases a Surrogate Recovery Standard (SRS) was added to each sample. In case of hedgerows foliage the adopted recovery standards were endosulfan-I-D4 and chlorpyrifos methyl, in case of vineyard and herbaceous leaves fenitrothion-D6 was selected as SRS.

Clean up. The clean up phase was carried up with a method developed starting from Barriada-Pereira et al. (2004): ENVI-Carb SPE conditioned with 5 ml of n-hexane, dried 30 min under a gentle nitrogen stream and eluted with 5 ml of a mixture 80:20 (volume: volume) of n-hexane and ethyl acetate.

Instrumental analysis. The GC-MS analysis was carried out with a gas chromatograph 6890N coupled with a mass selective detector 5973N with Electron Impact (EI) ionization mode. Compounds separation was achieved with a capillary column HT8-PCB of 60 m×0.25-mm i.D from SGE Analytical Science with the following temperature gradients: initial temperature 100° C (holding 1 min) at 30°C/ min to 280°C (holding 3 min) and at 10°C min to 300°C (holding 4 min) with 8 min of solvent delay, in case of herbaceous and *Vitis vinifera* samples. In case of hedgerows samples in which also endosulfan was a target compound the following gradient was adopted: initial temperature 120° C at 15°C/ min to 280°C (holding 4 min) with 12 min of solvent delay.
Helium was used as a carrier gas with a flow rate of 1 ml/min.
The injection volume was 1 µl and the injection was achieved in a splitless mode. A standard mixture of target compounds, SRS and IS was acquired in SCAN mode to selected the characteristic fragment ions of each compound (**Table 3.6**). Samples acquisition was performed in the Selected Ion Monitoring (SIM) mode for improved sensitivity.

Table 3.6: Fragment ions selected for the target compounds, the SRS (Surrogate Recovery Standards) and the IS (Internal Standard).

compound	m/z_1	m/z_2	m/z_3	m/z_4
chlorpyrifos	197	199	286	314
chlorpyrifos methyl (SRS)	125	286	-	-
α endosulfan	195	339	-	-
β endosulfan	195	339	-	-
endosulfan-I-D4 (SRS)	201	345	-	-
fenitrothion D6 (SRS)	115	131	266	283
PCB 30 (IS)	186	256	258	-

Quantification. The SRS were added before the extraction to each sample and the IS (PCB 30) added just before the analysis and used for the quantitative analysis. Quantification was carried out with the internal standard calibration method adding a known amount of IS (final concentration of 1 µg/ml) both to reference standard solutions and to the samples. Internal standard quantification was done manually with Enhanced Data Analysis ChemStation software (Agilent Technologies).
The recovery percent were for target compounds and SRS were between 70 and 110% with generally a relative standard deviation below 15 % for all the compounds as required in quality standards (SANCO/10232/2006).

Soil samples

Sample preparation. Soil samples, stored at -20°C were lyophilised for 48h before the extraction as reported for leaves materials.

Extraction. Freeze dried soils sub-samples (1-5 g) were extracted with ethyl acetate for 45 minutes divided in three cycles (15 min each). Before the extraction to monitor losses during extraction and clean up phases SRS (endosulfan-I-D4 and fenitrothion-D6) were added to each sample.

Clean up. The clean up phase was carried up as reported for leaves materials.

Instrumental analysis and quantification. The GC-MS analysis was carried out with a gas chromatograph 6890N coupled with a mass selective detector 5973N with Electron Impact (EI) ionization mode Compounds separation was achieved with a capillary column HT8-PCB of 60 m×0.25-mm i.d. from SGE Analytical Science with the following temperature gradients: initial temperature 120° C at 15°C/ min to 280°C (holding 4 min) with 12 min of solvent delay.
Helium was used as a carrier gas with a flow rate of 1 ml/min. The fragments ions for quantification purposes are reported in **Table 3.6.**
The recovery percents were checked for different levels of concentration obtaining a mean recovery of 105% (RSD=7%).

3.3 RESULTS AND DISCUSSIONS

Vineyard. In **Table 3.7** concentration of chlorpyrifos (µg/g dry weight) on crop leaves measured in *Pinot Grigio* and *Prosecco* are reported.

Table 3.7: Concentration of chlorpyrifos (µg/g dry weight) in *Pinot Grigio* and *Prosecco* samples collected in two transect (1 and 2) and 4 rows (1-4).

	Pinot Grigio		***Prosecco***	
Row	**1**	**2**	**1**	**2**
1	/	30.7	26.5	19.9
2	59.7	61.9	29.7	37.0
3	56.6	61.8	31.2	23.9
4	55.9	93.9	28.8	40.2

Generally, concentration of chlorpyrifos is higher in *Pinot Grigio* than in *Prosecco* leaves, this should be linked to the differences in the application

pattern. Within the same *cultivar* the concentrations are relatively homogeneous, with a mean value of 60±19 for *Pinot Grigio* and of 30±7 for *Prosecco* leaves. The variability could be linked to the pattern of application and to the position of the vines rows (contribution of the nearest row). From the mean values calculated for each *cultivar* the total amount intercepted by vine plants was calculated as reported in **Equation 3.11** and compared with predicted total amount intercepted estimated with FOCUS scenario foliage interception (0.70).

$$A_{fol.m} = \left[(\overline{m} * dw/fw) * SLW * 10^{-6} * (LAI * r * h)\right] \quad (\mathbf{3.11})$$

Where $A_{Afol.m}$ is the total amount intercepted (g), $\overline{m}$ is the mean concentration (μg/g dry weight), dw/fw the ratio between dry weight and fresh weight (evaluated for each sample weighting it before and after lyophilisation with a precision balance), SLW is the Surface Leaf Weight (g/m^2,**Table 3.5**), LAI the Leaf Area Index (m^2/m^2, **Table 3.5**), r the row length (m) and h the row height, 10^{-6} is the conversion factor (μg to g).
In this case the maximum LAI from FOCUS scenario "Piacenza" was used to calculate the mass (g) of pesticide intercepted by foliage. This may led to an over estimate (e.g. in Pergher et al. 1997, a LAI of 1.94 was reported for *Pinot Grigio* full canopy) for both the cultivars, mostly in case of *Prosecco* plant which were young plants of 1 m of height.

In **Table 3.8** the predicted and measured total amount (g) are reported.

Table 3.8: Total amount intercepted (g) and foliage interception (%) of vine plants predicted (from FOCUS, 2003) and calculated from mean concentration measured for each *cultivar*.

	Predicted	Measured	Amount applied	% Intercepted (FOCUS)	% Intercepted (from measured)
Pinot Grigio	1000.2	619.1	1428.9	0.7	0.4
Prosecco	367.5	335.1	525.0	0.7	0.6

In case of *Pinot Grigio* the amount intercepted obtained from analytical data is substantially lower than those predicted from FOCUS scenario. Consequently the foliage interception predicted from FOCUS is almost the double with respect to the measured one. In case of *Prosecco* cultivar the obtained interception fraction is similar but lower with respect to the predicted. As reported in Baldoin et al. (2008) the pesticide target loss during the application is still a problem. The maximum amount of spray recovery almost never exceed the 60% also when modern sprayers are in

use, with traditional blast sprayers only 15-35% of pesticide is placed on foliage.
The prediction of foliage interception play an important role in term of pest control, if the adequate dose is not intercepted pest control could be ineffective, but also in term of environmental pollution. For this reasons it is crucial to evaluate experimentally the foliage interception. In case of permanent crop these could be hampered by differences not only in seasonal morphology of the plant but also over years. Furthermore the presence of different kind of cultivars with sound differences in shape and morphology may determine variation in the intercepted fraction. Obviously also the equipment used for the application play an important role. As reported in Wang and Rauttman (2008), the nozzles type and the spray pressure have a percent of effect on drift, and consequently also on interception, respectively of 19.8 and 12.8 %.

Soil. In case of soil samples in the *Pinot Grigio* field, the concentration measured inside the field is almost double with respect to the concentration predicted, supporting the hypothesis that the plant interception fraction reported in FOCUS (2003) is not realistic in this specific case. In **Figure 3.7** the concentration on soil measured in samples collected in the field was compared with the concentration predicted starting from FOCUS scenario and concentration predicted from mean concentration on *Pinot Grigio* leaves samples.

$$C_{soil.e} = \frac{(T - A_{fol.m}) * 10^6}{s * p * d} \qquad (\mathbf{3.12})$$

where $C_{soil.e}$ is the concentration on soil (μg/kg d.w.) predicted empirically from the application rate and the experimental data on foliage, T is the total amount applied (g), $A_{fol.m}$ is the total amount intercepted calculated as reported in **Equation 3.11**, p is the depth reached by the pesticide (in this case the sampling depth 5-10cm), d the soil density (default value 1500 kg/m^3) and s the soil surface area (m^2) and 10^6 the conversion factor (g to μg). In this case the concentration is overestimated because it is assumed that all the pesticide that lose the target fall down on the soil. Actually part of the pesticide may reach non target area outside the field because of drift, in case of apple tree the fraction (F_{air}) that reaches air account for 0.1 (RIVM, 1998); considering that fruit crop generally show higher drift percent with respect to other crops typologies we could assume a comparable F_{air} for vineyard.

Considering that soil sampling depth was not precisely determined (between 5 and 10 cm) all calculation of soil concentrations were performed considering two depth scenarios and the data are reported as a range of concentrations. A high agreement between concentration predicted from foliage mean concentration (considering a soil depth of 5 cm) and measured concentration in soil could be observed (**Figure 3.7**). Also in this case the concentration predicted is affected (probably underestimated) by the LAI value used for calculating the total amount on leaves.

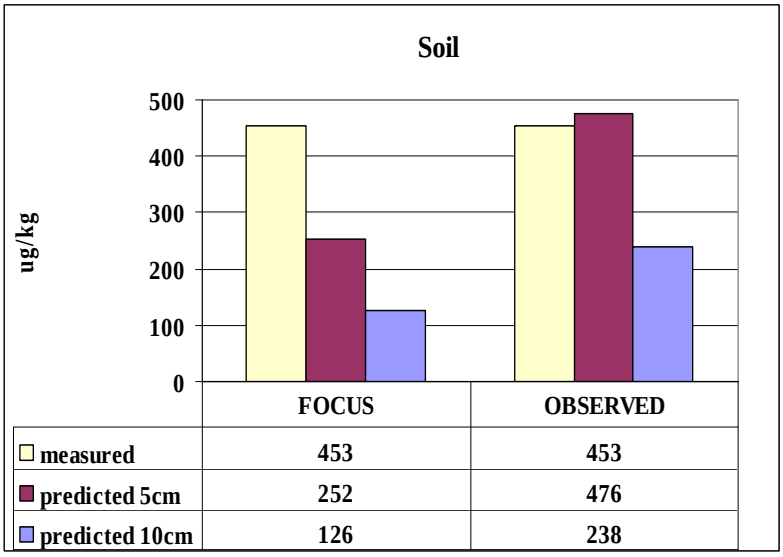

Figure 3.7: Concentration in vineyard (*Pinot Grigio*) soil samples; measured, predicted starting from FOCUS foliage interception (0.70) and from leaves (*Vitis vinifera*) mean concentration (considering a sampling range from 5cm to 10cm). All in µg/kg dry weight.

In **Figure 3.8** the predicted concentration in soil (µg/g dry weight) outside the field are compared with the measured concentration (µg/g dry weight). The predicted and measured concentrations outside the field show a good agreement at 4m. The prediction slightly underestimated the value at 10m.

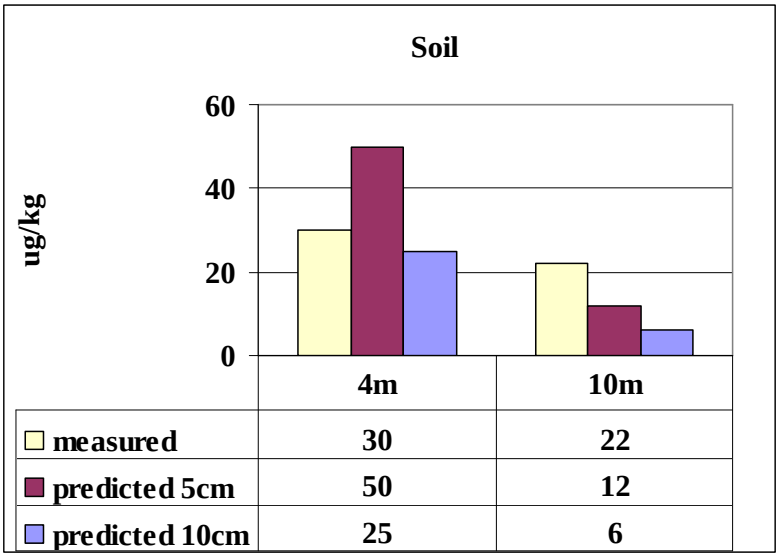

Figure 3.8: Concentration measured and predicted (considering a sampling range from 5cm to 10cm) outside the field (4 m and 10 m from the field margin). All in µg/kg dry weight.

Mass balance in the field. Starting from concentration measured on foliage samples and in soil, a mass balance for *Pinot Grigio* cultivar was carried out. The total amount of pesticide that reaches the soil was calculated considering two depth scenarios. Because of the imprecision of the sampling device a precise estimate of the sampling depth couldn't be made, as reported before. In **Table 3.9** results obtained are reported. A good agreement could be observed between the application rate predicted, in case of 5cm depth scenario, and application rate derived from farmer inquiry. The 10cm depth scenario overestimate the application rate, in any case the predicted plant interception is always substantially lower than those reported in FOCUS (2003).

Table 3.9: Chlorpyrifos mass balance in *Pinot Grigio* field. F_{int} = foliage interception, F_{soil} = fraction that reaches soil.

Soil[1] (g)	Leaves (g)	Soil+Leaves (g)	Rate (g/ha)	F_{int}	F_{soil}
1540[1]	619	2160	953	0.3	0.7
770[2]	619	1389	613	0.4	0.6
-	-	1429	630[3]	0.7[4]	0.3[4]

[1]10cm scenario
[2]5cm scenario
[3]Derived from farmer inquiry
[4]FOCUS (2003)

Non crop vegetation. In **Table 3.10** the concentration of chlorpyrifos predicted and measured in foliage of herbaceous species sampled are reported.

Table 3.10: Concentration of chlorpyrifos predicted and measured in herbaceous leaves, to predict concentration two LAI scenario were considered (1-4 m^2/m^2).

	Observed (µg/g dw)			Predicted (µg/g dw)
	Phragmites communis	*Urtica dioica*	*Fallopia convolvulus*	
TR. 1	12.8	/	/	6.7-26.8
TR. 3	8.1	1.0	/	6.7-26.7
TR. 2	4.7	1.8	3.7	2.1-8.5
TR. 4	2.6	/	1.2	2.5-10.0

In **Table 3.11** the concentration of chlorpyrifos and endosulfan predicted and measured in foliage of hedgerows are reported.

Table 3.11: Concentration of chlorpyrifos and endosulfan predicted and measured in hedgerows leaves (mean value of results obtained for both the hedgerows sides).

	Observed (µg/g fw)	Predicted (µg/g fw)
chlorpyrifos tr.A	0.08	0.09
chlorpyrifos tr.B	0.29	0.08
endosulfan tr. A	0.40	0.25
endosulfan tr. B	2.35	0.23

A relative good agreement between predicted and measured concentrations of pesticides on foliage can be observed. In case of herbaceous strip concentrations on *Phragmites* fall within the range of predicted concentrations as a function of LAI. The other plant species shows lower experimental values, anyway within one order of magnitude. The differences in plant and single leave morphology may have influenced the amount of pesticide deposited.
In case of hedgerows, the agreement is almost perfect in transect A, whilst in transect B predicted values underestimated endosulfan up to one order of magnitude. It must be considered that samples were taken about one week after application. Even if dissipation patterns on foliage had been considered, other mechanisms of distribution inside the hedgerows may have caused a distribution not homogeneous of pesticide on foliage. Therefore variability due to samples heterogeneity is also possible.

3.4 CONCLUSIONS

In this work a pesticide mass balance considering the main compartments involved in sprayed pesticides distribution at a field scale was carried out. Starting from available procedures, the distribution of some insecticides selected as tracers in experimental field was modelled and compared with analytical results. The results presented are preliminary and need further experimental effort in order to consolidate it, in any case the necessity of re evaluating the FOCUS (2003) foliage interception values comes from. In case of vineyard the seasonal changes in phenological stage could not be considered as the unique parameter to determine plant interception. Vineyard is a permanent crop that grows and changes over years, furthermore the high variability of the vines *cultivars* should be considered.
Pesticide risk assessment in terrestrial ecosystems suffers for the lack of suitable exposure assessment procedures for examples for hedgerows and natural patches. These structures represent the major source of biodiversity in agroecosystems. In this work the Ganzelmeier et al. (1995) approach was

modified and adapted to predict the amount of pesticide that drift off the field and reach non crop vegetation. The procedure is preliminary and need further validation. However, it represents a possibility for assessing pesticide exposure on non crop vegetation

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CHAPTER IV

Plant protection product risk assessment for pollinators I: exposure assessment and validation

One of the drawbacks in pesticide risk assessment for terrestrial ecosystems is the lack of procedures for assessing pollinator exposure. Therefore, the official risk assessment approaches are based on the Hazard Quotient (HQ) the ratio between the application rate (g/ha) and an ecotoxicological endpoint (LD_{50}, µg/bee). Exposure assessment in terrestrial ecosystems should start from data on the emission routes and environmental fate of pesticides but also to the behaviour and biology of target organisms. All these issues should be considered while modelling exposure in agroecosystems. The exposure matrices may vary as a function of the exposed target, its behaviour and life cycle. In case of pollinators, exposure take place mainly on vegetation. The fraction of pesticide that reaches non crop species is strictly linked to the applied crop, the formulation and application patterns. In this work a GIS based procedure to model exposure is proposed as a starting point to elaborate an exposure index function of the treated crop and the applied active ingredient. The elaborated index was preliminarily applied and validated in two experimental areas of 4x4 km located in North-East Italy. The predictive capability of the approach, as a function of the resolution of the data set, was assessed, in order to evaluate the possibility of up scaling, with a sensitivity analysis.

Keywords: Exposure, pollinator, index, terrestrial ecosystems

4.1 INTRODUCTION

Risk assessment tries to estimate the probability of adverse effects to occur. When assessing the potential effect of chemicals on ecosystems, the characterisation of risk involves the comparison of expected exposure level vs. the toxicity of chemicals to the exposed species, population and communities (Tarazona and Vega, 2002). European legislation requires that environmental risk assessment is carried out according to four main steps: hazard identification; dose (concentration)/response (effect) assessment; exposure assessment and risk characterisation. Different approaches are proposed for risk characterization: quantitative PEC/PNEC estimation and qualitative procedure. Exposure assessment should be based on both measured data, if available, and model calculations (European Commission, 2003).

Exposure assessment is a critical step in terrestrial ecosystems: chemical fate and transport in the environment determine the contaminant bioaccessibility whilst specie-specific natural history and behavioural traits play an important role in the likelihood that exposure pathways, from source to receptor, are complete (Smith et al., 2007). In terrestrial ecosystems the concentration of pesticides is distributed along a gradient from the treated field to the outside area and organisms may move and feed in areas with different concentrations of pesticides. For this reason exposure assessment should consider the specific behavioural issues of the target organism and not only the pesticide environmental emission and fate. As reported in Smith et al. (2007) a taxa-specific assessment is needed to evaluate exposure in terrestrial ecosystems. In case of pollinators, exposure to pesticide may arise from oral route (contaminated food ingestion) or from contact route (contact with contaminated plant tissues). The possibility of exposure depends on the attractiveness of plant species (presence of flowers). In the meantime, the distribution of pesticides in exposure matrices depends on the emission pattern which determines the Predicted Environmental Concentration (PEC) on target crop and non crop vegetation. A precise estimate (based on sound models) of concentration in the matrices involved in exposure in the forage area of pollinators (that could reach some kilometres) is impossible due to the variability of PECs on the territory. As a consequence, official procedures for risk assessment on pollinators (OEPP/EPPO, 2003) are based on the *ratio* between an application rate and an ecotoxicological endpoint (Hazard Quotient; $HQ=AR/LC_{50}$) without any quantitative assessment of exposure.

Up to the authors knowledge, there are a few studies on PEC estimation at a large scale in terrestrial ecosystems. A procedure to assess exposure specifically addressed to systemic insecticides is proposed in Halm et al. (2006). The PEC intended as the “amount to pesticide a honeybee might be

exposed to" is evaluated starting from known and validated concentration of active ingredient (in this case imidacloprid) in pollen and nectar considering five contamination ranges from low to high in function of the land use (location of the hive). In Villa et al. (2000) a tool for comparative screening of risk for pollinators based on physico-chemical properties, persistence and application rate was proposed and a comparison with Hazard Quotient was carried out. The necessity of a risk assessment method based on exposure estimates is underlined in both the approaches.
The aim of this work was to elaborate a semi-quantitative index to assess exposure in terrestrial ecosystems as a starting point to elaborate risk assessment procedures for epigeous terrestrial organisms with a relevant forage range area like pollinators. The elaborated index was applied and validated in experimental areas of 4x4 km located in North East Italy. A sensitivity analysis was carried out.

4.2 MATERIALS AND METHODS

Index development. The development of an exposure index for terrestrial ecosystems should be based on a conceptual model of the emission/exposure scenario. Specific behavioral traits of organism target of the assessment (e.g. feeding behavior, life cycle, behavioral characteristics) should be integrated to physico-chemical and emission data of considered chemicals, in order to establish the main exposure matrices. The geographic unit of exposure assessment depends on the maximum forage range of the considered target.
In **Figure 4.1** an outline of a general procedure to assess exposure in terrestrial ecosystems is reported.

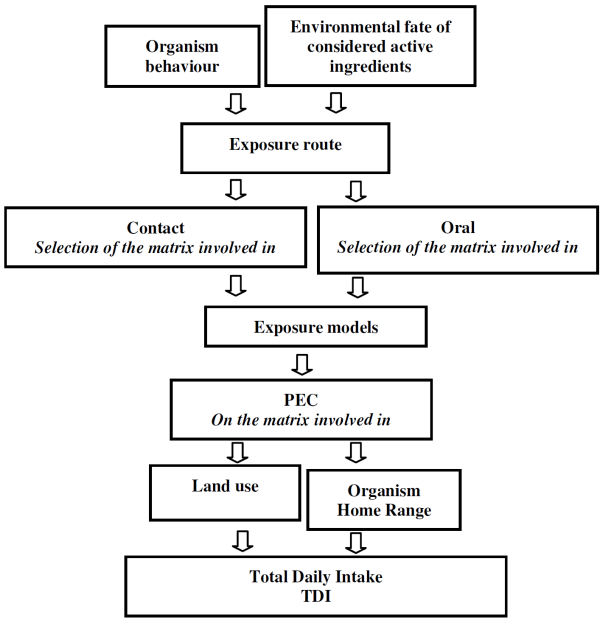

Figure 4.1: Outline of the exposure assessment steps for terrestrial organisms (Vaj et al., 2009, modif.)

In case of pollinators, plant tissues of species in bloom during pesticide application period could be considered as the main matrices involved in exposure. Application during flowering period is generally avoided, for this reason non target plant species could be considered as the main exposure compartment for pollinating insects. The main mechanism by which pesticides may reach non target areas in terrestrial ecosystems is *droplet drift*, defined as the fraction of spray carried off-target by the wind during application (Gauvrit, 1988; Vicari et al., 2001; Lazzaro et al., 2008).
Available drift models (Ganzemeier et al., 1995) permit to evaluate the amount of pesticide that reaches the field margin or the ditches within fields. These models could be adapted in order to predict the amount of pesticide that reaches vegetated areas at a field scale, as reported before (Chapter III). At a larger scale, because of the complexity of the landscape scenarios, the PEC could be predicted only in a semi-quantitative way considering the contribute in total exposure of all the fields in a specific area (**Figure 4.2**).

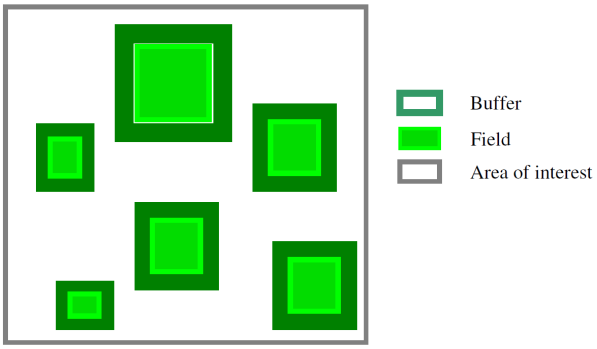

Figure 4.2: Outline of the index development assumptions.

In particular, exposure assessment on agricultural situation should start from the definition of the area out side the field affected by agricultural chemicals (*buffer area*). Once determined this parameter the total amount of pesticide drifted off in a specific area depends on different factors. Particularly the perimeter of the *field* determine the dimension of the buffer area and the applied crop determine the drift percent in function of the distance. The PEC in the entire area of interest is so calculated as the total amount of pesticide drifted off each field divided by the total surface area considered.
As reported before (Chapter III) for vineyard, the amount of pesticide that drift off a field could be described by a curve, derived from Ganzelmeier et al., 1995 data (**Table 4.1**).

Table 4.1: Drift percents with respect to the application rate, as a function of the distance from the field, from Ganzelmeier et al., 1995.

		Drift %	
Distance (m)	**Field crop**	**Vineyard**	**Fruit**
1	5.0	-	-
2	1.8	-	-
3	1.4	7.5	15.5
4	1.0	-	-
5	0.7	5.2	10.1
7.5	0.5	2.6	6.4
10	0.4	1.7	4.4
15	0.2	0.8	2.5
20	0.1	0.4	1.4
30	0.1	0.2	0.6
40	-	-	-
50	-	-	-

In **Figure 4.3** the curves obtained for three crop typologies (all in full canopy stage) are reported. It could be noted that, for comparable distances,

the drift percent is higher for fruit crop, followed by vineyard. Field crops determine the lowest drift percent. This is linked mainly to the structure of the considered crops and to the application pattern: tall crop generally determine higher drift percents for comparable distance.

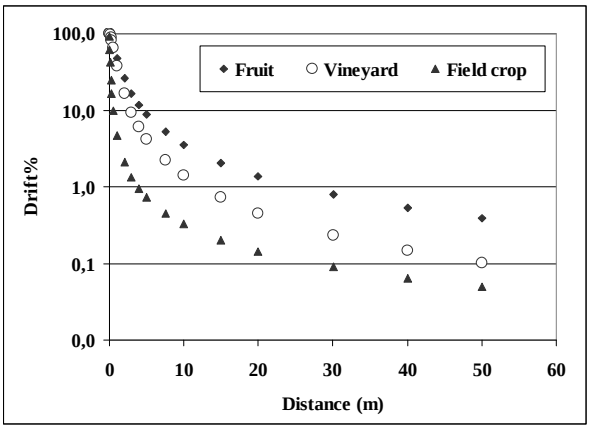

Figure 4.3: Drift % in function of the distance from the field and of the applied crop.

The Index was, then, elaborated as an algorithm (**Equation 4.1).** This algorithm permits to predict the mean concentration in an area starting from the application rate, the crop typology (fruit-vineyard-field crop), the field perimeter and area and a default LAI (Leaf Area Index, averaged value for herbaceous and hedgerows plants).

$$FC = \frac{\sum_{i=1}^{n}\left[AR \cdot \left(ax_{(0...50)}{}^{-b}\right) \div 100\right] \cdot 10 \cdot p_n}{St \cdot LAI} \qquad (\mathbf{4.1})$$

where FC_s is the foliage concentration (µg/cm^2), AR is the application rate (µg/cm^2), p_n is the perimeter of the field (cm), LAI (cm^2/cm^2) is the Leaf Area Index, S_t is the surface area considered (cm^2), n is the number of fields treated with a given active ingredient, x (m) is the distance from the field, 10 is the buffer width (cm) and *a* and *b* the coefficients of the curves (**Figure 4.3**) derived from Ganzelmeier et al. (1995), specific for each crop. typology. In **Table 4.2** the coefficients obtained for fruit, vineyard and field crop (all in full leaves stage) are reported.

Table 4.2: Coefficient *a* and *b* for three different crop typologies, derived from Ganzelmeier et al., 1995.

	Fruit	Vineyard	Field crop
a	19.481	12.409	3.340
b	0.708	0.896	0.973

FC_s can be transformed in weight/weight concentration (FC_w) by multiplying it by the Surface Leaf Weight (SLW: weight of a surface unit of foliage, cm^2/g).

Index application. The approach was applied in two 4x4 km field sites located in North East Italy with data collected during the productive seasons 2007-2008. These sites were selected in an intensive agricultural area (Meolo River basin). The main crop cultivated in these areas was vineyard. Data on land use and pesticide application were collected in both the site, anyway the level of resolution of the data set was different.
In 2007 it was not possible to obtain land cover maps and precise application data for all the field of the square. In **Table 4.3** the land use data for Meolo 2007 are reported.

Table 4.3 : Land use in Meolo Field Site (season 2007)

Land use	%
Maize	27.4
Soy	10.3
Vineyard	24.2
Wheat	10.3
Other	27.8

The most used insecticide was identified and selected as a tracer in order to apply the developed index. Chlorpyrifos was identified as the most used active ingredient in term of amounts and hectares applied (68% of the vineyard surface area were applied with this compound). In **Table 4.4** collected application data for chlorpyrifos are reported.

Table 4.4: Amount applied, surface area treated and main application period in Meolo Field Site in 2007.

Data	Amount (kg)	Surface area (ha)	Application periods
Not mapped	53[1]	103	25/06/2007-27/07/2007[2]
Mapped	80	133	17/05/2007-27/07/2007

1 From sale data
2 Derived from data on application in 2007

Also in the field site in study in 2008 one of the most relevant crop was vineyard (24% of the entire area). Data on pesticide application on vineyard were collected directly in the field during the productive season 2008. Position of each field was recorded and GIS based maps of the position of each field were produced using Arc View 3.1 software (ESRI). The amount of chlorpyrifos applied was determined starting from application data collected by farmers inquiry and sales data. Chlorpyrifos was the most used active ingredient both in term of hectares and amount applied **(Table 4.5)**.

Table 4.5: Amount applied, surface area treated and main application period in Meolo Field Site in 2008.

Data	Amount (kg)	Surface area (ha)	Application periods
Not mapped	63[1]	153	11/07/2008-09/08/2008[2]
Mapped	47	73	06/06/2008-08/09/2008

1 From sale data
2 Derived from data on application in 2008

Concentration of chlorpyrifos on foliage was predicted with **Equation 4.1** considering a default LAI value (3.5 m^2/ m^2, from Otto et al., 2009) and a mean SLW (0.02 m^2/g DW; Surface Leaf Weight; mean value from literature). The field perimeter and surface area was measured with Arc View 3.1 (ESRI). The vineyard patches were assumed as squares, for each patch, the buffers were constructed as reported in **Figure 4.4**.

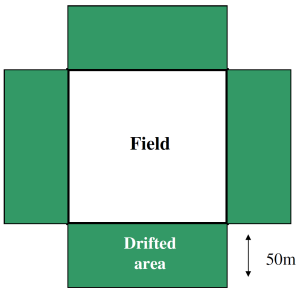

Figure 4.4: Outline of the drifted area determination for each field.

From **Figure 4.4** it is possible to see that exposure area is approximated and that part of the buffer is excluded from the calculation, considering that application pattern generally follow the crop rows, this approximation could be considered realistic.
In case of 2007 data a mean concentration in all the field site was evaluated starting from prediction in 2x2 km squares. In case of sales data the distribution of vineyards was assumed to be homogeneous in the four (2x2 km squares); 26 ha of vineyard were assumed to be in each 2x2 square. For 2008 the average concentration was determined in squares of 1x1 km. A "margin effect" was considered in both the cases by subtracting the parts of the buffer that fall out of the surface area assessed. From data collected in 2008 a sensitivity analysis was carried out. In order to assess the better resolution of the dataset in term of predictive accuracy of the approach, three different hypothetic data sets were simulated:

- percent of vineyard coverage on 1x1 km squares
- percent of vineyard coverage on 2x2 km squares
- percent of vineyard coverage on 4x4 km squares

In all the cases two main application dates, derived from actual application data, were considered:

- 11th July 2008
- 8th August 2008

An average application rate calculated from the application rates colleted in the field (510 g/ha) was considered. Temporal trend of concentration was predicted as reported in Leistra (2005). The concentration of chlorpyrifos was predicted in both the cases (2007-2008) for the sampling dates.

Index validation. In order to validate index predictions, samples of non crop species were collected in the productive seasons 2007 and 2008. In 2007 leaves samples were collected along the diagonal of the square in three point at 1 and 2 m of height (**Figure 4.5**; for more details on sampling method and analytical method see Chapter II).

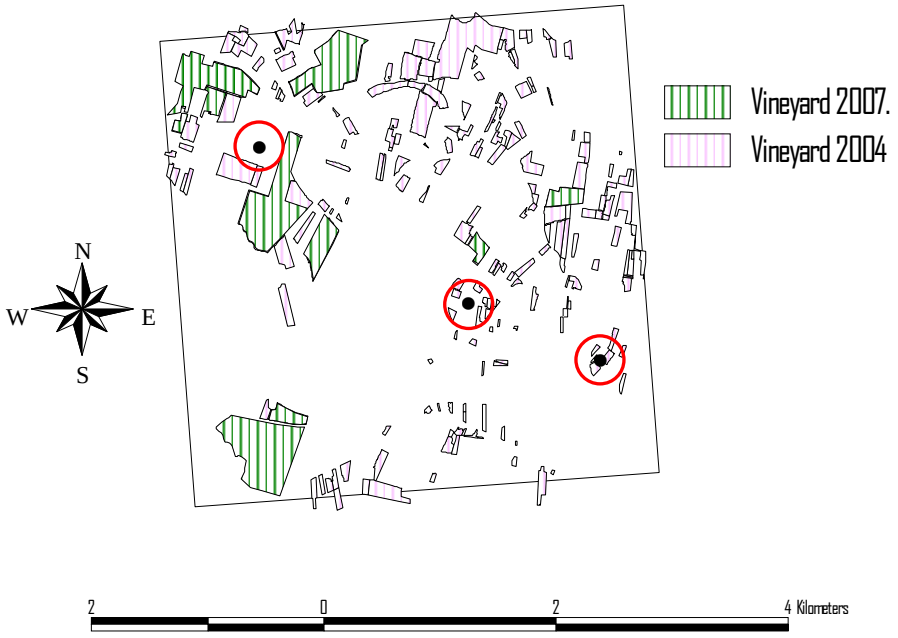

Figure 4.5: Sampling area 2007, analysed samples are circled in red.

In 2008 samples were collected from sixteen points selected from a 1x1 square grid of the considered site. Each sample was packaged in aluminium foil and stored at –20°C till the analysis. Sampling dates were selected in function of the main application periods, samples collected after the main application dates (16th July 2008) are here considered in order to validate the index. In this chapter only the results from the height samples analysed up to date are reported (**Figure 4.6**). Samples were extracted in two replicates by ultrasonication (3 cycles of 15 minutes), cleaned up with GCB SPE and analysed with GC-MS. The LOD of the method was 0.8 ng/g DW and the recovery percents evaluated for different level of contamination were 95% with a RSD of 13 (for more details see Chapter III).

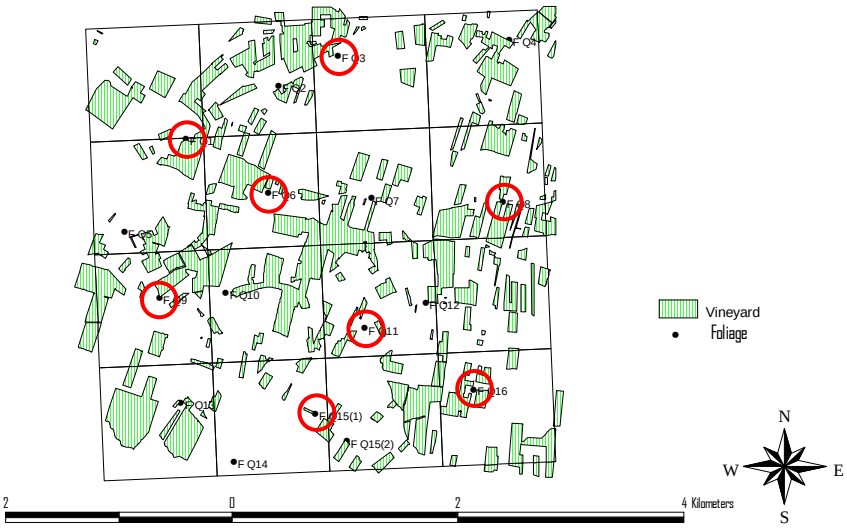

Figure 4.6: Sampling area 2008, analysed samples are circled in red.

4.3 RESULTS AND DISCUSSION

In **Table 4.5** the measured concentration of chlorpyrifos in samples collected in 2007 are reported. For each sampling date the mean concentration of chlorpyrifos at 1 and 2m of height was calculated.

Table 4.5: Measured chlorpyrifos concentrations in foliage samples collected in 2007. All in µg/g dry weight.

sample	14/06/2007		02/07/2007		09/07/2007[1]	
	1	2	1	2	1	2
1	0.0940	0.0340	0.0990	0.0610	-	-
2	0.0580	0.0380	0.0440	0.0320	-	-
3	0.0640	0.0520	0.1710	0.0750	0.0420	0.0430
mean	0.0720	0.0413	0.1047	0.0560	-	-
sd	0.0193	0.0095	0.0637	0.0219	-	-

1 In this case only one point was sampled because of a rain event during the sampling date

In **Table 4.6** the predicted concentration in each 2x2 square of the field site is reported.

Table 4.6: Predicted concentration of chlorpyrifos on foliage in Meolo 2007 field site calculated for squares of 2x2 km. All in µg/g dry weight.

	14/06/2007	02/07/2007	09/07/2007
a	0,010	0,056	0,009
b	0,000	0,034	0,005
c	0,021	0,027	0,004
d	0,000	0,024	0,004
mean	**0,008**	**0,035**	**0,005**
sd	**0,010**	**0,015**	**0,002**

A preliminary comparison between mean concentration measured in the 4x4 km field site with predicted concentration starting from mean value of 2x2 km squares data sets is reported in **Figure 4.7.**

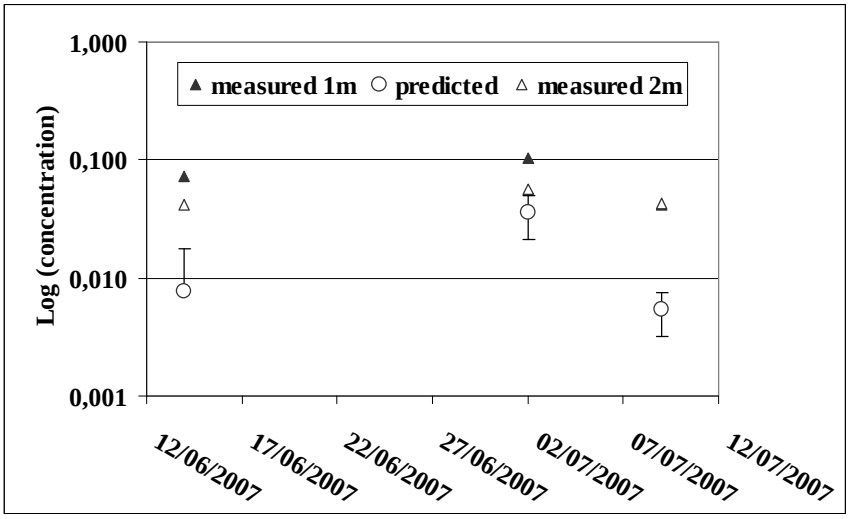

Figure 4.7: Mean concentration of chlorpyrifos measured (at 1 and 2 m of height) and predicted. All in µg/g dry weight.

Generally, for Meolo 2007 the predicted concentrations are lower of one order of magnitude with respect to measured concentrations in sampled leaves. This fact could be explained by a low resolution of the data set, both in term of land use information (even if the total surface area of vineyard was available, only big fields were mapped, see **Figure 4.5**) and in term of application date (for sale data two application periods were assumed arbitrarily). In any case, as reported in **Figure 4.7,** the temporal trend of concentration is well predicted.

In order to refine the approach and to evaluate the sensitivity of the index the same approach was applied to data collected in 2008. In this case the data set was completed in term of land use data. Furthermore, the number of sampling points was higher and distributed in all the 4x4 km square.
In **Table 4.7** measured (in samples collected after the main application periods, 16th July 2008) and predicted average concentrations of chlorpyrifos in square of 1x1 km expressed as µg/g dw are reported. Samples collected before the application period (20th May 2008) were also analysed, but in all the cases chlorpyrifos was not detectable.
Concentration of chlorpyrifos on foliage was predicted evaluating field by field the amount of active ingredient drifted off and estimating an average concentration on squares of 1x1 km. The dissipation of chlorpyrifos from the application date till the sampling period was evaluated as reported in Leistra, 2005.

Table 4.7: Measured and predicted concentration of chlorpyrifos on foliage expressed as µg/g dw. nd (sample 9) was not considered in average calculation.

Sample	Measured	Predicted
1	0.13	0.17
6	0.32	0.40
3	0.08	0.18
8	0.30	0.61
9	nd	0.18
15	0.21	0.33
11	0.26	0.35
16	0.25	0.34
mean	0.22	0.32
sd	0.08	0.15

A good agreement between predicted and measured concentrations could be observed. Except for two outliers (points 3 and 9), the ratio between predicted and measured data was within a factor of about 2 . Also the mean concentration predicted and measured are in agreement, demonstrating the predictive efficiency of the index when the resolution of the data set is high.
To assess the influence of the dataset resolution on predictive efficiency, the concentration of chlorpyrifos was predicted supposing to know only the % of coverage at 3 scales 1x1, 2x2 and 4x4 km (**Figure 4.8**).

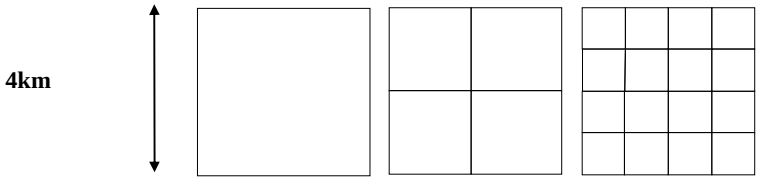

Figure 4.8: Hypothetic dataset scenario resolution

Results are reported in **Table 4.8** and **Figure 4.8**. A good predictive efficiency is shown in case of detailed datasets (predicted) and in case of small scale datasets (1x1 km), the higher scales (2x2 and 4x4) show a lower predictive efficiency. All these issues should be considered while assessing exposure.

Table 4.8: Measured and predicted concentration of chlorpyrifos on foliage at different scales of detail expressed as µg/g dw.

Sample	Measured	Predicted		
		1x1 km	2x2 km	4x4 km
1	0.131	0.102	0.062	
6	0.319	0.119		
3	0.085	0.102	0.057	
8	0.298	0.148		
9	nd	0.084	0.044	0.028
15	0.208	0.127		
11	0.258	0.079	0.059	
16	0.252	0.119		
mean	0.222	0.110	0.056	
sd	0.080	0.021	0.007	

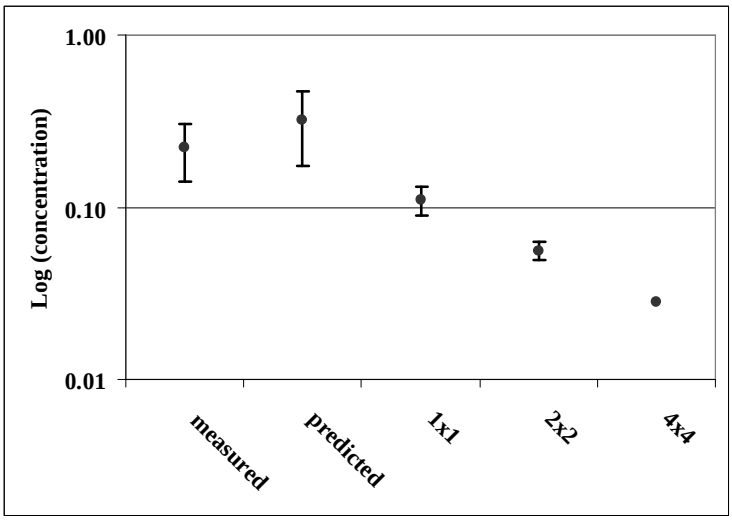

Figure 4.8: Mean and standard deviation of the measured and predicted concentration with different levels of detail of the dataset. *Predicted* = high level of detail (field by field maps).

4.4 CONCLUSIONS

The exposure index proposed would allow overcoming the concept of Hazard Quotient, allowing risk assessment for pollinators to be based on an estimate of actual exposure. Furthermore, the index would allow mapping pesticide risk for pollinators at different scale levels. Even if some more experimental data are needed, the preliminary validation seems to indicate a good agreement between the estimate concentrations and those experimentally measured. A sensitivity analysis allowed assessing the level of uncertainty due to the decrease of detailed information for large scale mapping. Finally, the proposed index could also have a general value in terrestrial organism exposure assessment and could be introduced and applied in method for other organisms *taxa* (e.g. bird).

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CHAPTER V

Plant protection product risk assessment for pollinators II: methods development

Pollination is one of the most important ecosystem services in agroecosystems and supports food production. Pollinators are potentially at risk being exposed to pesticides and the main route of exposure for is direct contact, in some cases ingestion, of contaminated materials such as pollen, nectar, flowers and foliage. To date there are no suitable methods for predicting pesticide exposure for pollinators, therefore official procedures to assess pesticide risk are based on a Hazard Quotient. Here we develop a procedure to assess exposure and risk for pollinators based on the foraging behaviour of honeybees (*Apis mellifera*). The method was applied in 13 European field sites with different climatic, landscape and land use characteristics. The level of risk during the crop growing season was evaluated as a function of the active ingredients used and application regime. Risk levels were primarily determined by the agronomic practices employed (i.e. crop type, pest control method, pesticide use), and there was a clear temporal partitioning of risks through time. Generally the risk was higher in sites cultivated with permanent crops, such as vineyard and olive, than in annual crops, such as cereals and oil seed rape. The greatest level of risk is generally found at the beginning of the growing season for annual crops and later in June-July for permanent crops.

Keywords: Pollinators, pesticide, risk assessment, procedure

Barmaz, S., Potts, S.G., Vighi, M. 2009. Plant protection products risk assessment for pollinators 1: methods development. Submitted to *Ecotoxicology*.

5.1 INTRODUCTION

The major challenge for modern ecotoxicology is to determine the consequences of the widespread occurrence of toxic substances in the natural environment (Baird and Van den Brink, 2007). A more ecologically sound approach in ecotoxicology is needed to evaluate risk arising from chemical exposure in natural communities. Recently the need for further research on the drivers of the 'pollinator crisis' was called for, and in particular the need to better understand the response of pollinator communities to complex environmental stressors (Ghazoul, 2005). Within ALARM Project (Settele et al., 2005) a specific activity (PACRAT, Pollinators and pollination in response to Agro-Chemicals and land-use as a Risk Assessment Tool) aimed to elaborate and validate a field risk assessment procedure for pollinators and pollination.

There is convincing evidence for the negative impacts of habitat loss and agricultural intensification on pollinator diversity for a range of taxa across continents (Ricketts et al., 2008; Winfree et al., 2009). The socio-economic value of pollinators is well documented; crop pollination by bees and other animals is an essential ecosystem service that increases the yield, quality and stability of 75% of globally important crops (Klein et al., 2007) and is estimated to be worth €153 billion per annum (Gallai et al., 2009). In particular, bees are considered to be the predominant, and most economically important group, among all the pollinators taxa (honey bees, bumblebees, solitary bees, wasps, hover flies and other flies, thrips, beetles and birds) in most geographical regions (Klein et al., 2007; Kremen et al., 2007). Any loss in biodiversity is a matter of public concern, but losses of pollinating insects may be particularly troubling because of the potential effects on wild flower reproduction (Biesmeijer et al., 2006) and crop plant productivity (Klein et al. 2007).

Agriculture may affect pollinators' communities in different ways: simplification of the landscape, habitat fragmentation, intensive use of agrochemicals (Winfree et al., 2009). These issues underline the need for procedures to assess risk for pollinating insects arising from agricultural practices. In terrestrial systems different compartments (air, soil and biota) may be involved in chemical exposure as a function of the target organism (EC, 2002). In the past, ecotoxicologists have focused on aquatic systems, so terrestrial risk assessments could only apply the aquatic model to soils, or have focused on specific targets such as risk posed by pesticides to birds, honey bees and beneficial arthropods (Tarazona et al., 2002).

The Official procedure to assess risk for pollinators is based on the EPPO guideline (OEPP/EPPO, 2003).

In this procedure the assessment is based on a three tier scheme comprising: early studies in laboratory condition followed by semi-field studies and completed by field studies. Preliminary characterisation of risk for sprayed

products is based on the calculation of the Hazard Quotient: the *ratio* between the application rate (g/ha) and the LD_{50} (μg/bee). Direct exposure to field spray is considered as the main route of exposure for pollinators, and only exposure while bees are foraging on treated fields crop is considered. The bee brood risk due to insect growth regulators is evaluated in a 'worst case' screening test. Further studies such as cage/tent/tunnel or field studies are required only in case of an effect predicted by the first tier (EC, 2002). This approach allows a comparison of the risk arising from different active ingredients and only requires a relative small amount of data, but quantification of the actual exposure is not assessed.
The aim of this work is to develop a method to assess risks for pollinators considering the emission routes of pesticides, land use information and pollinator behaviour. As a first step, this procedure specifically addresses to honeybees, however, later development and adaptation of the procedure to other pollinator groups will be undertaken and is linked to the availability of behavioural information on other taxa. The inclusion of other groups in risk assessments is essential if the aim is to understand the effects of pesticides on entire pollinator communities and manage pollination services in a sustainable manner. Honeybees are an important pollinator but other 'wild' pollinators may be more effective at pollinating some crops (Klein et al., 2007) and wild flowers; indeed Lonsdorf et al. (2009) state that "diverse wild bee communities provide both enhanced stability, quality and quantity of pollination services over space and time".

5.2 MATERIAL AND METHODS

5.2.1 Procedure development

Development of a conceptual model for exposure assessment. A conceptual model for ecotoxicological impact should consider the interaction between a stressor (in this case agrochemicals) and a resource of concern (in this case pollinator community) (Solomon et al., 2006). A conceptual model for agrochemical risk assessment should take into account the time table of pesticide application, the application patterns, the product formulation, the way of exposure and the organism behaviour. Therefore, an Emission scenario, a Fate scenario and an Exposure scenario should be integrated. The Emission scenario comprises: a georeferenced database: in our case land use data (European CORINE, 2000), and a non-georeferenced database: main active ingredients and relative products, application rate, surface area applied.
Uncultivated vegetation (such as herbaceous field boundaries and wooded areas) is the main exposure matrix for pollinators; application of insecticides during flowering of crop is generally avoided by Good Agricultural Practice principles and suggested as risk mitigation option (EC, 2002).

Direct drift is the main emission mechanism of pesticides on vegetation within fields. During pesticide registration procedures in the EU the following can be predicted according to Ganzelmeier et al.(1995) and Rauttmann et al. (2001): the concentration of pesticide (in soil) outside the field as a function of the crop, the phenological stage, and the distance from the field These empirical predictions may be adapted to estimate the concentration of pesticides in non-cropped vegetation by interpolating the proportion of deposited drift material as a function of the distance (95° percentile values) with a curve described by **Equation 5.1**:

$$y = ax^{-b} \quad (\mathbf{5.1})$$

where *y* is the drift % (% application rate), *x* is the distance from the field (m) and *a*, *b* the coefficients of the curve derived from Ganzelmeier et al. (1995) and strictly dependent on the crop type and the phenological stadium. For each crop type (grapevine, field crop, fruit crop, all in late growth stage phenological stadium) a specific equation was obtained and modified to obtain a maximum drift percent (100%) when the distance from the field converge to zero, **Equation 5.2**:

$$y = \frac{a}{x^b + \frac{a}{100}} \quad (\mathbf{5.2})$$

The following equations to predict the percent of drift as a function of the distance were derived from Ganzelmeier et al., 1995 data (**Equations 5.3-5.5**):

Fruit crop

$$y = 19.48x^{(-0.71)} \quad (\mathbf{5.3})$$

Vineyard

$$y = 12.54x^{(-0.89)} \quad (\mathbf{5.4})$$

Field crops

$$y = 3.34x^{(-0.98)} \quad (\mathbf{5.5})$$

Equations 5.3-5.5 could be applied at a field scale to predict the concentration on vegetation hedgerows close to field crop. At a larger scale, and in complex land use scenarios, a precise assessment of pesticide

gradients and of the actual distribution of concentration on foliage is impractical to estimate. So, an approximated approach, producing a 'weighted average' of pesticide concentration in a given area was developed. **Equations 5.3-5.5** were included in an algorithm to develop an exposure index capable of estimating pesticide concentration on plant tissues in a given surface area (S_t). The index, shown in **Equation 5.6**, has been successfully calibrated and validated in an experimental area (Barmaz et al., 2009). In **5.6** the amount of pesticide that reaches 0.1 m wide buffer strips is calculated, up to a maximum distance of 50 m from the field margin based on the perimeter of each field (p_n) and the application rate (AR). Summing the predicted amounts for each buffer strip for the *n* fields of S_t, the total amount of pesticide drift is estimated. The average concentration (weight/surface) is obtained by dividing this amount by the foliage surface area estimated as a function of the Leaf Area Index (S_t * LAI).

$$FC_s = \frac{\sum_{i=1}^{n}\left[AR \cdot \left(ax_{(0...50)}{}^{-b}\right) \div 100\right] \cdot 10 \cdot p_n}{St \cdot LAI} \qquad (\mathbf{5.6})$$

where FC_s is the foliage concentration (µg/cm^2), AR is the application rate (µg/cm^2), p_n is the perimeter of the field (cm), LAI (cm^2/cm^2) is the Leaf Area Index, S_t is the surface area considered (cm^2), n is the number of fields treated with a given active ingredient, x (m) is the distance from the field, 10 is the buffer width (cm) and *a* and *b* the coefficients derived from Ganzelmeier et al. (1995). FC_s can be transformed in weight/weight concentration (FC_w) by multiplying it by the Surface Leaf Weight (SLW: weight of a surface unit of foliage, cm^2/g).

In a preliminary approach it was assumed that, if application occurs during flowering period of non cropped vegetation, concentration on foliage, flowers and pollen are comparable.

Fate scenario. To date, and to the authors knowledge, empirical models to predict the temporal decay of actives ingredients on plants parts are available only for leaves (Leistra, 2005).

The decay after application can be estimated taking into account the rate of volatilisation, the rain wash-off and the rate of photodegradation of active ingredients on foliage. The rate of volatilisation can be described as a function of vapour pressure (**Equation 5.7**; Smit et al. 1998):

$$\log CV = 1.528 + 0.466 \log VP \qquad (\mathbf{5.7})$$

where *CV* is the cumulative volatilisation (% of initial mass on plant tissues) and *VP* is the vapour pressure (mPa). **Equation 5.7** allows determination of the cumulative volatilisation (in 7 days) as a percentage of the mass on

plant's parts. Generally pesticides show a volatilisation peak just after the application date, followed by a distinct decrease in volatilisation rate as a result of other competing process (uptake, wash off, photodegradation). Volatilisation behaviour is hard to quantify in a general rule, so for this reason in this study the volatilisation is assumed as a first order kinetics (**Equation 5.8**):

$$m_1 = m_0 * e^{(-kv*t)} \qquad (\mathbf{5.8})$$

where m_1 is the mass on plants after volatilisation (mg), m_0 is the initial mass on plant parts (mg), and t is the time (day). The CV of **Equation 5.7** allows calculating m_7 (i.e. tha mass after 7 days). **Equation 5.9** allows calculating the volatilisation rate K_v**:**

$$k_v = \frac{\ln m_0 - \ln m_7}{7} \qquad (\mathbf{5.9})$$

where m_0 is the initail mass on plant tissues, and m_7 is the mass remaing after 7 days from the application date. Photodegradation may compete with volatilization in plant tissues' pesticide mass balance. DT_{50} on foliage is rarely documented the in literature, but when available from literature, a photodegradation rate (*Kph*, day^{-1}*)* can be calculated to predict the mass remaining after a specific time according to the first order kinetics (**Equation 5.10**):

$$m_2 = m_1 * e^{(-kph*t)} \qquad (\mathbf{5.10})$$

where m_2 is the mass on plant (after photodegradation, mg).

The wash-off by a rain can be described by the equation (FOCUS, 2003):

$$m_3 = m_2 * e^{-(w*T)} \qquad (\mathbf{5.11})$$

where m_2 is the initial mass (after photodegradation, mg), m_3 is the final mass on the plant (mg), W the foliar wash off coefficient (mm^{-1}), T the rain fall (mm). The foliage wash off coefficient is calculated as (FOCUS, 2003):

$$W = 0.016 * Ws^{(0.3832)} \qquad (\mathbf{5.12})$$

where *Ws* is the water solubility (mg/l).

A possible competing process for all the decay mechanisms is foliage uptake, this process is difficult to quantify because of the lack of quantitative relationships with a general validity, therefore the extent of its influence is unknown (Leistra, 2003).
An *Exposure Scenario* should be developed based on specific behavioural features of the target organism. Pollinators communities are complex and therefore difficult to model in their entirety. Information for developing exposure profiles is available for some taxonomic groups (e.g. bumblebees, Thompson et al., 1999), however, toxicity data are not always readily available for these taxa. Therefore, *Apis mellifera* (honeybee) is assumed to be representative of all bees in this study. The typical forage distance of honeybees is within a 1km radius of the hive (Pinzauti et al.,1991). Exposure for honeybees involves both contact and oral routes in adults and immature stages, although the routes may not have the same relative importance in the two life stages (Alix et al., 2007). In a preliminary assessment, data on behaviour for an average honeybee, representative of the whole colony, may be used: total daily consumption of pollen 4.3 mg (derived from Seeley, 1985) and contact daily area of a bee ~5 cm^2 (Bernardinelli pers. comm.)
For contact exposure, it can be assumed, as a worst case, that the intake corresponds to the total amount of pesticide present on the contact surface. From these data and from the Predicted Environmental Concentration (PEC, calculated as reported before, **Equation 5.6**), the Total Daily Intake (TDI) of pesticide for each active ingredient applied in the forage area could be calculated as (**Equation 5.13-14**):

$$TDI_{oral} = C_w * 4.3 \qquad \textbf{(5.13)}$$

where TDI_{oral} is the Total Daily Intake (4.3 mg of pollen) and C_w is the concentration on plant tissues (μg/mg).

$$TDI_{contact} = C_s * 5 \qquad \textbf{(5.14)}$$

where $TDI_{contact}$ is the Total Daily Intake (5 cm^2) and C_s is the concentration on plant tissues (μg/cm^2).

Effect assessment. Honeybees are one of the indicator organisms selected for terrestrial risk assessment under regulatory processes. Therefore, different databases and source of toxicity data for this organism are readily available (e.g. Footprint, 2006, Agritox database assessed in July 2009, Tomlin, 2003). As a general rule, ecotoxicological risk assessment is based on data on a few selected indicator organisms and is hampered by the lack of information on sensitivity and vulnerability of the natural communities (De Lange et al., 2009). A more precise, site specific, assessment, should require

more information on the actual structure of the exposed community and its vulnerability. This would require a great deal of additional information that, at the present is almost impossible to obtain.

Risk characterisation. As reported, for non sprayed pesticides (e.g. mainly systemic pesticides; Rortais et al., 2005, Alix et al., 2007), risk assessment calculation for beneficial arthropods should be implemented with respect to the Official Procedures (Reference), in the form of either a toxicity exposure ratio (TER) or PEC/PNEC ratio, overcoming the concept of a Hazard Quotient. These is realised by calculating the ETR (Exposure Toxicity Ratio) starting from the contact and oral exposure TDI and from toxicity data taken from literature. With this approach the toxicological potency of mixtures can be predicted using the Concentration Addition (CA) model (Boedeker et al., 1993, Drescher and Boedeker, 1995); assuming this model as a reasonable worst case (Verro et al., 2009, Junghans et al., 2006, Finizio et al., 2005, Faust et al., 2003) gives (**Equation 5.15**):

$$TU_{MIX} = \sum_{i=1}^{n} TUi = \sum_{i=1}^{n} \frac{TDI_i}{LD_{50,i}} \qquad (\mathbf{5.15})$$

where *TDI* is the Total Daily Intake of the individual chemical 'i'; $LD_{50.i}$ is the effect concentration of the individual chemical 'i'; *TUi* are the toxic units of the individual chemical 'i' and TU_{MIX} are the toxic units of the mixture.

5.2.2 Application of the procedure

Area of study. The approach developed here was applied in 13 study sites (16 km^2 each) established within ALARM Focal Site Network (FSN), Hammen et al. 2009). The sites were: Catalonia (San Pere de Ribes), Spain (Quintos de Mora and Los Cortijos), UK (Chilterns and Lambourn), Estonia (Koeru and Vajke Maarja), Hungary (Tazlar and Soltvadkert), France (IDF-Marchais and Bonnelles) and Italy (Meolo and Livenza) (**Figure 5.1**).

Figure 5.1: Position of the field sites studied

These sites are representative of the main European biomes, climatic conditions and crop types. For each site an area with low intensity agricultural impact (U = undisturbed) and an area with high level intensity agricultural impact (D = disturbed) were selected with the exception of Catalonia field site in which only one site was considered. Full details of sites selection and characteristics are available in Hammen et al. (2009). Data on land use and pesticide application were collected during the growing season of 2007. Selection of priority compounds (Worst case scenario). **Figure 5.2** gives an outline of the entire procedure used here.

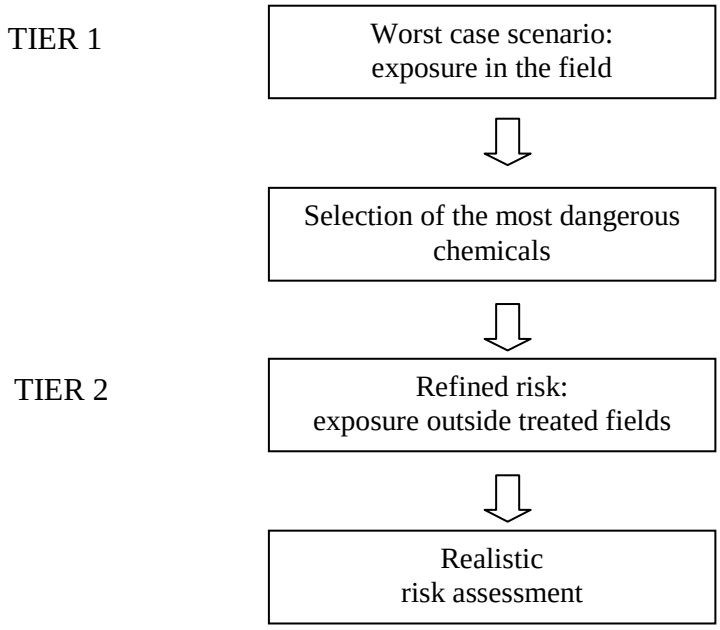


Figure 5.2: Outline of the entire procedure

A worst case scenario was applied in order to prioritise the compounds for inclusion in the models, with the assumption that all of the bee's feeding area is applied with the typical recommended application rate of each active ingredient (if not available from farmers' interviews, it was derived from Tomlin, 2003 or technical products labels). Herbicides and granular formulations were excluded as not relevant for pollinator risk, because of the low toxicity in case of herbicides or because of the low probability of exposure in case of granular formulation. A maximum plant interception was estimated from FOCUS (2003), and in addition to a seasonal change an annual trend is needed, based for permanent crops. For some crop, like vineyard, the cultivar type also influences the amount of pesticide intercepted. To evaluate the concentration on foliage weight, an average Specific Leaf Weight (SLW, 0.005 m^2/g) was obtained from the literature and the Leaf Area Index (LAI) for each period and crop was estimated from the maximum value reported in the FOCUS scenarios and from the percent of crop coverage of the different phenological stages (Becker et al., 1999). An outline of the worst case scenario is described in **Figure 5.3**.

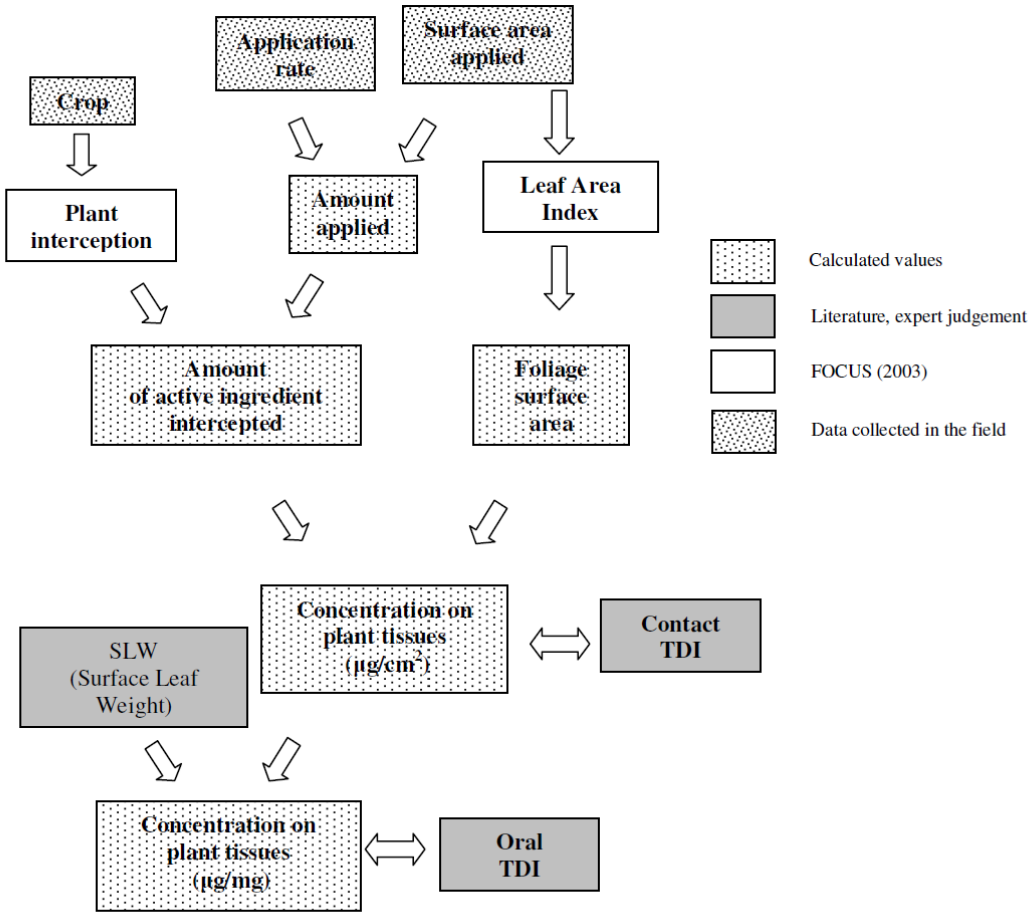


Figure 5.3: Outline of the worst case scenario

It should be noted that the TDI calculated in this case is not realistic, starting from the assumption that pollinators feed only in treated fields and that fields are treated during flowering (i.e. a misuse of pesticides). The objective of the worst case scenario was, therefore, the selection of potentially toxic active ingredients and not a realistic risk assessment. The potential risk was determined as reported in **Equation 5.16.**

$$ETR = \frac{TDI}{LD_{50}} \qquad \textbf{(}5.16\textbf{)}$$

A refined, more realistic, risk assessment scenario was applied using only the most dangerous compounds (see below).

Risk assessment refinement. The risk assessment refinement needed to consider the actual land use and pesticide application of each site. The European CORINE land cover (CLC; European CORINE, 2000) was collected for each 4x4 km square (**Figure 5.4**).

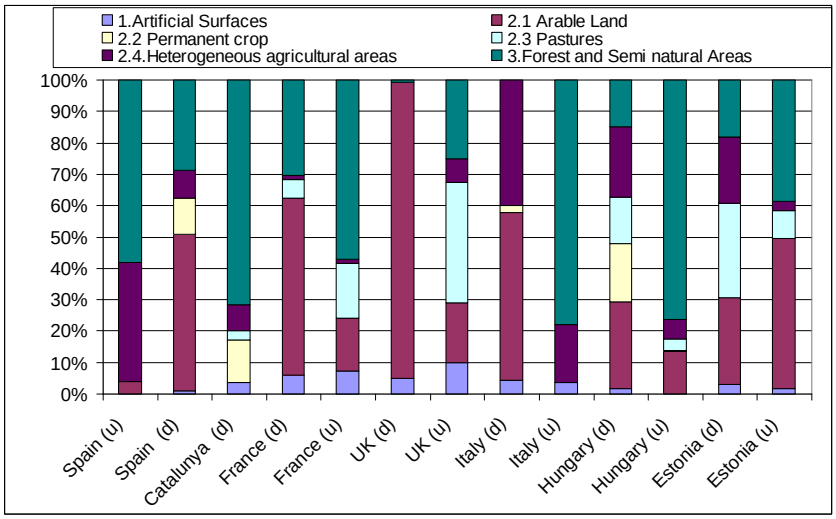


Figure 5.4: Land cover proportions for each site (European CORINE 2000)

Land cover data were compared with data collected directly in the field (ground-truthing) and integrated to obtain reliable land use maps. Each field site was divided in four 2x2 km parts representative of the forage area of *Apis mellifera* (**Figure 5.5**).

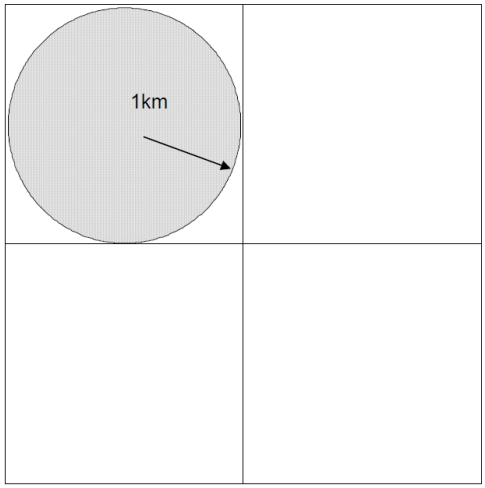


Figure 5.5: Outline of the forage area of *Apis mellifera* in a 4x4 km square.

In each square the land use patches treated with priority active ingredients were identified and the relative area and perimeter were measured with the software Arc View GIS version 3.1 (ESRI).

Equation 5.6 was applied to each 2x2 km sector of the field sites, assuming a bee positioned in the centre of the square and foraging in the whole 2x2 km area. In a first attempt a default average LAI derived from Otto et al. (2009) (for hedgerows and vegetation beneath the hedgerows: 3.5 m^2/m^2) was used to calculate the plant concentration.

In case of multiple applications, the contribution of each application to the total concentration on plant parts was calculated. *Apis mellifera* was assumed to be attracted only to full flowering vegetation. Therefore, exposure in the field was not considered because: fields are not treated during flowering (this was verified case by case in all the field sites), and if application is excluded during flowering, pollen of crop species is not exposed.

The trend of concentration was evaluated for the whole growing season using **Equation 5.7-12** to estimate the decay on plant tissues, **Equation 5.13-14** to assess the TDI, and **Equation 5.15** to characterize risk.

5.3 RESULTS

5.3.1 Selected active ingredients

In **Table 5.1** Molecular properties of the active ingredients selected for the assessment as priority compounds are reported. In **Table 5.2** LD_{50} of priority compounds are reported. In case of more than one value available, the lower value was used in the assessment in a worst case scenario approach. As expected all the selected compounds are insecticides, and two main classes of insecticides are included: pyrethroids (λ cyhalothrin and α cypermethrin), and organophosphates (dimethoate, chlorpyrifos, fenitrothion and malathion).

Table 5.2: Physico-chemical properties of the selected compounds (Tomlin, 2003)

Compound	**Vp (mPA) 25°C** [3]	**Water solubility (mg/l)** [3]	**DT_{50}**
chlorpyrifos	2.7	1.40	5[1]
dimethoate	0.250	23300.00	30[2]
fenitrothion	18	14.00	4 [3]
malathion	0.45	145.00	
α cypermethrin	0.00034	0.0040	
λ cyhalothrin	0.00020	0.01	10[4]

[1] Calliera et al. 2008 (on fruit)
[2] Pappas et al. 2002 (on fruit)
[3] Tomlin 2003
[4] Bostanian et al.1993

Table 5.2: Selected pesticides and toxicity data (LD_{50}) for *Apis mellifera* collected from different databases. In bold: data selected for risk assessment in a worst case scenario the lowest LD50 were consider

		LD50 contact			**LD50 oral**		
Compound	**Field Site**	**Agritox[1]**	**Footprint[2]**	**Literature**	**Agritox[1]**	**Footprint[2]**	**Literature**
chlorpyrifos	Italy/Catalonia	**0.059**	**0.059**	0.070[3]	0.250	-	**0.018**[4]
dimethoate	Hungary/Spain	-	-	**0.120**[3]	**0.120**	-	0.150[3]
fenitrothion	Catalonia		**0.160**	-	-	-	**0.071**[4]
malathion	Catalonia		**0.160**	-	-	-	**0.400**[4]
α cypermethrin	Estonia, France	**0.033**	-	-	**0.059**	-	-
λ cyhalothrin	UK	-	-	**0.909**	-	-	**0.038**[3]

[1] **http://www.dive.afssa.fr/agritox/php/fiches.php (assessed in July 2009)**
[2] **Footprint, 2006**
[3] **Tomlin, 2003**
[4] **Vighi et al. 1991**

5.3.2 Data collection

In **Table 5.3** the main crops, surface area treated, and the main application dates of priority compounds are reported. Selected field sites could be divided into two groups. The first group is represented by sites located in the Atlantic (UK and France) and Boreal (Estonia) biogeographical regions (EEA, European Environmental Agency, 2003) These sites are cultivated with annual crops (mainly cereals and oil seed rape) and characterized by relatively few insecticide applications (mainly pyrethroids) starting at the beginning of the growing season. For Estonian and UK sites, active ingredients and application dates were available. In the case of French sites, the application period was assumed to be similar to those observed in the UK, having similar agro-environmental conditions and the applied active ingredients (same target pest and crop). The land use data available presented different levels of detail: detailed land use map were available for UK field sites, whereas area (ha) or percent of coverage of each crop were available for Estonian and French field sites, and the position of the crop patches was obtained from CORINE.

The second group of sites comprises areas cultivated mainly with permanent crops (vineyard and olive) and located in the Mediterranean (Spain), Continental (Northern Italy) and Pannonian (Hungary) biogeographical regions. Only one application date in May was recorded for olive, but more than one pesticide application event occurred during the growing season, mainly in June and July for vineyard. These crops were generally treated with high amounts of insecticides, furthermore the application may generate higher drift percents (**Equation 5.3-5**). The field sites considered in the assessment show different percentages of crop coverage. The Catalan field site is characterised by an agricultural area of about 234 ha (14% of the total area), and the 73% is covered by vineyard (**Figure 5.4**). The priority active ingredients applied are chlorpyrifos, fenitrothion and malathion. In Italy data were collected in two different field sites: Meolo (disturbed) and Livenza (undisturbed). In the Meolo field site the main crops were maize and vineyard, covering the 27% and the 24 % of the entire site respectively. Only vineyard was sprayed during the study with high amounts of insecticides (e.g. chlorpyrifos). The Livenza field site is a natural area where agriculture is reduced to a few domestic fields and application of insecticide is negligible. There were two Spanish field sites: Quintos de Mora (undisturbed) is a protected area in which pesticides are not used whilst Los Cortijos (disturbed) is cultivated mainly with olive (12% of the total field site area) and cereals (34% of the total field site area). The only crop with insecticides applied was olive on which dimethoate was sprayed at the end of May. In Hungary, the disturbed site had an agricultural area of 70% of the total surface and vineyard represented the 50% of the agricultural area. In the

undisturbed field site the agricultural area is 14% and vineyard represented the 20% of that. The main compound applied was dimethoate.

Table 5.3: Main active ingredients applied, surface area treated and application rate and date in considered field sites (D: disturbed; U: undisturbed).

Site	*Treated Crop*	*Compound*	*Application Rate (g/ha)*	*Surface Area treated (ha)*	*Application Dates*[4]
Italy_D	Vineyard	chlorpyrifos	500	365[1]	17/05/2007 07/06/2007 16/06/2007 25/06/2007 14/07/2007 25/07/2007
Italy_U	-	-	-	-	-
Catalonia_D	Vineyard	chlorpyrifos	500	43[2]	15/06/2007 31/07/2007
	Vineyard	fenitrothion	750	43 [2]	15/06/2007 31/07/2007
	Vineyard	malathion	1250	43[2]	15/06/2007 31/07/2007
Hungary_D	Vineyard	dimethoate	380	549[3]	10/05/2007 20/06/2007 30/07/2007
Hungary_U	Vineyard	dimethoate	380	59[3]	10/05/2007 20/06/2007 30/07/2007

[1] Data collected in the field
[2] In this site the surface area applied with each active ingredient was estimated assuming that the main compounds applied were used in comparable surface areas. Land cover data was collected in the field.
[3] Estimated from the percent of land cover collected in the field and from the CORINE land cover 2000 data
[4] Estimated (expert judgement)
[5] General application period were collected in all the sites with a incertitude time window of 1-2 weeks.

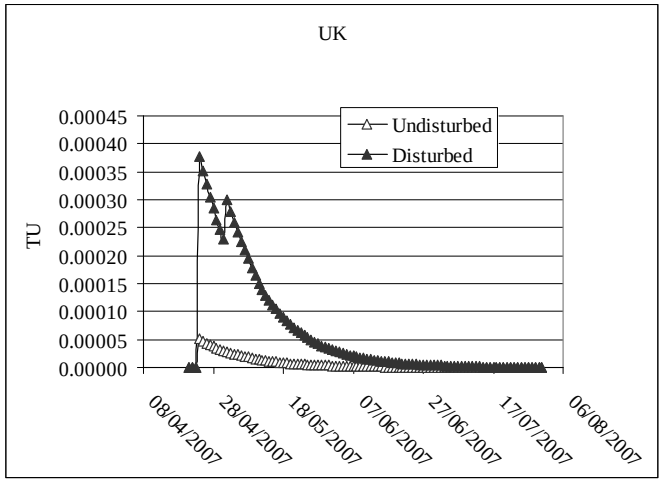
Table 5.3: (Continued)

Site	*Treated Crop*	*Compound*	*Application Rate (g/ha)*	*Surface Area treated (ha)*	*Application Dates*[4]
UK_D	Oil seed rape	λ cyhalothrin	75	309[1]	24/04/2007
	Bean	λ cyhalothrin	75	65 [1]	02/05/2007
UK_U	Oil seed rape	λ cyhalothrin	75	19 [1]	24/04/2007
Spain_D	Olive	dimethoate	720	199 [1]	30/05/2007
Spain_U	-	-	-	-	-
France_D	Wheat	λ cyhalothrin	6.25	453 [3]	24/04/2007[5]
	Oil seed rape	λ cyhalothrin	0.70	130 [3]	24/04/2007[5]
France_U	-	-	-	-	-
Estonia_D	Oil seed rape	α cypermethrin	10	147 [1]	04/06/2007 23/07/2007
Estonia_U	Oil seed rape	α cypermethrin	15	71 [1]	04/06/2007 23/07/2007

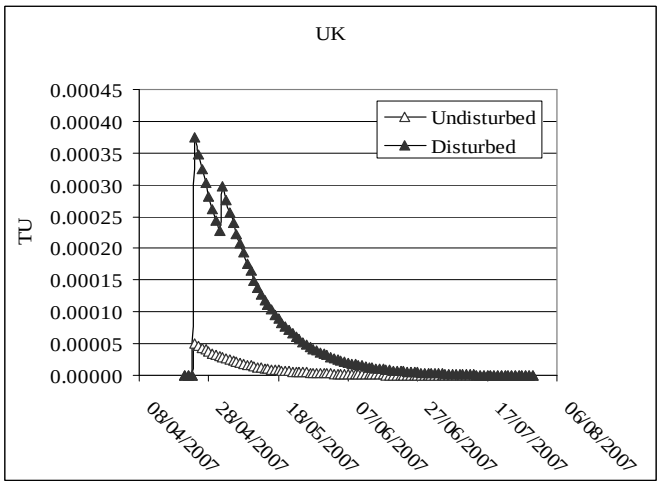
[1] Data collected in the field
[2] In this site the surface area applied with each active ingredient was estimated assuming that the main compounds applied were used in comparable surface areas. Land cover data was collected in the field.
[3] Estimated from the percent of land cover collected in the field and from the CORINE land cover 2000 data
[4] Estimated (expert judgement)
[5] General application period were collected in all the sites with a incertitude time window of 1-2 weeks.

5.3.3 Risk Trend

Figures 5.5-5.7 present the temporal of risk trends for contact and oral exposure in annual crop field sites. The data represent the average of the TUs calculated for the four foraging areas of the site.



a



b

Figure 5.5: Risk trend (Toxic Unit) in 2007. a) UK: contact exposure, b) UK: oral exposure.

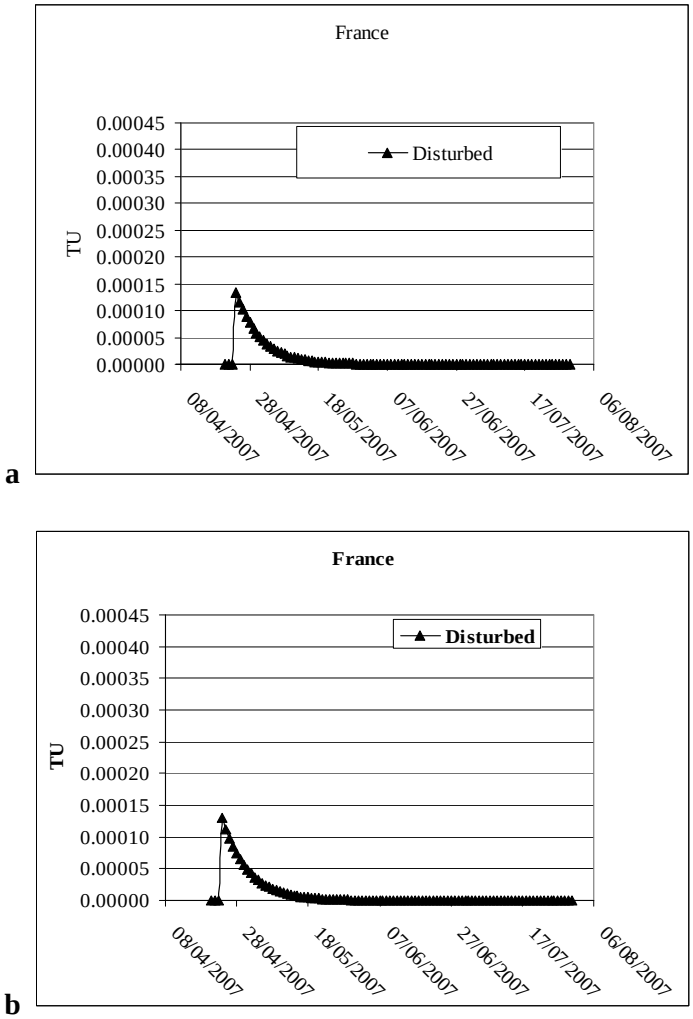


Figure 5.6: Risk trend (Toxic Unit) in 2007. a) France: contact exposure, b) France: oral exposure.

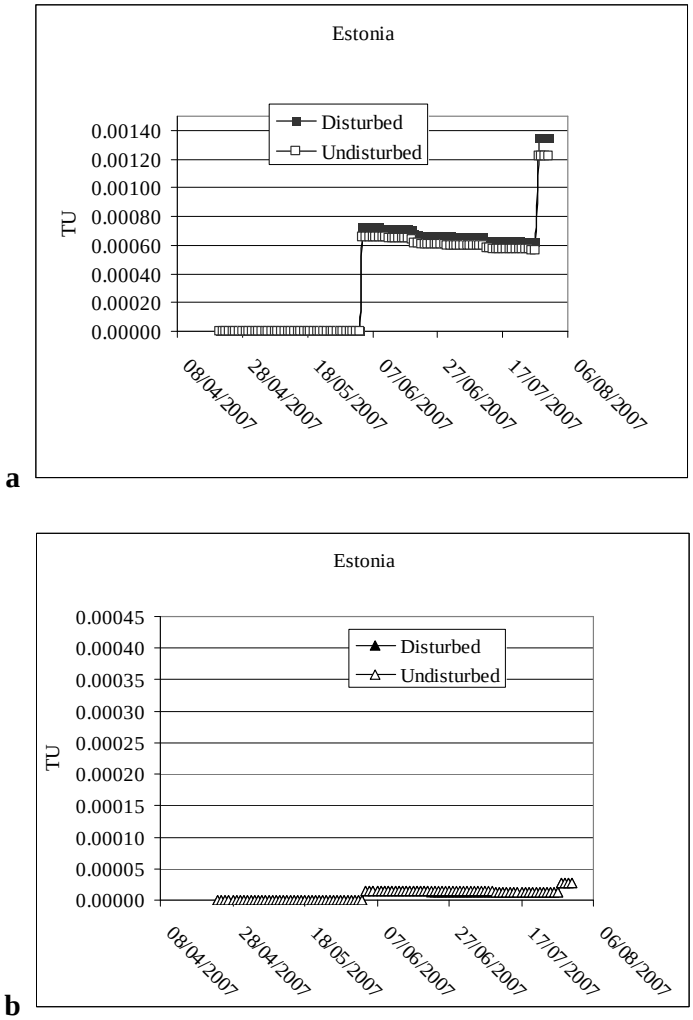
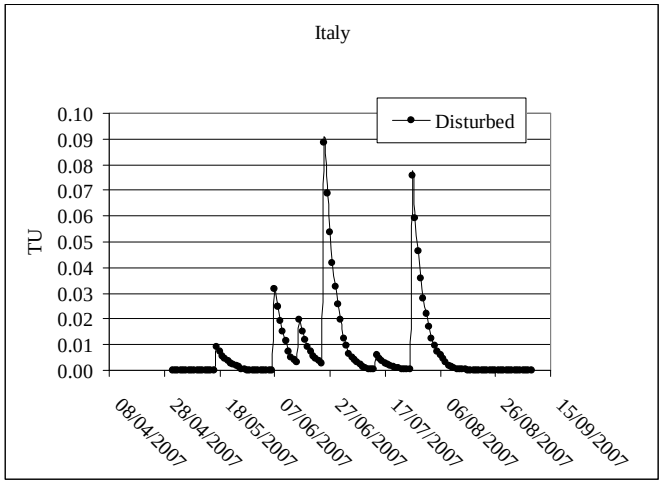


Figure 5.7: Risk trend (Toxic Unit) in 2007. a) Estonia contact exposure, b) Estonia oral exposure.

In **Figure 5.8-11** the TUs trend evaluated for permanent crop sites is reported. Temporal trends of risk in undisturbed sites is reported only for UK and Hungarian field sites. In the other cases (Italy, Spain) the remaining area was completely natural or occupied only by private fields with no or extremely low pesticide application. In case of the French field sites, no pesticides were applied in the undisturbed area, and in case of Catalunya (Spain), data were only collected in one site.



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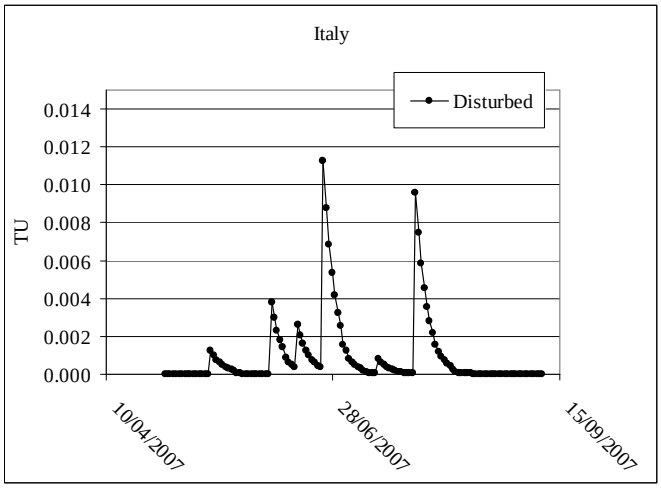

b

Figure 5.8: Risk trend (Toxic Unit) in 2007. a) Italy: contact exposure, b) Italy: oral exposure.

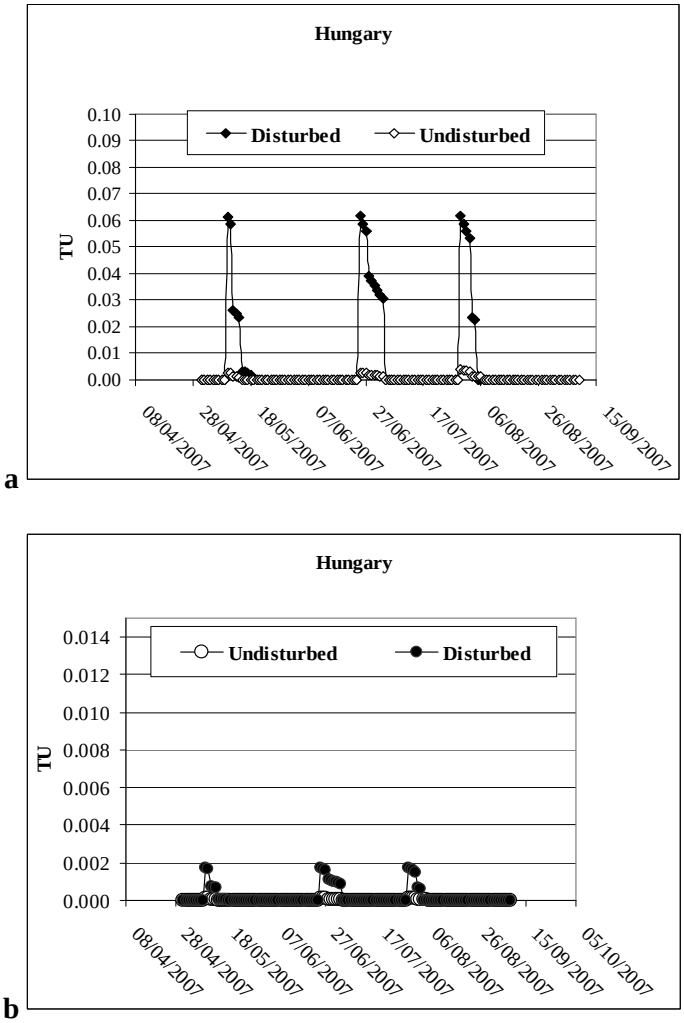

Figure 5.9: Risk trend (Toxic Unit) in 2007. a) Hungary: contact exposure, b) Hungary: oral exposure.

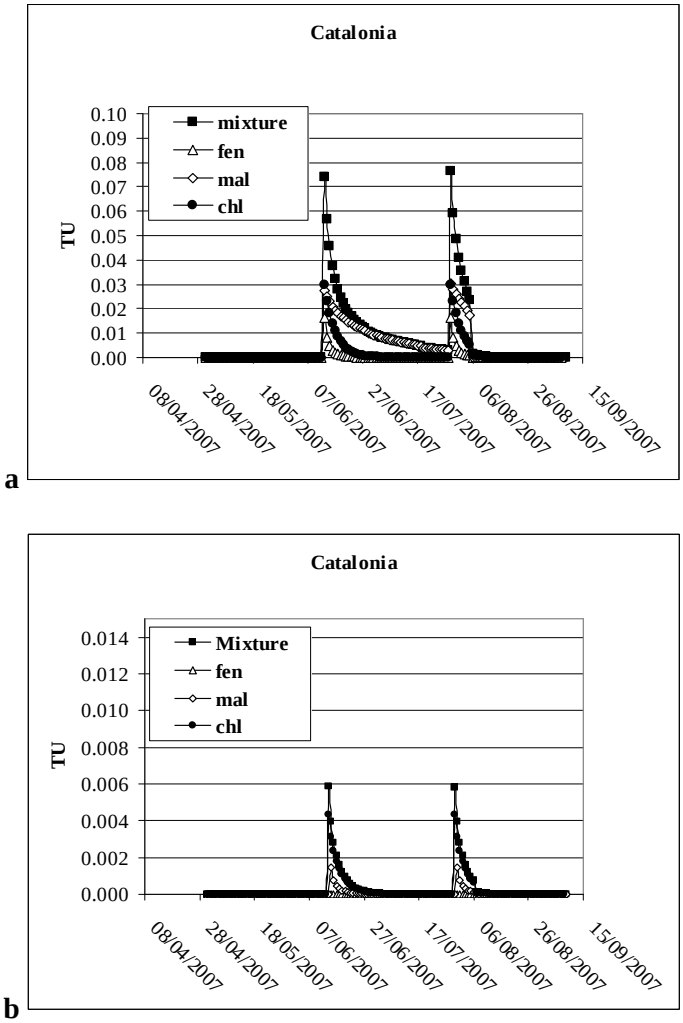

Figure 5.10: Risk trend (Toxic Unit) in 2007. a) Catalonia: contact exposure, b) Catalonia: oral exposure.

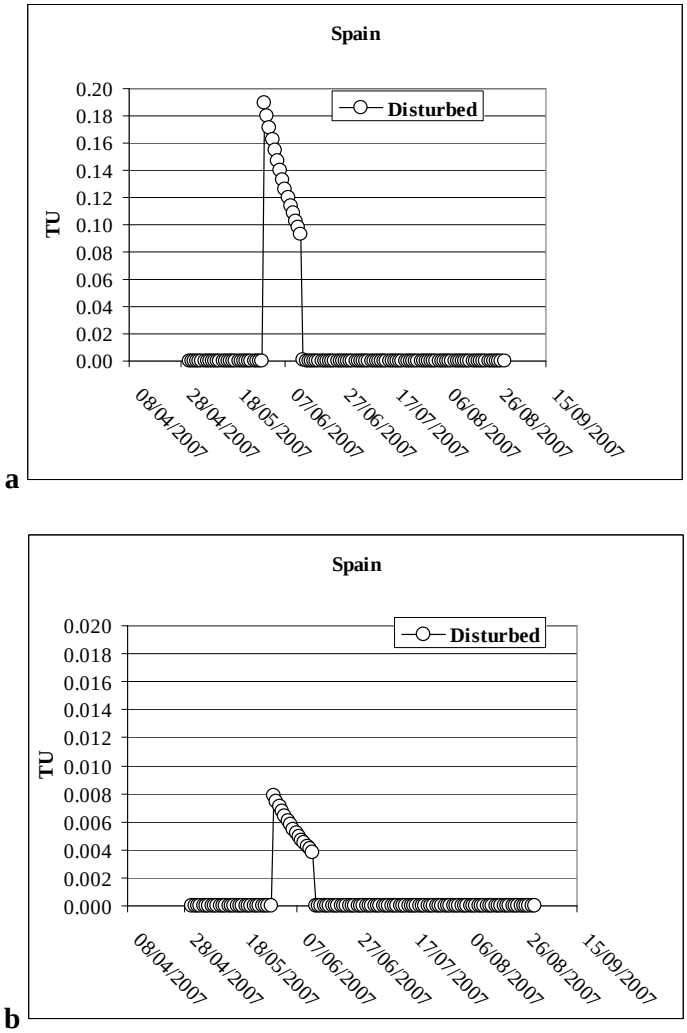

Figure 5.11: Risk trend (Toxic Unit) in 2007. a) Spain: contact exposure, b) Spain: oral exposure.

5.4 DISCUSSION

5.4.1 Risk trend

In annual crop sites (France, UK, Estonia) the risk produced by the selected active ingredients, both for contact and oral exposure, is at least three orders of magnitude below the threshold of acute effect (TU=1), and this may reasonably be assumed as a safe level even for sub-lethal effects. The temporal trend of risk for Estonian field site is inflated because of the lack of data on rain events and on photodegradation. The overall risk trend is therefore overestimated in the second date, but not in the first application date, when risk is negligible.
In the sites with permanent crops (Spain, Catalonia, Italy, Hungary), the greater amount of drift resulting from the application patterns, and the higher amounts of insecticides applied, produce a higher level of risk, in particular for contact exposure. The highest levels of contact exposure are reached in Spain (0.19 TUs), Italy (0.09 TUs) and, for the mixture of compounds, in Catalonia (0.08 TUs). Even if these are below the threshold of acute effects, a potential for sub-acute toxicity cannot be excluded. Moreover, vineyards are characterized by repeated insecticide applications in summer. The consequences of repeated applications are not fully known and should be further investigated to assess the possibilities for chronic effects, as well as the insurgence of resistance for some taxa.
In contrast, the Spanish field site, cultivated mainly with olive, is characterized by a unique peak that reaches the highest level of risk, but quickly decreases due to the high solubility of the active ingredient in water.
Generally, oral exposure determines a level of risk at least one order of magnitude lower than contact exposure.
The application has an important role for the risks to some pollinator groups; bumblebees, are considered particularly vulnerable at the beginning of the productive season when fertilised queens are founding nests. The life cycle of bumblebees in temperate regions differ from that of honeybees: only the queens over-winter, in spring mated queens emerge, feed and establish a new colony, so that the entire population depends upon the success of the queens in founding the new colony each spring (Thompson et al. 1999).

5.4.2 Data reliability and factors of uncertainty

The major factors of uncertainty of the proposed approach depend upon the availability and reliability of the input data. Data on agrochemical applications (product applied and surface area treated), land use and meteorological data (e.g. rain events) were collected directly in the field and present different levels of details.
The collection of data was in some case difficult, mainly because of the lack of official sources of pesticide application data. The information on pesticide

application from different sites is not homogeneous. In some cases precise information on active ingredients applied and surface area is available (UK, Estonia, Spain, Italy). In other cases the surface area treated was not known, but information on most used active ingredients was available (Hungary, Catalonia and France). In these cases, active ingredients were supposed to be applied uniformly across all the area covered with the target crop. In most of the cases, data on pesticide application was obtained directly from the farmers or from the local farmer cooperatives. The application rate was collected in the field or estimated form Tomlin (2003) and products labels. Information on rain events was readily available from the locals metrological offices that were able to provide most of required data. Rainfall data were collected from the meteorological centre nearest to each site.

One of the drawbacks of our approach is the lack of detailed land use maps. In most of the cases the only information available for land use was the CORINE (European CORINE 2000). This kind of maps gives a general overview of the main land uses (e.g. arable vs. perennial crops), but lacked specific detail (e.g. actual crop type) needed for risk assessment at this scale. Currently there is an update of the CLC 2000 underway, and a CLC 2006 will be produced covering 38 European countries and mapping the differences in land use from 2000 to 2006 (CLC 2006 technical guidelines). During this study data on land cover for 2006 were not available. A drawback of CORINE Land Cover Maps is the resolution of the land data, with only relatively big patches of land cover represented and this may result in a lack, or incomplete, information in particular in sites with a 'mosaic' structure of land use. For these reasons information provided by CORINE was integrated, where possible, with information collected directly in the field.

For the calculation of foliage concentrations some parameters on vegetation characteristics (LAI, SLW) are required. In this paper default values have been used. Some uncertainty could be reduced by using data more representatives of the characteristics of the site-specific vegetation.

The prediction of the temporal trend of the concentration requires the estimation of dissipation patterns which depend upon molecular properties (VP, solubility, DT_{50}) and various environmental parameters. The availability of photodegradation data may be problematic. Moreover, models to assess decay of pesticides on pollen are not available and dissipation patterns in the hive may be not be comparable with those occurring outside the hive (Tremolada et al., 2004). Therefore, for pollen the same dissipation rate as for foliage has been assumed.

Finally some uncertainty may arise from the variability of toxicity data reported in literature (e.g. LD_{50} for oral exposure for chlorpyrifos ranged from 18 ng /bee to 360 ng /bee).

5.5 CONCLUSIONS

The approach developed here represents a first attempt to overcome the concept of Hazard Quotient. An index of exposure in terrestrial ecosystems was applied here in order to quantify exposure in a more realistic way. This approach permits an evaluation of the temporal trends of risk over the entire growing season and allows the assessment of the potency of agrochemical mixtures. Using this approach also allows the spatial trends of risk to be mapped using appropriate spatial units based on the known forages area of the target organism. The scale of the final assessment depends mostly on the resolution and availability of pesticide application and land use data. We are aware that several assumptions need to be better tested, and that refinement of exposure assessment needs to consider the different behaviours of pesticides in plant tissues. However, in spite of these assumptions and uncertainties, the procedure for exposure estimation has been successfully experimentally validated (Chapter IV), indicating that, even in the present form, the approach proposed could be a potentially useful tool suitable for estimating risk exposure with an acceptable level of approximation.

The validation of the risk assessment for whole pollinator communities will be much more complex. A first step will be to extrapolate this approach to other bees and pollinator taxa. Community complexity could be reduced by modelling a series of pollinator guilds which are representative of a number of individual species sharing common traits of body size, dispersal and feeding behaviour.

Within the PACRAT framework, experimental studies on pollinator communities have been performed in the same field sites considered here. The results of these studies may represent an useful opportunity for the improvement of the approach and for the validation of the theoretical procedure.

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CHAPTER VI

1. CONCLUSIONS

The aim of this research was to analyse the main critical issues of agrochemicals risk assessment in terrestrial ecosystems. Different steps of agrochemicals risk assessment were considered and evaluated coupling field studies with predictive approaches. Different scale levels of risk assessment, with particular attention on exposure evaluation, were considered. Risk for pollinators was selected as a specific case of study and a procedure to assess exposure and risk for these organisms was developed.

In 2000, Linders reported that "there has not been enough research to facilitate accurate estimates of spray interception for a large number of crops under growing conditions in various part of the word", eight years later Baldoin et al. (2008) reported that "the pesticide target loss during the application is still a problem". The open problem of pesticide mass balance was, therefore, analysed in this work. The prediction of foliage interception plays an important role both in term of pesticide efficacy (the effect concentration should be reached on target plants) and in term of environmental pollution (the fraction not intercepted may affect non target ecosystems). Starting from official exposure models adopted in EU registration process (FOCUS, 2003; Ganzelmeier et al., 1995), the mass balance of sprayed insecticides was modelled in two different situations: vineyard-hedgerows and vineyard-herbaceous strip system. The distribution of agrochemicals was evaluated considering the plants (vines rows and non target vegetation) and soil (inside and outside the field) compartments. The fraction intercepted by target crop (vine row) was calculated and compared with experimental results obtained from soil and crop leaves samples. Preliminary results obtained showed a substantial difference between measured intercepted fraction and foliage interception values reported in FOCUS (2003). In case of vineyard it appears that the seasonal changes in phenological stage could not be considered as the unique parameter to determine plant interception. Vineyard is a permanent crop that grows and changes over years, furthermore the high variability of the vines *cultivars* should be considered. A necessity of re evaluating the FOCUS (2003) foliage interception values comes from this study.

On the other hand, the predictive approach adopted for exposure assessment at a field scale for non target compartments outside the field showed, generally, a good predictive efficiency both for soil and plants compartment.

From these results a terrestrial exposure index applicable at different scale levels was developed, applied and validated. The aim was to produce a semi-

quantitative index evaluating exposure for epigeous organisms with a relevant forage area like pollinators. The preliminary validation of the index demonstrates its good predictive capability when the detail of data is high. The sensitivity analyses permitted to estimate the uncertainty of the approach linked to the dataset detail level. The elaboration of exposure methods like those here proposed represent the first step to overcomes the concept of Hazard Quotient in pollinator risk assessment method, in order to obtain results more representative of the risk posed by agrochemicals to natural communities, actually one of major challenge for modern ecotoxicology (Baird and Van den Brink, 2007).

Developed exposure assessment approach was adapted to the specific case of pollinator community and integrated in a risk assessment method applied in 13 field sites selected within the Field Site Network of ALARM project. Considered sites were representative of the main European meteo-climatic conditions and cultivated crops. Risk trend was evaluated for the productive season 2007 in a site specific GIS based approach. The method is now under validation.

Predictive approaches application and validation was supported by agrochemical analytical methods development and validation in collaboration with a research group of the CSIC (IDAEA), of Barcelona. Particularly, an analytical method to determine insecticides commonly applied in EU in solid matrices was developed and validated.

In conclusion this research represents an important overview of the main critical issues in risk assessment for terrestrial ecosystems. Starting from the general postulate that an ecological sound approach is needed in ecotoxicology (Van Straalen, 2003), alternative approaches with respect to official ones, are suggested as a preliminary first step forward a better understanding of the risk posed by agrochemicals to natural communities

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