METHODS AND TECHNIQUES USED FOR THE ASSESSMENT OF ENVIRONMENTAL EXPOSURE TO PESTICIDES AND THEIR POTENTIAL APPLICATION IN A NEW COHORT IN MOLINA, CHILE.

Master Thesis

Date 20-08-2014

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SUMMARY

Pesticides are widely used in the world due to their importance in agricultural production, especially in developing countries, where their use is high. Different health effects such as adverse reproductive outcomes, neurological disorders and cancers have been associated with pesticide exposure. Populations can be exposed to pesticides via different pathways, such as their occupation or via the environment, when pesticides applied to agricultural fields are transported from their intended treatment sites to neighboring residential areas. Environmental exposure to pesticides can occur through several pathways, such as spray drift, volatilization and dispersion, while take-home pathway and personal use can also contribute to pesticide exposure within the living environment. There are different methods and techniques described in the literature, which are used to assess environmental exposure to pesticides. Measurement techniques, such as bio-monitoring and the collection of environmental samples have been used to determine environmental exposure to pesticides. The main advantage of bio-monitoring is that the actual total pesticide exposure (the dose) of a subject is measured, but short half-lives of most biomarkers limit their application to short-term health effects and small study populations due to high costs. Modelling techniques, making use of geographic information systems, have been developed recently and have become one of the preferred techniques when researchers want to study long-term health outcomes related to environmental pesticide exposure in large study populations. However, validation studies are generally lacking for these models, which represents a problem for the use of these techniques.

Currently, a new prospective cohort study in the city of Molina, Chile, has been initiated to study chronic health effects related to environmental factors (such as pesticides). The aim of the Maule Cohort (MAUCO) is to enroll 10,000 subjects aged 38 to 74 years living in the city of Molina and follow them up for 10 years. As this region is heavily involved

in agricultural activities and this cohort focusses on chronic, long-term health effects, past environment exposure to pesticides is one of the main interests. Geospatial modelling, making use of the subjects' residential history, data on agricultural land-use, and (historical) pesticide use would be the most suitable in this context to determine (past) environmental exposure to pesticides. In addition, a sub-study, making use of biomarkers and environmental samples, is recommended to determine validity of the modelled environmental pesticide exposures.

LIST OF ABBREVIATIONS

FAO	Food and Agriculture Organization
US-EPA	United States Environmental Protection Agency
OP	Organophosphate
CA	Carbamate
PYR	Pyrethroid
DIT	Dithiocarbamate
GLY	Glyphosate
TRI	Triazines
PD	Parkinson’s disease
PUR	Pesticide Use Report
GIS	Geographical information system
ACCDiS	Advanced Center of Chronic Diseases
MAUCO	Maule Cohort

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INTRODUCTION

Pesticides are chemicals and biological products which are widely used throughout the world. According to the Food and Agriculture Organization (FAO), it is estimated that 5.4 million tons of pesticides were used worldwide during 2010 with an amount of $23 billion spent only in pesticides’ export (1). 1.8 billion people who engage in agriculture use these compounds to protect crops and products they produce, with especially high volumes used in developing countries. Countries that, in addition, often lack programs to control exposure to pesticides (2).

Exposure to pesticides has been associated with several acute and chronic health outcomes in humans. Among these, there are reproductive diseases such as decreased fertility, demasculinization and elevated rate of miscarriage (3); cardiovascular diseases such as hypertension and atherosclerosis (3); respiratory diseases such as asthma (3); and neurologic diseases, such as Parkinson’s diseases, Alzheimer’s diseases and Amyotrophic lateral sclerosis (3, 4). Furthermore, associations between pesticide exposure of the mother and adverse birth outcomes such as decreased birth weight, shorter pregnancy length, decreased cognitive abilities, attention and reflexes problems in children have been reported in a number of studies (5, 6). Moreover, psychiatric disorders such as depression and suicide have been also described in relation to pesticide exposure (7), as well as many types of cancer in adults and children such as breast, prostate, lung, colorectal, stomach and pancreatic cancer in adults; and Leukemia in children (3, 4). Health outcomes that can occur due to short- or long-term exposures, depending on the time lapse between the exposure and the occurrence of the health outcome.

Although research has traditionally focused on pesticide exposure in occupational settings, people can be exposed to pesticides via other routes. One of these is the environmental pathway, and environmental exposure to pesticides has also been associated with adverse health outcomes (e.g. Parkinson's diseases, low fertility and many types of cancer) in a number of studies (3-5, 7).

Environmental exposure to pesticides involves the exposures that do not occur in an occupational setting, but originate from the (outdoor) environment and result in subjects being exposed to pesticides in their living environment. The pathways involved can be related to pesticide drift from pesticides application on neighboring agricultural fields, atmospheric contamination and deposition. Other pathways such as the residential (personal) use and take-home pesticides can also contribute to the exposure of pesticides within the living environment, however these are not considered as true environmental exposure (8-14). Since environmental exposure is not only restricted to the population that is occupationally exposed to pesticides (e.g. farmers), the number of people potentially affected is larger, including a potentially more susceptible part of the population, e.g. children and elderly people.

Over the past years, several epidemiological studies have investigated the potential associations between environmental exposure to pesticides and health outcomes. However, the most important limitations and considerations in these epidemiological studies are the methods used to assess environmental pesticide exposure in the study population.

Several methods and techniques have been described to assess environmental exposure to pesticides. There are methods that measure exposure directly, either via bio-monitoring (15-36) or environmental (37-44) samples of the study population's living environment (***Measurement methods***); and methods that model the environmental exposure to pesticides, based on data on regional pesticide use and geographical characteristics of the residences from the study population (***Modelling methods***) (45-52).

The following thesis will present a literature review on the assessment of environmental exposure to pesticides. First, a brief introduction will be given about the processes involved in contamination of the environment with pesticide products and environmental exposure to pesticides (Chapter 1). Second, it will explain the main methods used in scientific literature to assess environmental exposure to pesticides (Chapter 2 and Chapter 3). Finally, it will describe the aims and characteristics of a new prospective cohort study which is located in Molina, a rural city in Chile, and discuss which of the exposure assessment methods described would be suitable to study health outcomes potentially related to environmental exposure to pesticides in this cohort (Chapter 4).

CHAPTER 1. PESTICIDES AND ENVIRONMENTAL EXPOSURE

1.1 Pesticides

According to FAO, pesticides are defined as "any substance or mixture of substances of chemical or biological ingredients intended for repelling, destroying or controlling any pest, or regulating plant growth" (53).

Pesticides are used in agriculture for the control of insects, fungal diseases and weeds in crops. They bring many benefits since they increase agricultural yields and decrease manual work (54). These benefits are of great interest nowadays due to the intensification of agricultural production to meet the increasing demand for food, feed and fiber; and the need to comply with quality standards commanded in international trade. Therefore, pesticides play a key role in crops production (55).

Different types of pesticides are used in agriculture and can be grouped based on their main function into herbicides, insecticides, fungicides and others (56). According to the United States Environmental Protection Agency (US-EPA), 40% of the pesticides used in the world during 2007 were herbicides, 18% insecticides and 10% fungicides (57). A second way to group pesticides is by their chemical group or structure such as organophosphates (OP), organochlorine (OC), carbamate (CA) and pyrethroids (PYR) insecticides, phenoxy, urea, triazine (TRI) and glyphosate (GLY) herbicides, or dithiocarbamate (DIT) fungicides, among others.

1.2 Pesticides and environmental exposure

Environmental exposure to pesticides can be defined as the exposure that occurs through non-occupational routes of exposure, especially via the (outdoor) environment (48). Different pathways have been described via which environmental exposure can occur and potentially affect the human population. Among these pathways are pesticide drift, atmospheric dispersion of pesticides and deposition. Other routes such as take home pathway and residential (personal) use of pesticides can also contribute to the environmental exposure to pesticides in the living environment and should be considered as well, however these do not classify as true environmental exposure to pesticides.

1.2.1 Pesticide drift and atmospheric dispersion and deposition

According to the US-EPA, pesticide drift is define as the movement of pesticide dust or droplets through the air at the time of application or soon after, to any site other than the intended treatment area (8).

During pesticide application, pesticide products are often sprayed in a liquid form and are usually atomized by hydraulic pressure nozzles, producing different droplet sizes. Droplet size depends on factors such as the type of application and equipment used (e.g. type of sprayer and nozzle type); which in the end are important in terms of the droplets' movement (drift). Larger droplets settle to the ground sooner than smaller droplets, which have a greater tendency to drift downwind (58). In addition, this drift can be influenced by a variety of meteorological factors such as wind speed and direction (14).

Up to 30-50% of the amount of pesticide product sprayed can be lost to the air during and after application (9) being deposited in off-target lands. Besides, pesticide drift and drift deposition, sprayed pesticide products can be also transported in the environment via other (secondary) routes; they can be volatilized, transformed and transported over shorter or longer distances, and eventually deposited as dry deposition or precipitation (wet deposition) on soil, water and other off-target crops. These secondary processes not only depend on droplet size and meteorological conditions, they also depend in specific properties of the compounds and other factors (58).

Pesticide volatilization is more likely to occur from particles that are deposited on plants, water and soil than from the spray droplets themselves (58). Vapour emissions from water, plants and soil can be influenced by the physical-chemical properties of the compounds (e.g. solubility and vapour pressure), meteorological conditions (e.g. air temperature), agricultural practices (e.g. application rate) and the special properties of each type of the surfaces; for instance the water content and pH in the case of soil; and amount of foliage in the case of plants. Moreover, emissions from soil not only involve volatilization, but also wind erosion of soil particles with absorbed pesticides. All of these emissions routes have shown to be significant in experimental data and are therefore an important source of pesticides in the atmosphere (10).

After pesticides get into the atmosphere, different processes such as transport and transformation take place before the pesticides are deposited again. Different kinds of transformations (e.g. photolysis or reactions with free radicals such as OH) can occur in the atmosphere depending on the phase of the pesticide (either gas or particle phase) (10).

Removal of pesticides from the atmosphere can occur via deposition. This can be dry deposition of particles with adhered pesticides onto different surfaces (soil, water, plants) or wet deposition (precipitation) through the rainfall with pesticides present in the water droplets. Just as for the transformations, both processes depend on the phase of the pesticide. For instance, wet deposition often occur when pesticides compounds are as a particle, while either dry or wet deposition can be produced when compounds are in a gas phase (10).

Transport of pesticide compounds through the atmosphere can range from 1-1000 m distance from the source of application (short-range transport), to even more than 1000 km (long-range transport), depending on chemical and meteorological factors, and the layer or height in the atmosphere where the compounds are. Short-range transport usually involves dispersion of particles through the wind without transformation and deposition processes, while long-range transport involves transformation and the mix of the substance over the highest layers of the atmosphere (10). Finally, via these processes mentioned, pesticides can be transported and deposited into the living environment, resulting in environmental pesticide exposure. A picture summarizing these processes can be seen in figure 1.

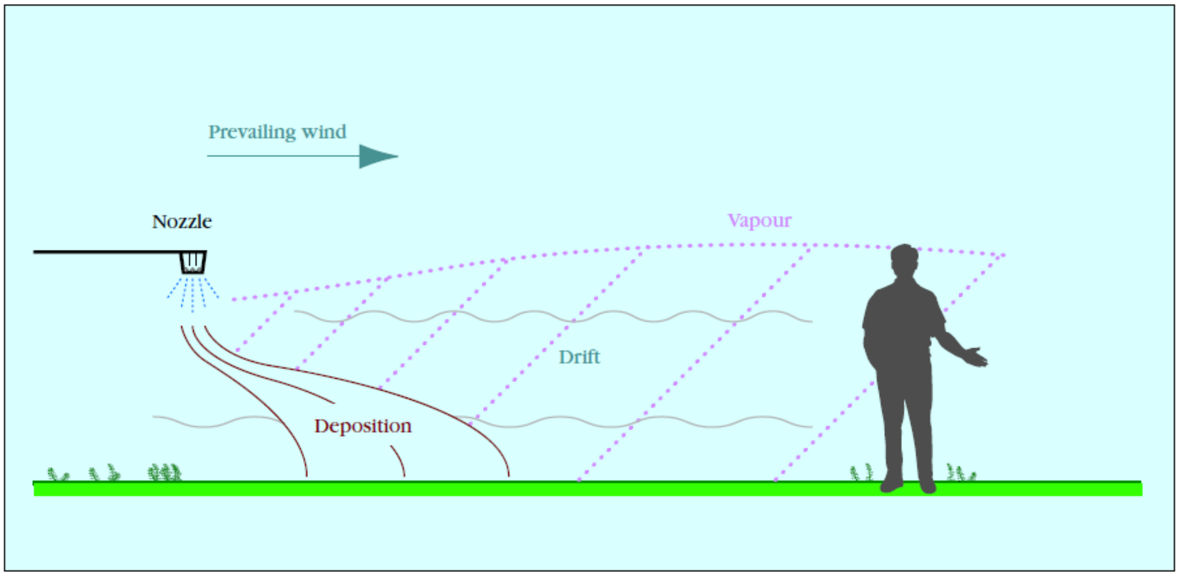

Figure 1. Scheme characterizing the processes of pesticide spray drift, volatilization (vapour), transportation and deposition, which may result in environmental exposure to pesticides (10).

Several studies have investigated the process of pesticide drift and its relevance for environmental pesticide exposure. In a study by Garron *et al.* (2011), significantly higher air concentrations of pesticides were detected in locations at 0 and 30 m away from the sprayed area during 2 hours after spraying compared to the pre-spray concentrations. On the contrary, for the locations 100 m away, no significant difference between the concentrations measured at pre-spray and 2 hours post spray were found. No effect of wind speed on pesticide concentrations was found in this study (59). Similar experimental studies have investigated drift from crop fields using different types of spraying equipment and nozzle types (60-62), showing that pesticide drift is higher closer to the fields edge, and that concentrations depend on the crop treated and the spraying equipment used.

As pesticide concentrations are clearly dependent on distance from the application source, many epidemiological studies investigating the association between health outcomes and environmental pesticide exposure use the proximity to agricultural land as an indicator of environmental pesticide exposure (45, 50, 51).

1.2.2 Take-Home pathway

Pesticide exposure via the take-home pathway involves agricultural workers who are occupationally exposed to pesticides and take these compounds into their homes by contaminated working clothes and shoes (12, 13).

The take-home pathway is believed to be an important pathway for exposure of pesticides in the living environment, especially for children's exposure to pesticides. Small children tend to spend large amounts of time on the floor and have a high hand-to-mouth frequency. Thereby they can come into contact with pesticide residues on the floor or in house dust, tracked into the home by household members (13).

In a literature review by Coronado *et al.* (2011), the researchers reported higher dust concentrations of pesticides in homes of agricultural workers (e.g. threefold higher mean concentrations of herbicides) and higher urine metabolites of pesticides in children of agricultural workers as compared to children of nonagricultural workers in many studies (e.g. 6,72 vs. 2,72 $\mu g l^{-1}$, respectively). They concluded that there is mounting evidence that this route is an important source of pesticides in the living environment if a subject is employed in agriculture (13).

1.2.3 Residential or personal use of pesticides

Finally, the residential or personal use of pesticides within or around the home is another source of pesticides in the living environment. Personal or home pesticide use can includes pet treatment, extermination of household pests, removal of lice, and garden and lawn treatments. These kind of pesticide treatment are common in the general population, however they can be different from those applied in agricultural fields (12).

CHAPTER 2. MEASUREMENT METHODS TO ASSESS ENVIRONMENTAL EXPOSURE TO PESTICIDES

Measurement methods to assess environmental exposure to pesticides can be divided in methods that involve bio-monitoring of the study population, and methods that involve measurement of environmental media from the residences or areas were the study population lives or spends the majority of their time.

2.1 Bio-monitoring

Bio-monitoring involves the measurement of the parent pesticide, its metabolite or reaction product in biological media, such as blood or urine, to determine if exposure to this pesticide has occurred (15).

Following environmental exposure, pesticides can be absorbed through the skin, respiratory, and gastrointestinal tract. In this context bio-monitoring is of importance since it provides data reflecting the cumulative dose absorbed by the body by all routes (16). However, to successfully use these techniques, information about the target pesticides are needed such as their pharmacokinetic properties (15).

Below, the main types of biological samples used in the assessment of environmental exposure to pesticides are described, which pesticides or metabolites can be measured in these samples and examples of studies that used these techniques to assess associations with health outcomes.

2.1.1 Biological samples

Many different biological samples can be used to study environmental pesticide exposure. From those samples, blood (plasma and serum), and urine are the most widely used. According to Barr *et al.* (2002), blood samples have the advantage that in most cases the parent compound is measured, instead of the metabolite. Therefore, it yields more accurate information about the specific exposure. Furthermore, the concentrations of pesticides measured in blood/plasma/serum are often more stable than in other biological fluids and do not depend on external factors such as water intake. However, sampling of blood is an invasive technique for epidemiologic research which can limit the subjects participation rate (17).

On the contrary, urine samples are an easy sample to obtain, enabling repeated sampling and will suffer less from decreasing participation rates compared to studies using blood samples. Furthermore, according to Barr *et al.* (2002), the concentrations of pesticides or metabolites in urine have typically higher values than in blood due to rapid metabolism and excretion; and are detectable for a longer period of time (17, 25). However, in urine, metabolites are usually measured instead of parent compounds, and the concentrations may be influenced by factors such as dilution due to differences in water intake (17).

Beside blood and urine samples, other fluids or human products can be used to measure pesticide exposure. For instance, Koutroulakis *et al.* (2014) describe a method to assess OP insecticide exposure using amniotic fluid during a critical period of fetus development (between the 16^{th} and 20^{th} week of pregnancy), however its invasive characteristic may be an important disadvantage for using this type of biological sample (19). Another method currently used is measuring pesticides in hair, which enables the detection of pesticide exposure over a longer time-period, as pesticides appear to be stably fixed in hair, compared to blood and urine (20). However, it requires more complex pre-analytical procedures (36). Breast milk is a biological sample which has been successfully used to study the presence of OC insecticides in a Japanese study (15). Finally, meconium is another sample which has been used for the bio-monitoring of pesticide exposure, especially OP compounds. It can be used to assess exposures of the fetus from the week 16^{th} of the gestation and it is a non-invasive and easy sample to collect (22).

2.1.2 Biomarkers

Several biomarkers can be measured in bio-monitoring samples. Within blood samples, parent compounds are often measured. OP, CA and PYR insecticides and some TRI herbicides such as atrazine can be measured in serum or plasma using a method described by Barr *et al.* (2002) (17). Similarly, OC insecticides can also be measured as parent compounds in blood, serum or/and plasma (15).

In urine, it is possible to measure the parent compounds or their metabolites. For example, when taken up by the human body, OP insecticides are hydrolyzed to a dialkyl phosphate moiety (DAP), which is excreted in the urine. Six DAP metabolites are most commonly measured in urine in epidemiological studies: DMP, DMTP, DMDTP, DEP DETP and diethyldithiophosphate (15). However, these analyses measure the cumulative exposure to all OP insecticides a subject was exposed to. There are some specific OP insecticide metabolites which can be measured in urine, such as TCPy, a specific metabolite from chlorpyrifos or malathion dicarboxylic acid (MDA) a metabolite that reflects malathion exposure specifically (18).

Other insecticide metabolites measured in urine are y-HCH, a metabolite of endosulfan and lindane (18), 1-naphtol, a non-specific metabolite of CA insecticides, or 3-Phenoxybenzoic acid (3PBA) a metabolite of PYR insecticide (15).

For GLY herbicides, the parent compound can be measured directly in urine (28), while for TRI herbicides the metabolite mercapturate can be used as a biomarker of specific exposure to atrazine (29).

2.1.3 Analytical techniques

Gas chromatography-high-resolution mass spectrometry (GC-HRMS) is one of the main techniques described to detect pesticides in the different biological samples described above. This technique is used by Barr *et al.* (2002) (17). Furthermore, other techniques make use of gas chromatography (GC) to detect pesticides or metabolites in urine or blood samples, for examples GC with electron capture detection (GC-ECD), GC with mass spectrometry (GC-MS), GC coupled with flame photometric detection (GC-FPD), GC with flame ionization detection (FID), GC with mass spectrometry or tandem mass spectrometry. These techniques vary in costs and sensitivity to detect the pesticides of interest (15).

For samples such as hair, since it is a solid matrix, additional pre-analytical procedures are needed compare with other biological fluids, for example liquid-liquid and solid phase extractions (LLE and SPE) and gas or liquid chromatography tandem mass spectrometry (36).

2.1.4 Biomarkers half-life

The use of bio-monitoring to assess (environmental) exposure to pesticides involves accurate knowledge about pharmacokinetic of the pesticides of interest in each type of biological sample.

In terms of blood samples, compounds are usually no more than 24 hours available in blood. A study conducted by Busby-Hjerpea *et al.* (2010) found a half-life of 6.3 hours of the compound chlorpyrifos in blood of rats; which after 24 hours was not detectable anymore (23). Moreover, for another OP compound (malathion) the half-life in blood was found to be only 12 minutes (18), and for atrazine, a TRI herbicide, most of the parent compound and its metabolites were excreted within the first 24 hours in a study conducted in mice by Ross *et al.* (24). This reflects an important consideration when using bio-monitoring to investigate pesticides in blood samples, especially for pesticides which tend to be rapidly metabolized and excreted in urine within the first days after exposure (25).

In urine, pesticides or their metabolites can typically be detected over a longer time window after exposure. Roca *et al.* (2014) reported that DAP metabolites are usually excreted within 24 to 48 hours after exposure, while for PYR this excretion occurs within 4 to 13 hours and for herbicides such as TRI and GLY this time can vary from 12 to 72 hours (33).

As described in the previous paragraphs, bio-monitoring of pesticides is often only suitable when studying exposure in relation to short-term health outcomes. Epidemiological studies that use bio-monitoring to assess pesticide exposure mainly focused on reproductive outcomes, which can be considered short or mid-term outcomes. Perera *et al.* (2003) assessed the effects of trans-placental exposure to environmental pollutants, such as OP insecticides, on birth outcomes. This study measured chlorpyrifos levels in the blood of women within one day postpartum and umbilical cord blood collected at delivery, and assumed that one measurement during delivery was enough to assess overall maternal environmental exposure during pregnancy (26). Wolff *et al.* (2006) assessed the association between OP metabolites levels (DEP, DMP and DAP) in the urine of future mothers and birth outcomes (31). This study took two urine samples during pregnancy to measure OP metabolites concentrations, assuming that the exposure was constant during the whole period, to determine the environmental exposure to pesticides during pregnancy and its potential association with birth outcomes.

2.2 Environmental sampling methods

As bio-monitoring is not always feasible, or as a complement for bio-monitoring, the presence of pesticides can be measured in the living environment or any other defined environment of the study population (e.g. a school). Often house dust is analyzed, which can be obtained from carpets or floors located in strategic areas, or from other surfaces in the environment of the study participants. Furthermore, pesticides or their metabolites can also be measured in air samples and soil samples, among others.

2.2.1 Dust samples

Dust samples are considered to be a good medium for assessing long-term exposure to pesticides in the home, since pesticides tend to be protected from degradation and transformation by sunlight, moisture and microorganisms within it (37). Furthermore, it is thought that pesticides levels in such environment may be more temporally stable than those in bio-monitoring samples (41). Moreover, house dust can be collected in large quantities, enabling the analysis of a large number of different pesticides (38).

Dust samples are of importance in studies that relates pesticide exposure to health outcomes in many types of populations, but especially in small children, which are usually in close contact with dust on the floor. Some studies described the use of a specially designed vacuum cleaner to sample dust as it has the ability to collect particles < 5 microns in diameter and to achieve a constant dust removal efficiency across different types of carpet (41). However, a recent study by Colt *et al.* (2008) demonstrated that the use of a household vacuum cleaner is a reasonable alternative, since there is a good correlation (0.6 or higher) between the pesticides concentrations detected by using either a specially designed device or a household vacuum cleaner (38). Usually, the dust is collected in the rooms where the subjects spend most of their time. Alternatively, samples can be collected from rooms located on the side of the home which face agricultural crops due higher likelihood of pesticides' detection. Most studies collect dust from an area of at least one m^2 within a pre-defined room, with a total sample of 10 mL. Carpets are mainly used for this collection, however smooth surface floors and furniture attachment can also be sampled (38, 39, 41). Methods similar to those used to detect pesticides in bio-monitoring samples are also used to detect pesticides in dust samples, such as GC-MS (39).

Deziel *et al.* (2013), evaluated how stable pesticide concentrations were in multiple dust samples taken over a period of two years. The researchers found that one sample of dust within the two-year period was a reasonable surrogate for the average exposure, (median intraclass correlation coefficients between samples was 0.73) (38). The latter is important in terms of epidemiological studies that try to assess associations of environmental exposure to pesticides and long-term health outcomes such as cancer or neurodegenerative diseases.

Pesticide concentrations measured in carpet dust samples were significantly associated with the proximity of the residence or school to agricultural fields (e.g. 83% increase in dust concentrations of chlorpyrifos insecticide and 19% increase in dust concentrations of tetrachloroterephthalate herbicide for each kg applied per day, near participant homes) (11, 37, 41), indicating that the use of pesticides in vicinity of such location results in environmental exposure to pesticides in these homes.

2.2.2 Other environmental samples

There are other environmental samples described in the literature which can be used to determine environmental exposure to pesticides. However, these samples are not as widely used in epidemiological research, as they may not be as stable as the dust samples mentioned before.

Samples from soil and air have been used to assess the presence of pesticides in residences, schools and playgrounds. Air samples can be collected by passive and active methods. Dalvie *et al.* (2014) used an active method to sample air in and around schools located near crops fields and vineyards in South Africa, and measured pesticide concentration in these samples. Researchers found five different types of pesticides within these samples (11). Another study by Wofford *et al.* (2014) also sampled air using an active method in three elementary schools in the city of Parlier, United States. They measured concentrations of 35 different pesticides and used the concentration over 24 hours, the average concentration every two weeks, and the average concentration for the year as a proxy of acute, subchronic and chronic exposure (42). However, to study long-term effects of environmental exposure to pesticides the degradation rates of the pesticides of interest in air should be considered, as pesticides can degrade or metabolized in air as well (10).

Soil samples can also provide information on environmental exposure to pesticides, and different studies have used such samples in the past. Morgan *et al.* (2014) collected one sample of soil by scraping surface near a playground area of children or within 500 m of the school and public areas of the children's home. Their main objective was to study the exposure to pesticides in a population of small children in a quantitative way (40). However, the persistence of pesticides in soil varies depending on the specific compound. A study conduct by Sharma *et al.* (2014) showed that in field trials following good agricultural practices, the insecticide flubendiamide and its metabolite were no longer in the soil 15 days after application (43), while in a laboratory study with the fungicide tetraconazole, it showed a half-life greater than 66 days (44). The latter reflects that, similar to biomarkers and air samples, the use of soil samples to study environmental exposure to pesticides requires knowledge about the persistence of the pesticides in this environmental media, to determine if these samples can be used to study short- or long-term exposures and health effects.

Many methods can be employed to measure or approximate environmental exposure to pesticides. All have their advantages and disadvantages, and researchers will need to evaluate these in relation to the design of their study to decide on the feasibility and most appropriate methods.

CHAPTER 3. MODELLING METHODS TO ASSESS ENVIRONMENTAL EXPOSURE TO PESTICIDES

During recent years, modelling methods to assess environmental exposure to pesticides have been increasingly used and improved. Nowadays, such techniques have become one of the preferred methods used by researchers to study environmental exposure to pesticides. These methods can enable investigating long-term exposures and long-term health outcomes such as cancer (45), which is often not feasible using bio-monitoring or environmental measurements.

The modelling of environmental exposure to pesticides has progressed rapidly over time and increasing numbers of variables and exposure determinants have been incorporated to improve exposure assessment. The simplest methods described in the literature are those which involve the distance between the source where pesticides were applied, and health outcomes (*Modelling methods using distance to the source*). Other methods use administrative areas (i.e. regions), with an exposure estimate based on regional agricultural land-use or pesticide use practices, and the analysis of health outcomes within each area (*Modelling methods using an ecological approach*). Finally, more complicated methods involve geographic information systems (GIS) in which data on the location of residences of the study population, spatial and temporal data on agriculture and pesticide use, and geographical and meteorological data are combined to model environmental exposure to pesticides (*Modelling methods using land use data and GIS approach*).

3.1 Modelling methods using distance to the source

Early studies investigated associations between pesticide exposure and health outcomes by using proximity of certain pesticides sources, such as specific crops fields or waste sites where pesticides were disposed, as a surrogate for environmental pesticide exposure (45). Aschengrau *et al.* (1996) studied the association between different types of cancer and the proximity of the patients' residences to cranberry crops in a case-control study. The exposure assessment entailed calculating the distance of each residence to the nearest edge of the fields by the use of aerial photographs and maps prepared by the Department of Forestry and Wildlife Management at the University of Massachusetts. Based on previous studies of spray drift, any house within 780 m of a cranberry field was considered exposed (46). Bell *et al.* (2001) studied the association between environmental pesticide exposure and fetal death due to congenital anomalies. These authors used data from the Pesticide Use Reporting (PUR) dataset, available in California, which contains information on the application of all restricted-use pesticides, including timing and location of each application. Researchers were able to assess the proximity of the mothers' residence to locations where specific pesticides were applied. A subject was considered exposed to a particular pesticide if the residence was within an area of 1 square mile or within one of the adjacent square miles next to a pesticides' use location (47).

Nowadays there are studies still using distance between residences and pesticide exposure source to approximate environmental pesticide exposure, but most studies are using more complex ways to estimate environmental exposure, using additional geographical and meteorological data.

3.2 Modelling methods using an ecological approach

Another method to model pesticide exposure is the ecological approach. In epidemiology, ecological studies make use of groups or populations defined by their geographical area, on which exposure status is also based. Exposed and un-exposed populations are compared to determine if there is an association between the exposure in the regions and the health outcomes of interest.

This type of design was used in two recent studies by Parrón *et al.* (2011 and 2013), who studied the risk of cancer and neurodegenerative diseases in relation to pesticide exposure. The authors selected health districts with high agricultural activity and used data from the Andalusian Council of Agriculture to determine which of these districts had a larger number of hectares devoted to intensive agriculture, using this variable as a surrogate for pesticide exposure (48, 49). Another interesting study using this approach is the one by Moisan et *al.* (2011). In this study, the researchers identified PD patients from a farmer dataset in five districts in France, and estimated their exposure based on the density of 16 farming types within each district, using five different categories. Interestingly, in this study, researchers were able to use individual information of the population, which is often lacking in ecological studies (52).

3.3 Modelling methods using land use data and geographic information system approach

Over the years, more sophisticated methods to model environmental exposure to pesticide have been appearing in the literature. These new methods make use of GIS and elaborate on the proximity of an pesticide exposure source, by adding other spatially (and temporally) resolved data related to agricultural land-use, pesticide use, geographical characteristics of the location where the study population lives, or even meteorological variables. One of the first studies describing this type of method was conducted by Ward *et al.* (2000). These authors developed a model to estimate potential environmental exposure to agricultural pesticides in a population by using historical pesticide use data and satellite images in GIS. They created historical land cover map of the region of interest from satellite images of 1984, where they identified specific crops cultivated through remote sensing. Remote sensing is a technique that determines the type of land cover and vegetation (crops) from the colors of the satellite image (see Figure 2). Ward *el al.* (2000) calculated probabilities of pesticide for each crop and added these to the land-cover map. They located the study participants' residences and determined the proportion of major crop types located within a 500 m buffer distance from the residences. Subjects were considered exposed to pesticides if one or more major crop type was present within the 500 m buffer (50). A similar approach was used in a study on birth outcomes (birth weight) (51). The authors created crop maps for the years 1991 until 1993, which were matched to the mother's residences at the time birth, and information on the birth outcomes. The percentage of area covered by crops in a 300 m and 500 m buffer around the residences and during the year of birth was used as a proxy for environmental pesticide exposure.

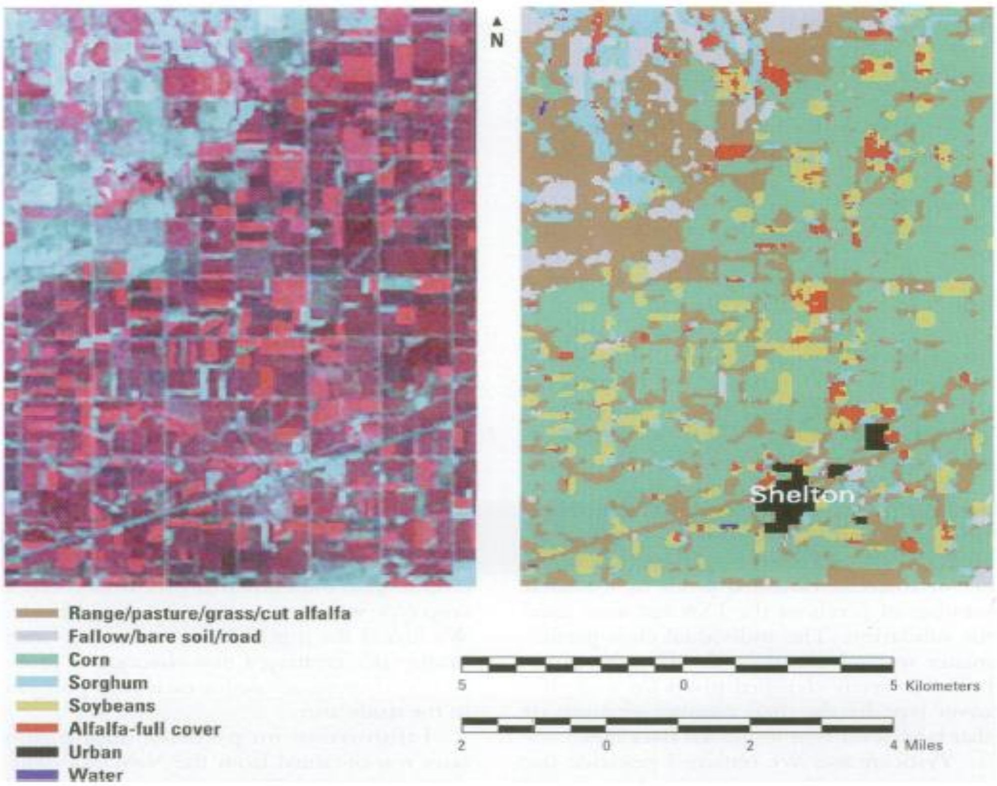

Figure 2. Original Landsat image used (left) and the land cover map (right) developed by Ward *et al.* (2000) to estimate environmental pesticide exposure (50).

These studies made use of land use data (based on satellite images) and geographical information on the subjects' residences to assess environmental exposure to pesticides. However, pesticide dispersion in the environment may be influenced by other factors such as meteorological conditions. Brody *et al.* (2002) took into account meteorological factors in their study aiming to develop a historical model to reconstruct residential exposure to large-scale pesticide applications. They used historical data on pesticide use for the last 40 years within the study area. In GIS, this data was combined with land-use coverages from past years and other datasets on hydrography, power lines, property boundaries, etc. The researchers developed a computer program (The Spatial Proximity Tool) to link subjects' addresses with this environmental data to calculate the relative intensity and duration of the historical pesticide exposure in the study population. Even more, after the relative exposure calculation, the authors adjusted the estimates from aerial applications with The Spray Drift Task Force AgDRIFT model, a model that took into account the meteorological conditions to predict in a better way the spray drift (45).

In conclusion, proxies of exposure and limited modelling using either dispersion modelling or land use regression are used to model environmental exposure. Limited data on agricultural land-use and residence location can be sufficient for simpler approach, while the more sophisticated modelling of environmental pesticide exposure requires much more input, such as local pesticide use and meteorological conditions at the time of the application. Depending on data-availability, researchers need to determine which approach is feasible in their study.

CHAPTER 4. COHORT IN MOLINA, CHILE

Molina is a city located in Curicó Province of the Maule Region, in central Chile (see Figure 3). It is a city with a population of 38,521 inhabitants which are highly dedicated to rural living and agricultural work. In fact, the Maule Region is the region of Chile with the highest percentage of the population living in rural areas (33.6%) (63), and a high density of agriculture, accounting for the 16% of the total number of agricultural fields in the country. A total of 266,371 hectares is dedicated to agriculture in this region, from which cereals, fruit trees and vineyard are the most important, with an area of 73,719, 54,749 and 45,514 hectares, respectively (64).

Figure 3. Map of Chile and the location of the city of Molina.

Due to the large number of agricultural fields in the region, pesticides are widely used. During the year 2008 a total of 5,491,947 kg/l of active ingredients were sold in Maule, making it the third region in the country with the most pesticide use. Fungicides and bactericides where the most sold pesticides during this year, followed by insecticides. The main insecticides, herbicides and fungicides used in agriculture were OP, CA and PYR insecticides, GLY and TRI herbicides and DIT fungicides (65).

Nowadays, Molina is of importance to scientific research in Chile because a group of researchers within the Advanced Center of Chronic Diseases (ACCDiS) decided to conduct the first cohort study in Chile on chronic diseases in this city: The Maule Cohort (MAUCO). The aim of the MAUCO is to investigate chronic diseases such as cardiovascular diseases and cancer, which are believed to be related with chronic inflammatory processes, and identify their (environmental) risk factors, biomarkers of diseases, mechanisms and potential preventive measures (66).

Molina was selected because recent studies showed a high mortality rate for stomach cancer in the Maule Region, especially in Molina (67). Furthermore, the city also has significantly higher rates of mortality due cardiovascular diseases than the rest of the country (68). In addition, logistic reasons were also considered such as proximity to the capital, transport connections, proximity to a University and quality of health care services (69).

With the founding of the "Fondo de financiamiento de centros de investigación en áreas prioritarias (FONDAP)" by the Government of Chile, and the joint work of the major universities in the country, the MAUCO aims to enroll 10,000 adults between 38 and 74 years living in the city of Molina and follow them up for 10 years. The recruitment will involve two phases: the first by incorporation of volunteers by invitation letters, second by visiting the homes of the residents of Molina. Subjects are eligible for inclusion if they 1) are adults between 38 and 74 years old, 2) intent to remain living in the city for the next three years, 3) do not have any terminal disease (e.g. cancer), and 4) can give an informed consent at the moment of the enrollment. Data will be collected at the baseline and will involve a face-to-face interview were information will be collected on health status, lifestyle habits, nutrition, occupation and residential history, among others. In addition, biological samples (i.e. blood, urine and hair) and environmental samples (i.e. soil, food and house dust) will be collected and stored for further analysis (69).

The MAUCO project will start with a pilot study in August 2014, involving the enrollment of 3,000 subjects. The total sample size of 10,000 subjects is anticipated to be reached in December 2015. Between January 2016 and December 2018, re-examinations of the study population will take place every 24 months. The researchers hope to further extend the follow-up period of 10 years in the future (69).

Exposure to pesticides is one of the main exposures of interest for this cohort (69). Occupational exposure, personal use of pesticides and dietary intake of pesticides can be addressed via the sections in the main questionnaire, either directly by self-report (personal use and diet), or potentially indirectly by asking for an occupational history to assign pesticide exposure to via job-exposure-matrices, minimizing recall bias. To determine environmental exposure to pesticides, other methods will need to be used.

There will be a large number of study participants involved and the aim of the MAUCO is to study chronic diseases such as cancer. Therefore long-term exposures to pesticides, going back in time for at least 10 years, need to be investigated. The most suitable technique to assess these exposures retrospectively is modelling environmental pesticide exposure by the use of agricultural land-use maps, historical pesticide use data, and geographical data on the location of the subjects' residences, among others. The subjects' residential history can be collected via the main questionnaire.

Since 1997, Chile has a registry of land vegetation which is managed and updated by the National Forestry Corporation of Chile (CONAF) (70). The system has information about the registry of native forest, vegetation and land use for the whole country. However, for the Maule region, this information has only been updated for the years 1999 and 2009. There is no information available for every year and more importantly, this registry provides no information on which specific crops were cultivated in the region. Nonetheless, other sources of information on crops are available. Satellite images are freely available for several years in the past with a resolution of 30 meters (see Figure 4). Satellite images with better resolution (5 and 0.5 meters) are also available, however these are not for free. Satellite images can be used to construct crop-maps for the Maule region by using remote sensing techniques, similar to the studies from Ward *et al.* (2000) and Xiang et al. (2001).

Figure 4. Satellite image (Landsat) of the city of Molina (available in: http://earthexplorer.usgs.gov/)

There is only limited information on pesticide use available in Chile. There are no farmers' surveys or registrations such as the PUR in California. However, the Agriculture Service of Chile (SAG) has a registry on pesticide use which is not public, and this database might be obtained in the future. Alternatively, crop protection specialists can be interviewed to determine (past) pesticide use on the main crops in the region, similarly to what was done in the study by Brouwer *et al.* (71). The aforementioned authors used expert assessment to reconstruct historical pesticide use in The Netherlands and compared it with self-reported pesticide use, collected from framers surveys. The agreement between the experts and the data was reasonable (Inter-expert agreement K_w=0.25 to 0.69; agreement between experts and the data K_w=0.32 to 0.69). Another alternative is conduct a survey in a sample of agricultural holdings within the city of Molina and surroundings to collect information about current pesticide use and use it as a proxy for past exposure.

Effort will be needed to collect and/or create land-use maps and pesticide use information for the Maule region, in order to model environmental exposure to pesticides for MAUCO. Furthermore, the biological samples and environmental samples that are already contemplated in the study design should be used to validate this modelling work performed. However, with these samples only modelling of recent exposure can be validated, leaving some uncertainty on the models performance back in time.

DISCUSSION AND CONCLUSIONS

Measurement and modelling techniques to assess environmental exposure to pesticides are widely available nowadays. Different biological and environmental samples can be used to measure pesticides or its metabolites (15-44), while many variables such as agricultural land-use, historical pesticide use, geographical characteristics and meteorological conditions can be used to model environmental exposure to pesticides in GIS (45, 50, 51).

However, to study associations between environmental exposure to pesticides and health outcomes, not every method is suitable. For instance, bio-monitoring of pesticides or metabolites in blood or urine samples is often only suitable if short-term outcomes are of interest, since most pesticides have short-lives in the body and can only be detected during a short time period after exposure (23, 24, 33). Measuring multiple samples during the study period could partly overcome this disadvantage (assuming that exposure is relatively constant during this period), although this could further decrease the participation rate within the study, as biological samples are often considered invasive and burdensome by the study participants. Furthermore, the costs of sampling and analysis are high, which limits the number of subjects that can be included in the study using these techniques. Nonetheless, bio-monitoring also has some important advantages. These measurements reflect the actual total pesticide exposure of an individual subject (the dose), independent whether the exposure originates from occupational, environmental or dietary pathways. Moreover, these are objective methods to assess pesticide exposure, not susceptible to recall or information bias (15, 18, 25).

Environmental samples such as air, soil and house dust samples, can also be used to estimate environmental exposure to pesticides, but generally these are also only used in studies investigating short-term health effects. Pesticides in air and soil will also degrade and/or metabolize and half-lives in the environment differ depending on the environmental compartment and the pesticide studied (11, 40, 42-44). Pesticides in house dust are considered to be more stable over time as they are protected indoors against several types of degradation (37, 41). The presence of pesticides or their metabolites in the environment around or within residences or schools does not equal personal exposure of the subjects, but rather an approximation of their exposure. Detection of pesticides in the environment indicates their presence but does not take into account the intake by the study subjects and subsequent processes. Nonetheless, studies have shown that the presence of pesticides in house dust within the residences of farmworkers and their children correlates well with pesticides measured in urine of these subjects, and therefore their intake, especially during the spray season (72). Moreover, according to Diezel *et al.* (2013) one sample of house dust within two years could be sufficient to represent residential exposure to certain pesticides during that period, which reflects the potential use of this method to study pesticide exposure in long-term periods (38). Measurements of dust samples within a residence could be used to study long-term health outcomes if sampling is repeated over time. Similar to bio-monitoring, the costs of these type of measurements could be high, limiting the number of subjects that can be included.

Recently, methods to model the environmental exposure to pesticides have gained more interest. These methods are suitable to study long-term outcomes when historical data on agricultural land-use and pesticide use is available, and they can be used in large populations due to limited costs and minimal requirements from the subjects (residential histories). Moreover, these methods rely on independent dataset and are therefore objective, avoiding recall and information bias. The downside is that these (GIS-) modelling techniques make use of many different datasets on agricultural land-use, pesticide applications, etc., which might not be available in all studies for the desired region and/or time periods. The exposure estimates generated by these models do not equal personal exposure or the actual absorbed dose of pesticides. And, in the case of ecological approaches, individual information is often not collected. In addition, these modelling techniques usually make assumptions on the main exposure route (e.g. pesticide drift) and often ignore part of the factors involved such as meteorological conditions, application techniques, pesticide transformation or degradation, chemical properties, etc., often due to limitations in the data available. The most important issue with these modelling approaches is that validation studies are often lacking. Validation studies are needed and bio-monitoring and environmental sampling can be used to validate modelled environmental exposure to pesticides to determine accuracy of the methods and potential misclassification.

In conclusion, the methods to study environmental exposure to pesticides depend on the aim of the study, the long- or short-term health effects studied, the pesticides of interest and the (spatial) data available on regional agricultural practices and pesticide use. For the new Chilean cohort MAUCO, modelling techniques in GIS would be the most suitable to investigate past environmental pesticide exposure of the study subjects. Efforts are needed however to obtain information on agricultural land-use and pesticide use in the region for the period of interest. Validation of this modelling work with biological and environmental samples is important.

REFERENCES

1. FAO. FAOSTAT database. 2014. Available at: http://faostat3.fao.org/faostat-gateway/go/to/home/S.
2. Alavanja MC. Introduction: pesticides use and exposure extensive worldwide. Rev Environ Health. 2009;24:303-9.
3. Mostafalou S, Abdollahi M. Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. Toxicol Appl Pharmacol. 2013;268(2):157-77.
4. Alavanja MC, Hoppin JA, Kamel F. Health effects of chronic pesticide exposure: cancer and neurotoxicity. Annual review of public health. 2004;25(0163-7525 (Print)):155-97.
5. Koureas M, Tsakalof A, Tsatsakis A, Hadjichristodoulou C. Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. Toxicol Lett. 2012;210(2):155-68.
6. Stillerman KP, Mattison DR, Giudice LC, Woodruff TJ. Environmental exposures and adverse pregnancy outcomes: a review of the science. Reproductive sciences. 2008;15(7):631-50.
7. Freire C, Koifman S. Pesticides, depression and suicide: a systematic review of the epidemiological evidence. Int J Hyg Environ Health. 2013;216(4):445-60.
8. US Environmental Protection Agency. Fact Sheet: spray drift of Pesticides. 2014 [updated May, 2014; cited June 27, 2014]. Available at: http://www.epa.gov/opp00001/factsheets/spraydrift.htm.
9. Gil Y, Sinfort C. Emission of pesticides to the air during sprayer application: A bibliographic review. Atmospheric Environment. 2005;39(28):5183-93.
10. FOCUS. Pesticides in Air: Considerations for Exposure Assessment. 2008. Report of the FOCUS Working Group on Pesticides in Air, EC Document Reference SANCO/10553/2006 Rev 2 June 2008. 327 pp.
11. Dalvie MA, Sosan MB, Africa A, Cairncross E, London L. Environmental monitoring of pesticide residues from farms at a neighbouring primary and pre-school in the Western Cape in South Africa. The Science of the total environment. 2014;466-467(0):1078-84.
12. Bouvier G, Blanchard O, Momas I, Seta N. Pesticide exposure of non-occupationally exposed subjects compared to some occupational exposure: a French pilot study. The Science of the total environment. 2006;366(1):74-91.
13. Coronado GD, Livaudais J, Hanisch R, Tekeste T. Take-Home Route of Pesticide Exposure. In: Nriagu JO, editor. Encyclopedia of Environmental Health. Burlington: Elsevier; 2011. p. 312-24.
14. Coronado GD, Holte S, Vigoren E, Griffith WC, Barr DB, Faustman E, et al. Organophosphate pesticide exposure and residential proximity to nearby fields: evidence for the drift pathway. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2011;53(8):884-91.
15. Barr DB. Biomonitoring of exposure to pesticides. Journal of Chemical Health and Safety. 2008;15(6):20-9.
16. Aprea MC. Environmental and biological monitoring in the estimation of absorbed doses of pesticides. Toxicol Lett. 2012;210(2):110-8.
17. Barr DB, Barr JR, Maggio VL, Whitehead RD, Sadowski MA, Whyatt RM, et al. A multi-analyte method for the quantification of contemporary pesticides in human serum and plasma using high-resolution mass spectrometry. Journal of Chromatography B. 2002;778(1-2):99-111.
18. Barr DB, Angerer J. Potential uses of biomonitoring data: A case study using the organophosphorus pesticides chlorpyrifos and malathion. Environmental health perspectives. 2006;114(11):1763-9.

19. Koutroulakis D, Sifakis S, Tzatzarakis MN, Alegakis AK, Theodoropoulou E, Kavvalakis MP, et al. Dialkyl phosphates in amniotic fluid as a biomarker of fetal exposure to organophosphates in Crete, Greece; association with fetal growth. Reprod Toxicol. 2014;46(0):98-105.
20. Appenzeller BM, Tsatsakis AM. Hair analysis for biomonitoring of environmental and occupational exposure to organic pollutants: state of the art, critical review and future needs. Toxicol Lett. 2012;210(2):119-40.
21. Miyake Y, Tanaka K, Masuzaki Y, Sato N, Ikeda Y, Chisaki Y, et al. Organochlorine concentrations in breast milk and prevalence of allergic disorders in Japanese women. Chemosphere. 2011;85(3):374-8.
22. Wessels D, Barr DB, Mendola P. Use of biomarkers to indicate exposure of children to organophosphate pesticides: Implications for a longitudinal study of children's environmental health. Environmental health perspectives. 2003;111(16):1939-46.
23. Busby-Hjerpe AL, Campbell JA, Smith JN, Lee S, Poet TS, Barr DB, et al. Comparative pharmacokinetics of chlorpyrifos versus its major metabolites following oral administration in the rat. Toxicology. 2010;268(1-2):55-63.
24. Ross MK, Jones TL, Filipov NM. Disposition of the herbicide 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine (Atrazine) and its major metabolites in mice: a liquid chromatography/mass spectrometry analysis of urine, plasma, and tissue levels. Drug metabolism and disposition: the biological fate of chemicals. 2009;37(4):776-86.
25. Barr DB, Barr JR, Driskell WJ, Hill RH, Jr., Ashley DL, Needham LL, et al. Strategies for biological monitoring of exposure for contemporary-use pesticides. Toxicology and industrial health. 1999;15(1-2):168-79.
26. Perera FP, Rauh V, Tsai WY, Kinney P, Camann D, Barr D, et al. Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population. Environmental health perspectives. 2003;111(2):201-5.
27. Chevrier C, Warembourg C, Gaudreau E, Monfort C, Le Blanc A, Guldner L, et al. Organochlorine pesticides, polychlorinated biphenyls, seafood consumption, and time-to-pregnancy. Epidemiology. 2013;24(2):251-60.
28. Acquavella JF, Alexander BH, Mandel JS, Gustin C, Baker B, Chapman P, et al. Glyphosate biomonitoring for farmers and their families: Results from the farm family exposure study. Environmental health perspectives. 2004;112(3):321-6.
29. Chevrier C, Serrano T, Lecerf R, Limon G, Petit C, Monfort C, et al. Environmental determinants of the urinary concentrations of herbicides during pregnancy: the PELAGIE mother-child cohort (France). Environ Int. 2014;63(0):11-8.
30. Swaen G, van Amelsvoort L, Boers D, Corsini E, Fustinoni S, Vergieva T, et al. Occupational exposure to ethylenebisdithiocarbamates in agriculture and allergy: results from the EUROPIT field study. Human & experimental toxicology. 2008;27(9):715-20.
31. Wolff MS, Engel S, Berkowitz G, Teitelbaum S, Siskind J, Barr DB, et al. Prenatal pesticide and PCB exposures and birth outcomes. Pediatric research. 2007;61(2):243-50.
32. Eskenazi B, Harley K, Bradman A, Weltzien E, Jewell NP, Barr DB, et al. Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. Environmental health perspectives. 2004;112(10):1116-24.
33. Roca M, Miralles-Marco A, Ferre J, Perez R, Yusa V. Biomonitoring exposure assessment to contemporary pesticides in a school children population of Spain. Environ Res. 2014;131(1096-0953 (Electronic)):77-85.
34. Gonzalez-Alzaga B, Lacasana M, Aguilar-Garduno C, Rodriguez-Barranco M, Ballester F, Rebagliato M, et al. A systematic review of neurodevelopmental effects of prenatal and postnatal organophosphate pesticide exposure. Toxicol Lett. 2013(0).

35. Chevrier C, Limon G, Monfort C, Rouget F, Garlantezec R, Petit C, et al. Urinary biomarkers of prenatal atrazine exposure and adverse birth outcomes in the PELAGIE birth cohort. Environmental health perspectives. 2011;119(7):1034-41.
36. Duca RC, Salquebre G, Hardy E, Appenzeller BM. Comparison of solid phase- and liquid/liquid-extraction for the purification of hair extract prior to multi-class pesticides analysis. Journal of chromatography B, Analytical technologies in the biomedical and life sciences. 2014;955-956(0):98-107.
37. Gunier RB, Ward MH, Airola M, Bell EM, Colt J, Nishioka M, et al. Determinants of agricultural pesticide concentrations in carpet dust. Environmental health perspectives. 2011;119(7):970-6.
38. Deziel NC, Ward MH, Bell EM, Whitehead TP, Gunier RB, Friesen MC, et al. Temporal variability of pesticide concentrations in homes and implications for attenuation bias in epidemiologic studies. Environmental health perspectives. 2013;121(5):565-71.
39. Colt JS, Gunier RB, Metayer C, Nishioka MG, Bell EM, Reynolds P, et al. Household vacuum cleaners vs. the high-volume surface sampler for collection of carpet dust samples in epidemiologic studies of children. Environmental health : a global access science source. 2008;7(1476-069X (Electronic)):6.
40. Morgan MK, Wilson NK, Chuang JC. Exposures of 129 Preschool Children to Organochlorines, Organophosphates, Pyrethroids, and Acid Herbicides at Their Homes and Daycares in North Carolina. Int J Env Res Pub He. 2014;11(4):3743-64.
41. Harnly ME, Bradman A, Nishioka M, McKone TE, Smith D, McLaughlin R, et al. Pesticides in dust from homes in an agricultural area. Environ Sci Technol. 2009;43(23):8767-74.
42. Wofford P, Segawa R, Schreider J, Federighi V, Neal R, Brattesani M. Community air monitoring for pesticides. Part 3: using health-based screening levels to evaluate results collected for a year. Environ Monit Assess. 2014;186(3):1355-70.
43. Sharma KK, Mohapatra S, Ahuja AK, Deepa M, Sharma D, Jagdish GK, et al. Safety evaluation of flubendiamide and its metabolites on cabbage and persistence in soil in different agroclimatic zones of India. Environ Monit Assess. 2014;186(6):3633-9.
44. Alam S, Sengupta D, Kole RK, Bhattacharyya A. Dissipation kinetics of tetraconazole in three types of soil and water under laboratory condition. Environ Monit Assess. 2013;185(12):9819-24.
45. Brody JG, Vorhees DJ, Melly SJ, Swedis SR, Drivase PJ, Rudel RA. Using GIS and historical records to reconstruct residential exposure to large-scale pesticide application. J Expo Anal Env Epid. 2002;12(1):64-80.
46. Aschengrau A, Ozonoff D, Coogan P, Vezina R, Heeren T, Zhang Y. Cancer risk and residential proximity to cranberry cultivation in Massachusetts. American journal of public health. 1996;86(9):1289-96.
47. Bell EM, Hertz-Picciotto I, Beaumont JJ. A case-control study of pesticides and fetal death due to congenital anomalies. Epidemiology. 2001;12(2):148-56.
48. Parron T, Requena M, Hernandez AF, Alarcon R. Environmental exposure to pesticides and cancer risk in multiple human organ systems. Toxicol Lett. 2013(0).
49. Parron T, Requena M, Hernandez AF, Alarcon R. Association between environmental exposure to pesticides and neurodegenerative diseases. Toxicol Appl Pharmacol. 2011;256(3):379-85.
50. Ward MH, Nuckols JR, Weigel SJ, Maxwell SK, Cantor KP, Miller RS. Identifying populations potentially exposed to agricultural pesticides using remote sensing and a geographic information system. Environmental health perspectives. 2000;108(1):5-12.
51. Xiang H, Nuckols JR, Stallones L. A geographic information assessment of birth weight and crop production patterns around mother's residence. Environ Res. 2000;82(2):160-7.

52. Moisan F, Spinosi J, Dupupet JL, Delabre L, Mazurie JL, Goldberg M, et al. The Relation Between Type of Farming and Prevalence of Parkinson's Disease Among Agricultural Workers in Five French Districts. Movement Disord. 2011;26(2):271-9.
53. FAO. International Code of Conduct on the Distribution and Use of Pesticides – Revised version. Adopted by the 123rd session of the FAO Council in November 2002 (reprint 2005). Rome, Food and Agriculture Organization of the United Nations. 2002:32p.
54. Litchfield MH. Estimates of acute pesticide poisoning in agricultural workers in less developed countries. Toxicological reviews. 2005;24(4):271-8.
55. FAO. Guidance on Pest and Pesticide Management Policy Development. International Code of Conduct on Pesticide Management. 2010:39p.
56. US Environmental Protection Agency. About Pesticides: Types of Pesticides. 2012 [updated May, 2012; cited June 18, 2014]. Available at: http://www.epa.gov/pesticides/about/types.htm.
57. US Environmental Protection Agency.Pesticides Industry Sales and Usage 2006 and 2007 Market Estimates. . 2011:41p.
58. RECP. Crop Spraying and the Health of Residents and Bystanders. 2005. Royal Commission on Environmental Pollution. 184 p.
59. Garron C, Ernst B, Julien G, Losier R, Davis K. Concentrations and environmental risk of chlorothalonil in air near potato fields in Prince Edward Island, Canada. Pest management science. 2012;68(1):92-100.
60. Arvidsson T, Bergstrom L, Kreuger J. Spray drift as influenced by meteorological and technical factors. Pest management science. 2014;67(5):586-98.
61. Nuyttens D, De Schampheleire M, Baetens K, Dekeyser D, Sonck B. Direct and indirect drift assessment means. Part 3: field drift experiments. Communications in agricultural and applied biological sciences. 2008;73(4):763-7.
62. Wang M, Rautmann D. A simple probabilistic estimation of spray drift--factors determining spray drift and development of a model. Environ Toxicol Chem. 2008;27(12):2617-26.
63. INE. Censo 2002, síntesis de resultados. Instituto Nacional de Estadísticas. 2002. Available at: http://www.ine.cl/cd2002/sintesiscensal.pdf
64. INE. Censo Agropecuario y Forestal 2007, resultados por comunas. Instituto Nacional de Estadísticas. 2007. Available at: http://www.ine.cl/canales/chile_estadistico/censos_agropecuarios/censo_agropecuario_07_comunas.php.
65. SAG. Informe de venta de plaguicidas de uso agrícola en Chile, año 2008. 2008. Servicio Agrícola y Ganadero. División Protección Agrícola y Forestal. Subdepartamento de Plaguicidas y Fertilizantes. 116 p.
66. FONDAP. Centro de Estudion Avanzados de Enfermedades Crónicas (ACCDIS). 2014. Available at: http://www.conicyt.cl/fondap/centros-fondap/accdis/.
67. Icaza MG, Nuñez ML, Torres F, Díaz N, Varela D. Distribución geográfica de mortalidad por tumores malignos de estómago, tráquea, bronquios y pulmón, Chile 1997-2004. Rev Méd Chile. 2007;135:1397-405.
68. Icaza MG, Nuñez ML, Díaz N, Varela D. Atlas de Mortalidad por enfermedades Cardiovasculares en Chile 1997-2003. Servicio de Salud del Maule. 2006:92 p. Available at: http://pifrecv.utalca.cl/docs/Atlas_de_Mortalidad_por_Enfermedades_Cardiovasculares.pdf.
69. S. Cortés-Arancibia, PhD. Researcher from ACCDiS, personal communication (e-mail), 15-07-2014.
70. Conaf.cl, (2014). CONAF. [online] Available at: http://www.conaf.cl/nuestros-bosques/bosques-en-chile/catastro-vegetacional/ [Accessed 6 Aug. 2014].

71. Brouwer M, Huss A, Vermeulen R, Nijssen P, de Snoo G, Kromhout H. Expert assessment of historical crop specific pesticide use in the Netherlands. LID - oemed-2014-102189 [pii] LID - 10.1136/oemed-2014-102189 [doi]. Occup Environ Med. 2014;0:1-6.
72. Thompson B, Griffith WC, Barr DB, Coronado GD, Vigoren EM, Faustman EM. Variability in the take-home pathway: Farmworkers and non-farmworkers and their children. Journal of Exposure Science and Environmental Epidemiology. 2014:1-10.