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Pesticides

Toxic Aspects

Edited by Marcelo L. Larramendy and Sonia Soloneski

PESTICIDES - TOXIC ASPECTS

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Meet the editors

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Contents

Preface

Today, exposure to pesticides is one of the major concerns related to the safety of the environment worldwide. It is estimated that approximately 1.8 billion people engage in agricultural practices, and most use pesticides to protect the food and commercial products that they produce. Most people employ pesticides occupationally for public health programs and in commercial applications, while many others use pesticides in lawn and garden applications and for domestic protection. Although there have been attempts to decrease pesticide use through organic agricultural practices and the use of other alternative technologies to control pests, additional efforts must be made to find alternatives to chemical pesticides. At present, continued exposure to pesticides from a number of different sources, including, among others, occupational exposure, home and garden use, spray drifts, residues in household dust, food, soil, and drinking water, remains a serious health problem in both developing and developed countries. Risk assessment plays a crucial role in the process of decision making about the use of pesticides, both new and existing. Accumulating experience suggests that postmarket epidemiological surveillance of pesticide safety represents an essential method to ensure public health and the quality of our environment. Epidemiological studies have suggested that pesticides on the market currently may cause deleterious effects, e.g., neoplasias and other diseases in non-target species, including humans. Furthermore, many occupational and agricultural workers experience unintentional pesticide poisoning each year worldwide. In addition to causing environmental damage, wild non-target species are frequently affected by pesticide exposure because they possess physiological or biochemical similarity to the target organisms.

This book, "Pesticides - Toxic Aspects," is intended to provide an overview of toxicology that examines the hazardous effects of common chemical pesticide agents employed every day in our agricultural practices. We aimed to compress information from a diversity of sources into a single volume. The chapters include a large variety of pesticide-related topics about the effects of several methods of control on undesired weeds and pests that grow and reproduce aggressively in crops, as well as their management and several empirical methodologies for study. The topics considered include details of the effects of pesticides on target and non-target organisms; the behavioral consequences of exposure to toxic chemicals, with a focus on the nervous system; the study of the action mechanisms of pesticide toxicity in individuals exposed, with emphasis on the interaction of pesticides with the DNA molecule; the lethal and sublethal effects of the herbicide glyphosate in freshwater organisms; a discussion of pesticides as economic poisons and the balance between the economic effects of pesticide use and their adverse effects on the environment and non-target organisms; a description of field observations and laboratory measurements of pesticide concentrations in wild species; a comparative analysis of two overwhelmingly different agricultural settings after pesticide exposure; and, finally, a detailed study of alternative types of pesticide use in non-agricultural areas.

Many researchers have contributed to the publication of this book. The combination of experimental and theoretical pesticide investigations of current interest will make this book of significance to researchers, scientists, engineers, and graduate students who make use of those different investigations to understand the toxic aspects of pesticides. The chief objective of this book is hence to deliver state-of-the-art information for comprehending the toxicity of several pesticides in target and non-target organisms. We hope that this book will continue to meet the expectations and needs of all interested in the different aspects of pesticide toxicity.

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Chapter 1

Efficiency of Pesticide Alternatives in Non-Agricultural Areas

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Additional information is available at the end of the chapter

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1. Introduction

Global pesticide use is increasing, and such growth is recognized as stemming from agricultural needs in response to global food stress. However, pesticides are used on other areas than agricultural fields. Even if agricultural consumption of pesticides is undoubtedly the main use, the transfer from other, more impervious surfaces is regarded as a key point in understanding the fate and the global impact of pesticides, named biocides, when used for nonagricultural purposes. In the overall environment these chemicals are combined with those applied to agricultural areas, leading to confusion and thus a probable underestimation of nonagricultural pesticides. Numerous information campaigns have targeted agricultural users. The high remaining level of background contamination of rivers highlights that minoring even obliterating urban consumers precisely stultify the considered information campaigns. The ambiguous situation of port contamination will also be discussed in the present chapter.

However, nonagricultural uses mainly involve the same chemicals (e.g., herbicides) as agricultural uses. In the present chapter, the main biocides used will be listed, and then the differences in consumption depending on countries and legislation. The environmental traces of the main pesticides will be summarized with the confounding uses for watershed scale interpretation. The consequences of pesticide use depend on transfer rates, themselves conditioned by the type of surface where these chemicals are applied and their imperviousness. For highly artificialized urban areas, where biocides are mainly used, such information is pivotal: they explain a minor but significant part of aquatic environment contamination.

Alternatives to pesticide uses have been developed for decades, some even before the advent of pesticides, primarily herbicides. The present chapter will detail the alternative types, the respective efficiency depending on substratum and vegetation type. The discussion on the shortcomings of each alternative, the development level and the risks for humans or resulting from hazardous techniques (for both the environment and substratum) will distinguish promising techniques from those that have shown to be inapplicable. The authors will explain why technological impasses are patent and the possible ways to improve such technology to make them applicable. The mechanical techniques studied are mowing, brushing, rotative clogs, sweeping, and harrowing. Thermal techniques include solarization, high-pressure steam, foam mix, gas flames, infrared and to a larger extent, laser, electrocution and UV, microwave, and γ-radiation.

2. A semantic obstacle: How should pesticides and biocides be distinguished?

Far from being trivial, this question needs to be raised prior to examining international data. Indeed, if pesticides are used for crop protection, biocides are pesticide chemicals, i.e., poisonous compounds targeting pests, but non-agricultural pests. Incidentally, molecules involved and prophylactic control molecules could be the same.

Concerning the urban context, where the excessive use of pesticides is complicated by population density and the use of pesticides by local authorities is the most aggressive which targets healthy and ostentation goals both. Pesticide inputs could combine the two environmental contamination pathways. Considering urban use except for prophylactic campaigns, pesticides are spread in kitchen gardens and around ornamentals, i.e., gardens, golf courses, parks, including plant protection. However, use of the same chemical on roads and railways, facade building protection against termites, domestic and veterinary pest control all use biocides. In the present chapter "pesticide" will be used indifferently for pesticides and biocides, unless otherwise specified.

Use of sodium chlorate and iron sulphate should be precisely evaluated and taken into account: their amount is fourfold greater than non-mineral pesticides and could explain the discrepancy of the quantities reported. As for aquatic environment contamination, this controversy seems pointless: whatever the source, the chemical impact is the sole pragmatic yardstick.

3. Pesticide use

3.1. The pesticides used

First of all, inquiries on sales and applications of biocides should be considered carefully: some herbicides are indicated as being found in the urban watershed but could result from non-agricultural pesticide use in urban areas. For example, Gerecke *et al.* (2002), Devault *et al.* (2007), Gilliom (2007), and Botta *et al.* (2012) mention atrazine as consistently polluting the urban watershed without any homologation as a biocide.

Whatever the misuses, *glyphosate* and *diuron* are the most widely applied pesticides worldwide for biocide use. The main use of biocides is for weed control in developed countries, so glyphosate, whatever the geographical region, accounts for about half of non-agricultural use in terms of quantity, one-eighth of glyphosate sales (Hanke *et al.*, 2010; Blanchoud *et al.* 2007). Glyphosate is also used for agricultural ways to sign urban input, despite its increasing urban use. This trend is due to the ban of biocide use such as diuron (Okamura *et al.*, 2003; Gilliom, 2007), despite several marginal agricultural uses (vineyards and sugarcane in Australia (Haynes *et al.*, 2000)). Although diuron is banned for outdoor applications, it is found in veterinary devices and in antifouling paint additives (Irgarol 1051®), an emerging concern because of first-order kinetic façade leaching process (Burkhardt *et al.*, 2011; Wittmer *et al.*, 2011b). Even if Coutu *et al*. (2012) proposed a model integrating rainfall conditions depending on façade exposure, other climatic events (the effects of frost or sun) on building coatings and additives are not currently studied when examining the fate of façade chemicals. Non-agricultural use is reported to be equal or dominant for diuron (Bucheli *et al*., 1998; Gerecke *et al*., 2002; Wittmer *et al*., 2011).

Aminotriazole (also shortened to *amitrole*) for Europe (Blanchoud *et al.* 2004 and 2007) and *prometon* for the United States (Kimbrough & Litke, 1996; Bruce & McMahon, 1996; Hoffman *et al.*, 2000; Philips & Bode, 2002, 2004, Ryberg *et al*., 2010) are respectively the third most referenced chemical in urban areas and marginal or even ignored in the US. For Ryberg et al. (2010), prometon use may be the most widespread biocide in the US Northeast and Midwest.

In the US, urban pesticide use seems to involve other pesticides than in Europe. Braman *et al*. (1997) noted the substantial use of pendimethalin (41%) in their study area near Atlanta but no diuron or amitrole, as Glozier *et al*. (2012) also noted. Amitrole and pendimethalin are not mentioned as urban pesticides contaminating US streams by Gilliom (2007). Even if pendimethalin is known in the European market, residues of pendimethalin are not indicated in European monitoring. Similarly, prometon is indicated (Kimbrough & Litke, 1996; Bruce & McMahon, 1996; Hoffman *et al*., 2000; Philips & Bode, 2002, 2004; Ryberg *et al*., 2010) as a major herbicide used in the urban environment. Philips & Bode (2004) highlighted that prometon concentrations in rivers were proportionate to the population density in the corresponding watershed, Gilliom (2007) listed it as the most frequently detected of the seven herbicides used in urban areas, and Ryberg *et al*. (2010) reported that, although prometon contamination was dominant in the US Northeast and Midwest, it was the most homogeneously represented herbicide for urban areas in the country as a whole. It is the most commonly used soil sterilant in urban areas (Kimbrough & Litke); locally unavailable to homeowners, it continues to be used in these areas by licensed applicators (*Ifid*.).

Only Gerecke *et al.* (2002) mentioned DEET (insect repellent) and diazinon (used by individual gardeners). Gilliom (2007), summarizing pesticide data in US streams and groundwater, showing that, between the six most relevant insecticides, five are significantly more frequently detected in urban streams than in agricultural streams. Four of them are significantly more detected in urban areas than in agricultural areas: diazinon, carbaryl, chlorpyrifos and malathion, despite substantial climate, biocoenosis and legacy diversities. Kimbrough & Litke (1996) already highlighted that urban insecticide use was greater and less diversified than agricultural insecticide use. Consequently, urban streams are more contaminated by such chemicals than agricultural streams. Diazinon, carbaryl, chlorpyrifos and malathion were the only insecticides used in urban areas and were noted by Whitmore *et al*. (1992) in the top 10% of the most frequently used pesticides by homeowners and certified applicators out of 312 compounds identified.

In the Croton Lake watershed, near New York City, Philips & Bode (2002 and 2004) also inventoried diazinon, in addition to carbaryl and imidacloprid. However, diazinon (with prometon) is the only one indicated as being present in densely populated watersheds (Philips & Bode, 2002, 2004). In a Californian urban context, Walters *et al*. noted carbaryl contamination due to *Homalodisca coagulata* (Say) infestation.

For urban use, Moran (2010) and Jiang & Gan (2012) reported that, in California, pyrethroids are the most widely used pesticides for urban areas. Weston & Lydy (2012) focused on pyrethroids due to their representativeness.

Considering "urban streams" as coming from watersheds whose land use was at least 25% urban and at the most 25% agricultural, Ryberg *et al*. (2010) listed pesticide residues and trends: if prometon was the herbicide the most frequently found in US rivers, herbicide trends are described as mixed. Although s-triazines are the main monitored pesticides, simazine and atrazine are more often found in rural areas. Neither a downward nor an upward trend seems to dominate, even if atrazine metabolite DEA is increasing compared to active chemicals.

3.2. Quantities

Wittmer *et al*. (2011a) noted that urban biocide consumption is within the same range as agricultural pesticides in Switzerland (1300–2000 t each), like Pissard *et al*. (2005) in Belgium. Similarly, Lassen *et al*. (2001) conclude that Denmark has high biocide consumption. This question is pivotal and could explain the clear differences between authors: Blanchoud *et al*. (2004) consider nonagricultural chemicals as approximately 1% of the total amount in the Marne River watershed (France), in accordance with several authors (Chauvel, pers. comm.), whereas Aspelin (1998) estimates it at about 25% for the US and previous authors (Lassen *et al*., 2001; BLW, 2007; FriedliPartner *et al*. 2007) at about 50%.

Approximately 10% of pesticide quantities spread stem from nonagricultural use in developed countries (Hanke *et al*., 2010; Kristoffersen *et al*., 2008). Municipalities maintain recreational gardens and playing fields. Even if athletes and the young are more exposed in such places (Harris & Solomon, 1992; Bernard *et al*., 2001; Chaigneau, 2004), this contamination pathway is not identified as a major one. Sports fields are roughly counted because of their heterogeneity: villages could present turf areas as a sports field that cannot be compared with large cities' equipment. That said, about 30,000 sports grounds have been inventoried in France: about one per town, as in all developed countries. Amenity use accounted for approximately 0.19% of pesticide use in Denmark, about 2.7% for the Netherlands and the United Kingdom, less than 3.4% for Germany, 0.6% for Finland and 1% in France (Blanchoud *et al*., 2004).

These results, considering minor surfaces with regard to local and even global land use, is due to greater urban use of pesticides, in comparison to the same surface treated, than in rural areas (Barbash & Resek, 1996; Devault, 2007). Barbash & Resek (1998) considered that lawns received 7.4 kg/ha (insecticide: 2.4 kg/ha; herbicide: 5 kg/ha), golf courses 18.8 kg/ha (insecticide: 13 kg/

ha; herbicide: 5.8 kg/ha), whereas agricultural areas received 2.3 kg/ha (0.9 kg/ha herbicide and 1.4 kg/ha insecticide).

It is also valuable to compare these results, from survey questionnaires, completed on a volunteer basis with the estimation from Aspelin (1997) and UIPP (2000): even if pesticide use is tending to decrease, other biases should be put forward: (1) hidden pesticide use such as flea collars), (2) the spontaneous trend to minimize one's own pesticide use, and (3) the lack of pesticide traceability.

3.2.1. Trends in developing countries

Developing countries' urban areas form a related context (Ecobichon, 2001), the subject of increasing concern. To provide the least expensive off-season fresh fruit (Forget *et al.*, 1993), more acutely toxic and persistent pesticides are used in developing countries (Schaefers, 1996). The trend is similar for biocide use of pesticides: pyrethroid esters are used for household spraying to repel or kill tropical disease vectors (mainly biting insects), which are nine times more expensive than DDT (Webster, 2000): without international sponsoring, poorer nations often limit or abandon control programs. Older but restricted pesticides are not patented: local or regional chemical synthesis could occur because international bans are not applied, despite the Stockholm and Basel conventions. Thus, the main pesticide intoxications occur in developing countries: data are biased by unreported cases, but the World Health Organization reported 3 million severe poisonings, including suicides and 220,000 deaths for 1990. Such results, which have since been corroborated, are caused by careless handling and home storage (under beds, on kitchen shelves (Ecobichon, 2001), lack of protective equipment (possibly due to discomfort), and individual, collective or governmental actions (Gomes *et al.*, 1999), consumption of food or beverages stored in pesticide containers for improper uses (water or food storage). Commuters may produce food in kitchen gardens but male handwork is mainly employed in cash-paying jobs in plantations surrounding cities or in industries: once planting has been completed, crop care is in the hands of women and older children, along with child care and domestic tasks. These tasks induce frequent comings and goings between indoors and the garden, enhancing pesticide exposure risks. Kitchen garden care and maintenance is so devoted to inattentive and overbusied female or infantile handwork As in developed countries, but more acutely, the long-term solution to pesticide problems is education (Ecobichon, 2000), but developing countries lack the regulatory framework, due to insufficient awareness, means and trained personnel for these controls (Ecobichon, 2001).

In all countries, more than 50% of private gardens are treated with pesticides; Hanke *et al.* (2010); in Switzerland the percentage is estimated at 90%, 60% are total herbicides for terraces and about 30% are selective herbicides for grass, shrubs and trees. Fungicides, insecticides and other pesticides (against rodents, mollusks, etc.) account for about 4% each. Thus, the main individual consumption is for esthetics, not for kitchen gardens. Twenty percent of the Swiss population spread pesticides on walkways and garden paths, although this is strictly forbidden in Switzerland (Hanke *et al.*, 2010).

In France, where about 1,100,000 ha are grassed, 605,000 ha are residential, including 23,000 ha of apartment buildings: gardens remain a status symbol. Consequently, the main grassed surface is under private control without adequate training, subjected to unclear application protocols, and receive about 5000 t of pesticides every year. For example, park treatment information is conventionally provided for a 600-m² applications, due to large rural gardens and parks: the indicated quantity to use could be scrupulously determined but is often interpreted incorrectly: many users only spread pesticides on a limited surface, i.e., a few square meters, but use the dose for 600 m² because they do not understand the instructions for use. This information base could also be lacking because 20% of individual gardeners say they are unaware of the impact of pesticides on health and the environment (French Ministry of Ecology, 2011).

In the US, Voss *et al*. (1999) identified diazinon, 2-4D, and mecoprop as the main pesticides polluting streams during rainstorms and successfully compared them to sales for residential use.

3.2.2. Ports and economic activities

Historically, human settlements were inferred to abundant and potable water resources in order to palliate technological paucity. Handworks labor and population should be supplied, resort to highly polluting techniques involved (tannery, slaughterhouses, clothiers, etc.) and wastewater treatment had not yet been invented (Leguay, 1999).

Developing landlocked cities were consequently located near large rivers, but this indispensable water could represent a major threat: even the early civilizations soon learned to protect themselves from floods. Upstream dams and channelling were beyond their ability for large streams but were rapidly set up for minor rivers.

Moreover, hydrologic droughts, historically mainly due to lack of precipitation, had dramatic consequences: even if the water supply was the main problem, maintaining a navigable depth was progressively more difficult when the size of boats increased: particularly during the 19th century, large cities accommodated their ports with low dams in order to allow barge circulation and dug artificial coves for barge mooring. Combined with the industrial era's perception of shoreline development (i.e., clear-cut logging of riparian trees), numerous cities interconnected them to an anthropized fluvial network whose shoreline erosion accounted for about half of the sediment load of urban streams (Trimble, 1997), which accumulated upstream of the urban dam.

For coastal cities, sedimentation could be due to urban activities and, as for dams, to lentic areas bought for naval security reasons. Old-named "heavens", such places could be connected to estuaries but were more often built on the shore for long-term mooring and in order to provide a calm harbor. Such conditions enhanced suspended matter deposition. Moreover, sediment could receive water or wastewater from the shore. However, boating and other naval activities induce additional pesticide consumption: antifouling is mainly performed by using pesticides against algae and shellfish. Numerous publications provide information on past and modern pesticide use, from tributyltin and its derivate to current mixes. For example, Okamura *et al*. (2003) mentioned Irgarol use in Japanese ports, where the highest Irgarol concentrations are observed. Carbery *et al*. (2006) noted the same pattern in the Caribbean harbors of the Virgin

Islands and highlighted amateur mixes made with Irgarol and diuron. Their sampling included sediment, where the maximal concentrations were obviously found.

Whether river port or sea port, sediment accumulation has been observed, and sediment is very well known for accumulating metallic (Cooper & Harris, 1974) and organic (Karickhoff *et al*., 1979) contaminants: sedimentation due to human activities induces contaminant storage in populated areas (Devault *et al*., 2007), where pesticides are only one of several contaminants. Because of the urban context, such sediment could reach high biocide concentrations leading to contamination hotspots and, for river ports, contaminating the aquatic environment downstream during major floods.

Chauvel (2006) asked industrialists, including the transport junction, about their pesticide consumption. In descending order, industrialists consider pest control to be useful to:

- limit fire risks (herbicides against brambles and thickets), completed by the third item in this list.
- close behind fire risk, esthetic considerations are brought up: weeds are a sign of disorder, decrepitude, inactivity and, finally, abandonment. On the contrary, business and work areas have to impress competitors, customers, suppliers and employees with an image of organization, hygiene, and activity.
- Weed development could be an obstacle for rescue operations. A practical argument could be based on risks from animals on legacy obligation or on inner safety committee requirement.
- Equipment and structure alteration. Depending on the equipment and structures involved, esthetic concerns could predominate. The risk from animals is the main risk: electrical installations (power plants, airports, etc.) are sensitive to damage by animals.
- Risk of pest invasion. Some of the industrialists surveyed were in the food processing industry, but this could be redundant with the previous item.
- Health risk. Only 6% consider this risk as sufficiently pertinent to justify pesticide use (Chauvel, 2006).

4. Aspersion of pesticides and the consequences of pesticide transfer

In the urban context, use of aspersion depends on the substratum. Agricultural practices could be adapted to lawns and parks. To a lesser extent, clay sidewalks and paths could be treated with the same equipment. However, considering impervious substratum such as asphalt, pavement, concrete slabs or roofs (Van de Voorde, 2012), using the same techniques is not viable: urban pesticide spraying occurs in “tiger stripes” on impervious surfaces, which does not facilitate comparison with agricultural uses.

The example of railways should be cited: high-speed trains, whatever their model (the TGV in France, the Shinkansen in Japan, the ICE3 in Germany) could be struck by weeds growing on embankments and because of the high speeds attained by these trains, this could damage the rolling stock. Consequently, railway companies are identified as potentially significant polluters.

To avoid aquaplaning, rainwater should be rapidly evacuated. Roads are therefore directly connected to sewers. Even if safety imperatives prevail, this direct surface runoff could generate serious consequences (see below).

In France, 190 airports, covering between 50 and 2000 ha (Chauvel, 2006) have paved ground totaling more than 50% of nonagricultural use. Approximately 1 million km of highways and freeways cover France, combined with all types of roads covering approximately 713,500 ha, including 145,000 ha of grassed surfaces (Chauvel, 2006), about 6% of France's total surface area.

Considering railways, information is still heterogeneous except for systematic control embankments: Schweinsberg *et al*. (1999) estimated pesticide input at approximately 8–10 t/ha, but the French railway company only declares 3 kg/ha (Blanchoud *et al*., 2004). This result highlights how linear to surface expression could bias reasoning: in France, cumulated railways are about 85,000 km long (Chauvel, 2006).

Indeed, considering maintenance of impervious surfaces, users try to control weeds growing in fissures or interfaces between impervious surfaces. This type of application also depends on fissure/interface location: along a wall, weeds could be considered as less anaesthetic or impeding than along a gutter (Zadjian *et al*., 2004); grassed suburban sidewalks are regarded with more tolerance than city center sidewalks. Narrow cracks in the substratum are sprayed, targeting weeds, including the impervious surroundings, a practice that is more widespread than in the agricultural context, considering weed biomass as well as surfaces: a survey of the Californian Department of Pesticide Regulation (Fossen, 2009) noted that 60% of pesticide use in urban areas occurred on impervious surfaces.

4.1. Runoff transfer

Blanchoud *et al*. (2004) estimated pesticide runoff from agricultural areas to be between 0.1% and 2.4% depending on runoff conditions. Concomitantly, under the same rain conditions, runoff in urban areas was between 0.8 and 6.7%. These results are confirmed by Wittmer *et al*. (2011a) who observed that rural pesticide runoff was between 0.4% and 0.9% when pesticide runoff in urban contexts was about 0.6–15%. The transfer rate in agricultural contexts is in agreement with Clark & Gloosby's review (2000), who estimated agricultural exportation between 1 and 4%, Leonard (1990), who estimated agricultural runoff at about 2%, and Bro-Rassmussen (1996), who determined maximum runoff in field conditions from about 0.5 to 5%. It also integrates pesticide losses from plots where storm events occurred such as highlighted by Louchart *et al*. (2001) and Revitt *et al*., 2002. Thus, Wittmer *et al*. (2011a) propose that pesticide runoff from urban areas could be considered as one order of magnitude greater than in agricultural areas. This estimation seems to be in accordance with the literature. The agricultural maximum transfer rate observed in blind conditions (for diuron) is close to the urban maximum transfer rate, to our knowledge, at the watershed scale (45.1%, Revitt *et al*.,

2002) but is clearly much rarer than in the urban context. The 6% transfer runoff integrating agricultural and urban areas of a whole watershed proposed by Blanchoud (2001) seems to be consistent.

Apart from runoff, pesticides spread on limited-adsorption surfaces will be exposed to other processes. However, to our knowledge, no study has specifically detailed the abiotic fate of pesticides in these conditions. Indoor conditions will be detailed in another chapter.

4.2. Lixiviation transfer

Less studied and less obvious, the impact of urban areas on lixiviation remains significant (1) because the lixiviation volume is minimized and (2) because pesticide transfer to groundwater differs comparatively to other land uses (Trauth & Xanthopoulos, 1997).

As previously indicated, water cycles in urban areas are modified: the contribution to groundwater is halved compared to the natural water cycle. Compared to urban pesticide use, groundwater could be more contaminated than under agricultural land. Thus, it is possible to identify the urban impact on groundwater just as it is possible to identify the urban impact on surface waters.

Bruce *et al*. (1996) distinguished residential, commercial, and industrial areas in the urban impact on groundwater. Commercial areas have a greater impact on groundwater because of ornamental plants as well as roads and parking lots, while residential areas are more marked by the needs of ornamental plants and industrial areas by impervious surfaces. Residential areas showed higher contamination levels than industrial areas. However, Trauth & Xanthopoulos examined this segregation: urban areas mix industrial plants (i.e., point source contamination), roads and sewers (linear contamination), and allotment areas (surface contamination): groundwater contamination is not the faithful reflect of the surface one. Nevertheless, statistical results on studies on wells have shown that pesticide concentrations were higher in urban areas than in rural areas. Even if some pesticides are found more frequently in urban areas, statistical consistence is impacted by the number of wells. Malaguerra *et al*. (2012) outline groundwater contamination via the groundwater table and sediment from contaminated streams caused by enhanced runoff in urban areas. Inversely, because of less vertical water transfer due to impervious surfaces, leaching could be slowed, favoring degradation and lateral water transfer, mixing groundwater contaminants (Malaguerra *et al*., 2012).

5. Resident exposure to pesticides spread in urban areas

Pesticide use in urban areas is a major concern for the aquatic environment as well as for human health (Van Maele-Fabry *et al*., 2011). The influence of water contamination resulting from urban pesticide runoff is greater on an aquatic environment than on human health, and food contamination is due to agricultural applications of pesticides. Consequently, the main exposure of urban residents by pesticide spread in urban areas stems from air contamination

(Ragas *et al*., 2011). Moreover, except for pesticide use in urban areas compared to agricultural areas, the urban context favors human contamination by atmospheric pesticides. Due to hydrophobic patterns of the majority of pesticides, contamination by dust is the main source of contamination by air. The aim of the present chapter is not to propose a review of the abundant literature on contamination by pesticides and associated dusts. Appropriate reviews exist, e.g., Schneider *et al*. (2003), Bradman & Whyatt (2005), Garcia-Jares (2009) Kanazawa & Kishi (2009) and Karr (2012). The aim is rather to explain why the urban context encourages human contamination.

In short, buildings are enclosed, windless, sunless, partly septic spaces where dusts can be trapped and accumulate, particularly in fabrics such as carpets (Obendorf *et al*., 2006): 80% of pesticides found indoors are found in clothes, particularly shoes (Quiros-Alcala *et al*., 2011). Moreover, degradation occurs less indoors than outdoors (Roberts *et al*., 2009). Other variables independently associated with dust levels included temperature and rainfall, storing pesticide products in the house, housing density, imperfect housecleaning, and air conditioning (Harnly *et al*., 2009). Farmworkers expose their families more than other professional categories (Quiros-Alacala *et al*., 2011); consequently, in suburbs, municipal service employees and private gardeners could be considered as possible vectors to their relatives. Weschler & Nazaroff (2008) outlined the relationships between gaseous organic chemicals, including pesticides, and dust contamination: solubility and Koa (partition coefficient between octanol and air for chemicals) successfully describe gaseous pesticide contamination, in contrast to other molecules (Schoeib *et al*., 2005). Clothes abrasion and other contaminations (paint coating) occur indoors, promoting indoor pesticide content and some of the organic matter in dust, such as cotton linters, may differ substantially from octanol in terms of sorption of gas-phase Semi-Volatil Organic Compounds (Weschler & Nazaroff, 2008). Direct contact of dust with polluted surfaces seems to be enough to pollute dust (Clausen *et al*., 2004). Moreover, high indoor temperatures induce chemical volatilization, and the difference between the laboratory temperature for Koa determination and the private indoor temperature could be significant. Passive air sampling does not efficiently inform about long-term contamination because of passive samplers (Weschler & Nazaroff, 2008) and quantification thresholds.

Blanchoud (2001) estimated agricultural pesticide amounts used on the Marne watershed at about 5200 t/year, urban use at about 62.5 t/year and atmospheric amounts at about 0.5 t/year. But global contamination should not be ignored: MCE (2003) estimated that Rhine valley inhabitants, by breathing, were twice as contaminated by pesticides than if they drank 1.5 L of water with close to 0.5 μg/L total pesticide concentration, i.e., the maximum allowed concentration by surface water quality norms. Moreover, gaseous pesticides are directly bioavailable compared to pesticides associated with particles, which sequester more than 99% of the main pesticides (Koc and Kow>2). Studies on pesticide exposure mainly target farmers and pesticides used in agricultural areas (Mercadante *et al*., 2012). Considering the issues at hand, data on public exposure to urban pesticide use are rare, even if studies are currently in progress.

Population exposure to contaminated particles or volatile pesticides is more than ever an issue because this exposure occurs as much at home as at work, and because enclosed living spaces affect every age group.

Air contamination data is still too rare and incomplete, and would benefit from further study.

To pesticides designed to protect crops, one must add a large number of biocides designed for heath or esthetic uses (household products, paints containing algaecides, etc.): the nature of these pesticides is not well understood by users. Because the sense of sight prevails over the other senses, the most readily perceived pollution is air pollution, associated with transport. Coupe *et al*. (2000) assert that high oxidative conditions in urban areas compared to rural areas (Finlayson-Pitts & Pitts, 1986) promote pesticide oxidative degradation. There is no evidence of a significant urban influence.

6. Progressive pesticide awareness of urban pesticide use

For Denmark and the Netherlands, the first monitoring programs demonstrated evidence of water contamination. Depending on its groundwater for drinking water, in 1995 Denmark discovered its groundwater pollution level. For the Netherlands, water pollution was striking because of the Meuse River contamination, which resulted in a ban, forbidding water intake for 7 weeks (1993 and 1994), while this country depends on surface water for 40% of its drinking water, soon to rise to 50%. In wooded Sweden, the threat to human health was the main driver because Swedish forestry and roadway services air-applied Agent Orange, a 2,4-D and 2,4,5-T formulation known for its mutagenic potential. Concerned by Agent Orange use and air-spraying, the population continued to debate about the daily place of pesticides after Agent Orange's definitive ban (1977). The first monitoring campaigns were carried out in 1985 and revealed water resource contamination, leading to early and radical directives (Ulén *et al*., 2002).

Pesticide awareness differed in the largest countries. For agricultural countries such as the United States and France, pesticide awareness came early but was mainly associated with agricultural use. In Germany, the negative effects of pesticides were avoided by early plant protection legislation: the first legislative provision was decreed in Germany in 1919 and was implemented in 1968. Thus, weed control by herbicides is forbidden on hard surfaces without local authorization and only if there is no runoff risk. In this case, plant protection control programs determine the few available chemicals. For the US and France, pesticide contamination evidence dates back to the 1960s. Associated with agriculture, pesticide use was rarely reported in urban areas until the 1990s when extensive monitoring, directives in other countries, and early scientific publications (Cole *et al*., 1984) awakened awareness. The Nationwide Urban Runoff Program, prepared between 1978 and 1980, carried out between 1980 and 1982, provided the first public information on urban contamination in the US. In comparison, the first French publication on urban pesticide contamination is Chevreuil *et al*. (1996), and was still focused on agricultural contamination; even if the Water Law was decreed in 1992, compatible urbanization was taken into account in 2004 (Diren, 2010). The European

Union is a key factor in French pesticide awareness. Like the above-mentioned countries, the UK did not experience a catalyst event leading to massive pesticide awareness. Without previous legislation as in Germany, and without agriculture importance like in the US and France, urban pesticide use would have been more evident given Greater London's importance in UK land use. However, British awareness seems limited and related publications are scarce (only Rule *et al*., 2006, and Stuart *et al*., 2012).

The virtuous pesticide approach is performed in agreement with the European Union: at the same time, legislators follow European directives such as the Council Directive 91/414/EEC (EEC 1991) and the Water Framework Directive (EEC, 2000), and support forums on amenities or pesticide representations. However, this process is more efficient in countries with leading governance such as France: British self-regulation practices and the tradition of voluntarism make them less easy to apply (Grundy, 2007).

Initiatives could be combined as is done in the US. First of all, professionals are involved, then private applicators. Consequently, since 1993, the US has established a 2-year license for spraying pesticides depending on member states' initiatives, presenting different process levels. For example, Idaho, Georgia, and Minnesota have established a voluntary program for the publication of a pesticide sale and use database. Idaho and Georgia follow Urban Pest Management Programs in order to involve individual applicators.

Although Germany's 1919 plant protection decree was a notable base for environment protection, the country continues to strengthen its pesticide reduction policy even if detailed data for urban herbicide do not exist. Parenthetically, annual consumption of pesticides used in part in urban areas is about 230 t. Kristoffersen *et al*. (2008) estimated glyphosate, the main pesticide used in urban areas, at about 2 t/year. Finland's use is estimated at about 5–6 metric tons of active molecules per year (*Ibid.*); the substances allowed are limited and use of very toxic pesticides is limited to qualified persons.

Pesticides used in urban areas are limited; for example, diuron is often forbidden in Europe Union countries and the Canadian province of Ontario (decree 63/09, 4 March 2009, enforced 22 April 2009) banned all pesticides use for esthetic purposes, but some limited uses, such as on golf courses, are allowed. Golf courses require intense pest control and artificialized surfaces, with pesticide transfer close to urban areas. In France, greens cover about 20,000 ha. Considering the 550 golf courses in France, the average surface of a green is about 36.4 ha. Even if economic arguments, ecological concerns, and society's growing awareness are influencing golf course managers taking these concerns into account, the results from such sites should be considered with caution, due to divergent goals or the risk of different interpretations.

The ultimate level of urban pesticide use awareness is differential taxation and alternative innovation. The Netherlands and Denmark are the most forward-looking countries for alternative development, followed by Sweden, the leading European countries for environmental issues.

In addition to legislation, the importance green political parties or related are a better reflection of the population's awareness of ecological concerns, as expressed in elections in the number of deputies for a given population: Germany sent 22 ecologist deputies to the European Parliament, France 19, Sweden, the Netherlands and the United Kingdom 5, but, in contrast to others, the United Kingdom's deputies are mainly autonomists. With these results, the political interest in the environment could be considered as moderate (Kristoffersen *et al*., 2008). Thus, adoption of an ethical attitude will be limited until citizen support is expected. For example, despite its legislation strictly controlling urban pesticide use since 1954, Finnish people do not show a willingness to complete its legislation by greater amenity pesticide control (Kristoffersen *et al*., 2008). Moreover, hard surfaces or the status of amenity areas could curb initiatives: in the United Kingdom, administrative land fragmentation results in local authorities being responsible for weed control (Grundy, 2007).

7. Technologic alternative

Road shoulders were mowed in certain places up to the 1950s, although hay production was declining at this time. Green shoulders limit soil erosion and consequently prevent road sap, helps drivers see curves and anticipate the course of the road, allows a good visibility of signs, protect from wind, and prevent monotony for drivers and eyesores for residents. However, walkers, wildfowl and rain require road shoulders to be flush cut. Margoum (2003) highlighted how ditches could enhance pollutant retention. Considering pesticide costs and low user solicitation, highway companies could notably reduce their biocide budget using alternatives to pesticides (at least 50%, Mahe (2007)).

Based on Table 1 (Marque & Chabaud, 2006), in order to control at least 8- to 10-cm-high weeds, mowing seems to be the best alternative. Mowing does not induce soil or root lifting, and cutting at an appropriate height could avoid passage: flush cutting weeds too short could harm low-growth perennial plants, which inhibit high-growth annuals such as allergen ambrosia. Moreover, perennial weeds are often more endemic than annual weeds, contributing to biodiversity promotion. Mowing seems to correspond to private and public professionals' financial means and satisfaction surveys highlight its popularity (Mahe, 2007).

In Germany, a system has been developed, the Rotofix, a hand-operated roller sprayer as an alternative to spray a zone for a single plant. Appropriately used, it could reduce herbicide volume by 75–95% (Hermanns *et al*., 2006).

Instead of using pesticides, public authorities could employ other molecules, such as acetic, citric and pelargonic acids on hard surfaces. Although it is used in Germany, acetic acid is also prohibited in 50% of Swedish municipalities and is only allowed in the Netherlands when there is no runoff risk.

Ground cover could be an alternative (Table 1), if the ground use is amenable (Marque & Chabaud, 2006). Mulching and covering with plastic drastically limit weed growth, but could be too aleatory (vegetal wood and cloth covers), temporary (vegetal and cloth), fire-prone (vegetal, polypropylene and cloth), unaesthetic (polypropylene, vegetal cover with time), difficult to deploy (minerals are heavy, vegetal covers need time), and require maintenance (vegetal and mineral covers).

Technique	Material	Advantages	Disadvantages	Raw material cost	Productivity cost	Number of input (/year)	Cost/m²	Durability
Mulching	Vegetal matter	Ease of use Natural visual Water protection Matter organic input	Lengthy deployment; Volatile; Animal deterioration; Annual complement; Visual with time; Risk hazard; Dubious herbicide	0.2 to 2€/m²	3€/m²	0.1*	3.3 to 6.2€/m²	1 to 5 years
Mulching	Mineral matter	Heavy products	Ease of use but lengthy; Water protection; Animal deterioration; Needs complement; Herbicide	2.5 to 3€/m²		1*	5.5 to 6€/m²	5 years et +
Mulching	Plastic matter, polypropylene	Easy and fast to deploy; Water protection	Impervious; Visual controversy; Film eviction problems Risk hazard; Herbicide	0.25 to 0.70€/m²	0.70€/m² for plantation + for eviction	0.25 to 0.15*	0.95 to 1.40€/m²	5 to 7 years
Mulching	Ligneous slabs	Ease of use	Impervious; Dubious herbicide	0.5 to 2.5€ each	0.60€ to 1.10€ / each	1	1.10€ to 3.60€/m²	2 to 4 years
Mulching	Plant fibers and rags	Ease of use; Water protection; Recycling	Fragility products; Visual; Risk hazard; Dubious herbicide	1 to 4€/m²	1€/m²	0*	2 to 5€/m²	2 to 5 years
Ground cover	Plants	Natural; Esthetic; Good cover with time	Needs protection the first years; Maintenance	10m€/m²	3.5€/m²	Retouches*	13.50/m²	10+ years
Chemical	Anti-germinatives	Easy for granule; Fast use; Efficient;	Pesticide use; Follow good practices and framework legislation;	0.05€/m²		1	0.11€/m²	1 year
	Foliate	Selective products	Qualified staff	0.01€/m²	0.06€/m²	1 to 3	0.07€ to 0.21€/m²	Following regrowth

Table 1. Qualitative and economic analysis of alternative preventive methods (from Marque and Chabeaud, 2006).

7.1. Mechanical alternatives

Brushing can be used only on impervious and clean surfaces. At best, the rotation of the bristles extracts part of the roots. However, coated surfaces are abraded: asphalt near fissures could be snatched, deteriorating bristles. Pavements should be cohesive and regular but slipping could occur when wet. Moreover, steel brush tests have demonstrated the level of noise and vibration is incompatible with good working conditions and urban use (Hansson *et al*., 1992 in Rask & Kristoffersen, 2007). Brushes are only made in polypropylene. Despite brushing's efficiency, Lefevre *et al*. (2001) and Wood (2004) do not recommend it for long-term use but Lefevre *et al*. (2001) and Hein (1990) propose to use it for heavily weeded areas.

Rotative clogs comprise a heavy metal cylinder rolling on the ground and extracting roots. They are only used on pervious surfaces, which should be tamped after the application, an expensive step. The surface is severely abraded: rotative clogs could be limited to clay surfaces (Hamelet, 2004).

Sweeping, whether or not it is mechanized, even in gutters, could be useful, despite the number of sweepers required, and is a no n-hermal alternative (Hein, 1990; Parker & Huntington, 2002; Hansen, 2004): the advantages of cleaning could justify the price of optional engines or numerous teams. Lefevre *et al*. (2001) considered that seven to ten operations per year were very efficient for controlling weeds in temperate climates.

Harrowing is still efficient on gravel surfaces: easy to use, inexpensive to purchase, maintain, and deploy, in 1992 it led to banning herbicides for churchyard treatment in Denmark (Tveedt *et al*., 2002, in Rask & Kristoffersen, 2007).

Paradoxically, human mechanical work, whether it is used marginally or institutionally, seems to keep up for limited surfaces (Angoujard *et al*., 1999): the Versailles municipality organizes hoeing teams of seasonal workers (Mahe, 2007). The main obstacle is the cost of labor for developed countries with high labor costs, but this obstacle could be reduced in emerging or developing countries where sweeping appears to be a reliable alternative to herbicide use. However, such practices could be considered as retrograde and even degrading.

7.2. Thermal alternatives

Thermal alternatives use heat to scorch or burn off weeds. Heat could be obtained with sun (solarization), high-pressure steam, sugar foam, infra-red, freezing or gas flames (Table 2).

Globally, thermal uses require many passages (Rask, 2012) and are highly energy-consuming. Treatments are more effective on low-growth weeds and roots are scarcely damaged. The driving speed must be slow for an effective treatment. The main target of thermal alternatives is to expose pesticides to warm conditions, so as to degrade them. However, especially when the vector of the fluid, i.e., steam, warm water or a warm mix, the temperature reached should not be high enough to degrade or even mineralize the pesticide, but could enhance volatilization. This phenomenon is known to significantly affect the fate of some pesticide families. In the urban context, due to a lesser adsorption phenomenon leading to enhanced pesticide runoff, ground temperature, H Henry's constant greater than 10^{-5}, and the effects of Raoult's

Technics	Material	Advantages	Disadvantages	Investment in material	Productivity	Number of passages	Annual cost	Consumable
	Hoe	Ease of use; All weather; Handy; Very low investment	Number of passage; Labor-intensive; Limited to small surfaces.	20 to 30€	50m²/h	5 to 6	0.40€/m²	
	Rotative clog	Low investment; Ease of use	Number of passages; Only on pervious surfaces, even degradation					Gasoil
	Rotative brush	Moderate investment; Ease of use; Effective on pavement	Substratum and joint degradation; Number of passages	4k to 5k€	1000m²/h	4 to 6	0.36€/m²	Gasoil
Mecha-nical	Sweeper	Cleaning, Sharable cost	Average to very high investment; Number of passages; Joint degradation; Limited to gutters	2.65k to 90k€	2600ml/h	8 to 12	0.12 €/m²	Gasoil
	Mowing, small equipment	Moderate investment; Ease of use; All weather; Handy	Number of passages; Labor-intensive; Not for large surfaces; Fossil-energy dependent	200 to 600€	450 m²/h	3 to 4+	0.23 €/m²	Two-stroke gasoline
	Mowing/ mulcher, big equipment	All weather; Handy; Effective on ligneous	Average to expensive investment; Consumption of fossil energy; Visual on ligneous	2 to 20k€	3 to 6000 ml/h	1 to 2	0.12 €/m²	Gasoil
	Infrared	Medium investment; Ease of use; Handy; Useful on pervious as impervious; Manual or mechanized	Number of passages; Risk hazard; Only for seedlings and monocotyledons; Consumption of fossil energy; Greenhouse gases	3.3k to 9.5k€	2 km/h 1000m²/h	6 to 8	0.24 €/m²	Propane (1kg of gas/h/burner).
	Flame	Low to moderate investment; Ease of use; Manual or mechanized	Number of passage; Risk hazard; Consumption of fossil energy; Greenhouse gas generation	0.5 to 6.2€	3 to 5 km/h	4 to 6	0.22 €/m²	propane 2kg of gas/h/burner
	Steam	Efficient on impervious surface; Handy; Mechanized; Polyvalent; Cleaning effect	Expensive investment and water; Moderate efficacy on settled weeds; Number of passages	17.5k to 44k€	1600 ml / h 1100 m² / h	3 to 6	0.25 €/m²	Eau (4 to 500 l /h) gasoil
Thermal		Disinfection	Diesel fuel consumption; Ground speed 0,7 to 1 km/h					Gasoil (4 to 7L/h)
	Warm water	All weather; Handy; Polyvalent; Hose Effective on bryophytes	Average investment; Number of passages; Only on leaves; Water and diesel fuel consumption; Ground speed 1 to 2 km/h	15k to 18k€	1000 m²/h	4 to 6	0.25€/m²	Water (3 to 400 L/h) gasoil (4 to 5L/h)
	Warm foam	Useful on pervious and impervious surfaces; Handy; All weather; Number of passages	For rent only; Mainly effective on leaves; Water consumption; Diesel fuel consumption; Ground speed, 3 to 5 km/h	0.7k€/d*; 0.9k€/w; 3k€/m; 13k€/6m; 21k€/y;48k€/4y	350 m²/h	3 to 4	1€/m²	Water (15 to 50m³/ha); Gasoil (5 to 6L/h); Foam 100€/d; 0,2 to 0,4% additive
	Foliate	Low to moderate investment; Ease of use; Pervious and impervious surfacees; Manual or mechanized; Dose modulation following the weeds	Weather dependent; Run-off risk on impervious surfaces; Number of passages; Average efficacy on certain plants	50 to 5k€	1000 m² to 2000 m²/h	3 to 5	0.11 to 0.17 €/m²	Products 35€ Water Gasoil ***
Chemical	Foliate + anti-germinative	Low to moderate investment; Number of passaes; Manual or mechanized; Curative and preventive	Weather dependent; Pervious surfaces only	50 to 5k€	1000 m² to 2000 m²/h	1 + 1 retouch	0.12 to 0.18 €/m²	Products 400€ Water Gasoil ***
	Foliate+ residuary			50 to 5k€	1000 to 2000 m²/h	1 + 1 retouch	0.15€/m²	Products 150€ Water; Gasoil ***

Table 2. Qualitative and economic analysis of alternative curative methods (from Marque and Chabeaud, 2006). *Short-term rental including equipment+driver/technician **Equipment Rentals vehicle or technician without applicator ***If mechanized implementation.

law, pesticides could be exposed to enhanced volatilization (Burkhardt & Guth, 1981). For Scheyer *et al.* (2007ab) and Delaunay *et al.* (2010), high amounts of volatilized urban pesticides

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are notably observed in urban air but are too limited to induce long-distance contamination or to significantly pollute agricultural areas when farmland pesticides are found on the same order of magnitude in agricultural areas as in urban areas.

Considering *solarization*, two limits are identified. First of all, the weather should be sunny (at least 250 h/month) and shade should be avoided (due to other weeds). Also, a large amount of plastic waste is generated (Cheroux & Serail, 2006).

Due to the nature of impervious surfaces, i.e. mainly dark asphalt, solarization could lead to extreme temperatures (asphalt fusion temperature: between 90°C and 110°C). Many pesticides, particularly herbicides, could lyse at such temperatures, but no study has investigated this question. Even if the sunshine does not induce high temperatures, photolysis could occur but no direct evidence has been found in the literature for this special case. However, the long-term experiments conducted by Jorgenson & Young (2010), Jiang *et al*. (2011) and Jiang & Gan (2012) do not mention photolysis of urban pesticide, but experiments examined low photolysis-sensitive pyrethroids. However, the observed loss is far from being as fast as expected with less than 1 h DT50 photolysis at neutral pH (Fossen, 2006).

High-pressure steam application requires substantial quantities of water and a substantial financial investment. Its efficacy is poor (Daar, 1994) because of perennials. Foam could be used instead of water, made of coconut sugar and corn sugar, to enhance warming duration and subsequent efficacy (Daar, 1994). Numerous applications are required.

Gas flames alternative use has the advantage of being an intuitive and light (Rask, 2012). However, this alternative is expensive (substantial gas consumption) and may even be a source of fire danger (Wood, 2004). It is the most commonly applied thermal weed control method on hard surfaces. In Germany, a train equipped with flame weeders has been elaborated for railway embankments (Kreeb & Warnke, 1994).

Infrared radiation is the most effective non-chemical control method and economically comparable to herbicide treatment (Augustin, 2003, cited by Rask & Kristoffersen, 2007), but radiators are very expensive, brittle, and inoperative for dense vegetation (Ascard, 1998).

Other alternatives have been tried: laser (from Couch & Gangstad, 1974, to Heisel *et al*., 2002), gamma radiation (radioactive), UV radiation (nullified by mutagenic and fire hazards), microwaves (hazardous and need 1000–3400 kg diesel/ha for a significant effect according to Sartorato *et al*., 2006), electrocution (fire hazard for the surrounding terrain, electrocution risk for operators and passers-by, and a high amount of electricity needed, but this could be an alternative for railway pesticide uses). None of these methods currently presents consistent results.

8. Conclusion

The use of pesticides is still too directly associated with agriculture, a clear cultural barrier for those countries that are built on a strong dichotomy between the countryside and the city, the latter needing to be maintained. However, in developed countries, urban sprawl seems to be the main driver of water resource contamination. Outer urban areas are the most vulnerable to pesticide use: they do not have a water collection and treatment system as developed as those in city centers, while suffering from a greater level of pesticide pressure than is found in agricultural areas. However, studies on pesticide representation are mainly done in agricultural areas, and their urban equivalents are rarer.

Populations believe that the impact of indiscernible actors of pollution does not exist. Moreover, the fate of pesticides in urban substrates (asphalt, etc.) is not sufficiently known: recent studies on contaminated concrete and paint have shown gaps in our understanding of sorption, volatilization, and photolysis processes.

The descriptors of the pesticide pressure are lacking but also in need of improvement: comparing the surfaces covered by agricultural machinery spraying to urban “leopard spot” or “tiger stripe” spreading is not relevant. This lack of standardization can be found in studies seeking to highlight the performance of alternative techniques for spreading, which nevertheless seem capable of improvement.

Nonetheless, new curative or preventive tools could provide effective alternatives to pesticide use. The pivotal quality of alternative strategies lies in the choice of matching the tool to the substratum. However, this pas de deux is essential for limiting pesticide contamination.

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Chapter 2

Genotoxicity Induced by Ocupational Exposure to Pesticides

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Additional information is available at the end of the chapter

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1. Introduction

Pesticides are used to repel, kill or control certain forms of pests, e.g. animals or plants. These chemical compounds can be divided into three main classes: insecticides, which are used to control insects; herbicides, which are used to destroy unwanted vegetation; and fungicides, which are used to control fungi and their spores, preventing them from damaging plants (Maroni et al., 2000). Pesticides are employed extensively around the world and in recent years their use has even increased. On one hand, extensive use of pesticides in farming has lead to a higher production of pests that damage crops and, on the other hand, pesticide-resistant pests have emerged. Increased crop production demands increased use of pesticides (Mostafalou and Abdollahi, 2013).

The widespread use of agricultural chemicals in the food production and public health sectors has released large amounts of potentially toxic substances into the environment, most of which are unspecific and therefore potentially also target the human organism (Bolognesi, 2003; Dyk and Pletschke, 2011). Humans are exposed to the ubiquitous pesticides, e.g. in form of food contaminations through the production line, but also in the household, workplace, hospitals and schools (Bolognesi, 2003; Aprea et al., 2012).

Exposure to pesticides can induce two kinds of toxic effects: acute and chronic. The acute effects are immediate and include headache, nausea and/or other more serious effects, even death. Chronic health effects occur, when individuals are exposed continuously or repeatedly to foreign substances. In the scientific literature, the effects of acute exposure are more clearly described. In contrast to that, effects of chronic exposure still need to be further investigated, especially how they are triggered (Ray and Richards, 2001; Sanborn et al., 2007; Kortenkamp et al., 2007).

The degree of danger associated with chemical exposure can be evaluated by health risk assessments. Chemical exposure can be evaluated with respect to a single compound or to complex mixtures. Mixtures of toxins may influence and even amplify the toxicity of individual components through synergies, potentiation, antagonism, inhibition or additive effects (Muntaz, 1995; Reffstrup et al., 2010). The assessment of chronic exposure to mixtures of pesticides should improve the understanding of underlying intoxication mechanisms (Bond and Medinsky, 1995; Sanborn et al., 2007; Reffstrup et al., 2010). Indeed, the number of studies involving chronic exposure to pesticides and their consequences to human health (Muntaz, 1995; Mostafalou and Abdollahi, 2013) in the scientific literature is increasing. Individuals, who are in direct contact with and exposed repeatedly to low levels of pesticides (e.g. agro-chemicals) as part of their work (e.g. agricultural or cargo workers, etc.) may therefore provide a good opportunity to study the deleterious effects of chronic pesticide exposure to human health (Bolognesi, 2003).

2. Occupational exposure to pesticides: Toxicology and absorption pathways

Pesticides can be classified according to their chemical structures: carbamates (CBM), dithio-carbamates (DTC), synthetic pyrethroids, organochlorines (OC), organophosphorous (OP) compounds, thiocarbamates, phenoxyacetates (PHE), quaternary ammonium compounds and coumarins (Maroni et al., 2000). The individual toxicity of these compound classes is expressed by the dose inducing lethality in 50% of the specimens in tests with laboratory animals (LD50). During these LD50 tests, usually mice are exposed to a single given dose (Maroni et al., 2000; Suiter and Scharf, 2012). In practice however, the toxicity of pesticides should not be evaluated on the basis of a single dose, but by the absorption of small doses over a given time period (Bolognesi, 2003; Kortenkamp et al., 2007; Reffstrup et al., 2010). In addition, agricultural workers are usually exposed to a mixture of pesticides (Bolognesi, 2003; Kortenkamp et al., 2007; Aprea, 2012) and fundamental aspects such as type and duration of the exposure can severely affect the toxicodynamics of the pesticides (Gammon et al., 2012).

In laboratory tests, toxicokinetic models are important in order to determine the kinetic parameters of the active components and to understand chemical interactions between pesticides (Bond and Medinsky, 1995). Toxicokinetics refer to the route a xenobiotic takes to get into, through, and out of the body. It can be divided into several processes including absorption, distribution, metabolism, and excretion.

The effects of chronic exposure, which pesticides induce in humans, are highly sensitive to several parameters, e.g. dose, duration, and especially the absorption pathway (Aprea, 2012). In agricultural surroundings occupational exposure mostly involves absorption via dermal and/or respiratory routes (Leoni et al., 2012; Aprea, 2012). This type of exposure occurs predominantly during the period of the application of the toxins, e.g. through spraying

(Ranjbar et al., 2002). The penetration of the skin itself depends on several factors: type of pesticide, temperature, relative humidity, type of exposed unprotected area of the body (e.g. the back of the hand, wrist, neck, foot, armpit or groin), contact time, and the presence of wounds or skin lesions, which greatly facilitate absorption. Cases of absorption through the gastrointestinal tract are also known, albeit less frequent, because larger pesticide particles tend to be deposited in the upper airways of the respiratory tract (Aprea, 2012).

The knowledge about absorption pathways allows a more apt description of real doses of absorption (together with corresponding toxic effects), rather than a description of the dose, which is considered potentially toxic. For example, Ortiz and Bouchard (2012) demonstrated toxicokinetic effects for the fungicide captan after absorption. Unfortunately, it was impossible to isolate the toxic effects resulting from exposure, because the absorption pathways and toxic doses of this compound in humans are not yet known exactly. Several studies have reported a rapid absorption of organophosphates (OPs) via dermal routes, e.g. through connective and mucous membranes, but gastrointestinal and respiratory absorption routes are also known (Stallones and Beseler, 2002). Gammon et al., (2011) reported minor toxicity for CBMs when absorbed throught he skin, but more serious toxic effects after gastrointestinal incorporation. Pyrethroids are generally unstable under environmental conditions and tend to be rapidly absorbed after degradation through hydrolysis, but don’t accumulate in the body (Suiter and Scharf, 2012). In contrast, OCs are relatively stable under comparable conditions and can accumulate when absorbed; Absorption doses can moreover be cumulative, depending on the absorption route (George and Shukla, 2011).

Many of the toxicological effects of pesticides have been demonstrated to be mediated by induced redox signaling. Exposure to a wide variety of pesticides induces oxidative stress, as reflected in the accumulation of reactive oxygen species (ROS), lipid peroxidation and DNA damage. For some pesticides, the mechanisms leading to alterations in the cellular redox homeostasis are partially understood. Pesticides can alter cellular redox equilibria via different mechanisms, including their enzymatic conversion to secondary reactive products (e.g. ROS), depletion of cellular antioxidant defenses and/or impairment of antioxidant enzyme functions (Franco et al., 2009; Limon-Pacheco and Gonsebatt, 2009).

Nutrigenomics and nutrigenetics are recent research areas, which seek to understand the effects of diet and nutrients as genetic response modulators to pesticides. The effects of nutrient deficiencies or imbalances, as well as the toxic concentrations of some dietary compounds have been the subject of nutritional research. About 40 micronutrients are required in an optimal human diet, and their levels may vary depending on age, genetic predisposition, etc. (Ames, 1999; Ames 2001). Most interestingly, the genomic damage caused by moderate micronutrient deficiency is of the same order of magnitude as the levels of damage caused by exposure to high doses of environmental toxins (Kym, 2007; Dangour et al., 2010; Wald et al., 2010). Folates and other B-complex vitamins perform key functions in biological processes pivotal to a healthy constitution. Even moderate folate deficiencies may cause genomic damage in the general population. Folates maintain genome stability by regulating DNA synthesis and repair as well as methylation processes. Deficiencies in folic acid can therefore increase chromosomal instability (Beetstra et al., 2005). A major co-factor in the folate metabolism is vitamin B_{12} and clinical evidence suggests that the inappropriate intake of vitamin B_{12} may result in damage of the DNA. Moreover, chromosome repair mechanisms may be compromised, when vitamin B_{12} concentrations are too low (Swanson et al., 2001). Age and gender are other factors, which may possibly influence the level of DNA damage. Fenech and Bonassi (2011) showed that the damage to DNA increases with age, probably due to a combination of several factors such as inadequate nutrition, occupational or environmental exposure to genotoxins, and a wider variety of other unhealthy lifestyle factors.

3. Pesticide metabolism

Metabolism is one of the most important factors in the toxic profile of a pesticide. During the first steps of the metabolism, chemical compounds are bio-transformed by phase I enzymes, usually the cytochrome P450 (CYP) system. Phase II conjugating enzyme systems, which are present in the glutathione complex, subsequently transform these reaction products into more soluble and excretable forms (Guengerich and Shimada 1991; Eleršek and Filipič, 2011). These enzymatic reactions are generally beneficial, since they help to eliminate compounds from the body. Sometimes however, these enzymes transform otherwise harmless substances into highly reactive forms – a phenomenon known as "metabolic activation" (Guengerich 2001; Abass et al., 2010).

The metabolism reacts towards these xenobiotics in phase I by generating functional and/or polar groups, with the goal to create a substrate for enzymatic reactions in phase II (Hodgson and Goldstein, 2001; Parkinson, 2001). The CYP system comprises a large family of multigenes, which are important in the metabolic phase I of xeno- and endobiotics. Their reactions occur predominantly in the liver, which is the place of subsequent eliminations (Abass et al., 2010). Beyond the hepatic tissue, CYP multigenes can be found in the lung (Lawton et al., 1990), brain (Bergh and Strobel, 1992), and kidney tissue (Hjelle et al., 1986), as well as in the gastrointestinal tract (Peters and Kremers, 1989), and dermal (Khan et al., 1989) and mucous membranes (Eriksson and Brittebo, 1991). The result of these catalytic reactions depends on the type of pesticide and can range from induction to enzyme inhibition (Patil et al., 2003). The toxicity of OPs and CBMs for example, can be monitored after exposure by measuring the esterase activity (Chambers et al., 2001). The high toxicity of OPs and CBMs is attributed to their ability to mimic esters (natural compounds present in biological organisms), such as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) (Ray et al., 1998; Chambers et al., 2001). Metabolic phase I reactions take place in the liver, where chemical bonds between phosphorus and carbon atoms are cleaved by alkylations (methyl- or ethylation), resulting in the formation of active enzyme centers (Wild, 1975). Trough these phosphorylation processes in the esterase enzymes (AChE and BChE), complexes are formed between the enzymes and the pesticide (Ray et al., 1998; Kamanyire and Karalliedde, 2004; Gupta, 2006). Moreover, the phosphorylation of hydroxyl groups inactivates the enzymatic activity towards substrates and the esterase enzymes lose both stability and function (Pullman and Valdemoro, 1960; Wild, 1975; Ray et al., 1998; Kamanyire and Karalliedde, 2004; Costa, 2006). These interactions can result in the formation of reversible and irreversible complexes, depending on the pesticide and the recovery time of the esterase. OPs tend to form more stable, sometimes irreversible, complexes, whereas CBMs usually form less stable and reversible complexes. The resulting complexes can be depleted by enzymes known as "oximes" (Ray et al., 1998; Kamanyire and Karalliedde, 2004). These metabolic depletion transformations can generate metabolites, which a far more toxic than the original foreign species (Abass et al., 2009; Eleršek and Filipič, 2011). During phase I of the metabolism, OPs and CBMs are involved in oxidation and hydrolysis processes. The oxidation reaction is important for the neurotoxicity of CBMs and OPs, since a desulphurization generates metabolites know as "oxons" through CYP enzymes. Oxons are also known as oxygen analogues of pesticides (Eleršek and Filipič, 2011). Usually, CYPs are relatively specific in the detoxification of chemical compounds, e.g.: diazinon is metabolized by CYP2C19; parathion by CYP3A4 / 5 and CYP2C8; chlorpyrifos by CYP2B6 (Eleršek and Filipič, 2011), or by CYP3A4 and CYP2C9 (Leoni, et al., 2012); atrazine, terbuthylazine, ametryn and terbutryn by CYP1A2 (Lang et al., 1997); endosulfan and carbosulfan by CYP2B6 (Abass et al., 2010).

Other metabolic enzymes, such as paraxonases facilitate hydrolysis reactions. Their function is to eliminate OP/CBM-generated oxons, which is achieved by cleavage of a dialkyl phosphate group. However, through this elimination reaction, highly reactive metabolites (e.g. ROS) can be generated. Eleršek and Filipič (2011) considered these to be genotoxic, since they can interact with DNA molecules. Paraxonases play a protective role against the toxic metabolite oxon, but the potential protection is specific to the type of pesticide and depends on the individual's genotype for the PON gene, which expresses these enzymes. Recent studies on animals, demonstrated an increased expression of PON1 as a result of the promotion, signal transduction and transcription factors on the expression of paraoxomase during the metabolism of OPs. However, there are no known relationships between genotypes, which efficiently detoxify through paraxomases, and/or the activities of AChE and BChE (Costa et al., 2012).

Chemical interactions between xenobiotics may cause saturation of enzymes involved in the metabolism (Bond and Medinsky, 1995). Moreover, evaluations involving low doses during exposure to pesticides may not alter metabolism enzymes, which operate without saturation, and therefore mask a possible effect of intoxication (Bolognesi, 2003; Dyk and Pletschke, 2011). Also, the efficiency of conjugation, a process involved in the glutathione complex during phase II of the metabolism, should be proportional to potential excretion (Eleršek and Filipič, 2011). Accordingly, individual genetic variability, involved in the metabolic transformations of pesticides, can influence the observed pathophysiological effects.

4. Pathophysiology of pesticides

Pyrethroids, OPs, CBMs and OCs represent different classes of insecticides (George and Shukla, 2011). The main effect on human health they share can be attributed to neurotoxicity (Dyk and Pletsche, 2011). Pyrethroids, which lack (type I) or contain a cyano group (type II) in the phenoxybenzyl moiety of their chemical structure (Maroni et al., 2000; Nasuti et al., 2003), interfere with the opening and closing of sodium channels, extending the time of entry for Na^+ cations into the cell (Narahashi, 1996; Spencer et al., 2001). Type II pyrethroids interfere moreover with the chloride channels, blocking the neurotransmitter glutamate receptor (GABA) in the postsynaptic nerve. As a result, the binding of GABA at the receptor site is inhibited and the influx of Cl^- anions into the nerve cell is suppressed (Manna et al., 2006; Suiter and Scharf, 2012). GABA is the major inhibitory neurotransmitter in the central nervous system (CNS) of vertebrates and the absence of synaptic inhibition leads to a CNS hyperexcitability. The same effect can be observed through the incoporation of OCs, especially as the active ingredient in fipronil (Suiter and Scharf, 2012).

OPs and CBMs are neurotoxic due to their inhibition of cholinesterases (AChE, BChE), which interfere with the function of the neurotransmitter acetylcholine (ACh) and long-term effects can be observed (Maroni et al., 2000; Mansour, 2004). AChE and BChE are responsible for hydrolyzing ACh, which is widely distributed in the nervous system of vertebrates (Ray et al., 1998; Chambers et al., 2001). In order to regenerate cholinergic synapses, ACh must be rapidly hydrolyzed by AChE, producing choline and acetic acid after a neurochemical transmission (Namba and Hiraki, 1971).

The inhibition of AChE, caused by OP and CBM insecticides results in an accumulation of ACh at the cholinergic synapses and neuromuscular junctions, eventually causing various signs and symptoms (Maroni et al., 2000; Suiter and Scharf, 2012), Especially muscarinic and nicotinic sites as well as other areas of the CNS are severely affected. Usually, affected receptors are present on the surface of nerve cells (Ray et al., 1998; Kamanyire and Karalliedde, 2004). Due to the effects of AChE on muscarinic and nicotinic receptors, cardiac responses, such as tachycardia, sinus bradycardia, hypertension, hypotension, changes in heart rate and force of heart muscle contraction can be observed. Saadeh et al. (1997) also observed cyanosis and increased serum levels of creatinine and lactate dehydrogenase after OP poisoning. Cardiovascular symptoms occur most frequently after poisoning with pyrethroids, OPs and OCs. Cardiac sodium channel proteins are responsible for both rapid upstroke of the action potential and rapid propagation of nerve impulses through the heart tissue. Thus, their function is central to the origin of cardiac arrhythmias (Balser, 1999). Studies of ventricular myocytes in cats showed that deltamethrin increased the duration of the action potential. The kinetic changes produced in the cardiac sodium channels were similar to those induced by pyrethroids in the sodium channels of the nerve membranes (De La Cerda et al., 2002).

Neuropathy caused by exposure to pesticides is usually related to chronic poisoning cases, since the neurological damage in patients with acute intoxications can be reversed and controlled with adequate treatment (Ray et al., 1998; Ray and Richards, 2001; Costa, 2006; Jayasinghe and Pathirana, 2012). OPs are retained on the endoplasmic reticulum of the axons, promoting apoptosis and injury of the muscle spindle located in the center of the nervous system (involving the spine, spinal cord and cerebellum). This damage is manifested in symptoms such as lethargy, tingling, numbness and weakness of the hip (Ray et al., 1998). Furthermore, elevated risks of developing Parkinson's disease, psychiatric disorders and depressive memory disorders have been discussed (Calvert et al., 2007).

In a review, Rahimi and Abdollahi (2007) suggested hyperglycemia as another effect caused by chronic exposure to OP pesticides. OPs are able to alter the mechanisms involved in the glucose metabolism and thus potentially induce diabetes in exposed individuals. The risk of the general population to develop type 2 diabetes from exposure to environmental OP insecticides, especially in the form of residual contaminants of food supplies, has also been investigated by Rezg et al. (2010).

OP poisoning results in repeated stimulation of cholinergic nerves, which stimulate nerve fibers in the postganglionic parasympathetic muscarinic receptors. This can cause symptoms such as nausea, vomiting, abdominal pain, diarrhea, and tenesmus (Simpson and Schuman, 2002). The phenoxyacetic acid moieties of some herbicides have been associated with the development of gastric cancer. A study showed that chronic exposures to herbicides can result in a 70% chance to develop adenocarcinomas (Ekstrom et al., 1999). Xenobiotics are mainly metabolized in the liver and various types of enzymes, such as alanine aminotransferase, aspartate aminotrasferase (Gomes, 1999; Sarhan and Al-Sahhaf, 2011), and gammaglutamil transferase, as well as other amino acids and proteins (Gomes, 1999) may be affected by their presence. Forensic analysis in humans has also shown histopathological changes in the liver, e.g. necrosis, fat accumulation, and modified centrilobular sinusoidal dilatation (Seema and Tirpude, 2008). Studies conducted on rabbits showed that after the absorption of OPs, leukocyte infiltration occurred in the liver parenchyma, alongside cytoplasmic vacuolization, fatty degeneration and the emergence of pyknotic nuclei in the hepatocytes (Sarhan and Al-Sahhaf, 2011).

OPs are also able to inhibit enzymes, which are important for the metabolism of mitochondrial antioxidant defenses. These are in turn pivotal to the process of respiration and the generation of ATP (Kamanyire and Karalliedde, 2004; Shadnia et al., 2007). This way, pesticides can be directly linked to oxidative stress conditions via lipid peroxidation, which is a molecular mechanism involved in apoptosis (Rastogi et al., 2009). The mitochondrial ATP depletion leads to a stimulation of proteolytic enzymes and a subsequent DNA fragmentation, resulting in cellular death (Shadnia et al., 2007). Mutagenic effects could be observed through the frequency of micronucleus tests (MN), which - on average - were found to be increased after the exposure to OPs (Bolognesi, 2011). These results can be related to certain types of cancer such as Non-Hodgkin's Lymphoma and Leukemia (Bonner et al., 2010).

Mancozeb is a fungicide, commonly used for a wide spectrum of crops (especially soy) and contains a substance with important effects on human health: ethylene(bis)dithiocarbamate (EBCD). EBCD is easily metabolized into ethylenethiourea (ETU), which decreases the activity of tumor suppression proteins, thus facilitating tumor growth (George and Shukla, 2011; Paro et al., 2012). ETU has also been linked to congenital malformation and thyroid disorders (George and Shukla, 2011). Lower concentrations of ETU can affect the morphology and function of cells in the ovarian follicles of mammals (Paro et al., 2012). The effects of paraquat (1,1-dimethyl-4,4-chloride bipyridylium), which is a prototypical agricultural herbicide, have been described by Ranjbar et al. (2002). It promotes toxic effects mainly in the liver and kidney. The latter is predominantly affected by an unchanged excretion of paraquat in the urine (O'Leary et al., 2008). This results in increased tissue injury through lipid peroxidation, which is a secondary effect of the excessive generation of ROS and/or a depletion of the antioxidant defenses (Ranjbar et al., 2002; Samai et al., 2010). Nephrotoxicity processes can also be observed, usually as a result of oxidative stress and/or DNA damage (Samai et al., 2010). A recent study has investigated the effects of OPs on the neoplastic skin cells of rats. The main hypothesis was that the exposure to the herbicide glyphosate, a member of OP family, could lead to increased levels of oxidative stress and result in increased levels of DNA damage (George et al., 2010).

During or after the use of paraquat, triazines, OPs and thiocarbamates, the development of respiratory symptoms was observed among farmers (Hoppin et al., 2002). Paraquat can initiate pulmonary fibrosis through the generation of ROS, whereas glyphosate was associated with the development of chemical pneumonitis (Kirkhorn and Garry, 2000). After exposure to major dosage of OPs and CBMs, signaling in muscarinic receptors was affected and OPs were found in the nerves of post-ganglionic parasympathetic fibers, resulting in respiratory hypersecretion, rhinorrhea, bronchospasm, dyspnea, and cyanosis. These symptoms can evolve progressively and end - due to a complete lack of nerve signals - in apnea and respiratory paralysis (Gaspari and Paydarfar, 2007).

Pesticides interfere with the endocrine system and neurobehavioral development. Moreover, reproduction mechanisms are affected via the endocrine function of steroid hormones, which act as agonists/antagonists in the reproductive system (LeBlanc et al., 1996). The normal reproductive development depends on the interaction of steroid hormones with tissue specific receptors. Xenobiotics may affect the balance between androgen, estrogen and progesterone and hindered interactions between steroids and their receptors may have adverse endocrine effects. These interactions have been examined in studies regarding the exposure to CBMs, e.g. in fungicides. The biosynthesis of imidazole resulted in a deficient production of testosterone hormones (DiMattina et al., 1988), whereas chlordecone and endosulfan increased the testosterone metabolism (Le Blanc et al., 1966). These results contribute to the understanding of the causes of infertility in humans exposed to pesticides (Le Blanc et al., 1996). They also help to explain stillbirths, deformities during embryonic development, as well as congenital malformations caused by OPs (Garry et al., 2002; Maurizio et al., 2008). OCs may affect the function of the thyroid gland, especially regarding the level of thyroxine (T4) production (Le Blanc and Wilson, 1996).

Pesticides can also cause immune alterations, e.g. immunodeficiencies. However, these depend on various environmental factors, which are related to changes of cell functions, and the presence of sub-cellular and/or molecular components in the immune system. The immune response furthermore depends on the interaction between antigens and different cells in the immune system, e.g. lymphocytes or macrophages. Adverse effects can be triggered by direct or indirect immune responses, mainly because some pesticides are more selective than others and may not involve all cell types of the immune system. More specifically, an inhibition of esterases could induce a degranulation of mast cells, thus triggering the release of histamine, which could result in allergic reactions of exposed human individuals. The enzyme phospholipase A2 is involved in the signaling of inflammatory processes, interfering with the humoral and cellular immune system and with T-type lymphocytes (Li et al., 2000; Kamanyire and

Karalliedde, 2004; Li, 2007; Li et al., 2009). It can also produce antibodies, autoantibodies and inhibit natural cell killers such as CD5 and CD26, which promote cytotoxicity (Li, 2007; Li et al., 2009). Most of the pesticide metabolites generate free radicals, which are involved in the generation of oxidative stress conditions. The main mechanisms of the immunotoxicity of pesticides therefore usually involve homeostasis of the pro-oxidant agents and antioxidant defenses.

5. Genotoxic damages of pesticides

Exposure to pesticides has been associated with an increase in the occurrence of non-Hodgkin's lymphoma (Hardell and Eriksson, 1999), multiple myeloma (Khuder and Mutgi, 1997), soft tissue sarcoma (Kogevinas et al., 1995), and lung sarcoma (Blair et al., 1983). Pancreatic, stomach, liver, bladder, and gall bladder cancer have also been reported (Ji et al., 2001; Shukla and Arora, 2001). Moreover, relations to Parkison's disease (Gauthier et al., 2001) and reproductive influences (Arbuckle et al., 2001) have been examined. Several reports are concerned with chromosomal aberrations (CA) (Au et al., 1999; Zeljezic and Garaj-Vrhovac, 2001; Jonnalagadda et al., 2012), sister chromatid exchange (SCE) (Shaham et al., 2001; Zeljezic and Garaj-Vrhovac, 2002), micronuclei (MN) (Falck et al., 1999; Pastor et al., 2003; De Bortolli et al., 2009; Da Silva et al., 2012a; Benedetti et al., 2013) and Comet cells (Grover et al., 2003; Zeljezic and Garaj-Vrhovac, 2001; Da Silva et al., 2012b; Benedetti et al., 2013) as a result of pesticide exposure. In general, significantly increased levels of these biomarkers were found, suggesting severe genotoxic effects of these pesticides.

Various studies have reported significant incidences of cytogenetic damage in agricultural workers, floriculturists, vineyard cultivators, cotton field workers and others (Bolognesi, 2011). Studies involving biomarkers of exposure are usually used in order to assess occupational exposure, i.e. to correlate exposure to chemical reagents with health effects (Aprea, 2012). For this purpose, different biomarkers regarding exposure, effect or susceptibility towards xenobiotics are used to express a specific measure of interaction between a given biological system and a genotoxin (Bolognesi, 2003; Aprea, 2012). The influence of genotypes on the cytogenetic damage is the specific ability of individuals to influence genotoxic biomarkers, i.e. to activate or detoxify substances with respect to their potential to induce mutations, cancer and other diseases (Hagmar et al., 1994; Hagmar et al., 1998). A variety of enzymatic isoforms have been suggested to influence the individual's risk of contracting cancer after exposure to genotoxins (Sulbatos, 1994; Clapper, 2000). Genomic stability has moreover been linked to several dietary micronutrients, nutrient imbalances, dietary deficiencies, as well as excessive exposure to environmental mutagens or carcinogens, all of which can potentially increase genetic damage. As previously discussed, deficiencies of folic acid or other vitamin B cofactors (e.g. B_{12} and B_6) may cause impaired DNA repair.

In view of these diverse and complex findings, the investigation of humans exposed to pesticides constitutes to be a highly important research topic. MN tests and comet assays are accurate and practical analysis tools, complying with most of the criteria used in human biomonitoring (Fairbairn et al., 1995; Grover et al., 2003; Moller et al., 2000). In order to assess, if a prolonged exposure to complex mixtures of pesticides could lead to an increase in cytogenetic damage, our group has examined individuals occupationally exposed to agricultural pesticides in Rio Grande do Sul (Brazil), and the public health workers occupationally exposed to agricultural pesticides in Piauí (Brazil). We were also interested in the potentially important effects of gene polymorphisms, which encode proteins involved in the xenobiotic metabolization/detoxification of phase I or II. These should influence the DNA repair pathways, which should allow an evaluation of the genetic predisposition of individuals towards their xenobiotic metabolizing capacity, i.e. the individual susceptibility towards genotoxic effects of pesticides. Therefore, we also investigated the polymorphism of the PON, GSTM1, GSTT1, CYP2E1, OGG1 and XRCC1 genes. Apart from observing the occupational exposure of individuals towards pesticides, we were also interested in the influence of micronutrient intake (vitamin B_{12}, B_6 and folates), as well as the influence of MTHFR C677T polymorphism on the observed DNA damage.

In order to study all of the aforementioned aspects, our group conducted an investigation on vineyard workers, which involved a total number of 173 individuals (Rohr et al., 2011). Of these, 108 were agricultural workers exposed to pesticides and 65 were control individuals. As evident from MN tests, the individuals exposed to pesticides showed a high rate of DNA damage ($P<0.001$; Mann-Whitney U test), relative to the control group. In addition, some of the MN results of the exposure group suggested genetic polymorphisms of PON, GSTM1, GSTT1, and CYP2E1. OGG1 and XRCC1 are examples of important proteins in the base excision repair (BER) pathway (Au et al., 2004; Goode et al., 2002; Hao et al., 2004; Muniz et al., 2008). In another study, we evaluated two BER polymorphisms: OGG1 Ser326Cys: rs1052133 and XRCC1 Arg194Trp: rs1799782 as well as the combined genotypes of these polymorphisms with PON1 Gln192Arg. The modifications of the genotoxic susceptibility as a function of pesticide exposure was measured by MN tests and DNA damage induction in the peripheral leukocytes of the vineyard workers. Our study demonstrated that the polymorphisms in the BER pathway could modulate the susceptibility to DNA damage caused by the pesticides. Since this repair pathway is the major cellular defense against oxidative DNA damage, our results corroborate existing evidence, which suggests an involvement of oxidative damage in the pesticide-induced genotoxic effects. Our study also reinforces the importance of considering combined effects of metabolism and repair-variable genotypes on the individual susceptibility towards DNA damage. It seems feasible to conclude that these two processes act cooperatively in determining the final response to pesticide exposure.

Brazil is a major producer of soybeans, which are planted in several federal states, but especially in Rio Grande do Sul (RS). The increasing agricultural use of the land is hereby concomitant with an increased use of pesticides. Soybean workers in this region are increasingly exposed to a wide variety of herbicides and insecticides (especially OPs). A study originating from our research group investigated a total of 127 individuals, of whom 81 were exposed and 46 were not exposed to pesticides (Benedetti et al., 2013). Both groups consisted of residents from the city of Espumoso (RS-Brazil), whose main economic income relies on soy crops. We evaluated comet assays of the peripheral leukocytes and buccal micronucleus cytome assays (BMCyt; micronuclei and nuclear buds) in exfoliated buccal cells. We observed significant increases in DNA damage in the pesticide-exposed group relative to control group. We also found the gene PON1 to express the enzyme paraoxonase, which is believed to be involved in the protection against oxidative stress in the OP metabolism. The metabolizing genes PON1, GSTM1, GSTT1 and GSTP1 were evaluated in order to analyze the influence of individual susceptibility in response to exposure. The genetic polymorphisms obtained from the exposure biomarkers showed no influence of the genotype on the DNA damage in the farmers' cells. The exposure to pesticides increased DNA damage and did not change the evaluated metabolizing genes.

Occupational risks for tobacco farmers involve the exposure to very large amounts of pesticides, which are applied to the crop fields. Contact with the pesticides is normally established via the contact to green leaves during the tobacco harvest and through the additional exposure to nicotine. Nicotine poisoning could also lead to "Green Tobacco Sickness" (GTS), which occurs, when workers absorb nicotine via the skin as they come in contact with the leaves of the mature tobacco plant. GTS is characterized by nausea, vomiting, headache, muscle weakness, and dizziness. Our group examined the occupational risk of tobacco farmers, involving 167 individuals, of whom 111 were exposed, and 56 were not exposed (Alves, 2008; Da Silva et al., 2012a; Da Silva et al., 2012b). Subjects were recruited from Venâncio Aires and Santa Cruz do Sul (RS-Brazil) between July and February in the years 2008-2010. Blood and buccal cells were collected twice during the tobacco crop cycle of every year. Once during the distribution period of the pesticides and again during the harvest period. Blood and buccal cells were also collected from a non-exposed control group (office workers, who were living in the same region as the exposed individuals). Our study evaluated exposure biomarkers indicative of early biological effects and susceptibility. Genotoxicity and mutagenicity in the tobacco farmers were investigated by comet assays and micronucleus tests of buccal cells and binucleated lymphocytes, respectively. In order to detect a potential impact of these chemicals on the farmers, superoxide dismutase (SOD), catalase (CAT) and plasma cholinesterase activities, as well as levels of thiobarbituric acid reactive substances (TBARS) were evaluated. Total contents of chemical elements in the blood were examined by particle-induced X-ray emission (PIXE) and cotinine levels were analyzed in plasma samples. In order to establish a possible correlation between a potential genetic predisposition of the metabolism of xenobiotics / repair of DNA damage and individual susceptibility towards genotoxic effects of pesticides and nicotine, farmers were genotyped for several genes. The evaluation of the DNA damage also considered the following secondary parameters: use of protective measures, time after exposure, age, and gender. As tobacco farmers were exposed to complex mixtures of pesticides during the application period, significantly higher levels of DNA damage were found in the exposed group relative to the control group. For the exposed group, the damage to the DNA was three times higher during the application period and four times higher during the harvest period relative to the control group. However, no significant difference in the activity of serum cholinesterase was observed between exposed and control group. Prior studies, examining pesticide workers, were unable to identify any correlation between chronic exposure to OPs and BChE inhibition. During the exposure period, all individuals showed symptoms related to pesticide poisoning and GTS, e.g. headache, abdominal pain, nausea, and vomiting. We observed in our study that the serum cotinine levels among the non-smoking section of the exposed individuals during the harvest period were significantly increased, suggesting absorption of nicotine through skin contact with tobacco leaves. Nuclear anomalies in the buccal mucosa cells of exposed tobacco farmers (both during the application and harvest period) showed mixtures of genotoxic and cytotoxic substances. A minor discrepancy concerning the mutagenicity was noticed between the two different periods of the tobacco cycle. During the harvest period, higher MN values were observed in buccal cells, relative to the application period. In addition, effects on the extent of pesticide-induced DNA damage and cell death as a result of the genetic polymorphisms of PON1 and CYP2A6*9 were observed. Binucleated lymphocyte responses to genetic damage were evident from higher MN levels in the exposed group (mainly during the application period) relative to the control group. Workers employed in the production of pesticides and farmers who used pesticides showed a higher risk/level of exposure and hence, were more prone to the potential deleterious health effects of pesticides. Besides, many pesticides, which are commonly used on tobacco crops, contain inorganic elements, including Mg, Al, Cl, Zn, and Br, which are known to cause DNA damage. In our study, absolute inorganic element levels in the blood samples of the exposure group (application period) were found to be increased.

Elevated levels of DNA damage in the exposure group were also observed during the harvest period, presumably via contact with green tobacco leaves and tobacco plants during the various cultivation processes and concomitant dermal nicotine absorption. Nicotine has been implicated in the generation of free radicals in human cells, directly addressing the relationship between ROS induction and observed DNA damage. Thus, synthetic and natural pesticides may induce oxidative stress, and lead to increased generation of free radicals as well as subsequent alterations in antioxidants, free oxygen-based radicals, lipid peroxidation, and the quenching of enzyme systems. In the exposure group (application period), only the antioxidant enzyme SOD showed increased activity, relative to harvest and control groups. In the harvest group, levels of body-defending antioxidant mechanisms (SOD and CAT) were increased, in order to overcome the induction of oxidative stress. These results indicate that the level of lipid peroxidation was significantly different in the harvest group relative to application and control groups. It is feasible to assume, that the internal antioxidant stimulation in the body were insufficient to scavenge all the free radicals and thus compensate for the increased levels of lipid peroxidation. Age and personal protective equipment (PPE) also showed an influence onto the results obtained from the MN tests. An increase of MN levels corresponding to age was observed for both groups (exposed and control). Interestingly, significant differences were observed between exposed individuals (application period) with complete PPE, relative to those without.

The effect of individual genotypes of metabolism genes on the level of the different biomarkers (comet assay and MN tests in binucleated lymphocytes) was examined in the exposed group. Increased damage of GSTM1 in the application group and an increased damage of CYP2A6*1/*1 in the harvest group were observed. The individual genotype of DNA repair genes in the exposure groups did not show any influence on the different biomarkers analyzed in this study. Our study demonstrates once more the importance of occupational training for farm workers, regarding safe working practices and safe working environments. Developing countries should use such data to establish occupational safety rules when using pesticides (especially in the context of tobacco crops) in order to minimize occupational risks for the workers involved.

We also evaluated the influence of micronutrient intake (vitamins B_{12} and B_6, folates) and MTHFR C677T polymorphism on DNA damage in the exposed individuals (Fernandes, 2012). We examined 110 individuals of both genders (average age: 42.3 ± 13.3 years), living and working in the city of Venâncio Aires (RS-Brazil). The examined exposure time was 30.3 ± 15.6 years. The results showed increased levels of MN in lymphocytes and modified consumption of folates and $B_{12,}$ ($p = 0.030$ and $p = 0.014$, respectively). No significant correlation between DNA damage (MN frequency, comet assay) and age, gender, smoking, years of exposure or BMI could be observed. Similar results were obtained for the genetic polymorphism of *MTHFR C677T.* A diet with appropriate folate and vitamin B_{12} supplements was able to facilitate adequate DNA repair.

Another study originating from our group followed, for over 30 years, a group of public health workers, concerned with endemic diseases. During their work, these individuals have been exposed to considerable amounts of genotoxic and mutagenic pesticides, which are used in vector control programs. Our study with this group therefore aimed at the evaluation of the mutagenicity (MN tests in buccal cells) caused by the occupational exposure to pesticides in the "Território Entre Rios" (Piauí-Brazil) (Fianco, 2013). The study included 129 individuals, of whom 66 were public health workers (exposed group), and 63 individuals without occupational exposure to pesticides (control group). Mutagenic events were manifested through the presence of significant increased numbers of MN (14.7 ± 2.7), binucleated cells (5.9 ± 1.1) and nuclear buds (10.2 ± 1.8) in the exfoliated oral mucosa cells of the workers, relative to the control group (4.2 ± 0.9, 2.8 ± 0.8, and 3.9 ± 0.9, respectively; Mann-Whitney test). However, age, gender, exposure time, smoking, drinking, or diet did not influence the DNA damage parameters examined. According to these results, the occupational exposure of public health workers to pesticides induces mutagenic damage. Even though public health workers should be aware of the risks they are exposed to, the proper use of personal protective equipment could still be improved.

6. Conclusions

Our findings show in general that agricultural workers exhibit higher levels of DNA damage in somatic cells, suggesting that pesticide exposure is a potential health risk for these workers. In addition, it was possible to correlate these results to specific genetic susceptibility, to the absence or inappropriate use of PPE and to dietary habits. It became evident that continuous education is very important for exposed workers, in order to minimize the deleterious effects of the occupational exposure and the risk of contracting work-related diseases. Chronically exposed individuals were more susceptible to the clastogenic effects of pesticides. Significant differences in the cytogenetic damage were detected in individuals with symptoms of chronic intoxication (Zeljezic and Garaj-Vrhovac, 2001). Furthermore, others studies observing agricultural workers demonstrated an increase in chromosomal damage during the spraying/ application season, when pesticides were used intensively (mainly in workers not using PPE). The use of PPE seems to be beneficial for the workers, which is evident from reduced cytogenetic effects (Shaham et al., 2001). The DNA damage (CA, MN and SCE) could be correlated with the exposure duration in many of these investigations (Bolognesi et al., 1993; Joksic et al., 1997; Shaham et al., 2001; Bolognesi et al., 2002; Bolognesi, 2011), and moreover seem the clastogenic effects to be cumulative for a continuous exposure to pesticide mixtures (Bolognesi, 2003; Bolognesi, 2011).

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Chapter 3

Flagship Species Conservation and Introduced Species Invasion : Toxic Aspects Along Loire River (France)

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Additional information is available at the end of the chapter

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1. Introduction

In France, the Loire river basin is a unique river system, because of its size, variety of aquatic and wetland habitats and its exceptional diversity of living communities. Succeeding fresh-water aquatic biological communities belong to all types of water habitats, from the area of spring stream to estuarine area.

Aside from the great variety and richness of the living communities they host, aquatic ecosystems composing the Loire river system are exposed to many sources of pollution and anthropogenic disturbances affecting water quality and ecosystem functioning throughout the food webs.

Xenobiotic substances in the water are a wide range of contaminants, both organic and metallic and radioactive (nuclear power plants), which focus, by bioaccumulation in the tissues, organs and fat of living beings, and by biomagnification through all trophic levels. This is why some animal species, including predators, can play the role of "biosensors" of contaminants, particularly interesting to study and monitor the health of ecosystems and provide a tool for identification of these elements, then restore water quality and preserve the diversity of environments.

To better understand the pathways of contamination, the phenomena of biomagnification and potential degradation of contaminants during the trophic transfers across the whole Loire river basin, a group of indicator species representing different trophic levels was analyzed. Among them (see figure 1 and table 1 below) are three top-predators, two polyphagous fish (one sedentary, the other diadromous), three predator and scavenger crayfish species, and a species of benthic bivalve. These invertebrates, accidentally (*Corbicula*) or voluntarily introduced for aquaculture (crayfish), quickly located in the basin of the Loire River and developed in recent years a marked invasiveness, now causing major imbalances of the whole hydrosystem. Consequences like transmission of pathologies to local species, competition for habitat or resource and direct predation on local or rare species were observed. This selection of bio-indicators, or their trophic counterparts in case of absence in pre-selected study sites, should enable to understand the phenomena of water pollution and contamination of food webs by persistent biocides and toxic xenobiotics at different scales of spatial perception from the permanent station (bivalve) to intercontinental vital area (migratory fish-eating birds), through the local scale of a few kilometres (confined fish and / or mammals).

Fixed Sedentary	**Confined / mobile sedentary**		**Nomads**	**Migratory**	**Migratory breeding / wintering**
Asiatic clam Corbicula fluminea	Red swamp crayfish Procambarus clarkii	Chub Squalius cephalus	European otter Lutra lutra	Thinlip grey mullet Liza ramada	Osprey Pandion haliaetus
	Signal crayfish Pacifastacus leniusculus				Great cormorant Phalacrocorax carbo carbo/ sinensis
	Spiny-cheek crayfish Orconectes limosus				

Table 1. Indicator species used in this study.

2. Methods

2.1. Study area

Study area corresponded to the whole Loire River and main tributaries catchment in France. 9 study sites were used for the sampling, especially concerning mussels, crayfish or fish (see figure 2). Loire River catchment (117000 km^2, total length of rivers and tributaries of about 40000 km) is characterized by an important diversity of habitats and species, and is considered as one of the most preserved large hydrosystems in Western Europe. A national and European action plan, “Plan Loire Grandeur Nature”, is running since 1994 to study and conserve this diversity, but also to protect inhabitants from floods and to maintain economic activity.

Figure 1. Map of France

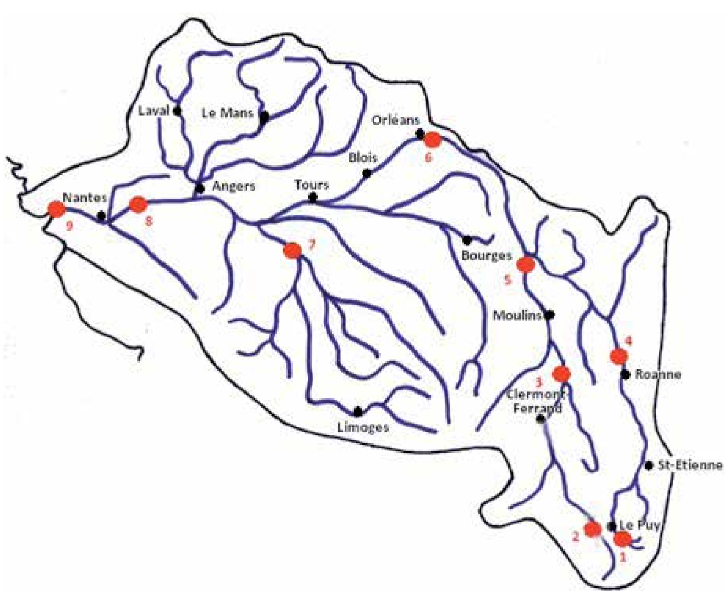

Figure 2. Detail of the study area showing sampling sites (numbers 1 to 9).

2.2. Sampling

Concerning rare species like otter or osprey, it is difficult to obtain sufficient sample material from enough individuals to support analysis and statistics. Otter and osprey are fully protected by national and international laws, and listed as species of interest by the European Community (Habitats Directive 92/43/EC, Birds Directive 79/409/CEE. So, for legal and ethical reasons it was not imaginable to trap or kill otters or ospreys for analyses. To avoid any vital risk related to handling, capture and bleed of animals were not considered. All operations were therefore entirely conducted under appropriate authorizations by a non-invasive approach. A large network, constituted by people in charge of otter and osprey studying and monitoring in mainland France was built to organize and enhance sampling under the coordination of the Muséum d'Orléans.

The national agency for game and wildlife (ONCFS), hunting federations (FDC), the national agency for water and aquatic environments (ONEMA), health centres of the national union (UFCS) and of the birds protection league (LPO – French representative of Bird Life International), the national research centre on birds population biology (CRBPO, associated with the French national museum of natural history MNHN), the French Ministry of Environment (MEEDDM and DREAL Centre), the national agency for forests (ONF), private land owners and companies, museums, associations ("Loiret Nature Environnement") and regional naturalists were contributors for this study. Great cormorant, another protected species in France, is however concerned by legal shots, in order to limit the fishing impact of the species in fish farms and pounds. These shot birds were used for toxicological analyses, to avoid useless destructions.

Concerning otters, only road-traffic killed individuals and those found dead in the wild in study area were collected. Based on visual observation, carcasses found more than 24h (during summer) or 48h (during winter) after road collision were considered too degraded and not taken into account for post-mortem examination and toxicological analyses. Concerning ospreys, non-hatched eggs and dead young in nests were collected during chicks ringing operations. As scientists and birdwatchers monitor a majority of osprey nests in continental France, non-hatched eggs and dead young in nests were reported and sampled as soon as possible. France is also a major crossing area for migrating osprey from different populations [1,2]. Due, in one way, to the extreme rarity of this species in continental France (less than one hundred reproductive individuals), and in an other way that "foreigners" individuals (*i.e.* born in neighbour countries, but potentially breeders in France) are able to be found dead within the national territory (naturally or after illegal shots, electrocution on power cables, or drown in fish farms), migrating individuals flying towards reproduction areas elsewhere in Europe (Germany, Great-Britain, Scandinavia) completed sampling.

Other animals studied here (great cormorant, fish, crayfish and mussels) were sampled under authorizations after legal shots near fisheries or pounds (great cormorants), or using fishing license (fish and crayfish), near sampling sites where possible.

All samples were deep-frozen (-40 °C) prior to analyses. For each carcass, a necropsy was performed, and about 20 g of liver was sampled (preferred to fat because of the very low fat content of ospreys, especially at the end of spring migration). Sex and weight were determined; animals were measured and aged according to criteria like total size, teeth development or plumage characteristics (total and head, body, foot and tail lengths). Non-hatched osprey eggs were drilled and emptied; eggshell was conserved for future studies on shell thickness. Each animal is characterised by a specific case-record gathering discovery circumstances, clinical and biometrical data. After necropsies, carcasses were conserved for further showing or collection in museums or, if too degraded, systematically destroyed according to law.

2.3. Organochlorine pesticides analyses

2.0-8.0 g of tissue were sampled and 30 ml of hexane/acetone 75/25 mix was added. Each sample was blended twice with an Ultraturrax® (Ika, Werke, Germany) and filtered trough a phase separator membrane. The extract was evaporated at 60 °C in a rotary evaporator. The dry extract was dissolved in 10 ml hexane. Two ml of fuming sulphuric acid (SO_3 7%) were added, and after centrifugation at 4x *g*, 1 ml of the supernatant was used for OC pesticides quantification by gas chromatography with electron capture detection material. Temperature program and injection conditions are described in [3,4]. Each sample was run in duplicate. Organochlorine pesticides concentrations were calculated by using different mix standards. Recovery level on standard mixtures was always greater than 92%. All standards were purchased from CIL (St Foy la Grande, France), and purity was > 99%. Linearity was determined between 5 and 100 $ng.g^{-1}$ ($r^2 > 0.99$ on standards and spiked samples, 5-point calibration curves). Limits of detection were between 0.5 and 1.0 $ng.g^{-1}$ lipids for individual PCB congeners. Cod liver oil (BCR349) certified material was used as a regular quality control.

2.4. Organophosphate pesticides analyses

5 g of tissues sample was shaken with 60 ml dichloromethane and 10 g anhydrous sulfate. Mix was then filtered trough a Whatman 1 PS membrane, and evaporated under vacuum at 40°C. Dry samples were diluted in 3 ml ethanol, and underwent an ultrasonic step. Extract was then purified with a Sep pack R 300 (Silica Waters, 020810; 500 mg) column conditioned with 2 ml methanol and 2 ml ethanol. 2 ml dichloromethane were used for column elution. Purified samples were dried and diluted with 3 ml dichloromethane. Organophosphate (OP) and 2 carbamates (CA) pesticides (Dichlorvos, Carbofuran, Mevinphos, Phorate, Phorate oxon, Phorate sulfone, Methiocarbe, Terbufos, Diazinon, Disulfoton, Chlorpyriphos methyl, Chlorpyriphos ethyl, Fenitrothion, Pyrimiphos methyl, Malathion, Fenthion, Parathion, Methidathion, Disulfoton sulfone, Triazophos) concentrations were determined by GC/MS in SIM mode (OP + carbofuran and methiocarbe). A 5973N MS coupled with a 6890 GC (Agilent®) was used, with a 30m HP5-MS column (0.25 mm ID, 0.25μm thickness). For each samples standard and spiked sample, 2 μL were injected. The temperature program was 100°C (2 min), 55°C/min up to 200 °C (held for 5 min), 50 °C up to 220 °C (held for 3 minutes), followed by 60 °C/min up to 300°C. A final, post-run time of 2 min at 300°C was maintained. Total run time was 13.55 min. Injector was set at 250°C and the He flow was set at 2.5 ml/min.

Each OP or CA was identified based on the following criteria: retention time and 3-4 fragmentation ions with pre-defined relative amounts and 20% variability acceptance for each ion. Linearity was confirmed between 25 and 500 $ng.g^{-1}$ with 5 point calibration curves and $r^2 > 0.99$. Recovery was determined between 76% and 104% for all spiked samples and repeatability was considered acceptable with coefficients of variation <15%.

2.5. Pyrethroids pesticides analyses

5 g of tissue sample was shaken in 60 ml ethanol and 10 g anhydrous sulfate, and then filtered trough a Whatman 1 PS membrane. Extract was dissolved in 5 ml methanol and underwent a second filtration procedure. Concentrations were determined by GC / ECD and confirmed by GC/MS according to a modified method of the French Food Safety Authority (Anses Met AFSSA). An Agilent GC-ECD 6850 with a 30m HP1 column (0.32 mm ID, 0.25µm film) was used. For each samples standard and spiked sample, 2 µL were injected. The temperature program was common to OC and pyrethroids pesticides (initial temp: 100°C, first ramp 6°C/min up to 220 °C held for 10 min, 2nd ramp 7 °C/min up to 285°C, held for 1 min, total run time 42.29 min) Injector was at 230°C, detector at 300°C. Total He flow was 9 ml/min. Pyrethroids were identified according to their retention times. Linearity was confirmed between 10 and 100 $ng.g^{-1}$ with 5 point calibration curves and r^2 >0.99. Recovery was determined between 82% and 94% for all spiked samples and repeatability was considered acceptable with coefficients of variation <15%. For all positive samples, a confirmatory analysis was performed with GC/MS in SIM mode. Identification was based on retention times and 3 or 4 ions.

2.6. Herbicides analyses

2 g of tissue sample was shaken during 5 minutes in 8 ml acetone, and then centrifuged at 4x g; supernatant was placed in separate tubes, and this extraction was performed twice. Samples were evaporated under nitrogen, and dry extract was dissolved in 1 ml aceton/methanol (50:50) solution. Extract was then purified with a SPE C18 500 mg column conditioned with 2 ml acetone and 2 ml methanol. Column was vacuum dried and purified samples were diluted in 3 ml acetone. After drying under nitrogen, samples were diluted in 1 ml methanol. Herbicides (Trifluraline, Atrazine, Simazine, Terbuthylazine, Diuron, Alachlor, Metolachlor, Cyanazine, Epoxyconazloe) concentrations were determined by GC/MS spectrometry. A 5973N MS coupled with a 6890 GC (Agilent®) was used, with a 30m HP5-MS column (0.25 mm ID, 0.25µm thickness). For each samples standard and spiked sample, 2 µL were injected. The temperature program was 85°C held 1 min, followed by 6°C/min up to 170°C (held for 12 min), then followed by 20°C/min up to 280°C, held for 4.33 min (total run time 37 min). Injector was at 250°C and in the splitless mode. Each herbicide was identified based on the following criteria: retention time and 3-4 fragmentation ions with pre-defined relative amounts and 20% variability acceptance for each ion. Linearity was confirmed between 100 and 500 $ng.g^{-1}$ with 5 point calibration curves and r^2 >0.99. Recovery was determined between 67% and 98% for all spiked samples and repeatability was considered acceptable with coefficients of variation <15%.

2.7. Anticoagulant rodenticides

Analyses for anticoagulant rodenticides (8 compounds marketed in France: bromadiolone, chlorophacinone, difenacoum, difethialone, warfarin, coumatetralyl, brodifacoum, flocoumafen) contamination were completed using high-performance liquid chromatography according to [5,6]. Briefly, 1.0-2.0 g of liver were used. All solvents and reagents used were of the highest purity available. 50 µl of each sample or standard solution were injected in a C18 column (10 nm pores, 5 µm granule size), 250 x 4 mm (Chromcart Nucleosil, Macherey-Nagel, Strasbourg, France). The HPLC system used was made of an isocratic pump (L6000), an automatic sampler (AS2000), and a fluorimetric detector (F1000). Specific software (D7000 HSM) was used for data acquisition (Merck, Nogent-sur-Marne, France). Linearity was determined on standards and spiked liver samples between 0.05 and 1 $mg.kg^{-1}$ (r^2>0.99%). Percentage of recovery varied between 80.1% and 89.2% for bromadiolone, chlorophacinone and difenacoum (the three most common rodenticides in France). Limits of detection were 0.02 $mg.kg^{-1}$ for all anticoagulants tested.

2.8. Calculation methods and statistical analysis

Geometric means of *p,p'*-DDE, *p,p'*-DDD and *p,p'*-DDT were added to calculate the sum of DDTs (Σ DDTs). Geometric means of lindane, endosulfan, DDE, DDD, DDT, heptachlor, heptachlor epoxyde, aldrin and metoxychlor were summed to provide the sum of pesticide concentrations (Σ Pesticides). All these were chosen by the National Veterinary School of Lyon (VetAgro Sup Campus Vétérinaire de Lyon, France) standard protocol [7,3,4,8]. The Mann-Whitney test was used to compare two independent samples, Kruskall-Wallis for *k* comparisons, Spearman correlation rank test to quantify associations between two variables. Statistics were performed using *R.* [9].

3. Results

3.1. Contamination of osprey

3.1.1. Organochlorine pesticides

Contamination results of osprey harvested in France by organochlorine pesticides are presented in Table 2 and figure 4. Organochlorine pesticides were detected in 20 samples out of 27 (74%) who have undergone analysis. Among the compounds tested, only residues of DTT (mainly p, p'-DDE) and methoxychlor were detected, with one single case of contamination with lindane, though forbidden to use since 1998. DDE was detected in 12 samples (44%), including 5 eggs from different nests in France. DDE concentrations, averaging 0.92 $mg.kg^{-1}$, remained generally low (between 0.1 and 8.2 $mg.kg^{-1}$ fresh weight, this extreme value being high, however), and overall comparable to levels found in the literature concerning other prosperous population [10,11]. On unhatched eggs, the maximum concentration of DDE was 4.6 $mg.kg^{-1}$, which is slightly above the threshold of 4.5 mg kg^{-1}, beyond which the shell of the egg, fragile, can break under the weight of the incubating adult [11,12]. This egg was not damaged, but these significant results suggest a relatively recent use of DDT in the basin of the Loire River, probably after its ban in France (1973). Indeed, even if the species is a migratory one, contamination of osprey eggs was documented as more indicative of contamination of breeding then wintering areas [13].

Figure 3. Osprey (*Pandion haliaetus*). Photo C. Lemarchand.

Methoxychlor was meanwhile detected in 8 samples, or 27%, again with relatively low concentrations. The average concentrations of methoxychlor reached 0.04 $mg.kg^{-1}$, significantly lower than the values found in the literature [14]. In France, no significant changes in concentrations of organochlorine pesticides were observed, depending on the sex or age of ospreys, this result can be explained by the small total sample size. Aldrin, heptachlor and endosulfan were never detected in the tissues analyzed ospreys, and lindane only once, in contrast to what was observed in previous studies conducted overseas [11,15,16,17]. This difference could be explained by greater exposure of ospreys to contaminants in America, where the species declined but not disappeared, than in continental France, where ospreys disappeared after direct destruction and before massive use of pesticides in the environment. So accumulation pattern of organochlorine was probably different among ospreys from America, which were heavily affected and locally literally decimated by the accumulation of pesticides. The ban of DDT and other organochlorine pesticides, has led to a slow decline in levels observed in tissues of osprey to the current situation which has seen numbers recover. In France, organochlorine pesticides were banned and declined in the environment decades before the return of the species, resulting in a lower and declining exposure and accumulation.

Considering the natural expansion of the species in France [18] and reproductive success of most couples, organochlorine pesticides do not appear to threaten the osprey as breeding species in France in the short term, or affect the stability of European populations. Some relatively high observed values, however, does not rule out any risk for individual survival, and it should be noted that the relatively small sample size and lack of perspective, given the

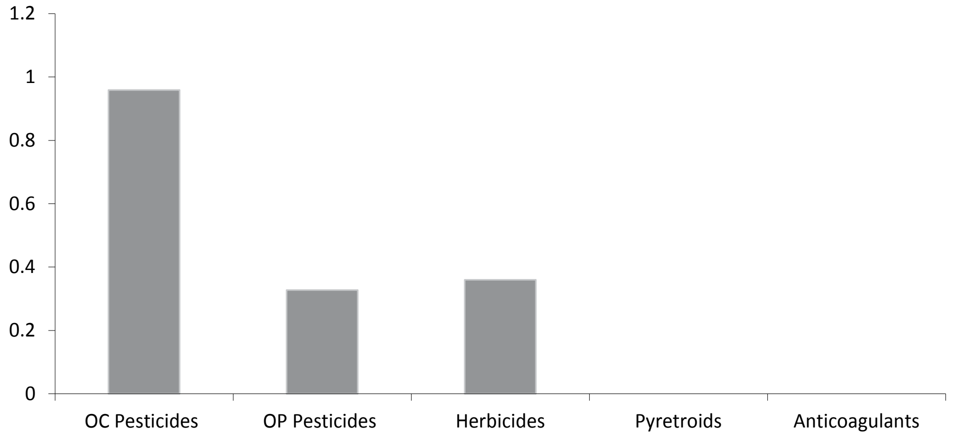

Figure 4. Contamination of ospreys by pesticides (mg.kg^{-1}).

recent nature of the toxicological study of case, require some caution and further research programs will give more relevance to these results.

3.1.2. Ospreys contamination by organophosphate pesticides, carbamates and pyrethroids

Among the highly toxic cholinesterase inhibitors measured in this study (mevinphos, phorate, malathion, phorate sulfone, chlorpyrifos-ethyl, parathion, fenitrothion, methiocarb, methidathion, disulfoton sulfone and triazophos) several were quantified in tissue ospreys (see Table 2 and figure 4). Contamination of ospreys by organophosphate pesticides appeared weak and dispersed, only a few individuals being affected. Organophosphate pesticides were detected only in adult and subadult tissues, and never in juveniles or eggs (see Table 1.1). For contaminated individuals, differences by age, gender or geographical origin of birds is not significant. No individuals from the breeding population of mainland France analyzed here have revealed contamination by organophosphates. Triazophos, disulfoton sulfone and mevinphos were the most frequently detected compounds in Osprey. As described above, the recovered ospreys were generally in good physical condition and had normal values of total weight and overweight, they showed no clinical signs manifest organophosphate poisoning during the review post mortem, such as diarrhea, pulmonary edema, or tighten clows. In addition, some of them were recovered during migration, where energy reserves are mobilized and may lead to phenomena of acute intoxication, and no evidence of this type has been observed, the causes of death. Measured concentrations remained well below thresholds for toxic doses of cholinesterase inhibitors (5 to 10 mg.kg^{-1} ww) and did not constitute a lethal agent for individuals.

Carbamate pesticides were not detected in tissues of osprey in this study (see Table 2 and figure 4), in contrast to recent work on other raptors in France, such as the red kite (*Milvus milvus*) [19], showing a wide poisoning of wildlife by these elements. It can be assumed that the diet of the osprey, specialized and based on consumption of fish has little exposure to the accumulation of carbamates compared to other species, especially opportunistic scavengers like kite. Given these observations about the osprey and the evolution of the regulation of organophosphate and carbamate pesticides, the measured concentrations of the first, relatively small and not likely to increase very slightly, are not likely to pose a constitute a threat to the conservation of the species.

Pyrethroids have not been detected (see Table 2 and figure 4), in any Osprey analyzed in this study. Given the extreme rarity of investigation of these compounds in the literature on wildlife, further studies are probably needed to really set the overall contamination of aquatic systems by pyrethroids, despite weaker toxicity compared to organophosphates and carbamates for mammals and wild birds [20,21].

3.1.3. Contamination by herbicides

The results for herbicides analyzed in this study are shown in Table 2 and figure 4. As observed for organochlorine pesticides, herbicide contamination appeared weak and undiversified. 10 Osprey showed detectable concentrations of herbicides. Terbuthylazine, cyanazine and alachlor were the only herbicides measured, with low concentrations (from 0.01 to 0.28 $mg.kg^{-1}$) and the observed variations according to age, sex or geographical origins of birds were not statistically significant. Only one individual from the breeding population of mainland France showed a measurable concentration of herbicides. As in the case of organochlorine pesticides and carbamates, herbicide concentrations remained low, and probably have very little impact on the conservation of the species, but the total sample size is too small for a definitive conclusion. Moreover, insofar as herbicides are rarely analyzed in comparable studies of the literature, very few items are available for comparison [21].

3.1.4. Contamination by anticoagulant rodenticides

Anticoagulants have not been detected in tissues of osprey in this study (see Table 2 and figure 4). These results can probably be easily linked to the diet of strictly fish-eating species [22], limiting exposure to elements mainly used against the proliferation of rodents that ospreys do not capture. But given that in one hand, the possibility for the Osprey to capture and eat rodents for periods where fishing is difficult or even impossible [23] and on the other hand the regular presence of anticoagulants residues in various environmental compartments (including aquatic ones), highlighted by the decreasing thresholds of analytical detection, the risk of poisoned ospreys by anticoagulants can not be totally excluded.

	Total	Eggs	Juveniles	Subadults	Adults	Males	Females
OC Pesticides	0,96	1,38	0,11	<d.l.	1,61	1,23	0,28
OP Pesticides	0,33	<d.l.	0,56	<d.l.	0,80	0,66	0,58
Herbicides	0,36	0,04	0,24	<d.l.	0,99	0,29	1,21
Carbamates	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Pyrethroids	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Anticoagulants	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.

Table 2. Data concerning osprey intoxication by pesticides ($mg.kg^{-1}$; n.a.: not analyzed ; d. l. : detection limit)

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3.2. Contamination of European otters

3.2.1. Contamination by organochlorine pesticides

3.2.1.1. Upper part of the basin

The results concerning contamination of otters from upstream Loire river basin (Limousin and Auvergne regions) by organochlorine pesticides are shown in Table 3 and figure 6 below. Organochlorine pesticides were detected in all individuals analyzed, the amount of pesticides reaching a maximum of 9.4 $mg.kg^{-1}$ lipid weight. Residues of DDT, DDE mainly constitute the dominant share (70-90%) of the total pesticides detected in the tissues of otters. DDT was detected in the tissues of both from the basin of the river Allier individuals, suggesting recent use of the pesticide, after legal prohibition in France, dating back to 1973. As observed in the preliminary study of otter scats [3], lindane is the most abundant organochlorine pesticide most frequently detected after the DDTs. The concentrations of aldrin and heptachlor remained weak, endosulfan and methoxychlor were never observed in the tissues. Concentrations of organochlorine pesticides measured in the liver of individuals were significantly higher ($p<0.05$) than those measured in spraints otters from the same population [3], suggesting a low elimination of these toxic compounds via the general metabolism. These results also emphasize the need to complement the study of otter scats by their tissues when they are available, in addition to a wider range of measurement of contaminants [24,25].

Figure 5. European otter (*Lutra lutra*). Photo C. Lemarchand.

These results emphasize the gradual decrease in concentrations of organochlorine pesticides in the environment after their ban. Females appeared to be more contaminated than males ($p <0.05$) by pesticides. So despite this significant contamination, prosperity observed population should be related to the availability of vacant habitats, like suggested above concerning osprey. Several studies [24,26,27] have already reported high concentrations of organochlorines in

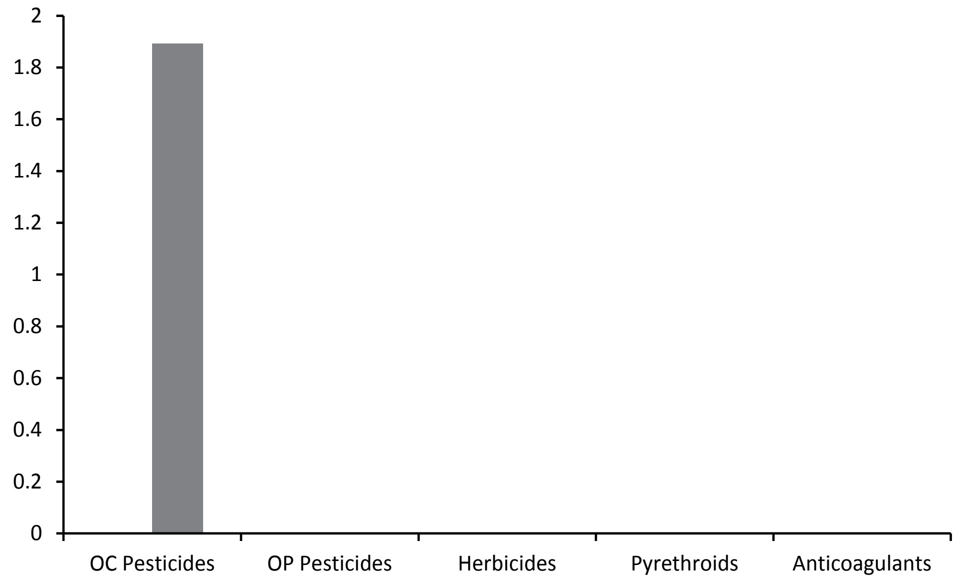

Figure 6. Contamination of otters by pesticides (mg.kg^{-1}).

increasing otter populations, a good reproduction pattern in unsaturated habitats offsetting losses du to intoxication [3,28,29].

3.2.1.2. Lower part of the basin

Results concerning the contamination of otters from the downstream part of the basin by organochlorine pesticides are presented in table 3 and figure 6 below, challenged prospects for comparison with data from the upstream, above. For this part of the basin, organochlorine pesticides were detected in all individuals, but concentrations remained under 0.5 mg.kg^{-1} lipid weight, significantly lower than in the upstream areas ($p < 0.05$). Residues of DDTs remained the most abundant elements. Unlike the upstream, the parent compound (DDT as itself) was not detected. Other pesticides remained at very low levels (mainly traces of lindane), or were not detectable. The variations with sex or age of the contamination of otters from the downstream part of the basin remained weak and insignificant. These data suggest a significantly lower contamination of this area and local food webs by organochlorine pesticides. It therefore appears that the contamination by organochlorine do not pose an immediate threat to the conservation of the species in the basin of the Loire, as noted elsewhere in Europe [4].

3.2.2. Contamination by organophosphate pesticides, herbicides, carbamates and pyrethroids

Of all the compounds tested, none of organophosphate pesticides, herbicides, carbamate pesticides or pyrethroids has been detected in tissues of otters, whether from the upstream or downstream or marsh western basin of the Loire river (see table 3 and figure 6). As the analysis has been carried out in the same series as those for other species in this study (including the

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osprey), any risk of an experimental bias detection can be excluded here. If the absence of carbamates and pyrethroids was observed for all three super-predators (see above and below) and seems to suggest a robust lack of accumulation of these compounds in aquatic food webs, however the absence of organophosphorus pesticides and herbicides in this population of otters seems surprising, since their presence (even if weak and dispersed) in both studied birds also highlights their environmental persistence and ability accumulative in food webs to higher levels. However, the bibliographic data for the European otter are rare on these compounds for possible comparison, and testing hypotheses to try to explain these results.

3.2.3. Contamination by anticoagulant rodenticides

Of all the otters from the Loire River basin (all periods and all collection sites combined) analyzed for their potential contamination with anticoagulants residues, only two individuals involved bromadiolone concentrations that reached 0,40 and 0,85 $mg.kg^{-1}$ fresh weight respectively (see table 3 and figure 6; [4]). Chlorophacinone and difenacoum (often used in France) were never been found in any of the otters analyzed. These results highlight the risk of poisoning non-targeted species during rodent control campaigns [6]. The two individuals concerned were males from the same sector of the Allier River, where the band of riparian vegetation and the banks have long been treated with bait (carrots or apples arranged on floating rafts) poisoned with anticoagulants for the removal of raccoons and muskrats. If these treatments "air" are banned in France since 2006, the practice remains active locally, and the use of anticoagulants in the form of buried wheat grains poisoned bromadiolone, especially against the proliferation of land voles (*Arvicola scherman*) can result for the otter by secondary poisoning due to predation on non-target rodents such as amphibian voles (*Arvicola sapidus*, now a protected species in France).

Due to the absence of clinical evidence of intoxication anticoagulants such as severe anemia or bleeding, and the relatively small number of individuals involved, anticoagulants do not seem to pose a threat to the conservation of the otter, especially as current practices no longer allow the use of anticoagulants in the aquatic area. However, lowering the thresholds regular analytical detection puts increasingly highlight the significant environmental release anticoagulants, and some care must be set in the future about their illicit use and monitoring.

Pesticides	Total	Juveniles	Subadults	Adults	Old	Males	Females
Organochlorine	1,83	1,63	1,92	2,01	1,77	1,2	2,1
Organophosphate	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.
Herbicides	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.
Carbamates	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.
Pyrethroids	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.
Anticoagulants	0,62	n.a.	0,62	n.a.	n.a.	0,62	n.a.

Table 3. Data concerning otter intoxication by pesticides ($mg.kg^{-1}$; n.a.: not analyzed ; d. l. : detection limit)

3.3. Contamination of great cormorants

3.3.1. Organochlorine pesticides

The results of contamination of cormorants by organochlorine pesticides are shown in Figure 8 below. OC Pesticides were detected in all individuals for all study sites. The observed values were generally low, less than 0.1 $mg.kg^{-1}$ of fresh weight, less than those observed for ospreys and otter (see above; [4,8]). The most abundant organochlorine pesticides are DDE, metoxychlor and lindane. The set of the species logically reflects the overall trend of contamination of watersheds. The measured differences in the contamination of cormorants by pesticides according to the study site, the subspecies (*P. c. carbo* or *P. c. sinensis*), age or sex of the birds were found non-significant (see figure 4.1). The different species of cormorants were often used as models for studies in toxicology [30,31,32,33]. The concentrations of organochlorine pesticides identified by these authors in different tissues of great cormorants were generally higher than those observed in the basin of the Loire River, with no impact on populations, such as cases of direct mortality or reduced expansion of the population. Following these authors, and given the strong dynamic of great cormorants population observed in France, organochlorine compounds are probably not, for the moment, a direct threat to the species.

Figure 7. Great cormorant (*Phalacrocorax carbo*). Photo C. Lemarchand.

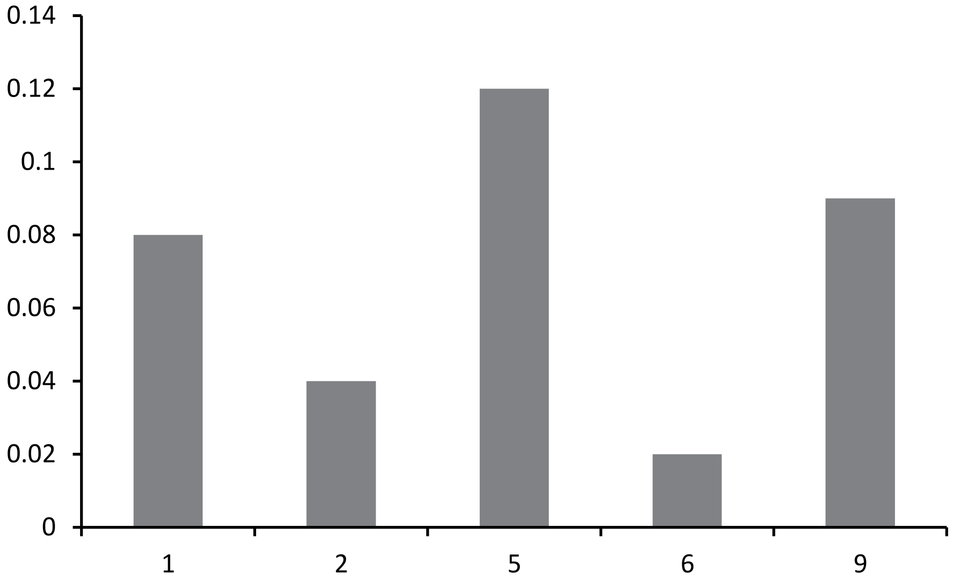

Figure 8. Contamination of great cormorants by organochlorine pesticides (mg.kg^{-1} ww).

3.3.2. *Contamination by organophosphate pesticides, carbamates and pyrethroids*

As in the case of the osprey, carbamates and pyrethroids pesticides were not detected in tissues of cormorants in this study. In this case also, the diet of cormorants, based on aquatic prey (fish and crayfish) probably explains the low or the absence of transfer of carbamates to the predator and seems to confirm the very low accumulation of pyrethroids in aquatic systems, at least in predators and top predators food compartments [20,21]. It is likely that these values situated below the detection limits of current analytical methods and not likely to increase in the future won't constitute a threat to the conservation of the species. However, given the relatively recent nature of the use of certain compounds (including pyrethroids) and the lack of experience and comparable data in the literature, research of pyrethroids and carbamates in cormorant tissues should be continued in order to determine the actual level of environmental contamination, or to assess the risk of interaction with other potentially toxic elements.

3.3.3. *Contamination by herbicides*

Contamination of cormorants by herbicides appeared weak and concerned a small number of compounds. Of all the individuals analyzed, 40% showed contamination by herbicides, alachlor and metolachlor were the only two compounds detected. The fungicide Epoxyconazol was also detected several times. Contamination by these three elements appeared on the completeness of the study sites, and values remained low, usually less than 0.1 mg.kg^{-1}. The differences between sites, sex, age or subspecies remained insignificant. Alachlor and epoxyconazol were also detected in osprey, other fish-eating bird, with orders of comparable size. Other herbicides detected in osprey (see above) were not, however, found in the tissues of the great cormorant, this may be related to differences in diet (and thus accumulation through food) between the two species or varying metabolic capabilities from one species to another. These low concentrations of herbicides probably have no or very low impact on the conservation of the species, especially in view of the expansion of cormorant species noted in recent decades. However, as in the case of the osprey, the lack of experience or comparable studies of these compounds in the literature limit any definitive conclusion [21].

3.3.4. Contamination of cormorants by anticoagulant rodenticides

Anticoagulants rodenticides were not detected in the tissues of great cormorants in this study. Like underlined for the osprey, these results may be related to the diet of this almost strictly fish-eating species [34], limiting exposure to the accumulation of anticoagulants. However, as noted for the osprey, the regular presence of anticoagulant residues in various trophic ranks, highlighted by the decreasing analytical detection limits can, do not exclude the possibility of contamination, and anticoagulants should be at least sporadically sought in future toxicological studies on the species.

3.4. Contamination of fish

3.4.1. Contamination by organochlorine pesticides

Among the contaminants of this family that have been researched in fish tissues (both species studied here) from Loire river basin, only three of them were found: DDTs (mainly DDE), endosulfan sulfate and lindane. DDE was only found in the downstream half of the basin, in chub and mullet tissues, upstream does not reveal any trace of contamination. The concentrations found when the molecule was detected were relatively high ranging from 0,4 to 1,6 $mg.kg^{-1}$ BW. Contamination of fish by DDTs (like those observed in the cormorant, osprey and otter) therefore indicates recent use of this compound, however banned since 1973 in France. Endosulfan was found on the same sites as DDT, with values ranging between 0,17 and 0,26 $mg.kg^{-1}$ ww. It therefore appears that the contamination of the Loire river basin with endosulfan sulfate is generally low to negligible, which may reflect an improvement over a previous situation where the compound was regularly detected in the environment, although no studies about it is available in the literature on the basin [35]. Lindane was the most prevalent organochlorine in geographical terms, and showed the highest concentrations, ranging from 0,04 to 3,42 $mg.kg^{-1}$ fresh weight. Its distribution within the basin, however, is heterogeneous, no significant difference between species, or sampling sites, or particular geographical gradient could be underlined. Contamination of the Loire river basin by lindane is comprehensive and relatively high despite the former prohibition of this compound, and it affects all trophic compartments, since apart from fish, lindane was also found in significant quantities in the tissues of otter [4].

Figure 9. Chub (*Squalius cephalus*, left), and Thinlip grey mullet (*Liza ramada*, right). Photo C. Lemarchand, drawing from www.fishbase.org

3.4.2. Contamination of fish by organophosphate pesticides

The detection of organophosphate in tissues of fish (whatever the species considered) is very punctual. The results demonstrate the absence of contamination gradient along the river. Compounds belonging to this family are banned from use in the European Union since 2000. They are recognized as non-persistent as quickly degraded in the environment (less than 4 weeks for most) and metabolized by organisms [35,36]. Finally, the fish are very sensitive to organophosphate acting rather a mode of acute toxicity. In other words, the fish exposed to organophosphate die very quickly, organophosphates and disappear in aquatic food webs and chronic contamination is difficult to detect.

3.4.3. Contamination of fish by herbicides

So similar to organophosphates, herbicides are not found at all sites. The three compounds detected throughout the watershed were Atrazine, the Terbuthylazine and Linuron. Atrazine was the most regularly detected compound in samples, values ranging between 45 $mg.kg^{-1}$ ww in chub from upstream areas and 210 $mg.kg^{-1}$ ww in both chub and mullet from the downstream part of the basin the Loire, intermediate areas reached 183 $mg.kg^{-1}$ ww. It may be suspected a concentration gradient of Atrazine in the tissues of fish along the Loire River, which proves coherent with the gradient use of this compound in agriculture [35].

3.5. Contamination of molluscs

3.5.1. Contamination by organochlorine pesticides

Table 4 below summarizes the data for contamination of mussels from the Loire basin. Asatic clam were not found in the downstream site (number 9). Of the eight OC pesticides analyzed, only lindane was detected consistently in the tissues of mussels, DDE is also common, this compound is indeed detected in the majority of sites.

DDT was found in the Loire basin, but more rarely in Asiatic clams from the upstream part of the basin and from the area situated close to the estuary. In mussels from the central part of the basin, concentrations were higher. The measured concentrations of DDT are low but as in the case of other species studied, this detection still suggests a relatively recent use of DDT, widely after the ban of this compound. For lindane and DDE concentrations were comparable across all sites sampled and are not significantly different. There are no significant differences between sites and between mussel species. Lindane is mostly met with 0.51 mg / kg lipid compound and then the DDE with 0.36 mg / kg lipid concentrations found in other European rivers [37,38,39].

Figure 10. Asiatic clam (*Corbicula fluminea*). Photo C. Lemarchand.

Sampling Site	Species	Lindane	DDE	DDT
1	Corbicula fl.	0,2 (0-0,3)	0	0
2	Corbicula fl.	0,72 (0,4-1,1)	0,4 (0-0,9)	0,006 (0-0,03)
3	Corbicula fl.	0,1 (0-0,2)	0,533 (0,4-0,7)	0
4	Corbicula fl.	0,8 (0,7-0,9)	0	0
5	Corbicula fl.	0,52 (0,4-0,7)	0,6 (0,4-0,9)	0
6	Corbicula fl.	0,52 (0,3-0,7)	0,1 (0-0,3)	0,024 (0-0,1)
7	Corbicula fl	0,41 (0,3-0,5)	0	0
8	Corbicula fl	0,67 (0,5-0,8)	0,29	0,012

Table 4. Mean concentrations (min-max) of OC pesticides in mussels from the Loire river basin ($mg.kg^{-1}$ lipid)

3.5.2. Contamination of molluscs by organophosphate pesticides, pyrethroids and herbicides

None of these compounds was detected in the tissues of Asiatic clams in the samples, regardless of the site in question. So it seems that the specific accumulation of these compounds modalities globally rare remaining in the tissues of species analyzed here, also check for molluscs, without, however, we can attest to the lack of toxicological effects induced due the lack of experience and references on this topic.

3.6. Contamination of crayfish

3.6.1. Contamination by organochlorine pesticides

Results of crayfish contamination by organochlorine pesticides are shown in Table 5 below.

Of the eight OC pesticides analyzed, only DDE was found in the whole homogenates of crayfish sampled (see table 5). DDE contamination therefore concerns all introduced crayfish species and all sites. DDT as itself was also detected twice, and values have been added to those of DDE (constituting DDTs).

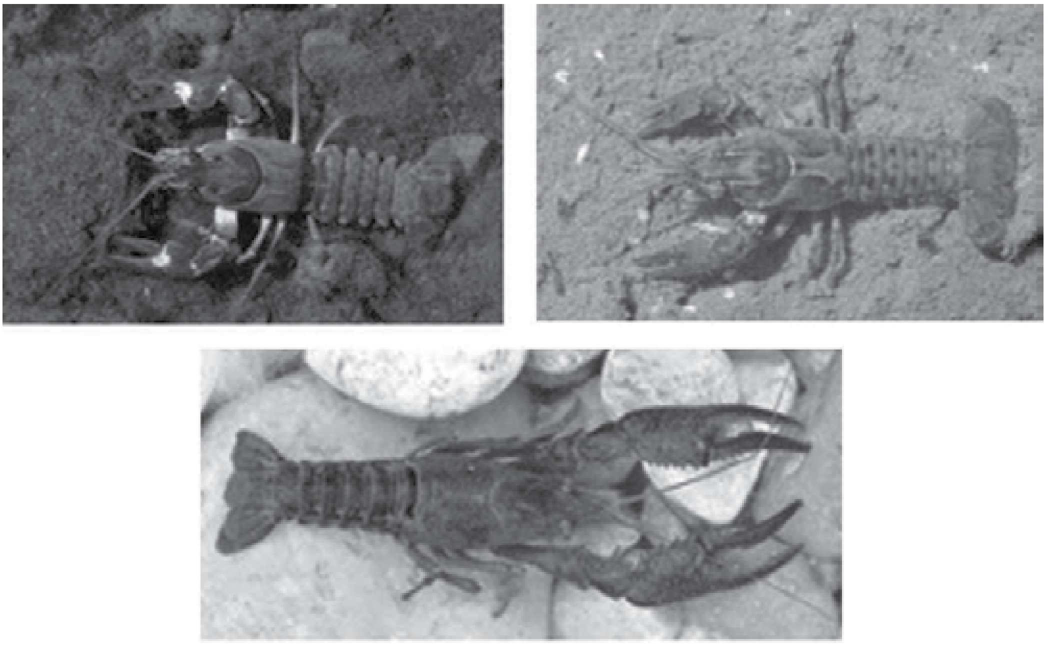

Figure 11. From left to right: signal crayfish (*Pacifastacus leniusculus*), spiny-cheek crayfish (*Orconectes limosus*) and Red swamp crayfish *(Procambarus clarkii)*. Photos C. Lemarchand

Other organochlorine pesticides, including lindane and endosulfan were not found in crayfish tissues, whatever the species and location.The concentrations of total OC pesticides, in that case DDTs (0,13 $mg.kg^{-1}$ of fat on average) are not significantly different from one site to another and appear to be higher than those found elsewhere in literature. Indeed, in the Meuse River, the tissues of *Orconectes limosus* had rates of DDTs ranging from 0,553 to 4,278 $ng.kg^{-1}$ dry weight [40]. However, the values recorded here are remote toxic levels can kill 50% of crayfish in 24 hours (DDT = 0,588 $mg.kg^{-1}$) established by Huner & Bar in 1991 (in [41]). Like molluscs, statistical analyzes conducted to compare the observed values within the different species apart from the effect of the sampling site, did not reveal any significant differences.

3.6.2. Contamination by other pesticides analyzed

As we observed for shellfish, organophosphate pesticides, herbicides and pyrethroids were not found in crayfish tissues, whatever the site or species considered. As suggested for other species of this study (see above), it remains difficult to certify the absence of toxicological effects induced given the lack of experience and references on this topic.

3.6.3. Comparison between crayfish, mussels and other species contamination

Comparison of concentrations of toxic elements studied here can assess the overall contamination of lower trophic level organisms, although their ecology and mobility are different, used to estimate the potential transfer to higher trophic compartments. Such an approach can only, however, be indicative, in the absence of local and complete knowledge of the diet of different predator species. Concerning organochlorine pesticides, comparison clearly shows a significant difference in contamination between molluscs and crayfish, the latter being much less contaminated ($p < 0.05$). Ecology and nutrition mode of filtering molluscs probably explain this higher accumulation of organochlorine pesticides, compared to crayfish, more mobile and whose diet is more diverse and variable during their development cycle. Organophosphate pesticides, herbicides and pyrethroidss were not found in the tissues of crayfish or mussels, as in the case of shellfish. The latter two were sparsely and irregularly noted in the tissues of fish, cormorants and otters (see above). This failure to detect organophosphate pesticides in species of the Loire River basin was also observed by [42], the short half-life of organophosphate limiting their entry into aquatic food webs via fish or invertebrates (*in* [42]). But this does not mean that these substances do not have any impact on the environment as aquatic ecosystems may be affected in the short term before these substances are degraded into non-toxic products [42]. Pyrethroids are little persistent compounds in the environment, and do not seem to bioconcentrate or biomagnify in organisms [43], although they are very toxic to fish and vertebrates [44]. The failure to find any molecule of the family of pyrethroids in our analysis would reinforce the idea that these compounds do not accumulate in food webs (see above). The absence of herbicides in the tissues of our specimen could be explained by a particular physico-chemical behaviour (biodegradability) or a very low absorbance of these molecules by fat tissue. The lack of perspective and tracks from similar work, however, requires some caution with respect to any final conclusion.

Study site	Species	DDTs
1	Pacifastacus leniusculus	0,09 (0-0,24)
2	Orconectes limosus	0,30 (0.05-0,8)
3	Orconectes limosus	0,07 (0-0,2)
4	Pacifastacus leniusculus	0,06 (0,02-0,1)
5	Orconectes limosus	0,04 (0-0,09)
6	Orconectes limosus	0,21 (0-0,8)
7	Orconectes limosus	0,06 (0-0,15)
8	Orconectes limosus	0,07 (0-0,19)
9	Procambarus clarkii	0,07 (0-0,26)

Table 5. Mean concentrations and range of organochlorine pesticides in crayfish from Loire River basin (mg/kg lipid weight)

4. Conclusion

This study, conducted during three years on Loire River basin has, on the one hand, confirmed preliminary data on some species, and on the other hand, very significantly supplemented the knowledge of the contamination of several species from different trophic levels. Thus, for the European otter, the results of contamination in Loire basin completed those acquired in other regions of France, and now cover a sample of individuals and geographical area of very important interest, at the international scale. Data on the great cormorants and ospreys are especially rare in Europe, and this work is therefore a standard concerning Western Europe knowledge of the contamination of osprey. Similarly, the results for the freshwater bivalves and crayfish are likely to improve the understanding of the toxicity of environmental contaminants on invasive species development.

Among the main results of this work, one should consider the universal nature of the contamination: no individual of any species from the whole basin appeared free of xenobiotics. Of the 54 elements systematically analyzed, organochlorine pesticides, were found most frequently. Work to identify and address sources of pollution will therefore affect the entire watershed, not just the most contaminated sites already known.

The results have often revealed a significant inter-and intra-specific contamination, which seems logical in view of the diversity of selected species, habitats and local diet variability. Despite this variability, trends can be highlighted: for example, the magnitudes of the main contaminants are the same for the three species of top predators (otters, osprey, cormorants), which highlights even for migratory species the existence of widespread contamination of all trophic compartments, and a non-linear but actual flow of contaminants.

The "modern" pesticides, *i.e.* those placed on the market after the progressive ban of organochlorine very persistent pesticides appeared much rarer than the latter in top predators. Thus, organophosphates, carbamates, pyrethroids, herbicides were scarce in ospreys and virtually absent in otters and cormorants. Lower toxicity and persistance of these compounds could be suggested, limiting their accumulation, but caution must be observed in this interpretation, since their relatively recent introduction into the aquatic environment have delayed their integration into trophic webs to top predators. It is therefore appropriate to continue standard measures of these pesticides in aquatic species, to confirm or refute this hypothesis.

None of the species studied here seems threatened with extinction in the short term due to contamination by xenobiotics. These observations are an improvement from the perspective of conservation of heritage species such as otters and osprey, which were directly threatened with extinction due to contamination by organochlorine pesticides 20 or 30 years ago. However, questions remain, particularly for species facing multiple causes regression in which contamination may be an aggravating factor, as European eel (*Anguilla anguilla*) or pearl mussel (*Mararitifera margaritifera*). The accumulation of some elements in the tissues of fish may result in restrictions on fishing activity (highly developed in France) detrimental to the economy, both for commercial fishing for recreational fishing. Improvement of water quality, must therefore take into account all the species, and not rely on requirements of the jewels of biodiversity. The gradual decrease of concentrations of certain elements (including organochlorine pesticides) in the tissues of the studied species reflects the positive effects of the discharge control or the prohibition of compounds. Finally, regular analyses of xenobiotic (pesticides but also metals and PCBs), pharmaceutical compounds or drug residues in various compartments of wildlife will provide information on the level of contamination in real time, but also the possible impact of unforeseen events or following measures controlling the flow of pollutions.

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Chapter 4

Pesticides, the Environment, and Human Health

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Additional information is available at the end of the chapter

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1. Introduction

This chapter discusses the intricate web of interactions between human beings, pests, and pesticides in the 21st century against the backdrop of good environmental stewardship and economic sustainability. Pests and pesticides are defined and their effects on human beings discussed. Pesticides are defined as economic poisons with equal emphasis on "economic" and "poison". Pest control is differentiated from pest management and the factors affecting the tolerance of human beings for pests also discussed. Pesticides are used in agriculture to prevent or reduce crop *injury* and *damage* but their application is based on bioeconomic principles that help maintain good environmental quality as well as improve economic returns in farm enterprises. Even though environmental concerns were the impetus for the development of these bioeconomic principles, their significant economic benefits have overshadowed their environmental beginnings and function. The Economic Injury Level (EIL) is a bioeconomic concept that refers to the lowest population of pests that will cause economic damage, and the Economic threshold (ET), is the point at which action need to be taken in order to prevent an increasing pest population from reaching the economic injury level (EIL). Environmental EILs have been proposed to replace economic EILs in order to re-focus attention on environmental concerns without losing sight of economic considerations. Concerns on the environmental impact of conventional pesticides have led to increased interest in less toxic alternatives including biopesticides. The fact that these compounds are generally not as fast-acting as their conventional counterparts makes early pest detection even more crucial than in cases where conventional pesticides are used. The notion that organic pesticides are harmless and therefore can be used without safety precautions is erroneous and dangerous. In fact nicotine pesticides have been discontinued in many countries because they are very toxic to humans and rotenone another natural active ingredient is very toxic to fish and other aquatic organisms. Pesticides have also been implicated in the Colony Collapse Disorder (CCD) of native populations of honey bees, a situation that has made it necessary for some farmers in the United States to purchase or rent pollinating bees to ensure high yield and quality of certain crops. Behavior-based control of pests generally results in more effective use of pesticides and thus the reduction of pesticides released into the environment to combat pests. In pest management, behavioral toxicology encompasses elements of behavioral science and toxicology and refers to the effect of pest behavior on the performance of toxicants deployed against them; "behavior" in this case refers to pest behavior before, during, and after exposure to the toxicant (pesticide). Proper understanding and use of the principles of behavioral toxicology will result in more effective use of pesticides and a reduction in the quantity of active ingredients deployed against pests worldwide. Elements of Worker Protection Standards (WPS) and Personal Protective Equipment (PPE) in the use of pesticides are discussed. Important safety intervals such as the one between the last pesticide application and reentry into a field (reentry interval [REI]) and the minimum number of days between the last pesticide application and harvesting (pre-harvest interval [PHI]) are also discussed. In order to further ensure that all these safety regulations and precautions are followed, a number of retailers in the United States including Wal-Mart (largest retailer in the USA), require their food suppliers to have two major types of certification namely: Good Agricultural Practices (GAPs) and Food Safety certification. Both of these certification programs involve training of farmers on the safe and proper use of pesticides, pest management practices that reduce pesticide residues in/on farm produce, proper storage of pesticides and, proper disposal of pesticide containers. Farm audits are carried out to ensure compliance with regulations pertaining to each certification program; farms that pass these audits are certified. Fruits and vegetables in the United States that recorded higher than acceptable limits of various pesticides in calendar year 2011 are listed and the underlying farming practices discussed.

2. Pests, pesticides, and tolerance thresholds for pests

The objective of this chapter is not to revisit all the basic definitions of pests, pesticides and their effects on the environment as well as human health; it however, seeks to discuss the real but intricate web of interactions between human beings and their environment based on the realities of life in the 21ST century; this entire discussion will be against the backdrops of environmental sustainability and economic returns to human beings. The judicious use of pesticides in ways that are consistent with good environmental stewardship and sound business practice requires information on the elements involved in decision-making pertaining to pest management.

In order to comprehensively tackle pesticides and their effects on the environment there is an absolute need to start the discussion from the very source: pests whether real or perceived. The world's current attitude to pests does not give enough room for distinguishing between real and perceived pests; this is because the mere fact that an organism makes someone uncomfortable or presents some element of "nuisance" (the definition of which is also very elastic) makes it a pest. This concept will be better understood by taking a close look at what is defined as a pest. Pests generally exhibit one or a combination of the following characteristics: they compete with human beings for resources such as crops, livestock, forests, health, and recreational resources; they reduce the availability, quality or value of a human resource; they transmit disease(s); they constitute a "nuisance". Based on this definition it is crystal clear that "pest" is an anthropocentric designation. Examples of pest groups include: agricultural pests; medical pests, veterinary pests, and urban pests. Now that the "pest" concept has been appropriately identified as an anthropocentric one, it is important to note that the level of tolerance that human beings have for pests vary based on factors that include cultural norms, economic status, level of education, gender, sometimes age and setting (i.e. whether domestic or field). Some organisms are deemed to be pests in some cultures and in other cultures they are either considered to be good or innocuous organisms. Irrespective of the setting anecdotal evidence points to the fact that the richer someone is the less tolerant they are of pests in both domestic and farm settings. Small-scale farmers with limited resources are more inclined to tolerate insect pests on their farms than large-scale commercial enterprises, mainly because of the cost of pest management efforts. The scarab/dung beetle in the family scarabeidae in the United States is considered a beneficial insect from an ecological point of view because they help to recycle the feces of animals but from a non-ecological point of view they are considered nasty beetles because of their close association with feces and rotting bodies. In Egypt on the other hand the dung beetle was associated with the sun god and some accounts indicate that it was worshipped as a god.

Pesticides used in the control and management of pests have been defined in a variety of ways but most of these definitions share certain themes and elements in common. One of the major elements is that these products are designed to act against an undesirable life form (pest). The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) of the United States defines a pesticide as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any insect, rodents, nematodes, fungi, weeds or any other forms of life declared to be pests; it also includes any substance or mixture intended for use as a plant regulator, defoliant, or desiccant. In 1959 FIFRA was amended to cover other chemicals in the category of economic poisons which is the legal classification for a substance used for controlling, preventing, destroying, repelling, or mitigating any pest. The term "economic poison" is an interesting choice of words because it aptly describes pesticides. They are indeed poisons that serve an economic purpose defined by the users. It is extremely important not to overemphasize economic benefits of pesticides at the expense of their toxic properties or vice-versa. The correct use of pesticides is thus a balance between two quests: one of which is to achieve high economic/aesthetic returns and the other to reduce adverse effects on the environment and non-target organisms. According to Pedigo and Rice (2009) it is difficult to imagine a technology that would produce the amount of food and fiber and maintain the level of public health that we have today without pesticides. The authors quoted Dover (1985) who gave pesticides an apt description: "As their hazards become more apparent, so does the need to use them. Although designed to kill, they are often life savers. Although increasingly costly, they bring economic benefits. And while they have opened up many possibilities for improving agriculture and public health, they have closed others, making us extremely dependent on them for our continued survival".

3. Bioeconomic principles governing the use of pesticides and the concepts of pest control/pest management

The terms "pest control" and "pest management" are often used interchangeably even in technical literature pertaining to pests. These terms however denote different levels of tolerance for pests. The appropriate use of the term "pest control" is in instances when there is zero tolerance for a pest. This is usually seen in domestic settings; total elimination of the pest is the aim of anti-pest activities in such settings. An example that illustrates the zero-tolerance situation is a situation where a homeowner (with a morbid fear of snakes) reports the presence of venomous snakes in his house and invites an exterminator/pest control company to eliminate them. The homeowner will clearly not be impressed if the exterminator upon completion of this assignment reports that the number of venomous snakes has been reduced from 30 to 2 and that the exact location of the two snakes is unknown. Such a homeowner has a zero-tolerance for venomous snakes in a domestic setting, especially when their exact location is unknown. This is a situation in which the zero-pest tolerance is understandable and the objective is complete eradication of the pest. Pest control is the appropriate mindset employed in household settings, at ports of entry into an area (e.g. country), with newly introduced pests, and with pests that transmit diseases to human beings. This zero-tolerance attitude however is usually counterproductive in agricultural settings. This is because this mind-set or attitude renders a farmer or gardener (pesticide) trigger-happy to the point where far more money is spent trying to eliminate a pest than the financial cost of the damage that the pest is capable of causing). This indiscriminate use of pesticides leads to high pesticide loads in the environment and on farm produce, development of pesticide resistance in pests, and pesticide-related health problems in non-target organism including human beings. Pest management is the appropriate term and attitude in agricultural settings. It involves activities that aim at keeping the population or severity of pests within tolerable limits (or within limits at which they do not cause more economic damage than the cost of eliminating them); the anti-pest activities are mainly suppressive. In farming especially crop production settings, eradication of pests may not be practically and/or economically feasible. In crop pest management, a number of factors are taken into consideration in determining the profitability of using pesticides. An important part of this calculus is the total amount of damage or economic loss that the population of pests is capable of causing (Flint and Gouveia, 2001). Another important factor is the unit price of the farm produce which provides information on the expected income from the sale of the farm produce. The decision on use of pesticides is based on a cost-benefit analysis; cost in this case is in financial terms. An aspect of the cost that almost always receives very little or no attention is the environmental cost of deploying toxic compounds into the environment; these compounds reduce environmental quality and sometimes kill non-target organisms that may be innocuous or even beneficial. A good scenario that brings this concept home is one in which it has been calculated that a known population of stink bugs in a cowpea field are capable of causing a maximum of $200 worth of damage; as a farmer the question is whether you would invest money in spraying the crops with a pesticide which will cost you a total of exactly $200. At the risk of being over-simplistic the answer is no (from a financial perspective); the answer may not be so clear-cut from a biolog-

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ical/ecological perspective. This is because if the farm enterprise is going to lose $200 either through crop loss or through cost of pest management then the farmer might as well sit at home and rest instead of putting in all the effort just to lose the same amount of money. The issue however gets a little more complex if the biology of that particular pest is such that some type of pesticide treatment is required in the current season in order to prevent a severe build-up of the pest populations in the next growing season or subsequent years. In this case spraying the farm will result in no short-term financial benefits but appreciable long-term benefits. The cost of pest management/control activities using pesticides and the market value of the produce are always major considerations in the decision-making process.

This is an appropriate place to segue into discussions on the bioeconomic principles of pest management by first of all defining some of the basic terminology in this subject area. Pest status is one of the concepts that are very crucial in the bioeconomics of pest management. Information on the mere presence or occurrence of a pest in a crop production environment without information on its status is a recipe for poor pest management decisions. On the basis of pest status there are 1) sub-economic pests 2) occasional pests 3) perennial pests and 4) severe pests (Pedigo and Rice, 2009). The main distinguishing feature between these is the population of the pest relative to the lowest pest population that is capable of causing economic damage (this damage-causing population is defined as the Economic Injury level [EIL]). Pests are defined as sub-economic if their average population is so far below the economic injury level that even peak populations neither reach nor exceed the EIL. Occasional pests have average populations that are close enough to the EIL that occasional population peaks reach or exceed the EIL. In the case of perennial pests their average population is very close to the EIL and peaks routinely reach and exceed the EIL. The average population of severe pests is always above the EIL. Economic threshold (ET) in insect pest management refers to the pest density at which management action should be taken to prevent an increasing pest population from reaching the EIL; controlling pests at densities below this level usually does not make economic sense and usually costs more (financially) than the damage the pest would have done to the crop if it had been left alone. Knowledge of fundamental pest management strategies cannot be overemphasized. In addition to EIL and ET, there are other concepts such as injury, damage, and Gain Threshold. The objective of this chapter is not to focus on these principles and how the various formulae were derived; instead it seeks to discuss the elements of these concepts, their shortcomings, impact on the use of pesticides and impact on the environment. As previously defined EIL refers to the lowest number of insects that will cause economic damage. Economic damage is the amount of pest-induced injury that justifies the cost of applying pest control measures. According to Pedigo and Rice (2009), gain threshold refers to the beginning point of economic damage which is expressed in terms of amount of harvestable produce. It is also defined as the time when the cost of suppressing pest injury equals the money to be gained from avoiding the damage. Economic threshold also known as action threshold refers to the pest density at which management action should be taken to prevent an increasing pest population from reaching the economic-injury level. Damage refers to a measurable loss of host utility; this most often includes yield quantity, quality, or aesthetic appeal. Injury refers to the effects of pest activities on host physiology that are usually deleterious. The economic threshold and economic injury level can be summarized in the following formulae:

$ET = EIL \times C^{-t}$

Where

C = factor of increase in pest (population/severity) per unit time

t = time period expressed in weeks

By design ETs are set below the EIL to afford farmers/pest management professionals enough time to respond to a pest problem before it reaches the EIL.

The economic injury level can be calculated using the following formula:

EIL = Gain threshold x (Loss per insect x Amount of loss avoided)-1

EIL is also calculated using:

$EIL = >C \times (V \times I \times D \times K)^{-1}$

Where,

C = Cost of pest management per unit area.

V = the market value per unit of produce

I = injury units per insect per production unit

D = damage per unit injury

K = Proportionate reduction in potential injury or damage

In crop production settings, the Economic Injury Level (EIL) concept is commonly used with insect pests but with a number of disease problems, preventive sprays are recommended instead because once the field is infected it becomes too late to prevent damage which may result in major economic loss. It is however important to note that cost-benefit analysis needs to be done against the backdrop of the market price of the produce and the cost of management efforts. Based on the formula above anything that causes the economic injury level to go up will result in more tolerance for pests and reduced used of pesticides. Factors that can raise the EIL include low market value, low number/amount of injury units per produce per pest, and a low level of damage per injury. Consequently farmers are less inclined to tolerate pest activities, injury, and damage to high value crops relative to low value crops because the quantum of economic loss due to injury by the pests is larger. Generally the lower the level of injury per pest the more tolerant producers are of their presence in the field. If the potential reduction of crop damage is low (with the application of pesticides) farmers are less likely to use them. Crop varieties that are healthier and more resistant to the pests will record less damage and or less injury resulting in higher tolerance of the pest due to the resulting high EILs. This shows that good agronomic and cultural practices that result in healthier crops will result in more resistance to pests and reduced need and use of pesticides. It is wrong to assume that farmers always make pest management decisions based on bioeconomic indicators or at least rough estimates of the cost and benefits of pest management actions or inactions. There is a however a pesticide application practice usually observed among small-scale, limited resource farmers in countries where pesticides are readily available. This practice has been aptly referred to as "revenge spraying" by some authors. It involves late detection or at least late action against pest outbreaks in fields which results in crop injury and economic loss; farmers attempt a revenge against the pests by spraying pesticides at a time when this action does not save the crop. This results in further economic loss (cost of pesticide and labor required to spray the product) as well as the release of toxic materials into the environment (environmental cost). This practice is neither consistent with conventional EILs nor is it consistent with environmental EILs (explained later in the chapter). Depending on the biology of the pest, sometimes it makes sense to reduce the pest population in order to avoid more severe infestations in the subsequent seasons but in a number of cases this does not form the basis for the decision to spray.

The element of environmental cost in pest management is most often overshadowed by the financial costs. The goal of pest managers is to achieve zero damage/injury which means a K value of 1.0. Attempts to achieve this goal however results in overuse of pesticides and thus the deployment and possible accumulation of pesticides in the environment; environmental quality is thus reduced. It must also be noted that in integrated pest management it is usually wise to leave a sub-economic population of pests in order to sustain the natural enemy population instead of completely eliminating the pest; this ensures the availability of natural enemies to deal with the pest next time there is an outbreak. The issue of how much pesticide should be applied against specific pests on specific crops is spelt out in the label rate. Pesticide manufactures in their quest to avoid lawsuits due to treatment failure and in order to compete favorably with alternatives on the market, set their minimum label rates to be higher than what is required. This has driven some authors to suggest the use of environmental EILs as opposed to conventional EILs where environmental protection or safety is taken into consideration. According to Pedigo and Rice (2009), the goal in using in developing an environmental EIL is to determine the lowest pesticide rate to achieve a K value that is virtually equal to 1.00. As indicated earlier use of environmental EILs make pesticide applications more compatible with natural and biological pest control methods.

Environmental EILs can be calculated as follows:

$$EIL = C + EC \times (V \times I \times D \times K)^{-1}$$

Where EC = Environmental cost and the other variables are same as defined earlier for conventional EILs.

The tricky part of including the element of environmental cost is its measurement. According to L. Higley and W. Wintersteen (1992) indirect environmental cost can be determined by assigning monetary value to non-market goods such as environmental quality. The method suggested by the authors involves analyzing levels of risk of pesticides to environmental elements such as surface water, ground water, and non-target organisms such as aquatic organisms, birds, mammals and beneficial insects. The element of how much producers are prepared to pay in production costs is also very instructive and important; cost here refers to both the additional cost of using more environmentally safe but expensive pesticides and/or tolerance of higher levels of crop loss. It will also be more informative to consider how much consumers in general are prepared to pay for organic produce and generally crops produced using less environmentally toxic pesticides. An important perspective in pest management is that a number of pesticides can get the job done against a given pest but the final choice of pesticide must include environmental safety as well as the financial cost of the pesticide and its performance against the pest. A choice based solely on performance sometimes results in deleterious effects on non-target organisms (environmental cost) which action sometimes becomes financially costly either in the short or long term. Conversely choice of a pesticide based solely on environmental safety or low price tag without reference to its efficacy falls far outside sound business management. The crux of the issue is that a large percentage of farmers in developing countries engage in farming as a way of life and not as a business enterprise. This attitude towards farming results in poor decision-making which results in a continuous cycle of poverty. According to Pedigo and Rice (2009) there are limitations to the use of the EIL concept. These limitations have to do with the types of pests or injury, the specific pest management tactics selected, research requirements, and desirability of multiple inputs in making decisions. It is important to note that EILs are not helpful in decision levels for management of certain types of pests; in fact these decisions levels cannot be determined using the EIL in certain instances. There is often a lack of (or weak) quantitative relationship between damage and injury caused by such pests. It is difficult or impossible to put an economic limit on the control of pests that are of medical importance.

In the preceding discussion on pest tolerance the appropriate decision/action point from an economic perspective is the economic threshold (defined earlier). It is very important to note that in a farm setting the decision to deploy pesticides is based on economic thresholds which is not the case in domestic settings and other places where pest presence and activities result in aesthetic damage or emotional distress; in this case aesthetic threshold is the operative decision point. Accurate determination of aesthetic threshold is difficult and sometimes impossible because the threshold is not set based on logical reasoning or calculation. It simply reflects how tolerant an individual is to that pest and its activities. There are insects that do not cause economic damage or compete with humans beings for common resources but their mere presence even in very low populations result in appreciable emotional and psychological irritation. There are indeed instances in which the presence of such irritants (pests) is imaginary. *Delusory parasitosis* is a good example of this; it is characterized by the feeling of insects or other organisms crawling over the skin. In fact this situation has resulted in sufferers selling houses and cars far below the market value simply because they felt they were disposing of property infested with insects or mites that the best pesticides could not eradicate or eliminate. Aesthetic thresholds by definition vary from individual to individual and can be raised through education (Flint and Gouveia, 2001). Pest management specialists are called in every now and then to address a pest problem only to discover that the target organism is actually a beneficial one. Sometimes the tolerance of homeowners to such organisms increases when they are informed about their beneficial nature but sometimes they insist on total eradication from their premises. Such homeowners are well within their rights because irrespective of how beneficial the organism is if it causes the homeowner to be uncomfortable in his/her own home then it is a pest by definition. It is also important to note that pest control is a business and pest control professionals are not averse to treating an entire house even in instances where localized treatment could have eliminated the pest. This is sometimes because the homeowners specifically ask these professionals to "nuke" the entire house or financial considerations prevent some of these pest control professionals from recommending more targeted treatments which translate to less money for their efforts. There are also instances where pest control professionals heighten the level of intolerance or fear of an already agitated homeowner so as to encourage treatment of an entire house even in instances where the biology/ecology of the pest as well as its actual distribution in the structure/house show clearly that spot treatment or localized treatment is the most cost effective way to handle the pest problem.

There are some fundamental principles of pest management that constitute an integral part of good environmental and financial stewardship. In crop production there are four fundamental strategies: a) do nothing b) reduce the population of the pest c) reduce the susceptibility of the host d) reduce both the population of the pest and the susceptibility of the host. The "do nothing" strategy is usually a good option in instances where the pest has a sub-economic status, or in instances where cost of pesticide application outstrips the quantum of potential loss that will be prevented. This option is imperative in cases where total or close to total crop damage has occurred and pesticide application will only serve to further exacerbate the financial plight of the farmer. There are also instances in which the crops are ready for harvest and recommended pesticides have long pre-harvest intervals that will require that crops stay in the field for several days or weeks before harvest. A decision to use such pesticides results in crops not harvested at the recommended stage with concomitant adverse effects on their quality and shelf-life. Reduction of pest populations is usually done using IPM practices which include cultural practices, biological control, and use of pesticides. The susceptibility of host plants can be reduced by selecting resistant varieties.

4. Effects of biopesticides and conventional pesticides on the environment/ non-target organisms

Natural pesticides are produced by processing natural substances. This group includes plant extracts (referred to as botanicals) and also mineral oils which are obtained when petroleum products are refined. These natural insecticides are classified into three broad categories: 1) biopesticides 2) botanicals and 3) biorationals (insect growth regulators). Biopesticides are pesticides derived from natural materials as animals, plants, microorganisms and certain minerals and have one or a combination of the following characteristics: 1) They have a natural occurrence 2) unique mode of action 3) low use volume and 4) have a narrow pest range.

A number of conventional pesticides are neurotoxins but most biopesticides have a mode of action that is unique. Low volumes of these products are usually effective against target pests; this reduces the amount of active pesticide released into the environment. This quality together with their biodegradable nature prevents the build-up of pesticides in the environment as is the case for some conventional pesticides. Possession of a narrow pest range reduces the probability of deleterious effects on non-target organism. In fact some microbial pesticides are so specific that they are affect only a target pest and closely related species. The relative specificity, the biodegradability, the low use volume, and the narrow host range render this group of pesticides more compatible in integrated pest management systems because of less impact on non-target organisms that may be beneficial. Issues of bioaccumulation and pollution of the environment are markedly reduced with the use of these products some of which are organic pesticides. This brings us to the point where the meaning of the word "organic" as used in "organic farming" or "organic pesticides" needs to be clarified. Clearly definitions of "organic" and "inorganic" from basic chemistry do not form the basis for classification of organic and conventional pesticides. In basic chemistry organic compounds are defined as carbon-containing compounds. This means that from a strictly chemical perspective, majority of conventional pesticides should be classified as organic pesticides because they contain carbon. Carbon-containing compounds such as DDT, chlordane and other cyclodienes are as conventional (inorganic) as pesticides come. It is important to note that the word "organic" in this usage refers to the view of the farm ecosystem as an organism with many functional parts working in harmony. The use of natural pesticides, biopesticides and botanicals is clearly consistent with this organismic view of the ecosystem/agro ecosystem; "organic" in this usage originates from this organismic concept. There is however, an erroneous impression that organic insecticides (some of which are biopesticides) are harmless and therefore do not require precautionary measures or protective clothing. This notion clearly needs to be dispelled because label instructions are for the safety of users and must be followed irrespective of whether the product is a biopesticide or a conventional insecticide. It is also important to note that some biopesticides such as those containing nicotine are very toxic to humans which has resulted in their discontinued use in many countries. Others such as rotenone are very toxic to fish and other aquatic organisms; the product has been used by South Americans as a fish poison since 1649. Some fish farmers use this poison to kill and clean out a pond prior to restocking them with new fingerlings. The use of pesticides containing rotenone as the sole active ingredient has been discontinued due to toxicity to fish and other aquatic organisms. There are however organic pesticides in the US market which contain rotenone as one of two active ingredients. Biopesticides are classified broadly into three main groups: a) microbial pesticides b) biochemical pesticides and c) plant-incorporated protectants. The positive attributes of biopesticides makes them popular with environmentalists and organic producers but it is important to note that not all biopesticides are compatible with certified organic production. Use of plant-incorporated protectants as is the case with transgenic crops (formerly referred to as Genetically Modified Organisms [GMOs]) renders them unacceptable as organic produce.

The use of more environmentally friendly products such as biopesticides and organic pesticides is associated with some drawbacks: generally organic pesticides are not as effective and or fast-acting as their conventional counterparts. Even though regular monitoring of fields and scouting for pests and other IPM practices are recommended for farmers who use conventional pest management methods, these practices are even more crucial for organic producers; this is because if pest issues are not prevented, reduced or detected early the pesticide options usually do not provide the quick and effective fix that conventional pesticides do. There are a few organic pesticides however, that compare favorably with their conventional counterparts in effectiveness and rate of action against pests. This situation introduces a tough choice

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between conventional pesticides that usually act faster, have longer residual activity, and are generally more effective and the more environmentally friendly natural pesticides (biopesticides, botanicals and mineral oils) on the other hand. The Environmental Protection Agency (EPA) in its quest to reduce pollution and toxic effects of pesticides on the environment, offered incentives to encourage the development of effective pesticides that had less adverse impacts on the environment. Generally it takes about a year or two to register a new biopesticide but it takes about 5-7 years to register a conventional pesticide. The reduced-risk pesticide initiative was introduced by the EPA to encourage the production of pesticides with less adverse impact on the environment; compounds with this designation receive priority in the registration process once they are approved. Given the millions of dollars that go into research into new active ingredients, formulation of pesticides, and efficacy trials (both laboratory and field), pesticide manufacturers are motivated to produce pesticides that are either biopesticides or reduced- risk compounds. Faster or expedited registration procedures for these pesticides offer pesticide manufacturers shorter periods between the development of the product and return on their investment. In the United States about 25% of pesticides are used in homes, gardens, lawns, parks, swimming pools and golf courses; lawns actually receive 10 times the pesticide dose that cropland receives. Heavy use of pesticides against pests on farms, in and around houses and recreational locations definitely has environmental and health costs on non-target organisms. Effects include morbidity and other behavioral changes that may not immediately culminate in death but affect the ecological r role of organisms in the environment; this sometimes lead to a cascading set of adverse effects that are sometimes difficult to trace back to pesticides.

5. Behavioral toxicology

Behavior has been described as the sequence of quantifiable actions involving cumulative effects of genetic, biochemical and physiological processes operating through the nervous system and aimed at maximal fitness and survival of the organism. It is a unique manifestation of the connection between the physiology and ecology of an organism and its environment (Little and Brewer 2001); this makes it a very important indicator of presence of toxicants and other environmental changes. Its usefulness as an indicator is further bolstered by what Kane et al. (2005) described as the nonrandom, highly structured and predictable sequence of activities associated with toxicity. To be relevant to toxicological assessments, behavioral responses must be: well-defined, measurable, ecologically relevant, and sensitive to a range of toxicants; the mechanism of response must also be understood. Behavioral endpoints that are represented across difference species of organisms and are capable of distinguishing between classes of insecticides with different modes of action are particularly ideal as indicators. The acceptance of behavioral endpoints as indicators of environmental toxicity in the United States began with the acceptance of avoidance behavior as legal evidence of injury to natural resources in 1986. This was under the proceedings of the Comprehensive Response, Compensation, and Liability Act of 1980 (NRDA 1986). The acceptance of other elements of behavior as indicators of toxicity marked an important milestone in the development of behavioral toxicology. Particularly noteworthy was the publication by the U.S. Environmental Protection Agency in 1991 listing behavioral response as a functional endpoint in neurotoxicity screening protocols. These behavioral endpoints have been used as early indicators of environmental pollution, but can be adapted for assessment of insecticide toxicity and performance. Behavioral toxicology refers to the impact of animal behavior/ecology on the effect of toxic compounds they come into contact with; it also refers to animal behavior after contact with toxicants. From the foregoing it is apparent that although the use of pest behavioral biology/ecology as the basis for successful pest management dates back several years, the development of the broader area of behavioral toxicology is relatively recent. The relevant elements of behavior span the period before, during, and after exposure to the toxicant. In the specific case of pests it refers to the effect of pest behavioral biology on the performance of pesticides deployed against them. It is important to re-emphasize the need to include behavioral symptoms of intoxication (i.e. behavior exhibited after exposure to the toxicant) in the broad definition of behavioral toxicology. A comprehensive definition of pest behavioral toxicology has to encompass exploitation of the natural behavior and ecology of pests to improve performance of pesticides; it should also include the use of well-defined and relevant behaviors for the assessment of pesticide performance. The contaminant does not necessarily have to be a pesticide and the exposure does not have to be deliberate. It is important to note that even though death is not a behavior most behavioral symptoms of intoxication culminate in death. This makes death induced by accidental exposure to toxic substances a very important indicator of environmental quality. In actual fact some organisms are so sensitive to toxic materials in their environment that their ability to survive in an environment is indicative of a low level of toxic materials in that environment. This is the basis for the use of organisms such as immature forms of mayflies as indicator organisms; their presence in a water body indicates a low level of pollution.

Behavioral toxicology is an aspect of both behavioral science and toxicology that is especially relevant in the control of subterranean termites and other social insect pests (Quarcoo, 2009). This type of termites present a good model for demonstrating the importance of pest behavior on the performance of pesticides deployed against them. The non-repellent termiticides that are commonly used in the United States are specifically designed to exploit various elements of termite social behavior to achieve optimum performance. These pesticides are typically slow-acting and non-repellent, allowing termites to continue their tunneling activities through pesticide-treated soil completely oblivious of the dangerous nature of the pesticides they are ingesting. The slow-acting nature of these pesticides serves the purpose of giving the foraging workers ample time to travel to their central nest to contaminate the queen who is responsible for laying the eggs. Foragers also groom each other and feed young termites, soldiers and the reproductive caste (i.e. King and Queen) through trophallaxis. Trophallaxis is the transfer of fluids including food by mouth-to-mouth (stomodeal) route or anus-to-mouth (proctodeal) route. These and other social interactions result in contamination of termites that have not had direct exposure to the pesticide. The behavior-based design of such pesticides results in a ripple effect that culminates in a high level of contamination/coverage of termites by the pesticide and thus results in better performance of these pesticides. Henderson (2003) studied the behavioral response of subterranean termites to treatment with two non-repellent termiticides:

Fipronil, and Imidacloprid. The neurotoxic effects of these pesticides and the underlying chain of reactions that result in the visible behavioral responses were discussed briefly. Su et al. (1982) compared the behavioral response of subterranean termites to three different categories of pesticides namely: repellent, slow-acting non-repellent, and fast-acting non-repellent compounds. Interestingly the termites sealed-off sections of their tunnels leading to areas treated with the repellent compound. Even though the same reaction was not reported for the fast-acting non-repellent compound, the high population of dead termites in the areas treated with this type of pesticide elicited avoidance behavior in the termite test subjects. Termites treated with the slow-acting non-repellent pesticide kept on tunneling into the treated zone and dead bodies were distributed all over the test arena as opposed to being concentrated in the treated zone as was the case for the fast-acting non-repellent compound. This resulted in the highest final mortality figures in the slow-acting non repellent treatment which was because there was neither avoidance behavior or sealing off of tunnels to the treated zone. The high mortality figures were also due to the slow-acting nature which afforded contaminated foraging termites ample time to interact physically and thereby contaminate other termites outside the treated zone. This study clearly demonstrated the effect of pest behavioral response on the efficacy of pesticides deployed against them. Another example of the importance of behavioral biology in pest management is when surveillance/sampling of pests is carried out during specific periods of the day when the pest is known to be more active which makes for easier sampling to determine the severity of the pest. The same principle is used in deploying contact pesticides which are usually sprayed during the period when there is a higher probability of direct contact between the pesticide and the targeted pest. Deploying a contact-type pesticide at a time of the day when the target pest is known to be hiding in a place that is either less or completely inaccessible (by the pesticide) renders a good pesticide less effective; this is especially so with pesticides that have a short residual activity. Lack of information on behavioral biology or lack of use of such information has resulted in treatment failures or the tendency of end-users to use higher than required quantities of pesticides to achieve desired results. Behavioral biology informs the choice of active ingredient and the best time to apply the pesticide formulations. It must be noted that the quest for higher levels of effectiveness and lower use volumes of pesticides involves targeting pests in their most vulnerable stage. Cockroaches that infest homes are generally known to like dark, humid, and warm environments (Pedigo and Rice, 2009) which explains their increased activity at night when the lights are off. A visual assessment of roach infestation carried out in a lighted room will result in an underassessment of the level of infestation. Behavior-altering chemicals such as female sex pheromones which are used by male insects to locate female partners for mating purposes is another tool that is employed against a number of insect pests. Typically it involves the production and use of synthetic analogs of female sex pheromones for a specific insect to attract its male counterparts into a receptacle where they are exposed to and killed by toxic strips (pesticides). Such pheromone are primarily used for pest detection but are sometimes used to cause significant reduction in certain pests through disruption of normal mating activities. The males spend so much time and energy looking for "superior or highly attractive females", as suggested by the concentrations of pheromones wafting to them. This leaves the males very little time to mate with actual females in the population. Some pheromone traps catch and kill sufficient male insect pests to affect the male to female ratio significantly enough to cause a reduction in reproduction resulting in significant dips in the pest population. It is important to recall that reduction in pest population is a fundamental strategy of pest management. Pheromone traps equipped with kill strips offer an environmentally friendly method of using pesticides without releasing them into the environment; as described these pesticides remain in the pheromone trap container. Hormoligosis is another interesting behavioral (mostly physiological) response to pesticides. It refers to reproductive stimulation of mites and some insects exposed to sublethal doses of pesticides; highest doses are recommended for such organisms (Pedigo and Rice, 2009). Low pesticides doses are used partly because the Environmental Protection Agency (EPA) regulations allow this practice primarily because of issues pertaining to the environmental fate, environmental cost, pesticide resistance, and financial cost of pesticides.

6. Safe use of pesticides and other pesticide-related good agricultural practices

There are two extreme views regarding pesticides but the most reasonable perspective is somewhere in the middle. One school of thought sees pesticides as products which cause havoc and must be avoided completely. The other end of the spectrum is the view that pesticides must be relied on solely to solve all pest problems and must be used every time there is even a hint of a pest problem. Those who hold this view either fail to understand the environmental cost associated with allowing pesticides to accumulate in the environment and/or hold the view that the ecosystem possesses such great recuperative capabilities to negate effects of intemperate release of pesticides into the environment. Neither the positive effects of pesticides on food production systems nor the health benefits derived from the control of disease vectors can be overemphasized. It is however extremely important that IPM methods are employed instead of the "identify and spray" method of pest management. Integrated pest management methods allow the use of other tactics in the management/control of pests so that even when pesticides become necessary the frequency of use and quantity deployed against pests is reduced.

Toxicity in all its forms (including environmental and direct effects on human health) should be reduced through the safe and judicious use of pesticides by following label and safety instructions. Restricted-use pesticides are available in most parts of the world but the enforcement of rules governing their use is lax in a number of countries (especially developing countries). Pesticide applicators (which are usually farmers) must be trained and licensed in order to qualify to buy and use restricted pesticides on their farms. These rules are enforced in a number of developed countries but same does not necessarily hold true in a number of developing countries. The situation is aptly captured in a publication by Eddleston et al. (2002) titled, "Pesticide poisoning in the developing world - a minimum pesticides list". The authors reported that pesticide poisoning is responsible for more deaths than infectious diseases in some developing countries. Poor regulation of pesticides, dangerous pesticide handling practices, and easy access to pesticides make them a popular method of self-harm including suicide. The Food and Agricultural Organization (FAO) attempted to address this issue in 1985 by developing a code of conduct for the pesticide industry. Apart from voluntary nature of the code, inadequate government resources in the developing world rendered it ineffective; this is evidenced in deaths which still continue today. A typical example is the death of 23 students in India in July 2013 after eating lunch contaminated with an organophosphate pesticide. Annie Banerji and Mayank Bhardwaj (2013) were informed by medical doctors treating affected students that they were poisoned by an organophospate compound. Initial reports from the police was that the deaths were caused by cooking oil that had been kept in a container previously used to store an organophosphate pesticide. In some parts of the world, empty pesticide containers are re-used to store water, beverages of all kinds, and vegetable oils. Pesticide poisoning due to improper disposal and re-use of pesticide containers occur more frequently in developing countries than media reports suggest Adherence to the rules on proper disposal of pesticide containers (which is one aspect of the pesticide training) could have averted this disaster. The World Health Organization (WHO) recommended that access to highly toxic pesticides be restricted; countries that followed this recommendation recorded lower suicide rates than what obtained previously. As indicated earlier in most developed countries, special licenses/permits are required in order to purchase and use restricted pesticides. Restricted-use pesticides are essentially a group of pesticides whose toxicities and modes of action render them too dangerous to be handled by untrained and uninformed people. A number of authors have advocated for this type of system to be put in place in developing countries. The development of a list of less dangerous pesticides for use in IPM systems is expected to result in fewer pesticide-related deaths in developing countries. In the United States, some pesticides are covered by a federal regulation called the Worker Protection Standard (WPS) which are designed to protect agricultural workers and people who handle pesticides from pesticide injury (EPA, 2013; Pedigo and Rice, 2009). WPS are used in addition to the specifications on the pesticide label. This law targets crop consultants/pesticide applicators; farm-owners/managers; and individuals/firms which contract and offer labor services on farms, forests, and greenhouses. The WPS provides specific instructions on personal protective equipment, Restricted-Entry Intervals (REIs), and other safety provisions all of which aim at protecting pesticide users from pesticide injury. REI refers to minimum amount of time that must elapse before workers can re-enter a field that has been sprayed with a pesticide. Personal Protective Equipment (PPE) for deploying pesticide include coveralls (or a long-sleeved shirt and long-legged trousers), neoprene boots and gloves, goggles/face shield, respirator, and a wide-brimmed hat. Trousers should not be tucked into boots but should be worn outside the boots to prevent direct assess of pesticides to the feet through the wide brims of these boots; pesticides can also roll off the trousers into the boots if they are tucked into the boots. Gloves should be unlined so that they can be properly washed.

In the United States the Department of Agriculture (USDA) has a set of guidelines on Good Agricultural Practices (GAPs) for farmers (USDA-AMS, 2013). The GAP guideline functions as a second level of impetus for farmers to follow recommended farming practices to ensure the production and supply of nutritious and wholesome food. Large retail shops including

Wal-Mart (which is the largest retail shop in the United States) insist that farmers who supply them with all kinds of farm produce to be GAP-certified. The GAP certification process involves training sessions to ensure that farmers understand the practices that lead to the production of safe and nutritious food for consumers. The aspect of GAP that is most relevant to the subject under discussion is training of farmers on the proper storage and use of agro-chemicals including pesticides. Another important aspect of the training covers the proper disposal of pesticide containers. USDA-GAP requires farm operations to use pesticides and other pre- or post-harvest materials in a manner consistent with prevailing regulations and the label instruction; this includes following state licensing requirements for pesticide applicators. Farm record-keeping is an absolute must for participation in the GAPs and Food Safety certification programs. Food safety audits are usually performed when crops are being harvested so that auditors can actually observe the range of farm activities to see if they tally with the food safety plan for each farm. The auditors inspect farm records pertaining to the type of pesticides used and date of application; with this information auditors can easily determine if harvesting is within the Pre-harvest Interval (PHI) or after the interval. PHI refers to the minimum numbers of days that must elapse before crops can be harvested from a field after they have been treated with a chemical product (pesticide). Unsafe pesticide residue levels result when PHIs are not adhered to. The market-driven requirement to use pesticides correctly and to test farm produce for pesticide has given farmers the economic impetus to get on board these programs. The increased popularity of pesticides in developing countries makes it imperative that regulations be put in place or existing ones enforced to ensure that consumers are provided with safe and wholesome food.

The practical definition for conventional pest management in developed and developed countries used to differ significantly; the increasing popularity of pesticides in developing countries is bringing the definitions a lot closer with time. The fact that food safety programs are neither enforced by Governments nor required by retailers in a number of developing countries puts consumers in a very unsafe place. In some countries there are no retailers of farm produce with the size and clout to economically enforce this food safety practices. This is partly because the marketing system for farm produce in these countries involves several very small-scale retailers or direct purchase of produce at the farm-gate. The large retailers in the United States require GAP-food safety certification of the farmers who supply them with produce. Food safety certification involves a complete audit of all farm operations to ensure the production of fruits/vegetables that are not contaminated with pathogenic organisms. Farms that engage in any type of irrigation are required to test the irrigation water for pathogenic organisms such as coliforms which indicate contamination with manure and harmful pathogens (Rangarajan et al. 2000). Farm produce are also sampled for pesticide residues to ensure that they are within acceptable limits. Pesticide residue values outside the acceptable limits are indicative of improper or excessive use of pesticides. These certification programs also involve unannounced post-certification audits/inspections to ensure that GAP and food safety practices are being followed. It has been observed that more and more retailers are requiring these types of certification in order to be eligible to supply them with farm produce. Other developed countries have their own versions of these certification programs but it appears as if a number of developing countries either lack such programs or fail to effectively use them. Wal-Mart recently started an initiative to buy fruits and vegetables from sources that are as close as possible to each outlet. In furtherance of this initiative, the retailer has been working very closely with researchers and Extension specialists at Tuskegee University in the United States, to train limited-resource farmers on GAPS, Food Safety, IPM and a range of other areas relevant to the production of fruits and vegetables. This trend of retailers taking the driver's seat on issues pertaining to safe and proper use of pesticides as well as pesticide residues on farm produce is a step in the right direction.

As indicated earlier, pesticides are economic poisons and must be treated as compounds that perform a great service when used properly; improper use on the other hand sometimes leads to losses that far outweigh their benefits. Adverse effects including death of non-target organisms including those beneficial in agroecosystems are some of the unintended effects of pesticides even when used correctly; improper use of these products exacerbates these effects. The colony collapse disorder (CCD) of honey bees has been attributed to the use of pesticides with some active ingredients receiving larger shares of the blame than others. Yang et al. (2008) reported that imidacloprid impairs the foraging behavior of honey bees. The authors exposed honey bees to different concentrations of imidacloprid (dissolved in dimethyl sulfoxide) and a control (50% sucrose solution [(wt: vol]). The study revealed a dose-dependent effect on the behavior of honey bees; they reported delays of at least 1.5 h in the return of some of the bees treated at low concentration whereas all the bees treated with higher concentrations of imidacloprid (i.e., 4,000 and 6,000 μg/liter) went missing. Lingering effects of imidacloprid-poisoning among returning bees resulted in foraging behavior that was markedly different from what was observed prior to treatment. Yang et al. (2008) also reported a positive relationship between concentration of imidacloprid and onset of abnormal foraging behavior and an inverse relationship between concentration of the pesticide and percentage recovery of bees. Certain concentrations of these pesticides somehow affect the homing system in bees. This is just one example of a practical demonstration of the effect of some pesticides on beneficial organisms but the jury is still out on whether CCD can be attributed exclusively to pesticides. Irrespective of the cause, the declining population of native bees has resulted in businesses in the United States which produce pollinating bees for sale to farmers. Some of these businesses rent out honey bees to farmers for crop pollination and still get to harvest the honey produced by the bees. Other businesses sell bumble bees for pollinating crops such as watermelons. Financial investment in pollinating bees in an evolving agro ecosystem where farmers can no longer depend on natural bee populations has forced farmers to pay closer attention to selection of pesticides that are compatible with plant pollinators. Some of these businesses have carried out their own research on the effects of various pesticides on bees which information is made available to customers. Using a symbol system, farmers are informed which pesticides are incompatible with the bees, which ones require that the hives be moved out of the field, which ones require that the hives be closed before spraying but opened a day later and which ones can be sprayed without even closing the hives.

First-hand experience with farmers has revealed a few mistakes that are sometimes made with respect to the use of pesticides. There are a number of erroneous views: first of all the fact that a product is labeled for use against a specific pest does not necessarily mean it is labeled for use on all crops attacked by that pest. The fact that a pesticide is registered for use in the United States does not guarantee that it can be used in the United Kingdom. In fact there are pesticides that are registered for use in some states in the US but are disallowed in other states in the same country. Some pesticides such as herbicides are registered for use on specific crops planted by direct seeding and are not registered for use on transplants. Use of these products for purposes for which they have not been registered constitutes off-label use of the product which is a crime in the United States; these are crimes irrespective of whether this is used inside the user's house, backyard garden, or commercial farm. In countries where this has not been declared a crime there is a need to consider that option; this option should be preceded by intensive public education on pesticide use and safety. Another observation is that some farmers wait too long to report pest problems to Extension specialists; this is usually because of failure detect the pest problem early due to lack of or infrequent pest surveillance (monitoring) activities. There are also instances where the problem is detected early but precious time is lost trying various recommendations from well-wishers who are not qualified to offer advice on these issues. This results in pest situations in which investments can either not be redeemed at all or the farmer is left no other option apart from the use of pesticide that are very effective but come at high environmental cost. This statement is not intended to discourage farmer to farmer education but to state that issues pertaining to the use of economic poisons need to be verified because once deployed they cannot be "unsprayed" and if the treatment fails then economic loss from the cost of pesticide treatment adds to the crop loss to result in an overwhelming vortex of economic loss.

7. Pesticide residues in fruits and vegetables and effects on human health

In order to effectively discuss the subject of pesticide residues a couple of terminologies must be defined. No Observable Effects Level (NOEL) refers to the level where no observable effects of the poison can be detected in experimental animals. The Acceptable Daily Intake (ADI) refers to the amount of chemical residue which is not thought to pose any appreciable risk to an organism even with a lifetime of daily exposure. This level is usually set a thousand fold or more less than NOEL (Pedigo and Rice, 2009).

In 1958 an amendment referred to as the Delaney clause, was made to the Food Drug and Cosmetic Act in the United States. The Delaney Clause disallows any cancer-causing chemical (carcinogen) on food for human consumption. In the quest to reduce exposure of consumers to pesticide residues, the Pesticide Data Program (PDP) was initiated in 1991 to collect data on Pesticide residues in food (USDA-AMS, 2013). The program currently plays an important role in the implementation of the 1996 Food Quality Protection Act (FQPA). The FQPA directs the U.S. Secretary of Agriculture to collect pesticide residue data on commodities most frequently consumed especially by children and infants (USDA-AMS, 2013). Two U.S. federal agencies namely the EPA and the Food and Drug Administration (FDA) use the PDP data. It is used primarily by the EPA to assess the dietary exposure during the safety review of existing pesticide tolerances (also called Maximum Residue Limits); the FDA uses it to assist in planning commodity surveys for pesticide residues which is done from an enforcement/ regulatory perspective. In the US, farm produce (mainly fruits and vegetables) with the highest pesticide levels have become known as the “dirty dozen”. The dirty dozen includes: apples, celery, cherry tomatoes, cucumbers, grapes, hot peppers, imported nectarines, peaches, potatoes, spinach, strawberries, and sweet bell peppers. Kale/collard greens and summer squash find their way onto the list when it is expanded to cover the 14 most pesticide-laden food items. Levels of pesticide residue exceeding the EPA tolerance levels are shown in (Table 1). These high pesticide residues are generally due to a variety of reasons including: inadequate knowledge or use of IPM practices. Farmers usually find themselves having to spray more than the recommended rates because pests are not targeted at their most vulnerable stage and so require higher quantities of pesticides (active ingredients). It is also possible that poor record-keeping by some farmers makes it difficult for them to follow pre-harvest intervals for the pesticides. When crops are harvested within the PHI, pesticide residues tend to be higher. Some of the farmers may not be calibrating their sprayers properly or may be mixing more than the recommended rate of the pesticides. Pesticide resistance by pests is another reason why high pesticide residues are recorded. This is because farmers feel compelled to continue using a product that has worked well for them in the past; this continues to the point where pesticide resistance develops and higher quantities of the product have to be sprayed in order to achieve the desired results. The demand for blemish-free fruits and vegetables contribute to the high pesticide residues in food.

Sometimes pest pressures are so high but so is the consumer demand for blemish-free produce. Some farmers spray more than the recommended amount of pesticides or spray more frequently than recommended in order to ensure blemish-free produce. There are also instances where high pesticide residues are due to drift of pesticides from aerial sprays (using aircraft) on neighboring farms during windy conditions; in these instances the farmers are not aware that more than the required amount of pesticides are getting to their crops. Excessively high rainfall periods also result in more fungal diseases which are usually dealt with using preventive (calendar) spray regimen which may sometimes be excessive. Metabolites of Captan fungicide on snap beans must be watched carefully based on the percentage of detections (9.2%). The relatively high percentage of detections of bifenthrin (19%) on cherry tomatoes and 5.7% detections each of dinotefuran and acetamiprid and sweet bell peppers deserve closer attention. Apart from bifenthrin on cherry tomatoes, the percentage detections are generally low but all these figures are an impetus to reduce the percentage of detections. In a number of developing countries restricted-use pesticides are imported with labels that show this designation very clearly but sale of these products is not restricted to people who have restricted-use pesticide permits; in fact these permits do not even feature in any discussion at the point of sale. Pesticide residue analysis also not the norm in a number of developing countries; there is therefore no way of telling the level of pesticide residue on farm produce in these countries. It must be noted however, that in some of these developing countries, pesticides are not used that much or in some rural communities they are not used at all resulting in farm produce that are basically organic.

Vegetable	Pesticide	Range of Values Detected (ppm)	EPA Tolerance Level (ppm)	Percentage of Samples with Detections
Cabbage	Acephate (Insecticide)	0.033	Not listed	0.1
Cantaloupe	Acephate (Insecticide)	0.017 – 0.054	0.02	0.3
Frozen Spinach	Acephate (Insecticide)	0.21	0.02	0.6
Sweet Bell Peppers	Acetamiprid (Insecticide)	0.002 – 0.22	0.20	5.7
Cherry Tomatoes	Bifenthrin (Insecticide)	0.007 – 0.16	0.15	19.1
Snap Peas	Chlorfenapyr (Insecticide)	0.004 – 0.034	0.01	0.8
Frozen Spinach	Cyhalothrin (Insecticide)	0.026 – 0.092	0.01	1.0
Snap Peas	Cypermethrin (Insecticide)	0.038 – 0.27	0.1	4.4
Snap Peas	Deltamethrin (Insecticide) Includes Tralomethrin	0.020 – 0.19	0.05	1.5
Sweet Bell Peppers	Dinotefuran (Insecticide)	0.010 – 0.81	0.7	5.7
Sweet Bell Peppers	Fludioxonil (Fungicide)	0.040	0.01	0.1
Hot peppers	Tetrahydrophthalimide (Metabolite of Captan Fungicide)	0.015 – 0.065	0.05	0.9
Snap Beans	Tetrahydrophthalimide (Metabolite of Captan Fungicide)	0.006 – 0.37	0.05	9.4
Snap Peas	Thiamethoxam (Insecticide)	0.003 – 0.12	0.02	2.2

Culled from the USA Calendar Year 2011 Annual Summary of the Pesticide Data Program (USDA-AMS, 2013)

Table 1. Fruits and Vegetables in the USA with Pesticide Residues above the EPA Tolerance Levels in 2011.

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In conclusion the human population in the world is continuously increasing and unless certain changes are made, the earth's resources (which are dynamic) may not be able to sustain this growing population indefinitely. Viewing and treating the earth's resources as "infinite" is erroneous, dangerous and unsustainable. In order to slow down or prevent our arrival at that carrying capacity it is important to achieve higher yields of crops and other food items per unit area of land. There are a number of ways to achieve higher yields including the use of transgenic crops (formerly known as GMOs) and pesticides. Transgenic crops have been developed to have characteristics including: drought resistance, pest resistance, pesticide resistance, and higher yields per unit area. Transgenic crops are however not accepted in all parts of the world for a variety of reasons but world food production needs to be increased one way or the other to feed the growing world population. The stance against transgenic crops in developing countries will become very untenable in the near future unless other improved farming methods are introduced to make up for the short-fall in food production. This is because in a number of these countries a large percentage of farmers lack the necessary managerial skills and technological capabilities to optimize the use of resources (farm inputs) in order to have high yields of good quality crops to feed their growing populations. In developing countries a combination of zero-tolerance for pesticides and zero-tolerance for transgenic crops without any improvements in the technical know-how and managerial skills of farmers as well as access to advanced farm equipment will only result in major shortfalls in food production. The fact that the use of pesticides will keep growing (at least in the foreseeable future) makes it imperative to continue research efforts to identify new pesticide chemistries with less adverse effects on the environment. It also makes it very important that pesticide users all over the world learn to use these products safely and properly in the spirit of good environmental stewardship. The use of IPM methods will help to greatly reduce the reliance on pesticides and hopefully slow down the rate of environmental pollution. The use of examples of regulatory framework for pesticides in the United States is not to suggest that it is the most perfect system in the word or the archetype for developing structures in other parts of the world; it is however important to take note of the level of effectiveness of these structures as well as the specific demographic, cultural and other characteristics of different parts of the world in order to design or improve existing regulatory structures for pesticides.

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Chapter 5

Pesticides and Agricultural Work Environments in Argentina

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Additional information is available at the end of the chapter

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1. Introduction

The use of chemical pesticides has brought benefits such as the increase of agricultural production, soil productivity and product quality, which is reflected in economic benefits, vector disease control and in general, in public health. However, given that only 10 percent of applied pesticides reach the target organism, a high percentage is deposited on non target-areas (soil, water, sediments) and, as well as affecting public health, impacts non-target organisms such as wild life [1]. Also, the extended use of pesticides commonly results in residues in foods [2] generating continued human exposure by different pathways, which has led to widespread concern over the potentially adverse effects of these chemicals on human health.

Pesticides are an important aspect of agricultural practice in both developed and developing countries and, despite the many technological advances brought by the modern intensification of agriculture, the increased yields were achieved primarily through the use of fertilizers and pesticides [3].

Argentina is one of the major crop producers in Latin America, with the export of cereals and oilseeds being one of the principal axes of the national economy. The frontiers of farming have expanded greatly in the past 30 years, from 15 to the current 30 million hectares, with an increase of the area planted for grain production, particularly for soybeans, from 34,700 ha in the 1969/70 season to about 18 million ha in 2011/12 [4]. Today, Argentina is the world's leading producer of vegetable oils, the fourth largest producer and second largest exporter of sunflower oil, and the fourth producer and leading exporter of soybean oil. The country has one of the highest yields in the world in soybean, corn and wheat [5].

Argentina's extensive production of cereals and oilseeds for the international market coexists with intensive horticulture and family farming, with wide geographical distribution, mainly close to urban centers, and diversity of cultivated species, occupying an area of about 230,000 ha [6], giving an annual production of over 10,000,000 tons, primarily for domestic consumption.

Crop production has been accompanied by a steady increase in the use of agrochemicals; pesticide marketing has grown strongly, from 155 million pounds in 1995 up to 700 m.p. in 2012 [7]. In technology used for spraying pesticides, the country has a wide variety of equipment ranging from self-propelled sprayers, which also involve high technological complexity, with filtered air cabins, to activated charcoal filters, spray drag and power and manual backpacks used particularly in intensive farming. Each of these different technological environments is associated with different health and environmental risks.

The Province of Córdoba is in the central region of Argentina, with a total area of 165,321 km². Its location, as well as its political and physical characteristics, make this province a hub of articulation between different natural regions of the country. It has a population of 3,304,825 inhabitants, 88.7% of whom live in urban areas and 11.3% in rural areas [8]. Among the inhabitants of rural areas, 45.9% live in towns of less than 2,000 inhabitants, and the rest dispersed in the open countryside [9]. The northern and western areas are less populated and are the ones which concentrate most indicators of structural and cyclical poverty. The agricultural roots of this province mean that the settlements are mixed with agricultural developments, increasing the risk of non-occupational exposure of communities adjacent to cultivated fields.

The rural area in Cordoba devoted to extensive crops (soybean, maize, sorghum, peanut, wheat and sunflower), has expanded from 3,397,050 ha in 1994/95 to 7,300,000 ha in 2011/2012 [4].

The country's extensive agricultural model, based on glyphosate-resistant transgenic soybean farming, no-till and the intensive use of fertilizers and pesticides [10], is highly dependent on modern technologies [11]. In contrast, intensive crops such as fruits and vegetables are characterized by high demand of labor per unit of output. Typically, this is a small-scale activity usually performed by the peasant family production unit [12, 13], with all its members participating. The incidence of pesticide poisoning in these agricultural settings includes non-intentional child exposure, occupational exposure of young farm laborers, para-occupational exposure of the farm workers and their families and the adjacent community, and exposure to banned pesticides [14].

In Córdoba Province, exposure to different pesticides linked to agricultural production has long been recognized [10, 15-16], as well as the unavoidable soil contamination even decades after its application [17]. Our previous results of a population-based study in the province of terrestrial applicators of pesticides in extensive crops (n=880), emphasized that workers were highly exposed to pesticides, and we studied various determinants of this exposure, including the pesticides most frequently used or still in use by the applicators. We also reported the negative health consequences associated with their employment status. The weakness of compliance with the rules governing the activity was also highlighted as a factor that increases the health risk of agricultural workers and the general population [10].

The greenbelt surrounding the provincial capital is a zone of fruit and vegetable farming, providing fresh food to the local urban population. Its extension includes neighboring towns, forming a strongly integrated commercial and productive system. Almost 90% of the fruits and vegetables it provides are produced within the urban area [17]. Horticultural smallholders and farmworkers are often immigrant workers from neighboring countries [18-20]: according to the Ministry of Education [21], sixty percent of them are Bolivian citizens, which increases their risk of environmental and occupational illness and injury, as well as the health disparities typically associated with poverty [22].

This chapter offers a comparative analysis of two widely different agricultural settings (extensive and horticultural crops) and characterizes the pesticide applicator populations in each, including the health conditions associated with occupational pesticide use. We introduce two pesticide exposure assessment proposals, consisting of intensity and accumulated exposure indexes for both scenarios. The proposals include new results about the pesticide applicators of extensive crops, including an update of the differential characteristics of worker populations in homogeneous ecological areas of the province. We also introduce a new scenario consisting of horticultural smallholders and farmworkers, and describe their working conditions. The study and comparison of these different work settings allows us to tailor the exposure indexes developed in our previous publication [10] to the particular pesticide exposure of greenbelt situations, as well as to develop proposals for preventive measures for the reduction of human exposure and environmental impact, according to each scenario.

2. Materials and methods

2.1. Population studies

We conducted a population-based study in Córdoba Province, Argentina, with two principal target populations: a) terrestrial applicators of pesticides of extensive crops; b) smallholders and farmworkers of the greenbelt of its capital city, Córdoba.

a. In the first case, all the applicators attending the mandatory courses for obtaining the applicator license, provided by the Agriculture, Livestock and Food Ministry, were asked to participate in the survey, during the period 2007-2012. A self-administered questionnaire was used to obtain demographic data, pesticides and technologies used, crops sprayed, workers' lifestyle and family health information, as already described in a previous publication [10]. From 1479 completed questionnaires, a consistency analysis for several responses was carried out, with a sample size of 1327 for further analysis. We also performed a stratified analysis taking into account the Homogeneous Ecological Areas (HEAs) divisions of the province in order to describe differences between these.

b. In the second case, 101 smallholders and farmworkers were contacted in Cordoba's wholesale fruit and vegetables market and in the greenbelt setting itself. The above questionnaire was adapted for this specific population, after an exploratory study through in-depth interviews during 2011. As described in the literature, the exploratory study shows that this is a difficult-to-reach population due to their migratory status and unstable working conditions.

2.2. Variables

2.2.1. Terrestrial applicators of pesticides of extensive crops

a. Social and demographic variables: age (in years, as from birth date), education level (highest level of educational attainment in the formal system) and marital status (married or cohabiting and others).

b. Technological and working practices variables: pesticide spray equipment (self-propelled crop sprayer with cab and activated charcoal filter; trailed crop sprayer with cab and activated charcoal filter), area worked (average hectares applied in the last year), seniority in the job (years mixing/applying), written pesticide prescription signed by an agricultural engineer (yes/no).

To assess the level of protection implemented by the terrestrial applicators, we adopt the proposal in [23], considering eight categories of personal protective equipment (PPE) used, alone or in combination: waterproof clothing, gas mask, chemical-resistant gloves, face shields or goggles, hat or helmet and other protective clothes (boots, apron, waterproof pants). The weighting of PPE elements is based on monitoring and measurement of occupational exposure during the task. A new measure called protection level was constructed [10]: unprotected (0% protection), partially protected (20 to 70% protection) and protected (90% protection).

These variables were analyzed comparatively between homogeneous ecological areas (HEAs) of the province, according to soil and climatic characteristics, land use and production activities, as described in [10].

c. Good agricultural practices: we considered two practices included in the local regulation aimed at reducing human risks and negative environmental impacts [24]: a) the triple washing of pesticide containers (yes/no). This practice consists in washing the empty container three times and draining for thirty seconds in upload position; and b) correct end use of pesticide containers (yes/no): properly cleaned containers must be transferred to an authorized registered storage center, to be destroyed in a pyrolytic oven; burial, burning, storage, sale or reuse are prohibited.

2.2.2. Smallholders and farm workers of the greenbelt surrounding the capital city of Cordoba

To highlight the particularities of the horticultural work scenario and its worker population, new variables were incorporated into the analysis when necessary.

a. Socio-demographic variables: age, education and marital status are described as mentioned above; origin (country and province of birth); household (members and their participation in horticultural work). Dwelling infrastructure and public services: running water installed (yes/no), bathroom installed (yes/no), domestic gas distribution network (yes/no); public service of urban solid waste collection (yes/no).

b. Work practices, technology and other exposure variables: pesticides sprayed, use of PPE (as described above); crops grown in the last year (type of crop and annual average harvests); greenhouse for crop (yes/no); household distance to the nearest crop (meters); extension of the productive unit in hectares: small: up to 10 ha; medium: between 11 to 40 ha; and large, more than 40 ha [25]: seniority in the job (years mixing/applying); pesticide spray equipment (self-propelled crop sprayer with cab and activated charcoal filter; trailed crop sprayer with cab and air intake activated charcoal filter or without air intake filter; trailed crop machine without cabin, manual and engine backpack).

c. Good agricultural practices (as described above).

2.2.3. Health worker conditions in both agricultural settings

a. Symptoms: Perception of acute and sub-acute manifestations: Irritative symptoms (skin, nose and eye irritation, nausea or vomiting, chest discomfort); fatigue/tiredness; nervousness or depression; headache; excessive sweating. Occurrence of symptoms: Never/Rarely/Sometimes/Frequently;

b. Medical consultations related to pesticide use effects: yes/no; and Hospitalization linked to tasks with pesticides: yes/no;

c. Workers' risk perception of different pesticides: not dangerous/slightly dangerous/dangerous/highly dangerous.

2.3. Exposure assessment

Based on proposed indexes of our previous work [10], the present study incorporates intensity level (ILE) and accumulated exposure (CEI) indexes into pesticide exposure, adapted to the smallholder and farmworker population of the greenbelt of Cordoba city, describing the principal differences among them. These indexes measure instantaneous exposure intensity and cumulative exposure taking into account the life years of worker exposure. To use these indexes in the horticultural worker population, we have carefully adapted the weighting score procedure to this particular context.

2.4. Statistical analysis for association

We used a modeling approach to check differences between ecological areas. Assuming counts or frequencies in each category of the variables as the outcome, we fitted Poisson and Gamma generalized models to estimate the parameters (effects). The latter was used since the empirical distributions of both indexes presented skewness. Association between two or three variables was studied through log-linear models in order to estimate the odds ratio as association measures.

3. Results

3.1. Population of extensive crops

In a previous work [10] we identified different agricultural settings in the province, based on homogeneous ecological area (HEAs) divisions (Figure 1). Differences in basic characteristics of this population, such as their average age, instruction level and length of occupational exposure to pesticides allow us to hypothesize the existence of diverse risk scenarios in the province. In this chapter, an update of the characterization of workers was performed with an increased sample size, n=1327.

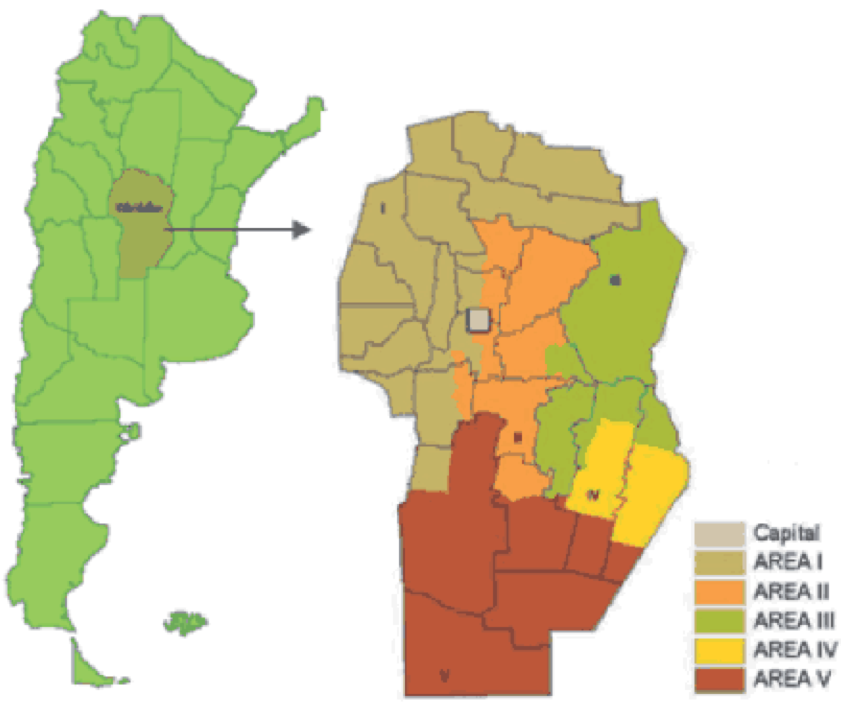

Figure 1. Homogeneous ecological areas (HEAs) of Córdoba province.

Significant differences among HEAs were found for age ($p<0.01$), education level ($p=0.03$) and marital status ($p<0.01$), as well as for seniority in the task ($p<0.05$), average/year of hectares sprayed ($p<0.03$), use of pesticides with written prescription signed by an agricultural engineer ($p<0.03$), and self-propelled crop sprayer with cab and activated charcoal filter ($p<0.03$) (Table

1). Protection level ($p<0.05$) also showed differences between HEAs I, II, III and HEA V ($p<0.05$), the latter having the fewest completely protected workers (31%). Only trailed crop sprayer with cab and activated charcoal filter results were similar in all the areas. It is important to highlight that HEA I showed the lowest percentage of applicators with complete secondary school level or higher (29.2%) and in subjects married or cohabiting (56.8%), but the most workers using complete protection (54%) and using pesticides with written prescription (58.1%) followed by HEA II (54.8%)

	AREAS					
	I	**II**	**III**	**IV**	**V**	**Total**
n	41	641	230	156	259	1327
Age (years)						
Mean	32.3	35.8	34.9	37.6	34.8	35.6
Standard Deviation	8.6	11.6	9.9	10.9	11.9	11.3
14 – 24	16.2	16.5	16.1	12.8	21.2	16.9
25 – 24	48.6	36.0	38.1	30.2	32.8	35.4
35 – 44	27.0	24.9	28.7	30.2	25.2	26.3
> 45	8.1	22.6	17.0	26.8	20.8	21.4
Marital Status (%)[1]						
Married or cohabiting	56.8	66.8	61.7	78.9	58.6	65.5
Unmarried, separated, divorced or widower	43.2	33.2	38.3	21.1	41.4	34.5
Education (%)						
Incomplete Primary	2.4	11.1	13.5	7.1	10.8	10.7
Complete Primary	29.3	27.9	27.8	29.5	32.0	28.9
Incomplete Secondary	39.0	26.6	21.3	22.4	23.9	25.1
Complete Secondary, Technical or University studies	29.2	34.3	37.4	41.0	33.2	35.2

[1]Percentage considering the total of responses.

Table 1. Sociodemographic characteristics of pesticide applicators by Homogeneous Ecological Areas. Córdoba, Argentina. 2007- 2012.

Pesticide with prescription signed by an agricultural engineer was used by only 33.7% of applicators in HEA V, which was different from the others ($p<0,03$); self-propelled crop sprayer with cab and activated charcoal filter was highest in HEA III (74,1%) and this was significantly different from HEAs II and V ($p<0,05$). No significant differences were found between areas in the use of the trailed crop sprayer with cab and activated charcoal filter, but this is a crop sprayer that is very little used in all the areas (Table 2).

	AREAS					
	I	**II**	**III**	**IV**	**V**	**Total**
N	41	641	230	156	259	1327
Protection Level (%)[1]						
Unprotected	12.2	12.5	12.2	9.0	17	12.9
Partially Protected	34.1	48.8	46.1	55.8	52.1	49.4
Protected	53.7	38.7	41.7	35.3	30.9	37.8
Average area/year applied (ha)						
Mean	9717	5226	9923	6535	7182	6767
Years personally mixed/applied pesticides (%)						
≤ 1	26.3	11.2	22.4	10.3	17.0	14.6
2 – 5	36.8	31.6	34.5	27.7	38.3	33.1
6 – 10	18.4	23.5	23.8	23.9	21.3	23.0
11 – 20	10.5	21.4	13.5	23.9	15.8	18.9
21 - ≥ 30	2.6	11.7	5.4	14.2	6.3	9.5
Use pesticides with prescription signed by an agricultural engineer (%)						
Yes	58.3	54.8	46.4	53.8	33.7	49.9
Apply with Self-propelled Crop Sprayer with Cab and Activated Charcoal Filter (%)						
Yes	63.9	49.7	74.1	67.9	63.2	58.7
Apply with Trailed Crop Sprayer with Cab and Activated Charcoal Filter (%)						
Yes	2.9	8.4	4.9	6.2	7.9	7.3

[1]Percentage considering the total of responses.

Table 2. Protection Level, Area/year applied Seniority in the Job and Technology in the different Homogeneous Ecological Areas. Córdoba, Argentina, 2007-2012.

Good agricultural practices were established to reduce the contamination that may be caused by empty pesticide containers and their geographical dispersion. Not all applicators carry out the triple washing of pesticide containers (89.9% do so), and only 10.5% are included in formally regulated programs to ensure the correct end use of empty pesticide containers; in many cases, empty containers of chemicals are burned, buried or reused.

3.2. Population of smallholders and farmworkers of the green belt around the capital city of Córdoba

The green belt, in place since the founding of the city, has seen its landscape transformed over time through a steady process of land use change [26], extending to the nearby towns.

Currently, the green belt is situated within an urban area with a sum of overlapping environmental hazards caused by agricultural activity and industrial activity (Figure 2).

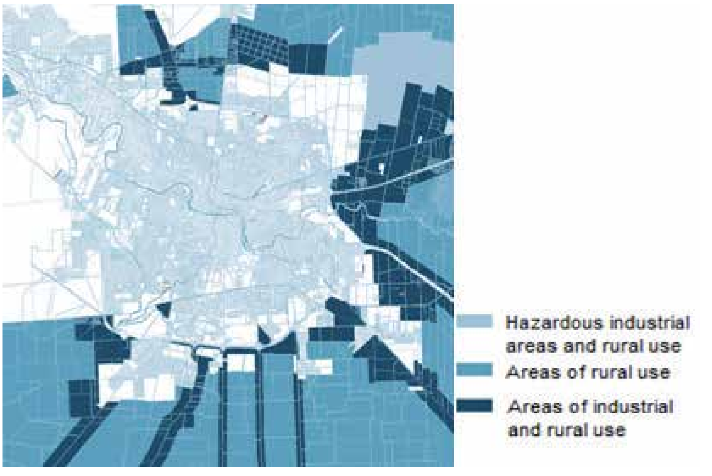

Figure 2. Land use map of the urban area of the city of Córdoba. Municipality of Córdoba, 2004 [17].

Our population consisted of male subjects, with only a single registered female. The mean age was 42.94 years (SD: 13.34), with 67% over 35 years (Table 3). 52% of subjects achieved low levels of education, with 24% who did not complete primary school and 28% who completed only this level. 71% were Argentine and 29% Bolivians. Of the Argentine farmers and workers, 13% were migrants from other provinces. One of the distinguishing characteristics of horticultural farms was their family origin, and this situation, with variations, was maintained over time [27]. 23% of respondents lived alone, while the remaining 77% lived with family members. Of these, 11% lived only with their partner, while 66% also lived with children and 14% with extended family members (older adults, uncles/aunts, cousins). In 31% of families, all took part in the horticultural work with different tasks and hourly loads (involving spouses, children and extended family members).

Among the job roles reported by the horticulturists are the owner, tenant, “mediero” and permanent or temporary employee, and combinations of the above. "Mediería" is a form of associative contract farming: the existence of a partner who provides land and part of the capital, while the other participant contributes labor and other inputs, sharing the product between them.

Part of the population of these small farmers and workers had unsatisfied basic needs, lacking such basic public services as a water network (23%) and a bathroom installed within the dwelling (13%). Precarious living conditions were associated with employment status and land tenure, with the “medieros” and employees having the highest chance of not satisfying these needs ($p < 0.048$), as well as with nationality, to the detriment of the Bolivian-born small farmers ($p < 0.014$, Table 3). An urban solid waste collection service was absent in 23% of cases and domestic gas network provision was lacking in large areas of the green belt (80%).

Sociodemographic characteristics	Number	Valid (%)[1]
Age (years)		
Mean	42.94	
Standard Deviation	13.34	
≤ 25	13	13
26 – 34	20	20
35 – 44	19	19
45 – 54	26	26
> 55	22	22
Education		
Incomplete Primary	24	24
Complete Primary	28	28
Incomplete Secondary	18	18
Complete Secondary, Technical or University studies	31	30
Marital Status		
Married or cohabiting	75	77
Unmarried, separated, divorced or widowed	22	23
Other members of the household working in crops		
Yes	33	31
Running water installed in the household		
Yes	75	77
Bathroom installed in the household		
Yes	85	87
Domestic gas distribution network		
Yes	20	20
Public service of urban solid waste collection		
Yes	66	67
Country of origin and internal migration		
Bolivia	29	29
Argentina	71	71
Born in Cordoba	62	87
Internal migrants	9	13

[1]Percentage considering the total of responses.

Table 3. Social and demographic characteristics of smallholders and farm workers of the Córdoba capital city green belt. 2012.

Table 4 shows that 58% of the productive units were classified as small in extension. Most of the smallholders and farm workers had long experience in the field, 61% with more than 15 years. 69% had their and their family's dwelling within the production unit where they work. 38% of the dwellings were located in close proximity to crops (less than 100 meters) and 50% within 500 m. The pesticide sprayer used by almost all the smallholders and farmworkers in the greenbelt was the backpack (85%), with the self-propelled crop sprayer with cab and

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activated charcoal filter reported by only one farmer. 13% of the productive units grew crops in greenhouses in the last year.

Work characteristics	Number	Valid (%)[1]
Extension of the productive unit (hectares)		
Small (≤ 10 ha)	57	58
Medium (11 to 40 ha)	34	33
Large (≥ 41 ha)	9	9
Area cultivated by worker (ha)		
≤ 10	70	71
11 - 20	11	11
21 - 40	9	9
≥ 41	7	6
Seniority in the horticultural work		
Average (years) 21.34 (SD: 14.58)		
≤ 5	15	17
6 - 10	13	14
11- 15	8	9
16 - 20	14	16
> 20	40	44
Dwelling distance to the nearest crop (meters)		
≤ 50	16	25
51 - 100	8	13
101 - 500	7	11
≥ 501	32	51
Greenhouse for crops in the productive unit		
Yes	13	13
Pesticide spray equipment		
Manual backpacks	77	77
Motor backpacks	7	7
Trailer crop sprayer without cab	28	31

[1]Percentage considering the total of responses.

Table 4. Work practices and technology used by smallholders and farm workers of the Córdoba capital city greenbelt. 2012.

The main vegetable crops cultivated in the green belt of Cordoba are leafy vegetables (Table 5): chard, lettuce, spinach, etc., with the particularity that they are grown throughout the year in a phased manner (Table 5). This means that on the farm at the same time there will be a patch prepared, a patch with the crop planted, another patch growing and another being harvested. These farms, located primarily in the northern greenbelt, are diversified with a large number of crops in many small lots. In these units, the farmer, tenant, and "mediero" work with their families or with hired laborers, carrying out the various farming tasks: transplanting, manual weed control, irrigation, pest control with manual (backpacks) sprays, harvesting, packing for market, loading and transport. The type of contract may be daily or for quantities.

In the southern area of the green belt, specialized farms have developed, devoted to potatoes as their main activity (22%) and rotation is incorporated into the production system with carrots (10%), wheat (9%) and soybeans (9%) variably according to the conditions of each crop year. These are large production units, with a greater degree of mechanization and automation. Most of the tasks are carried out with machinery, and pesticide application is performed with tractor-drawn and in some cases self-propelled machines. In these cases, labor is incorporated as needed, for example: chopping the seed potatoes and preparing them for planting, and harvesting at the manual collection stage. While potato harvester machines exist, they are not widespread in the green belt. The situation with carrots is similar.

Crops sprayed	**Number**	**Valid (%)[1]**	**Average harvests per year**
Chard *Beta vulgaris L. var. cicla*	71	75	3.75
Spinach *Spinacia oleracera L.*	70	69	3.28
Chicory *Cichorium intybus L.*	64	68	3.34
Scallion *Welsh onion Allium cepa L.*	68	67	2.79
Summer squash *Cucúrbita maxima*	66	65	2.46
Broccoli *Brassica oleracea L.*	65	64	2.93
Parsley *Petroselinum sativum Hoffm*.	62	61	2.98
White cabbage *Brassica oleracea*	61	60	3.22
Butterhead lettuce *Lactuca sativa L. var. Romana*	59	58	3.47
Lettuce *Lactuca sativa L. var. crispa*	58	57	3.83
Lettuce *Lactuca sativa L. var. capitata*	57	56	3.61
Leek *Allium porrum L.*	56	55	2.13
Beet *Beta vulgaris L.*	56	55	3.59
Purple cabbage *Brassica oleracea L. var. capitata*	55	54	3.11
Arugula *Eruca sativa L.*	55	54	4.08
Eggplant *Solanum melongena L.*	47	47	1.61
Cauliflower *Brassica oleracea var. botrytis L. subvar. Cauliflora*	41	41	2.40
Chinese cabbage *Brassica chinensis L.*	35	38	2.54
Radish *Raphanus sativus L.*	38	38	3.04

[1]Percentage considering the total of responses.

Table 5. Principal crops grown in Córdoba capital city green belt, 2012.

The most frequently used pesticides were herbicides (Table 6): glyphosate for 81% of the responses and metolachlor for 65%. In the group of insecticides, those most commonly handled were deltamethrin (72%), cypermethrin (65%), Imidacloprid (66%) and Chlorpyrifos (57%).

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The fungicides most frequently used were cabendazin (71%), mancozeb (63%), zineb (62%), and captan (50%).

Pesticides	(%)[1]
Insecticides	
Deltamethrin	72
Cypermethrin	65
Lambda-cyhalotrin	33
Cartap	40
Carbofuran	36
Carbaryl	34
Methiocarb	25
Chlorpyrifos	57
Dimethoate	50
Methamidophos	23
Imidacloprid	66
Endosulfan	46
Abamectine	35
Fungicides	
Azoxystrobin	47
Azoxystrobin + Ciproconazole	23
Carbendazim + Epoxiconazole	9
Mancozeb	63
Zineb	62
Maneb	15
Carbendazim	71
Captan	50
Chlorothanolil	38
Herbicides	
Glyphosate	81
Fluazifop p butil	46
Metolaclhor	65
2,4 D	19
Atrazine	12
Dicamba	14
Phenmediphan	27
Linuron	61
Metribuzin	31

[1]Percentage considering the total of responses.

Table 6. Most frequently used pesticides in the Córdoba capital city green belt, 2012.

The use (current or past) of banned pesticides was also surveyed: 33% reported having used Parathion, 16% Lindane, 10% Monocrotophos, 8% Methyl Bromide, 7% Malathion, 5% Aldicarb. Current use of Aldicarb was reported by two farmworkers; Monocrotophos and Aldrin were reported by a single case. Regarding good agricultural practices in this setting, 90% performed the triple washing of pesticide containers; but this was not accompanied by correct end use of the empty containers: 57% were stored, 17% were burned, 7% buried, and there were other misuses of contaminated containers.

3.3. Exposure assessment

In a previous work [10], we proposed two indexes to describe pesticide exposure in applicators. The Intensity Level of pesticide Exposure (ILE) index measures instantaneous exposure intensity and the Cumulative Exposure Index (CEI) takes into account the average period of exposure, including the previous ILE information. Both indexes were constructed based on the Dosemeci proposal [23], carefully adapting the weighting procedure to our own context, and particularly to local professional opinion. The expressions of these measures are as follows:

$$ILE = (mix * PPE) + (\sum_{i=1}^{n} \frac{meth * PPE}{\# meth}) + (repair * PPE) + house\_dist$$

$$CEI = ILE + (\sum_{i=1}^{n} \log(1 + \frac{Ha / year}{55})$$

where *mix* represents a dichotomic response about mixing pesticides, *meth* the category of the method used with a certain *PPE*, *repair* the binary variable for which success is the positive response, *house_dist* the score indicating the applicator dwelling proximity to the nearest crop, and *55* the average of ha treated with a single load in the crop sprayer. These measures were denoted ILE_{EC} and CEI_{EC} for extensive crop worker's population. Lantieri et al. [10] calculated both measures for all subjects in the opening sample of terrestrial pesticide applicators of extensive crops (n=880) and using Bootstrap and Monte Carlo resampling methods, identified the most suitable theoretical stochastic distribution for each measure. In the present work, we assessed the two indexes once again but on a larger sample of applicators (n=1327) and stratifying by HEAs.

The ILE_{EC} and CEI_{EC} indexes were adapted to assess the specific exposure conditions of the population involved of farmworkers and smallholders in the green belt of Cordoba city. The methodology and definition criteria for the preliminary version of these two indexes were as described in [10]. These indexes are presented bellow (ILE_{GB} and CEI_{GB}):

$$IL\ E_{GB} = \left[(mix / load\ * \mathrm{syst}) + \sum_{i=1}^{n} \frac{(meth\_apl)}{\# meth} \right] * PPE1 + (wash * PPE2) + (r\mathrm{ep} * PPE3) * h\,\mathrm{yg} * spill$$

$$CE\,I_{GB} = IL\ E_{GB} * Duration * Frequency$$

where *mix/load* represents a dichotomic response about mixing or loading pesticide; *syst* the sprayer system (open or closed); *meth_apl* the method of performing pesticide application; *PPE*1, the score of use of Personal Protective Equipment for spraying crops, as described before;

wash is also a dichotomic variable for washing the pesticide application equipment (backpack or machine); *PPE*2, the score of use of Personal Protective Equipment, as described before, for washing the machine and/or backpack; *Rep*, whether repairing application equipment; *PPE*3, the score of use of Personal Protective Equipment for repairing equipment; *hyg* hygiene mode after completing the task with pesticides; and *spill* the behavior during a pesticide spill on clothing, thus, whether the worker changes clothes immediately after the spill or not. The cumulative exposure index incorporates the intensity level of pesticide exposure, the *duration* (years) and *frequency* of exposures (number of days of applications per year).

Tables 7 and 8 show summary statistics for both the measures, constructed for exposure assessment in first population (extensive crops). As can be seen, mean values for both indexes were generally quite different from their medians, indicating empirical distributions different from the normal distribution. Significant differences between ecological areas were found for ILE_{EC} (p=0.013) and CEI_{EC} (p=0.003). For the former, areas I and III showed the lower and similar values (p=0.201) for exposure index, while area V had the highest average (p<0.01). As an intermediate group, there was no difference between areas II and IV (p=0.203), and these yielded higher values than those obtained in areas I and III (p<0.001).

	AREAS					
	I	**II**	**III**	**IV**	**V**	**Total**
n	41	641	230	156	259	1327
Statistics	**Exposure Index distribution**					
Mean	2.04	3.02	2.37	2.76	3.59	2.92
Standard Deviation	2.14	2.36	2.29	2.19	2.57	2.40
Median	0.94	2.61	0.94	2.49	3.24	2.6
Standard Error	0.29	0.09	0.13	0.17	0.15	0.06
p25	0.61	0.86	0.72	0.84	0.89	0.82
p75	2.61	4.47	3.66	3.74	5.66	4.34
Minimum	0	0	0	0	0	0
Maximun	8.80	10.15	9.23	8.94	10.93	10.93

Table 7. Summary statistics of Exposure Index distribution based on pesticide applicators of Extensive Crops (EI_{EC}) information, regarding to Homogeneous Ecological Area Classification. Córdoba, Argentina.

	AREAS					
	I	II	III	IV	V	Total
N	41	641	230	156	259	1327
Statistics	**Cumulative Exposure Index distribution**					
Mean	23.28	44.43	42.34	62.13	59.97	48.02
Standard Deviation	56.46	67.88	66.84	78.25	86.38	72.76
Median	2.18	16.18	13.80	28.52	27.56	17.83
Standard Error	7.61	2.61	3.70	6.17	5.14	1.88
p25	0	0	0	4.31	2.58	0
p75	21.48	56.98	50.62	89.15	84.61	62.36
Minimum	0	0	0	0	0	0
Maximun	383.3	534.877	383.34	370.96	514.2	534.87

Table 8. Summary statistics of Cumulative Exposure Index distribution based on pesticide applicators of Extensive Crops (EI_{EC}) information, regarding to Homogeneous Ecological Areas Classification. Córdoba, Argentina.

For the cumulative exposure index, the differences structure between areas was slightly different. Only areas II and IV were similar ($p=0.270$) showing intermediate values, while areas I and V yielded lower and higher averages ($p<0.001$) of the cumulative exposure measure. Figure 3 (first row) presents the box plots for both indexes for the five ecological areas.

When the log of applicator age was included as a covariate, the above results held. The estimate of regression coefficient (slope) for this covariate was equal to b=-0.40 (SE 0.15) and significant ($p=0.044$), showing that there is an inverse ratio between the exposure index and the log of age. Since the log is a mathematical monotone (increasing) function, this coefficient indicates that the younger workers have higher exposure. In contrast, the age pattern for the cumulative exposure index indicated a direct ratio: the coefficient estimate was 0.43 (SE 0.18), which means that, as expected, that older workers have higher values of cumulative exposure. Figure 3 (second row) illustrates this behavior.

Finally, personal protection was strongly associated with the differences between the areas for both indexes ($p<0.001$), indicating that in ecological areas with rural workers with lower cumulative exposure, the protection feature used was ideal (Figure 4). There was no association ($p=0.695$) between CEI_{EC} and the marital status of subjects.

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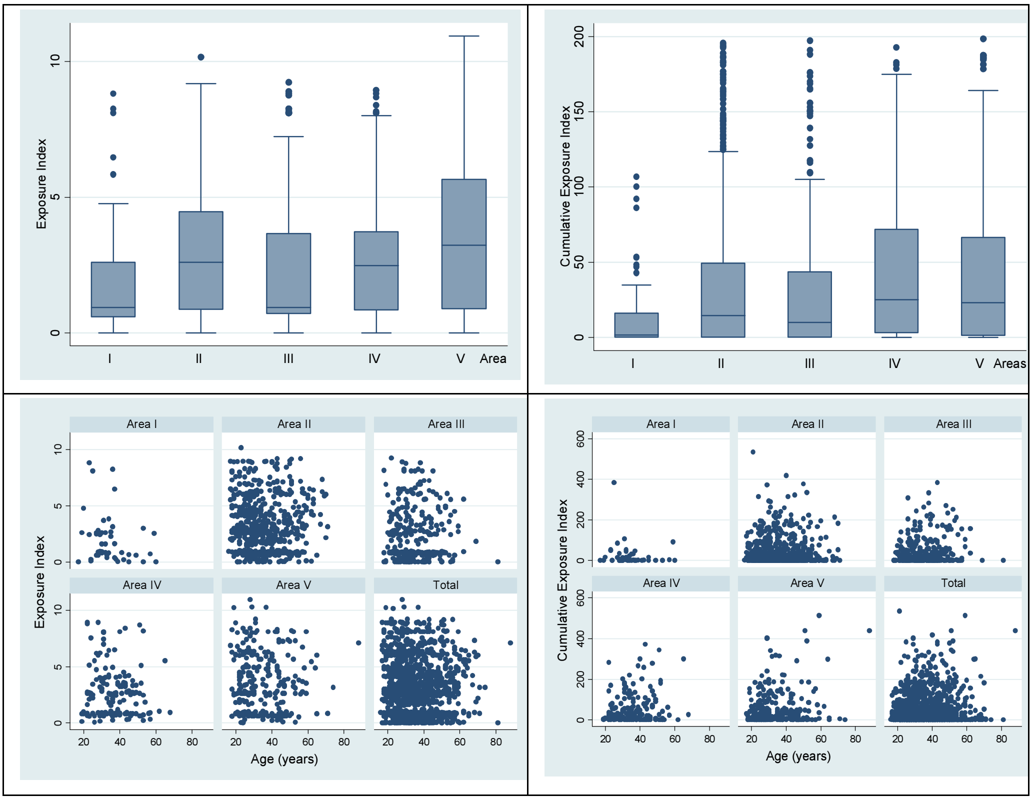

Figure 3. Box plots (above) of Exposure Index (EI_{EC}) and Cumulative Exposure Index (CEI_{EC}) and scatter plots (below) for these indexes *versus* age (years) of workers, for Ecological Areas.

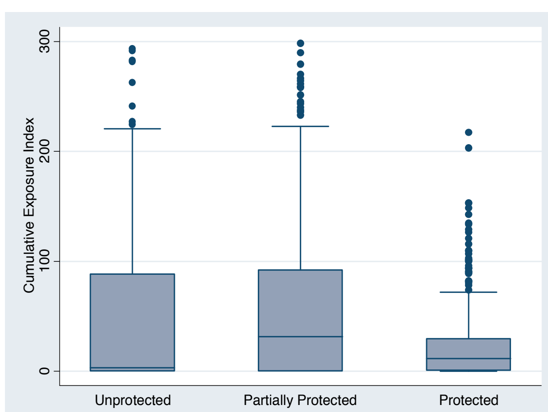

Figure 4. Box plot of Cumulative Exposure Index (CEI_{EC}) in terms of the personal protection category, Córdoba, Argentina.

3.4. Health status of workers related to pesticide exposure

a. Extensive crop pesticide applicator population.

A previous study reported a high prevalence of symptoms: 47.4% with occasional or frequent irritative symptoms, 35.5% fatigue, 40.4% headache and 27.6% anxiety or depression [1]. Increased frequency of medical consultation and hospitalization was associated with the use of chlorpyrifos ($p<0.001$ and $p=0.05$) and endosulfan ($p<0.001$ and $p=0.021$) insecticides, exposure to multiple pesticides ($p<0.001$) and seniority in the job ($p<0.001$). Only 32% of workers were adequately protected. The proper use of Personal Protective Equipment (PPE) (OR: 0.45, SE. 1.56) and marital status (OR 0.16, SE. 1.62) were protective factors for hospitalization.

Within HEAs, there was a difference between Homogeneous Ecological Areas II and III in the probability of medical consultation at least once for reasons related to occupational exposure to pesticides ($p<0.02$), with agricultural workers of HEA III having more probability of medical consultation. In the other health-related variables, no statistical differences were found.

b. Smallholders and farmworkers of the green belt population

In this sensitive population, occasional or frequent manifestation of irritative symptoms affected 49.3%, fatigue 35.6%, headache 52.6%, nervousness or depression 30.6%, dizziness 13.7% and excessive sweating 16.7%, and 18% had had an accident with pesticides. The prevalence of medical consultation and hospitalization was lower than expected: 22.2% and 4% respectively (Table 9). No statistical association was found between these two variables and exposure to specific pesticides.

Symptoms	Never / Rarely	Sometimes / Frequently	Number
Fatigue - tiredness	64.4	35.6	73
Nervousness ordepression	69.4	30.6	72
Headache	47.4	52.6	73
Irritative Symptoms	50.7	49.3	73
Dizziness or vertigo	86.3	13.7	73
Excessive sweating	83.3	16.7	72
Health assistance	**Never**	**Once or more times**	**Number**
Medical consultation	78.8	22.2	80
Hospitalization	96.0	4.0	75
Accident with pesticide			
Yes	82	18	95

[1]Percentage considering the total of responses.

Table 9. Prevalence of symptoms, health assistance and accidents related to occupational exposure among smallholders and farmworkers of the Córdoba capital city green belt. 2012

c. Workers' risk perception of different agrochemicals

We studied the perceived threat level of pesticides used. In the extensive crop pesticide applicator population, there was a high perception of danger (85.76 - 98%) only for insecticides, with the highest perception of danger for organophosphate. Herbicides and fungicides were considered less hazardous (35.09% - 91.54% and 49.07 - 58.55% respectively). Glyphosate, the most widely used pesticide in crops (98% use in the past year), was considered hardly or not at all dangerous. The level of protection used did not vary according to the perception of risk.

Among workers and smallholders of intensive crops, the insecticide group was also seen as presenting the highest perception of risk: between 33.3% and 86% felt that they are dangerous or very dangerous, with organophosphates and organochlorines seen as the most dangerous. Fungicides and herbicides were perceived as less dangerous (29% - 38% and 33% - 65%).

4. Discussion

This work presents an interesting update of our previous work on extensive crops of Córdoba province, stratified by the Homogeneous Ecological Areas (HEAs) [10]. It also includes, for first time in our country, a characterization of the horticultural smallholder and farmworker populations of the greenbelt of the provincial capital, both settings being recognized as vastly different in pesticide exposure determinants, based on professional judgment. The analysis of each agricultural scenario enabled groups with occupational exposure to pesticides to be identified in each particular labor context (extensive and horticultural crops), as well as the health conditions associated with occupational agrochemical use.

When evaluating the pesticide applicator population of extensive crops, we founded statistically significant differences between areas in age, education, marital status, seniority in the task, average/year of hectares sprayed, use of pesticides with prescription signed by an agricultural engineer, self-propelled crop sprayer with cab and activated charcoal filter, and protection level. Only trailed crop sprayer with cab and activated charcoal filter was similar in all the areas. Self-propelled and trailed crop sprayer combined showed an average 55.5% use in all areas, which means that a large percentage of workers used unsafe machinery, i.e., sprayer with no cab or cab without activated charcoal filter, and this was an important determinant of exposure and was more pronounced in HEA II (“Middle Agricultural and Livestock Area”), followed by HEA V (“South-eastern Agricultural and Livestock Area”). HEA I (“North-western Extensive Livestock Area”) was traditionally characterized by grazing cattle but it is now a newly developed agricultural region, due to the nationwide agriculturization process. This area's applicators had the highest level of personal protection and of using pesticides with written prescription, followed by HEA II. Others areas with a historical agricultural tradition, such as HEA IV (“South-eastern Agricultural Area”) and HEA V, did not have similar protective measures or a safe work environment; in fact, the highest rates of unprotected or partially protected applicators were found in these areas.

Our current results confirm previous works [10, 16] and MacFarlane's study [28] reporting no association between instruction level and personal protection. Indeed, HEA IV, with the highest percentage of applicators that had completed secondary school or higher, had only 35.3% of workers completely protected during the task. Likewise, we found no association between marital status and PPE use, as in the case of HEA IV, with the highest percentage of married or cohabiting subjects.

Based on two indexes proposed in previous work [10] for the assessment of pesticide exposure risk, the intensity and accumulated exposure indexes (ILE and CEI), the current assessment was performed in a larger sample of terrestrial pesticide applicators of extensive crops, stratifying by HEAs and showing significant differences among these for ILE_{EC} ($p=0.013$) and CEI_{EC} ($p=0.003$). The results reinforce the previous hypothesis of the emergence of different new risk scenarios in the province. As expected, HEA V yielded the highest averages for both indexes, followed by areas II and IV. It should be stressed that the differences between areas in both measures were strongly associated with the personal protection used (PPE).

As reported in a previous study, we continue to find a lack of enforcement of existing regulations (Law Nº 9164) in all the agricultural settings of the province, with low use of pesticide prescriptions signed by an agricultural engineer, and poor implementation of good agricultural practices such as triple washing of pesticide containers and their correct disposal. Burning, burying or reusing agrochemical containers, a common practice in the study populations, add other risk factors for applicators, as well as abiotic and biotic environmental contamination.

As expected, in contrast with extensive crop settings, wide differences were found in exposure determinants in the greenbelt population of Cordoba city, between their social and demographical characteristics and compared with other agricultural scenarios of the province, as shown above and in previous works [10, 16]. Horticultural workers had a greater average age, long experience in the task, lower educational level, and a high proportion of Bolivian workers and national migrants. Part of the population had unsatisfied basic needs: 23% lacked a running water supply and 13% a bathroom in the dwelling. Precarious living conditions were associated with being a "mediero" (see below), or an employee and a migrant, particularly Bolivian. It is thus a heterogeneous and highly vulnerable population, which favors lax labor structures for their work, leading to scenarios in which a higher rate of occupational health risk is to be expected. Seniority in the job was associated with higher cumulative exposure to pesticides, in turn associated with various deleterious effects on health [29].

The heterogeneity of this population is also seen in the different job roles, employment status and land tenure conditions of the smallholders and farmworkers. The agrarian structure has become dominated by family farms, giving rise to processes of social differentiation, concentration of land and capital, and the emergence of a new social actor: the "mediero", a kind of sharecropper that almost monopolizes the supply of labor by having their family take part in the work. This has transformed the social organization of horticultural work and is extremely functional [30] in that the existence of "medieros" often hides the figure of an unregistered employee, with the advantage for the farmers of transferring some of the risk, while avoiding compliance with labor legislation, social security and occupational risk prevention [31]. It enables them to turn fixed labor costs into variable costs, distribute downward the fluctuations in prices and profitability that are typical of fresh vegetable production, obtain a more stable workforce, delegate responsibilities and reduce the need for control, among others.

The active participation of the family (31%), as in the greenbelt, and the short distance from the home to the cultivation sites (38% less than 100 m), as also reported by applicators in extensive crops, (almost half of them live within 500 m of the nearest crop), leads to non-occupational exposure of the worker after work and para-occupational exposure of the other family members. McCurdy et al., Chaio-Cheng et al., Clifford et al., Loewenherz et al, and Lu et al., [as cited by 32] reported studies suggesting a take-home pathway for pesticides. Applicators and farmworkers accumulate chemicals on their clothing and skin, and can carry these into their homes. The homes of agricultural workers have higher pesticide concentrations in house dust than other homes in the same agricultural community. Children living there have elevated urinary metabolites of organophosphorus pesticides. Regarding dwelling location, higher levels of pesticides were found in dust samples in farmers' dwellings and non-agricultural reference homes closer to orchards [33].

In the greenbelt, the staggered mode in which a diversity of crops are grown allows farmers to grow a large number of crops in small plots, leading to a higher frequency of pesticide application. There is thus a heavy burden of pesticides in both scenarios: in extensive crops, due to the extensive areas sprayed, and in horticultural crops, to the process of spraying throughout the year. This also implies significant environmental pollution, with approximately 47% of the product deposited in adjacent soils and waters or dispersed in the atmosphere [34], depending on climatic conditions such as rain and wind direction, geological features such as soil type and the presence of water currents, and other factors such as the formula and presentation of the product as well as the application technique. Other phenomena promoting environmental spread are photodegradation and volatilization, leaching and surface soil washing, both related to streams and rainfall [35].

Other modern phenomena aggravate the level of pollution and affect the dynamics of farming in the greenbelt. The advance of crops such as cereals and oilseeds, mainly soybeans, over horticultural production, causes the greenbelt to shift towards other neighbouring districts [36]. Moreover, the increase of housing and of informal settlements in urban residential areas, coupled with inadequate planning and land management, further reduces and displaces horticultural production [37]. This is exacerbated by industrial development: the dominant industrial area (including dangerous industrial areas), increased from 8,000 ha (15.1%) to 12,000 (21%) between 2004 and 2012, while the predominantly rural area fell from 29% to 27.5% in the same period [38]. While about 40% of the area sown in the Capital Department is horticultural production [39], it is estimated that it has fallen from 11,000 hectares in 2004 [40] to an area of 5,500 hectares in 2012 [36]. Thus the greenbelt is now located in an urban area with a sum of overlapping environmental risks (caused by agriculture and pesticide pollution as well industrial pollution), making the Capital Department of Cordoba an area of high environmental risk [41].

The informality and precariousness of the situation endured by greenbelt workers is more complex than that of those in extensive crops, whose working conditions are more modern, regulated and safer. The wide diversity of greenbelt workers' tasks in contact with pesticides, the greater burden of insecticides resulting from the type of crops grown, and the application of risky technologies such as spraying with backpacks, also make this group of workers more vulnerable. The broad spectrum herbicide glyphosate, the most frequently used pesticide in this setting, is applied in the vicinity of the crops. Insecticides are also used several times during the crop cycle, as well as fungicides. The level of exposure and the likelihood of acute poisoning in these groups are thus substantially higher due to the continuous contact [34], which is for relatively short periods but is still intense and repetitive during the work day, causing toxic effects that vary depending on the type and amount of pesticide.

Work activity as a source of exposure to pesticides has been widely recognized in farm workers who mix, transport, carry, store or apply them [42]. The magnitude and severity of occupational pesticide exposure, its effects and consequences, cannot be measured exclusively by the classical indicators of mortality and morbidity. The apparent underreporting of cases of acute pesticide poisoning [43] hides the true extent of the problem in rural areas, where some authors report a deficit of up to 50% in reporting these events [44]. The adverse health effects reported in this study show a serious impact on exposed workers. The prevalence of acute and subacute symptoms reported in our study in both groups – extensive and intensive farming – with 47.4% and 49.3% irritative symptoms, 35.5% and 35.6% fatigue, 40.4% and 52.6% headache, 27.6% and 30.6% nervousness or depression, 35.6% and 22.2% rate of activity-related medical consultation, and 5.4% and 4% of hospitalization, respectively, show the high occupational exposure, and may be categorized as indirect indicators of the exposure level, unlike the recording of cases of pesticide poisoning. Argentina reported one of the highest indexes of agricultural accidents at work (94.8‰), with a mortality rate of 195 cases per million workers, only surpassed by the construction sector (229‰) [45]. The Province of Córdoba concentrates 88% of the labor sector in that area.

There are several factors involved in the occurrence of these high levels of accidents. The higher consumption of pesticides (kg/year), the toxicity and diversity of agrochemicals applied, the extent of the areas sprayed, the laxity of State monitoring, the prevailing weather conditions and, particularly, the everyday working conditions of applicators, are among the main variables that shape the patterns of occupational exposure to pesticides. This study provides evidence for this hypothesis and helps to analyze the risk. The association between the symptoms reported, as well as the increased hospitalizations and medical consultation among those exposed to certain insecticides, such as chlorpyrifos and endosulfan, as observed previously [46], provide evidence in this regard. Symptoms reported here, and the frequency of their occurrence, match other reports in Argentina and elsewhere showing a positive correlation between health effects and occupational exposure to pesticides [47-50].

Pesticide hazard perception can be associated with the occupational exposure risk prevention in agricultural settings. Our study found a low perception of hazard in relation to herbicides and fungicides and a higher perception to the group of insecticides in both populations, although the smallholders and farmworkers reported lower risk perceptions in all pesticide groups than terrestrial applicators of extensive crops. But it should be noted that the different risk perception reported in our study did not lead to variations in PPE. The hazard perception of insecticides may be explained by the acute toxicological data, and not by the volumes applied, the possibility of dispersal, environmental persistence and the likelihood of chronic health effects. Another explanation proposed for this behaviour is that the pesticide use in agriculture is not perceived as risky for the environment due basically to trust in the improvement of product quality, in the technological innovation that has taken place in the last few years and in the work of official agencies responsible for approving pesticides [51].

The absence of the agrochemical prescription, as well as the lack of implementation of formally regulated programs to ensure the correct end use of empty pesticide containers in both agricultural settings studied, indicate the weakness of compliance with the provincial regulations in force [24].

The results of the two subject groups present a picture of highly vulnerable populations, which must be considered in risk assessment, and in particular in the implementation of prevention strategies. Comprehensive knowledge of the study population is a priority in designing and strengthening protective measures for improving the health and safety conditions of workers and their families. The presence of highly vulnerable groups, such as women of childbearing age and children at all stages of growth, must be taken into account in assessing the problem, including approach strategies [14].

We proposed an analytical approach to assess workers' exposure to pesticides that takes advantage of existing comprehensive information about pesticide uses as well as about the main working habits of subjects, which is of relatively simple application. The information from assessing the indexes includes some observations relative to the specific local exposure scenario [10] in which the different variables that influence or determine exposure have been weighted and combined. Even though this approach does not give accurate estimates of individual exposure but rather pragmatic information on the risks faced by the workers and, consequently, of the presence or absence of a need for preventive interventions, we believe that these measures provide a valuable monitoring tool in our context.

There are some limitations to this study. Because of the complexity described in labor relations in the greenbelt, there is some selection bias in this study population due to difficulties in accessing directly exposed workers. The laxity in the employment relationship, the informality with which employment contracts are made, the uncertainty regarding operating times and the undocumented status of many of these workers [18], are some of the reasons for this, as has also been reported by other authors [52]. Secondly, information on pesticide use and on PPE, as well as some work practices, was based on self-reporting in the interview questionnaires. Thus, errors in recall and reporting may have occurred. A preliminary validation study was conducted, though only for the population of extensive crop workers (n=60), using a short version of questionnaire. Results (not shown here) indicated that the match between the volunteer farmers' questionnaire responses on both occasions was acceptable. Finally, the potential for differential exposure misclassification as reported by terrestrial applicators has been recognized in the present study by proposing the assessment of specific indexes describing the exposure. However, these measures weight, substantially, the use and the amount of pesticides applied in their usual work. Data from the National Cancer Institute studies found little evidence for differential recall of pesticides by farmers [53]. Since applicators are heavily involved in all aspects of pesticide manipulation/operation and this is practically their single occupation, they have a good memory for all the pesticides used. Further research will be carried out explore this in our populations.

5. Conclusion

The evidence presented describes a problem whose complexity is difficult to cover through the usual approaches. Exposure to pesticides in workers responsible for applying these is high. A variety of economic and socio-cultural factors affect exposure and only through a proper evaluation can its true dimension be identified and quantified. The assessment and monitoring of these populations allows us to obtain information about the risk factors associated with occupational exposure and the consequent health damage.

Recognizing the complexity of the processes underlying the vulnerability of these populations to pesticide exposure is a first step to significant change in preventive health. Adopting a comprehensive view of the different aspects of the problem will favor the reception of preventive proposals and their chances of application. The exposure reported here seriously conspires against this activity's desired goal of sustainability, creating serious health and environmental risks with costs that are underestimated in the balance of these operating models. From an economic perspective, action to reduce the risks of exposure and adverse effects of the use of pesticides and to contribute to maintaining and improving public health and the quality of life, supports economic development in all sectors of the country, especially in production. Workers and their families improve their quality of life and their family's economy and social security. Companies do not incur high costs of care for acute and chronic intoxication, disability and compensation. Employers benefit from a real decrease in absenteeism and staff turnover, and the country has a more dynamic and competitive work force. Consequently, such action is a factor that strengthens the development of the country.

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Chapter 6

Like a Canary in the Coal Mine: Behavioral Change as an Early Warning Sign of Neurotoxicological Damage

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Additional information is available at the end of the chapter

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1. Introduction

With more than 7 billion people in the world, global food production is of critical importance. Back in the 1960's, when population models predicted such a large human population, agricultural scientists and policy makers felt an urgent need to dramatically increase global food production and to radically change how food was grown and distributed. Science, business and governments collaborated to engineer a paradigm shift in how we feed our species. Galvanized by the efforts of Norman Borlaug to successfully develop high-yield and disease-resistant wheat, the Green Revolution led to an order of magnitude increase in global food production in fewer than forty years [1]. Both the Rockefeller and Ford Foundations worked with international governments to use a combination of agricultural and technological approaches to vastly increase the productivity of the major cereal crops that feed the world: wheat, corn, rice, and later other important crops.

The major changes in agricultural practice included the selective breeding of high yield crop varieties, changes to irrigation approaches, increased mechanization of farms and the use of fertilizers and pesticides. The high-yield varieties were selectively bred to produce large and many seeds and to have shorter and sturdier stems to support the seeds. They matured quickly and were much less sensitive to photoperiod, enabling a longer growing season or multiple growing seasons in a year. They were also bred to respond to externally applied fertilizers with fast growth and rapid maturation [1]. Farmers were eager to plant the super-crops and soon changed agricultural approaches to focus exclusively on a few or one variety, giving rise to the monoculture agriculture and large mechanized farming that dominates global agricultural practice today, particularly in the developed world. These varieties are dependent on plentiful irrigation and fertilizer application. To protect the new crops and further enhance yield, farmers applied a growing arsenal of chemical pesticides, some of them derived from chemicals produced in World War I and World War II as chemical warfare agents.

The Green Revolution prevented countless deaths due to starvation and created jobs and entire new industries. Huge mega-companies devoted to the production of pesticides, fertilizers and seeds now dominate big agri-business. More than one third of the world's workers are employed in agriculture. The costs are also enormous. Industrial agriculture has caused widespread damage to the land, water and air. Fertilizer run-off has damaged aquatic ecosystems; soils overplanted with monoculture crops are depleted of nutrients; soil, water and the air are contaminated with a complex mixture of pesticides and other toxic chemicals. What are the consequences of chronic exposure to low levels of mixtures of pesticides in the water we drink, in the air we breathe? How are other organisms affected? How are beneficial soil organisms influenced? There is growing awareness of the human health and environmental health consequences of our high-yield agricultural practices. An increasing number of studies indicate that most animals are affected deleteriously by the pervasive presence of pesticides in the environment, humans included.

This chapter focuses on the effects of pesticides and pesticide mixtures on unintended organisms. I explore the behavioral consequences of exposure to toxic chemicals in the environment, with a focus on the use of behavioral change as an early marker of potential or ongoing damage to the nervous system. Recent work in model organisms underscores the value of behavioral assessment of toxicity. Because of the substantial evolutionary conservation of the molecular targets of many pesticide chemicals, what we learn about in non-human animals can readily be applied to human health. We are all in this together.

2. The most common pesticides

The hundreds of pesticides in existence fall into five main categories: organophosphates, organochlorines, carbamates, glyphosates and neonicotinoids. Agricultural workers are often exposed to high doses, particularly over their lifetime. In addition, most rivers and lakes, non-agricultural soil and even the air contain measurable, albeit lower, concentrations of all these chemicals. The vast majority of pesticides in use to protect plants from herbivory target the control of muscle contraction, leading to paralysis and death of the intended target insects and other arthropods. Fungicides and herbicides target enzyme pathways that result in the death of harmful fungi and weeds, but the chemicals can have unwitting effects on animals as well. In order to better understand how these pesticides operate in animals, I will first briefly review the mechanism by which muscles are activated by nerves.

2.1. A Key target of pesticides: The neuromuscular junction

The connection between motor neurons that generate movement and the skeletal muscles they activate is called the neuromuscular junction (NMJ). This synapse is highly specialized to afford rapid and reliable activation of the muscle. In vertebrates, all skeletal muscles are activated by one type of neuron, the motor neuron, which uses the neurotransmitter acetylcholine (Figure 1). The presynaptic terminal of the neuromuscular junction contains hundreds of synaptic vesicles, each loaded with 5000- 10,000 molecules of acetylcholine. A single action potential firing from one motor neuron can cause the release of as many as 300 vesicles within a millisecond or so [2]. The 1.5 million molecules of transmitter rapidly diffuse across the cleft between the nerve and muscle cell and interact with 1000-2000 receptor channels that are densely clustered at the synaptic junction membrane [3], leading to a large depolarization that initiates a muscle contraction. The cytoarchitecture of this synaptic connection has a tremendously large safety factor, to ensure a 1:1 correspondence between an action potential in the nerve and a corresponding action potential (leading to a contraction cycle) in the muscle cell.

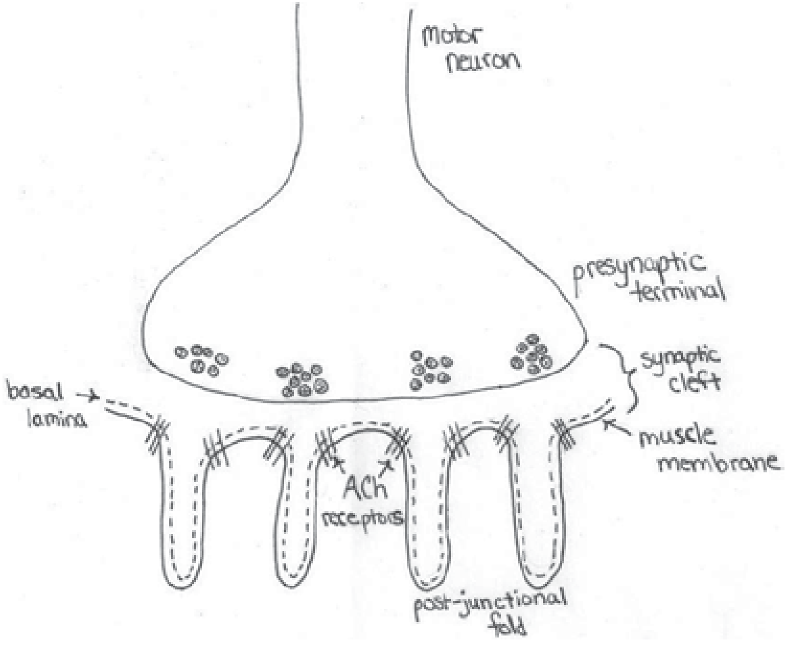

Figure 1. Vertebrate Neuromuscular Junction

At the vertebrate neuromuscular junction, the presynaptic nerve membrane and the postsynaptic muscle membrane are separated by a small space called the synaptic cleft that also contains an extracellular matrix-derived basal lamina that adheres tightly to the muscle membrane (Figure 1). Composed of structural molecules like collagen type –IV and laminins [4], the basal lamina also contains a form of acetylcholinesterase with a collagen tail (ColQ-AChE). The ColQ tail localizes this form of acetylcholinesterase to the NMJ [5]. Acetylcholinesterase is the product of a single, highly conserved vertebrate gene, and many different versions are generated by alternative splicing, co-translational and post-translational modifications and even the formation of oligomers [6], differing in tissue expression and subcellular localization. The catalytic region of the enzyme is very highly conserved across organisms. As a consequence, chemicals that generally affect cholinesterases can have effects on multiple tissues in multiple organisms.

There are hundreds of different proteins involved in the molecular mechanism of neurotransmitter release and the majority of these proteins are involved in the release of all neurotransmitters. The proteins govern the movement of synaptic vesicles to the presynaptic terminal membrane, the fusion of the vesicles with the membrane so as to release the chemical neurotransmitter, the recycling of the vesicle membranes back into the terminal and the packaging of neurotransmitter molecules back into vesicles. These important molecular players are highly conserved evolutionarily, being present in mammals, amphibians, flies and nematodes. In addition, all vertebrates utilize the same overall scheme for muscle contraction. Many of these proteins are found in all animals, including invertebrates. Nonetheless, there can be substantial genetic variation in the proteins, with differences in function and structure in vertebrate as compared with invertebrate neurotransmission proteins. The evolutionary divergence of these proteins is important in the design of pesticides that are selective for invertebrate target species, while at the same time being relatively less toxic to non-target animals. However, many of the pesticides currently in use do have considerable cross-reactivity with non-target organisms, depending on the dose and exposure methods used.

Invertebrates have an overall similar mechanism by which motor neurons excite muscles and many of the same proteins are critical for the release of neurotransmitter. There are a number of differences, however. While acetylcholine is the major excitatory neurotransmitter used by motor neurons in a large number of invertebrates including nematodes, annelids, arachnids and mollusks, insects and other arthropods utilize glutamate as a major excitatory neurotransmitter at the neuromuscular junction, with glutamate acting on postsynaptic muscle membrane glutamatergic sodium channels. Another key difference is that many invertebrates also control muscle movement via inhibitory motor neurons that utilize GABA or glycine as a neurotransmitter. These inhibitory motor neurons synapse directly on muscle fibers [7]. This local control of muscle function is quite different from the central spinal cord-level control in vertebrates [8]. Another distinction between vertebrate and invertebrate muscle control is that arthropods in particular modulate muscle function peripherally, rather than at the level of the central nervous system, via a wide variety of circulating hormones and neuromodulators [7]. Many invertebrate motor neurons release more than one neurotransmitter, achieving modulation of muscle force directly at the neuromuscular junction [7]. While ACh is an important neurotransmitter across all animals, the principle effect of pesticides directed at the acetylcholine neurotransmitter system is to alter central nervous function in arthropods.

2.2. Organophosphates

More than half of the insecticides in use across the globe fall into the organophosphorous group of chemicals. The major organophosphates in use today are chlorpyrifos, parathion, malathion and diazinon. Chlorpyrifos is the major compound used in the United States and Europe. Organophosphates were first developed as nerve gases in the 1930's in Nazi Germany [9]. After World War II, American chemists developed this class of insecticide based on those materials. It is estimated that more than a billion pounds are produced worldwide each year [9].

Organophosphates target the enzyme acetylcholinesterase, which is a member of the serine hydrolase enzyme superfamily [10]. This enzyme plays a critical role in nerve and muscle activity in vertebrates and some invertebrates (Fig. 1), and in central nervous system function in many animals, including arthropods. The organic groups of organophosphate pesticides can be modified to affect target specificity, penetration of the chemical internally, water solubility and persistence in the environment. All of the major organophosphorous pesticides in use for agricultural and residence purposes have a common mechanism of action, that of phosphorylating acetylcholinesterase, thereby inactivating it [11]. This covalent modification is permanent. The inhibition of acetylcholinesterase activity, particularly at the neuromuscular junction, reduces the clearance of acetylcholine from the synaptic, resulting in prolonged neuromuscular stimulation, which causes seizures and paralysis. In arthropods, the effect is primarily within central regulatory neurons and sensory systems [12].

2.2.1. Unintended targets of organophosphorus pesticides

When a pesticide is developed for use, it must be approved by governmental regulatory agencies in many countries. The Environmental Protection Agency (EPA) in the United States requires testing of toxicity to a variety of animals, usually assessing lethality at working doses recommended by the pesticide-producing company, as well as assessing potential cancer risk. The EPA requires pesticides be assessed for ecological as well as human health risks. A company seeking to gain EPA approval to sell and distribute a pesticide conducts the scientific tests and files the regulatory materials. Pesticides that have gained approval are periodically reassessed based on reports received from scientific study of risk and reports of problems submitted to the EPA. Ecological assessment data include toxicological studies on wildlife and plants that represent non-target organisms likely to be exposed unintentionally through runoff, aerial drift or bioaccumulation. For this testing, organisms are exposed to different concentrations of the “active ingredient” of the pesticide being assessed, usually in isolation, without other so-called inactive ingredients. Both short and long-term effects of the active ingredient are measured including lethality, growth and reproduction rates. In addition, residue measurements are conducted to determine degradation of the pesticide, possible toxicity of breakdown metabolites, persistence and ability to travel in the environment (soil, water, air, bioaccumulation). If the regulatory commission (like the EPA) believes that a particular type of ecosystem is not likely to be exposed to a candidate pesticide, then the registration materials stipulate that the pesticide is either “safe” or poses no “reasonable harm” to the organisms within that ecosystem, even if direct evaluation of risk or exposure has not been conducted. If the assessors predict a high likelihood a particular ecosystem will be exposed, then additional tests are often required [13].

Acetylcholinesterase, the major target enzyme for organophosphorus pesticides, is a member of a very large class of carboxylic esterases [14]. Most organophosphates interact with a serine group present at the active site of the enzyme. However, they can also affect other enzymes, including other serine hydrolases and even serine proteases [15]. As a result, many cell processes and cell signaling pathways can be disrupted, including serotonin and dopamine neurotransmission, growth regulation, hormonal regulation and other systems. Of particular concern to epidemiologists are the possible long-term consequences of exposure during key developmental critical periods. For example, 83% of pregnant women in the U.S. had detectable levels of an organophosphate metabolite in their urine [16]. At the same time, a 600% increase in autism incidence has been reported in California [17]. Mothers living in the California Central Valley who had been exposed to pesticides while pregnant gave birth to children who were 7.6 times more likely to be diagnosed with Autism Spectrum Disorder [18] and there was a 230% increase in maternally reported pervasive developmental disorders in children whose mothers had measurable organophosphate metabolites in their urine [19]. Cholinergic abnormalities have been reported in autism [20]. While not conclusive, studies such as these and others [20] indicate that gestational exposure to pesticides might well influence neural development. Direct experimental animal studies support these concerns. Exposure to chlorpyrifos during the early postnatal period altered rat memory function and spatial navigation in adulthood [21]. Chick embryos exposed to low levels of chlorpyrifos showed reduced head development [22] and axon development was disrupted in exposed zebra fish embryos [23].

Acetylcholinesterase (AChE) inhibition, affecting neural cholinergic signaling during development, is a likely cause of organophosphorus pesticide effects on behavior, growth, and reproduction. However, most organophosphorus pesticides can also affect other enzyme systems that involve serine esterases, serine hydrolases or serine proteases. This large and essential group of enzymes is important for overall cell and body metabolism, immune and endocrine system functioning, in addition to nervous system functioning. These enzymes differ in their sensitivities to different organophosphorus pesticides, from essentially insensitive to highly sensitive to inhibition. Doses of organophosphate compounds that exquisitely affect AChE activity might have little or no effect on other members of this enzyme superfamily. However, many unintended target enzymes are affected by concentrations of organophosphate compounds or their metabolites that have little effect on AChE. For example, several organophosphates that induce a delayed neuropathy are relatively less reactive with AChE [24]. Further, metabolites of organophosphorus insecticides can also exert effects by reacting with non-target members of the serine hydrolase enzyme superfamily [25]. Indeed, such interactions with serine hydrolases in liver are important means for detoxification [25]. Recent work on the environmentally relevant metabolites of chlorpyrifos and parathion demonstrated substantial inhibition of the activity of key liver carboxylesterases [25]. Several lipases important in brain function are sensitive to organophosphates. Neuropathy target esterase (NTE) is one such enzyme, first discovered as the target enzyme associated with a lethal neuropathy caused by a ginger extract substitute added to drinks during Prohibition in the 1930's [10], an insecticide that was also used as a lubricant for machine parts.

Over the past 15 years or so since the introduction of organophosphorus pesticides as a safer alternative to DDT and other organochlorine pesticides, increasing evidence indicates that organophosphates are harmful to immune function through both acetylcholinesterase inhibition and via noncholinergic mechanisms. Vertebrates and invertebrates share common cell types and mechanisms of innate immunity, namely the presence of phagocytic cells (monocytes and neutrophils), the production of cytokines [26] and a histocompatibility cell recognition system. The complex adaptive immune responses, in particular T- and B-lymphocyte antibody-mediated immune systems first appeared with the evolution of the vertebrates.

Serine hydrolases play important roles in innate immune function and could be unintended targets of organophosphates [27]. Malathion, at doses that inhibit acetylcholinesterase, suppressed humoral immune responses in mice exposed under laboratory conditions [28] and inhibited cytotoxic T-lymphocyte responses [29]. At low doses, below those that inhibit AChE, malathion stimulated immune activation in rodents [30]. Immunotoxic effects have also been reported in birds, fish, small mammals and soil invertebrates exposed to chlorpyrifos, malathion and diazinon [26]. Parathion, diazinon and chlorpyrifos have also each been shown to alter immune function [26]. Reports on earthworms have shown reduced immune function after exposure to organophosphorus compounds [31, 32]. Laboratory studies in fish also demonstrated an immunosuppression after sublethal organophosphate exposure to malathion [33] and diazinon [34]. A number of transcription factors important for cell division and differentiation are inhibited by chlorpyrifos [35]. These studies are complex and can suffer from interpretational difficulties and clearly much more work is needed. Nonetheless, the increasing reports of unintended effects on many different organ systems are cause for concern.

2.2.2. Organophosphorus pesticides act as endocrine disrupters (EDC's)

An endocrine disrupting chemical (EDC) is any chemical that alters an endocrine-regulated system or behavior. Early work centered on chemicals whose structural characteristics allowed them to be estrogen-mimics or estrogen receptor agonists or antagonists. However, more recent work has acknowledged that many chemicals, while not directly interacting with estrogen receptors, can alter endocrine-related functions and so produce long-term effects on growth, metabolism, reproduction and behavior [36].

Developmental exposure to organophosphorus pesticides has widespread effects on neural development, which influences behavior and physiology well into adulthood. Late pre-natal and early postnatal exposure of rats to low concentrations of chlorpyrifos caused impairments in synaptic function, with subsequent locomotory and cognitive dysfunction ([37- 39]). Exposure to very low levels of chlorpyrifos early in gestation, at the time of neurulation, a very early stage of brain development, resulted in locomotory and cognitive abnormalities that persisted into adulthood [40]. The impairments were not severe, suggesting some neurodevelopmental compensation or recruitment of alternate mechanisms to preserve functionality. Indeed, early developmental exposures to chlorpyrifos, parathion, malathion or diazinon have each been associated with alterations in serotonergic and dopaminergic systems [41]. The consequences of alterations in neurotransmitter systems during development, referred to as organizational effects, would have an impact on neuroendocrine system development, thus altering reproductive behavior, in addition to myriad effects on sensory function, cognitive function, predator/prey avoidance and other behaviors. For example, salmon exposed to diazinon were less responsive to predator cues [42]. Organophosphate exposure in development has also been linked to modified oxytocin and arginine vasopressin (AVP) activity [43], altered social behavior [44] and sex-specific behaviors [45].

There is sufficient evidence that developmental exposure to organophosphorus pesticides can cause permanent neurobehavioral and neurological impairments that chlorpyrifos has been banned in the US for residential use. Despite this compelling evidence, however, this and other organophosphorus pesticides are still heavily used in agricultural settings, exposing workers, nearby communities and the surrounding aquatic and terrestrial wildlife to these harmful and environmentally persistent chemicals. It is abundantly clear that, while recommended application doses of particular organophosphorus pesticides may not cause overt death, persistent exposure to these compounds, present at biologically active concentrations in water, soil and the food supply, can lead to neurobehavioral changes that influence wildlife and humans.

2.3. Organochlorine pesticides

Organochlorine pesticides are among the most environmentally persistent human pollutants, existing in soil, water and air for decades. Developed in 1946, the first commercial herbicides, including 2,4 D and 2,4,5, T were introduced to control broad-leaf weeds. 2,4,5 T was used extensively during the Vietnam war to clear jungle hiding places. Byproducts of that chemical production included dioxin and DDT, which are potent neurotoxins. In the 1940's, DDT was heralded as a potential eradicator of malaria and was sprayed widely as a residential and agricultural insecticide until alarming reports of bird and human morbidity and death in the 1960's. Other banned organochlorine pesticides include aldrin, dieldrin, chlordane and heptachlor. Despite recent bans on their use in some countries, measurable levels of many of these chemicals are still present, even in areas like the Arctic, where they were never used [46].

Others remain in use in the US and in the developing world, including lindane, endosulfan, dicofol, methoxychlor and pentachlorophenol. These chlorinated hydrocarbons are readily lipid soluble, which leads to bioaccumulation in wildlife and in the food chain. Measurable organochlorine residues are found in blood, adipose and breastmilk in humans. Most organochlorines interfere with ion channel function, predominantly in the nervous system. For example, endosulfan binds to and blocks the chloride channel portion of the GABA-a receptor, acting as a noncompetitive antagonist [47]. Lindane, an active ingredient in head lice treatments for children, also interacts with the GABA-a receptor, whereas methoxychlor, chemically similar to DDT, interacts with insect sodium channels, leading to their persistent activation [48]. Lindane and several other organochlorines have been banned from use in many countries because of unintended endocrine-disrupting effects on wildlife and humans (see below) but others, including endosulfan, continue to be used heavily in many parts of the world.

The primary target of endosulfan as an insecticide is to alter ion flux of sodium and potassium, thereby interfering with neuron activity and thus motor function in insects. However, it is a relatively nonspecific insecticide, which is in part why it is used widely for pest protection of many important crops worldwide, as well as for a preservation treatment for wood. Endosulfan, known to be toxic to fish and other aquatic organisms [47], blocks the GABA receptor ion channels, interfering with critical inhibitory neuronal function in vertebrates, resulting in hyperexcitability of many neuronal circuits. Endosulfan is highly toxic to fish and aquatic invertebrates [49] and has also been found to be genotoxic and neurotoxic to mammals [50]. Endosulfan can be transported by air and water and measurable and increasing levels have been reported in the air over the Arctic [50] and even within tissues of animals living large distances from regions that use pesticides, like polar bears in the Arctic [46]. As a result, endosulfan used in one nation will affect many other nations that might have banned its use. Organochlorines are found globally and in every ecosystem that has been studied [36].

2.3.1. Unintended targets of organochlorines

Organochlorines, like organophosphates, have a number of unintended effects, the nature of which varies with exposure dose/time, species, developmental stage and method of exposure. Chronic sublethal exposure of adult rats to endosulfan in food led to hair loss, enlarged kidneys and increased liver toxicity. Higher doses led to increased incidence of aneurysms [47]. Dogs exposed to endosulfan exhibited increased sensitivity to noise, jerky or tonic movements, excessive abdominal contractions and neurotoxicity [47]. These effects are not related to their intended effects on GABA-a channels and occur at lower concentrations.

In addition to the acute and pervasive neurotoxicity of the major organochlorine pesticides, the compounds in lower concentrations act as endocrine disrupting chemicals (EDC's). Low levels of endosulfan impaired sexual pheromone communication and mating success in newts (*Notophthalmus viridescens*) [51] and methoxychlor affected scent-marking behavior in mice [52]. Further, mice exposed to methoxychlor *in utero* exhibited altered exploratory behavior when adults [53]. Frogs exposed to endosulfan exhibited convulsions, followed by temporary paralysis [54]. Rats exposed to endosulfan as adults or early in development exhibit an array of neurological alterations, including impairments in learning and memory, increased spontaneous motor activity, reduced escape and avoidance behavior learning and increased fighting behavior [47]. Hormones modulate these behaviors, particularly during neural development. Thus, while endosulfan is not a direct endocrine disrupter [47], its potent effects on neural systems may indirectly influence many endocrine-regulated functions.

2.4. Carbamates

Carbamates, introduced in the 1950's, are organic insecticides and fungicides that were originally derived from the extracts of the West African calabar bean. This class of pesticide inhibits acetylcholinesterase activity much like organophosphate pesticides, but the effects are reversible. Carbamates degrade fairly rapidly in water, with half-lives ranging from 1 day to 4 weeks. The liver detoxifies carbamates and the metabolites are excreted via the kidneys. The major insecticides are aldicarb, carbofuran (Furadan) and carbaryl (Sevin). Carbamates are also found in cosmetics, as preservatives and in polyurethanes, so their presence in the home is quite pervasive [55]. Humans are exposed via skin absorption, ingestion and inhalation and acute toxicity symptoms include headache, nausea, and dizziness. Carbamates are the class of insecticides most prevalent for in-home use. Dithiocarbamates, in contrast, while still rapidly degraded, have little to no effect on acetylcholinesterase activity but rather bind divalent metal ions like manganese and zinc and are used as potent fungicides for home and agricultural use.

Carbamates, despite their rapid degradation and overall low persistence and mobility within the environment, are highly toxic to a wide range of vertebrates and invertebrates. Insecticidal carbamates cause the same symptoms of neurotoxicity as the organophosphate pesticides, with a similar wide range of LD50 values based on chemical modifications [55]. Because the chemicals have low persistence and low mobility, and because the actions are reversible, carbamates are generally considered safe for humans and wildlife, except for occupational or intentional exposure to high concentrations. Very little work on possible long-term consequences of brief or transient exposure to the chemicals has been done, nor has there been much work on possible genotoxic, carcinogenic or reproductive effects. A recent review of the available epidemiological studies of carbamates indicated variable cancer risk from occupational exposure to different carbamates [56]. A few reports indicate reproductive and developmental problems in children of exposed workers [57, 58], suggesting possible transgenerational effects.

There is growing concern about carbamates, particularly thiocarbamates, and in particular the associated metal toxicity. A recent human epidemiological study implicated the carbamate fungicide ziram in the etiology of Parkinson's disease [59]. The risk of Parkinson's disease was significantly higher when patients were exposed to the combination of ziram, maneb (another carbamate fungicide) and paraquat, for patients exposed in both occupational and residential areas, and for patients exposed at a younger age [59]. The manganese associated with several common dithiocarbamate fungicides is released when the organic carbamate moiety is degraded or metabolized which can enhance the toxicity of these compounds [60]. Manganese is a potent neurotoxin that is particularly harmful to dopamine neurons [61], causing toxicity via oxidative stress, mitochondrial inhibition and the production of reactive oxygen radicals. Dopamine neurons are particularly vulnerable because both extracellular and intracellular dopamine is enzymatically oxidized to a reactive species in the presence of manganese [61]. Mancozeb exposure, while not lethal to nematodes, inhibited larval growth, induced a heat shock response and altered gene expression [60]. These results suggest that, while dithiocarbamate fungicide might not be lethal or toxic in the short-term, it might cause long-term changes in neuron function that influence behavior and health later in life.

2.5. Glyphosate (Roundup)

Introduced by Monsanto in the 1970's, glyphosate is one of the most prevalent herbicides in use today. In 2007, the US applied 185 million pounds of Roundup for agricultural use alone [62]. Monsanto also developed glyphosate-resistant crops, so many farmers plant these crops and apply copious amounts of Roundup. It is also the herbicide most used residentially and on corporate campuses for lawn and garden maintenance.

The primary mechanism of action of glyphosate is to inhibit the enzyme 5-enolpyruvylshikimic acid-3- phosphate synthase, an enzyme found only in plants and microorganisms that is responsible for the synthesis of tyrosine, tryptophan and phenylalanine [63]. Because animals obtain these amino acids from dietary sources, glyphosate has been heralded as a safe and effective herbicide for general use. Glyphosate is considered non-carcinogenic and of very low toxicity to humans. It is less persistent in water than in soil, where it can be retained for over a year. Glyphosate has been considered only slightly toxic to amphibians and fish because of its 12-60 day persistence in water [64].

With its widespread use, glyphosate levels are measurable in most soil, water and even air. Low levels have been shown to inhibit steroidogenesis [65] and to alter testosterone levels and testicular morphology in rats exposed pre-pubescently to glyphosate [66]. There are also reports that the commercial formulations of Roundup, which contain inactive ingredients like surfactants to enhance persistence and ease of application, have teratogenic effects in amphibians and other vertebrates [67, 68]. Several studies have demonstrated that Roundup inhibits aromatase, which can have profound neuroendocrine effects [69], although these findings are controversial because these effects have so far only been demonstrated in isolated human cells. A recent report provided evidence that male offspring of female rats exposed to concentrations of Roundup in their drinking water that are found in the environment exhibited alterations in genes important for thyroid function [66]. These reports, taken together, suggest that glyphosate, or the combination of glyphosate and its inactive ingredients in commercial formulations, may cause changes in neural and neuroendocrine function that could have long-term consequences.

2.6. Neonicotinoids

In the 1980's and 1990's, largely in response to the toxicity caused by organophosphate and organochlorine pesticides, a new class of insecticide was developed based on the naturally occurring plant alkaloid nicotine, which is known to have a higher toxicity in insects than in mammals. Bayer Corporation introduced imidacloprid in the 1990's and it is now the most widely used neonicotinoid insecticide in the world [70]. Imidacloprid and two other prevalent neonicotinoids, clothianidin and thiamethoxam, are nicotine-related compounds that are photostable, water-soluble and environmentally persistent. Because they act selectively on the acetylcholine receptor, much like nicotine (Figure 1), it is thought that there are far fewer unintended targets for the compounds. Neonicotinoids are not metabolized by acetylcholinesterase and their clearance from tissue is slow and so their action on the acetylcholine receptor is essentially irreversible. The neonicotinoid insecticides have a much higher affinity for the insect nicotinic acetylcholine receptor than for other such receptors, leading to a lower toxicity in non-insect animals [71].

Neonicotinoids are of deep concern because they act with high potency against target insect pests as well as beneficial insects like honey bees and other important pollinators. A host of recent work has indicated that low-level exposures, of the magnitude measured in agricultural dust drift, pollen and nectar, impairs honey bee navigation, foraging and queen production [72], particularly when exposures occur alongside other pesticides [73].

Even though neonicotinoids are not lethal to non–insects, low, sublethal exposures impaired feeding behavior in a crucial benthic freshwater invertebrate, *Gammarus pulex* [74], and reduced exploratory and burrowing behavior in two different species of earthworm [75]. Further, male offspring of female mice exposed to chothianidin during gestation and lactation had long-term alterations in exploratory behavior and increased spontaneous locomotory activity [76]. These findings suggest that neonicotinoids are not safe for non-target organisms.

3. Assessing behavioral change rather than lethality

Determining whether a pesticide is harmful to humans or wildlife is a tricky business. Many different factors are important. How the chemical spreads through water, soil, and air affects the extent of pesticide exposure. How the chemical is applied (spraying, irrigation water, seed coating) also affects how it spreads through the environment. Temperature, pH, sunlight and other environmental conditions affect the persistence or breakdown of the chemical. The biological activity of the active ingredient can be altered by other so-called inert ingredients contained within the commercial formulation, like surfactants or solubilizers. The breakdown products might also carry risks in and of themselves. The action of a particular compound might also be different if it is present in a mixture of other pesticides. The concentration of the compound can also have different effects, both on different organisms and on different biological processes within the same organism. The time of exposure (long-term, chronic, repeated) as well as the time of life (early in development, in adulthood) also influence the kind or nature or extent of the biological effects. It is not surprising, then, that regulatory agencies focus on overt, direct toxicity or lethality of the active ingredient of a mixture. It is also not surprising that the focus is on major aspects like carcinogenicity or acute toxicity in humans and animals likely to encounter high concentrations of the compounds being tested for use.

Because so many pesticides have unintended effects and long-term consequences, it makes sense to develop assays of nonlethal effects on reproduction, behavior and nervous system function of exposure to field-levels of the compounds. A rational approach being taken by an increasing number of investigators is to develop behavioral assays in a number of model organisms that have proven to be useful in testing the efficacy of pharmaceutical compounds and that span animal taxa. Understanding the evolutionary similarities among these organisms can provide insight into not only the choice of the model organism, but also the types of behavioral assays that would be most useful. Further, appreciating the differences in mechanism of action or mode of interaction of particular pesticides across evolutionarily distinct taxa will also help inform toxicological testing approaches. While many of the current strategies try to take these kinds of approaches, the continued emphasis on lethality measures, particularly in the licensing approval process, quite often underestimates risks that might lead to long-term morbidity in humans and other animals.

Efforts to explore behavioral effects of pesticides and mixtures of pesticides have been quite successful. As early as 1958, Broley observed abnormal nesting, courtship and reproductive in the Florida bald eagle in areas that were sprayed with DDT [77]. Guppies exposed to the fungicide vinclozolin exhibited changes in body coloration and courtship behavior [78]. The courtship behavior of a number of different bird species, including ringed turtle doves, Japanese quail and Western gulls was adversely affected by exposure to DDT [36]. Many experimental studies in rodents have demonstrated changes in reproductive behavior, locomotory behavior and motivational behavior in response to pesticide exposure [36]. Organophosphates, organochlorines and carbamate pesticides have been shown to alter chemosensory behavior in salmon and mice [52, 79], social behavior in fish [80] and mice [81] and behaviors in tadpoles [82], birds [83] and mammals [84].

Behavioral changes can be more sensitive than other measures, like lethality or neurotoxicity. For example, swimming behavior in fish was more sensitive to toxic stress than either lethality or growth [85]. With the growing recognition that behavioral assays can be effective ways to determine possible sublethal and long-term effects of various pesticides, model organisms like the fruit fly *Drosophila melanogaster* are proving to be effective screens for pesticide sensitivity. Of course, because *Drosophila* species are insects, they may not be the best models for evaluating effects of pesticides, particularly insecticides, on unintended target organisms. But studies in closely related organisms are crucial for understanding the effects of pesticides on beneficial organisms, like honey bees and other insect pollinators that are also unintended targets of pesticides. *Drosophila melanogaster* has been historically used for evaluating genotoxicity of pesticides and heavy metals [86]. A number of straightforward behavioral assays, like locomotion, courtship behavior, and aggressive behavior, have proven quite useful in assessing the neurotoxicity and cellular mechanisms of toxicity of various chemicals.

3.1. *Caenorhabditis elegans* behavior

Caenorhabditis elegans (C. elegans), a free living soil nematode with a short life cycle, high fecundity and ease of maintenance in a laboratory, is a valuable model organism to use in neurotoxicology, genotoxicity or behavioral toxicology measures. Its genetic tractability also enables mechanistic studies. The *C. elegans* genome contains 60-80% of the genes found in humans and shares many of the same mechanisms of neuron function, development, gene regulation and signal transduction pathways [87]. Early work with this organism examined behavioral toxicity of heavy metals like copper, lead and mercury. Locomotory behaviors like head movement, feeding behavior and simple learning behaviors all showed sensitivity to heavy metal exposure [87- 89]. The herbicide paraquat (methyl viologen) has been extensively studied using *C. elegans*, particularly in response to emerging epidemiological evidence for an increased incidence of Parkinson's disease in humans exposed to paraquat [90]. Using *C. elegans*, several potential mechanisms involved in paraquat dopamine neurotoxicity have been described, including oxidative stress leading to reactive oxygen species production [91].

A growing number of studies are accumulating on the adverse behavioral effects on reproduction behaviors, general locomotion or feeding behaviors of sublethal exposure to various pesticides including aldicarb [92], juglone and paraquat [93, 94], organophosphorous compounds [95, 96] and carbamates [97, 61, 98]. The LD50 values for these pesticides are similar to those in rats and mice, further validating this model organism for studies of non-target animal toxicity. Sublethal, environmentally relevant concentrations of all of these different environmental contaminants have substantial effects on behavioral processes. Two organophosphorous insecticides, malathion and vapona, inhibit locomotion at sublethal concentrations [99]. Aldicarb, a potent acetylcholinesterase inhibitor, causes paralysis in *C. elegans* [92], likely because acetylcholine is the excitatory transmitter used at *C. elegans* neuromuscular junctions and is the neurotransmitter used by one-third of neurons in the *C. elegans* nervous system [100]. Cholinergic neurons are involved in many behaviors in *C. elegans*, including locomotion, egg laying, feeding and mating. Sublethal concentrations of the organophosphorous insecticide monocrotophos, still in use in developing countries, caused paralysis, reactive oxygen species production and reduced brood size in *C. elegans* exposed for 4 hours [95]. Chlorpyrifos, a potent acetylcholinesterase inhibitor, also affected brood size after a 72 hour exposure [101]. Dithiocarbamate fungicides like Maneb and Mancozeb, at sublethal concentrations, disrupt dopamine-mediated behaviors [98] and lead to dopamine neurodegeneration [97, 98], which supports epidemiological evidence for a link between carbamate pesticide exposure and Parkinson's disease. Exposure to Mancozeb inhibited locomotion in a dose-dependent manner after a 6 hour exposure [98]. In addition, dopamine-specific behaviors like the transition from swimming to crawling were much more vulnerable to Mancozeb than other behaviors mediated by distinct neuronal subtypes [98]. Dopamine-mediated behaviors were disrupted at the lowest concentrations of Mancozeb, whereas behaviors mediated by other neurons, like egg-laying behavior, mediated by serotonergic neurons, were disrupted at slightly higher concentrations of Mancozeb [98]. Importantly, the behavioral deficits are more sensitive to the compounds and the deficits precede overt neurodegeneration, suggesting that behavioral effects of pesticides serve as the earliest biomarker of pesticide toxicity. *C. elegans* have also been useful for determining gene expression changes due to pesticide exposure [86].

3.2. Long-term consequences

Why are behavioral biomarkers desirable when evaluating pesticides for toxicity? First, behavioral responses indicate that the organism is responding to a pesticide. Behavioral changes might well be the first indication of impending toxicity to cells and tissues. Second, some behavioral changes might be reversible, suggesting that eventual damage to the underlying cells/tissues/systems that underlie the behavior can be avoided. Third, behavioral effects, particularly in well-studied organisms, can shed light on mechanistic pathways and systems. Finally, behavioral effects provide insight into population and ecological consequences of long-term exposures.

Persistent changes in reproductive behavior, social behavior or predator avoidance behavior could have long-term consequences for an organism's overall fitness. In one study of the anthropogenic pollution effects on fish communication, researchers examined the turbidity of water on coloration and courtship behavior in Lake Victoria cyclids. Many fish use visual signals to initiate appropriate courtship behavior [102]. The coloration of scales is an important cue for reproductive fitness [102]. In turbid waters, caused by eutrophication and algal blooms or excessive turbulence or increased sediments suspended in the water, these visual communication cues are compromised, which can have an impact on reproduction and possibly overall population characteristics. Over several generations in the turbid water environment, the strong coloration patterns of male cyclids disappeared and the frequency of more mottled, intermediate coloration patterns increased [102]. In another study, endocrine-disrupting properties of a fungicide led to a reduction in the orange coloration spots of male guppies, which in turn reduced the mating success of those males [78]. Male stickleback fish exposed to pesticides showed reduced male nest building and aggressiveness [103], which compromised their reproductive success. Fish grown in waters polluted with mixtures of pesticides and other chemicals exhibited changes in aggressive behavior and predator avoidance escape behavior across generations, indicating heritable genetic or epigenetic mechanisms that could have very long-lasting consequences.

Behavioral changes can be due to sensory system dysfunction, to physiological responses or to developmental effects. These changes can differ depending on when an organism is exposed to the chemicals. Chlorpyrifos exposure, at low doses such as measured in the environment, during development of the nervous system, can profoundly affect behavior and susceptibility to disease later in life. Chlorpyrifos at levels below those required to inhibit the intended target acetylcholinesterase, affect the neuroendocrine regulation of development of sexually dimorphic behaviors in rodents. When exposed pre-natally to chlorpyrifos, adult female mice were less aggressively protective of their own nest, but exhibited enhanced maternal behavior towards the pups, as well as increased anxiety behavior [45]. Prenatally-exposed males exhibited increased aggressive behaviors and increased locomotory activity. These social behavior differences correlated with developmental differences in key neurotransmitter and neuroendocrine brain regions that persisted throughout the lifespan.

3.3. Courtship behavior

Pesticides can have substantial effects on reproductive success in non-target organisms, which could potentially have considerable negative impacts on wildlife. For example, the highly imperiled Buff-breasted sandpiper (*Tryngites subruficollis*) is a migratory shorebird that stops over in agricultural fields in the Rainwater Basin area of Nebraska [104]. During their spring migration stopover, these birds forage, rest and engage in social and courtship behaviors that could be impaired by exposure to pesticides. Migratory sea birds, including the albatross and petrel, have measurable levels of organochlorine pesticides in fatty tissues [105]. Flying ability was affected in homing pigeons exposed experimentally to environmentally relevant amounts of chlorpyrifos and aldicarb [106]. An experimental study of chronic low-dose exposure to the organophosphorous pesticide fenitrothion, affected several key reproductive behaviors in male three-spined stickleback fish [107]. Fenitrothion, which in low concentrations is a potent anti-androgen, caused a reduction in male nest-building and impairments in the zigzag courtship dance, both behaviors regulated by testosterone. Male spiders (*Rabidosa rabida*) exposed to agriculturally relevant concentrations of malathion were unable to perform key courtship behaviors effectively, leading to the males being killed by female spiders prior to copulation [108]. From birds to fish to non-target arthropods, increasing evidence suggests that non-target organisms suffer wide-spread behavioral impairments that could adversely affect population dynamics and success, which could broadly affect numerous ecosystems, even those far away from the site of use of the pesticides.

3.4. Maternal behavior

Low concentrations of many pesticides, including organophosphorous, organochlorine and carbamate compounds, affect diverse hormone-mediated systems, thus acting like endocrine disrupting chemicals. Even if a compound does not directly interact with steroid hormone receptors, many systems regulated or mediated by steroid hormones can be influenced via pesticide effects on signal transduction pathways, effects on gene expression or effects on metabolic pathways. Animals exposed to low, chronic levels of pesticides, far below the threshold for their intended effects, exhibit impairments in reproductive behaviors and other sexually dimorphic behaviors, particularly if animals are exposed to pesticides *in utero* or early in development. Chlorpyrifos exposure neonatally has been shown to inhibit DNA synthesis, neuronal differentiation and synaptogenesis in rats [109]. Long-term changes in sexually dimorphic behaviors measured in adult animals exposed perinatally to low levels of chlorpyrifos [110, 40] were associated with changes in the development of neural systems, including nontarget serotonergic systems [110]. Both males and females are affected, often in different ways. For example, adult female mice exposed to chlorpyrifos in utero had reduced anxiety behavior and reduced aggression, while adult males showed increased anxiety behavior and aggression. Pre-natally exposed adult females also showed changes in maternal behaviors towards their own young, including reduced nest defense, reduced anxiety in the presence of a strange male approaching the nest and increased licking and crouching of the litter [45]. Adult female mice exposed to the pesticide 2,4 D spent less time nursing their litters even while increasing licking of pups. As a result, litter growth was impaired by the overall reduced time spent nursing. Other animals show impairments in maternal behavior as a result of pesticide exposure: birds living in pesticide-contaminated areas showed parental neglect [111]. Impairments in maternal care can have long-term effects on the offspring, leading to trans-generational effects that are both physiological via developmental changes in the offspring and behavioral as a result of impairments in early socialization learning [45].

4. Acute versus persistent exposures

An important issue that has received little direct experimental attention is the differences in response to an environmental contaminant when exposed acutely as compared to living an entire lifespan in the presence of the contaminant. When organisms or populations of organisms are exposed to chemicals throughout their lifespan, physiological and genetic adaptations are possible that would reflect different behavioral effects than in organisms newly exposed to a compound. For example, we found that adult nematodes exposed for 6 hours to Mancozeb exhibited profound locomotory impairment, becoming paralyzed or barely moving and curling up tightly. In addition, their bodies appeared swollen. However, with a 24 hour exposure, those surviving worms exhibited normal locomotory behavior [98] and their bodies were not swollen or misshapen. Offspring exposed throughout development behaved normally as adults (Raley-Susman et al, unpublished observations). A similar result was seen in *Daphnia magna* exposure for two generations to an environmentally relevant mixture of pesticides [112]. Many studies have demonstrated that exposure during development to low levels of pesticides that can act as endocrine disrupters leads to life long changes in neuroendocrine function, reproductive and sexually dimorphic behaviors [36]. There is also increased risk of developing cancer in childhood or adulthood [113].

Several studies have reported persistent behavioral changes, changes that appear to be independent of targeted effects on acetylcholinesterase inhibition, in rodents exposed to low doses of organophosphate pesticides [114] or carbamate fungicides [115]. Repeated exposures to doses of chlorpyrifos that are below threshold for acetylcholinesterase inhibition caused attention deficits and increased impulsive behavior in rats [116]. Further, long-term exposure of zebra fish, *Danio rerio*, to sublethal concentrations of parathion in the water, led to an increase in general motor activity and food consumption [117]. The behavioral changes occurred in the absence of overt neurological impairment [118] in one study of chronic exposure of mice to rotenone.

5. Mixtures

Most organisms, including humans, are exposed to a complex mixture of many different pesticides at once, at varying concentrations and for varying lengths of time. Most freshwater sources contain hundreds of measurable human-produced chemicals. Of the sixteen contaminants present at the highest concentrations in Lake Michigan, eleven of them are pesticides, including diazinon, chlorpyrifos, endosulfan, melathion, atrazine, permethrins, dichlorvos, manganese, zinc, imidacloprid and naphthalenes [119]. Given that the Great Lakes provide 20% of the Earth's freshwater supply, the effects of these contaminants on the organisms that depend on this water are of critical importance to understand. Depending on the concentrations organisms are exposed to, the mechanisms of action can vary from effects on neuromuscular function to effects on other neurotransmitter systems, hormone systems, cell growth and metabolism. In addition, mixtures could act synergistically or additively. For example, a combination of three common fungicides used routinely in the wine industry acted synergistically at low concentrations to cause greater neurotoxicity than the compounds exerted individually [120]. In another study, long-term exposure to low dose concentrations of two different pesticides, chlorpyrifos and the pyrethroid deltamethrin, caused multiple effects in rat brain tissue. Some effects were additive and some were synergistic [121]. Similarly, azole fungicides have been shown to enhance the toxicity of pyrethroid insecticides to aquatic invertebrates both under laboratory and field conditions [122]. Further, atrazine has been shown to enhance the toxicity of organophosphorus pesticides [123]. Much more work is needed to understand the effects of the complex mixtures of different pesticides and other human contaminants on organismal behavior, fitness and health. It is abundantly clear, however, that the extensive use of pesticides is altering the behavior and possible fitness of many organisms across the globe and in all ecosystems. Because of the persistence of these compounds and the long-term consequences of exposure, some of which are trans-generational, we need to acknowledge that life in a world of pesticides is the new normal.

6. Summary and conclusions

Behavioral neurotoxicological and behavioral ecotoxicological approaches need to take prominence in evaluating the short and long-term consequences to wildlife and humans of pesticide use. Behavior can reveal much about the systems and processes affected by pesticides, as well as the mechanisms by which pesticides exert those effects. Behavioral change is often the earliest sign of harmful effects of pesticides, and even low doses of pesticides or pesticide mixtures can lead to long-term behavioral change, particularly when exposures occur during developmental sensitive periods. Because we are living in a world with measureable mixtures of pesticides and other human-produced chemicals, it is essential that more work be done to understand how animals, including humans, are living and behaving in this new surrounding milieu.

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Chapter 7

Lethal and Sublethal Effects of Pesticides on Aquatic Organisms: The Case of a Freshwater Shrimp Exposure to Roundup®

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Additional information is available at the end of the chapter

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1. Introduction

In the last few decades, rapid human population growth with its concomitant astronomical increase in urbanisation, industrialisation and technology has had its toll on natural resources of the world. Climate change, acid rain, nutrient enrichment of aquatic environments, pollution by pesticides, metals, and synthesised toxic substances on local, regional and global scales are the result of such anthropogenic disturbances. Recent events, as witnessed the world over such as large scale mortality of wildlife (e.g. sea mammals, birds), increasing menace to human health (e.g. cancerous cells, chronic respiratory disease, damage to organs such as brain, lung, heart, liver, kidneys) and algal bloom in many water bodies are all effects of the anthropogenic perturbations of the biosphere. The biosphere is part of the earth that supports life. It comprises of the lithosphere, hydrosphere and atmosphere. The hydrosphere is the total mass of water on planet Earth, which includes oceans, lakes, streams, groundwaters and glaciers. Saline water account for 97.5 % while freshwater accounts for 2.5 %. The bulk of freshwater, 68.7 %, is stored in ice and permanent snow cover, while 29.9 % exists as groundwater. Only 0.26 % is found in lakes, river systems and reservoirs [1]. However, among all the components of hydrosphere, freshwater ecosystems are the most vulnerable to pollution due to anthropogenic stresses [2-3]. Agricultural, industrial and domestic activities are the major sources of this pollution [4]. These activities use more than one-third of the Earth's accessible freshwater resources and have contaminated water with numerous synthetic and geogenic compounds [4]. For instance, about 300 billion kilograms of synthetic compounds used in industrial, consumer and agricultural products find their way into natural freshwater systems every year

[5]. Ten percent of the globally accessible runoff is used, generating a stream of wastewater, which flows or seeps into groundwater, rivers, lakes, or the oceans [5].

The use of agrochemicals is necessary to control pests and increase yields in order to produce adequate food for the global population, estimated at 6.8 billion in 2009 [5], and recently reported to have reached 7 billion [6]. Underdeveloped countries, where 1.02 billion people (15 %) are undernourished and 1.3 billion people (19 %) live on an inadequate diet [5], need an adequate food supply. However, the agricultural sector's annual application of over 140 billion kilograms of fertilizers and large amounts of pesticides creates massive sources of diffuse pollution of freshwater systems [4]. The presence of these toxic chemicals in both aquatic and terrestrial ecosystems has become an important issue globally. Growing research-based evidence shows that pesticides, metals and many industrial chemicals interfere with the health and normal functioning of the endocrine systems of a wide range of organisms, including humans [7-9]. It is believed that effects of these chemicals on the normal functioning of the endocrine system are responsible for a number of developmental anomalies in a wide range of species, from invertebrates to higher mammals [10-13].

2. Pesticides

2.1. Pesticide pollution in ecosystems

Pesticides are substances or mixture of substances designed to control, repel, mitigate, kill or regulate the growth of undesirable biological organisms. These undesirable biological organisms (pests) do not only compete with humans for food, transmit diseases and destroy property, but are generally nuisance. Pests include insects, plant pathogens, weeds, molluscs, fish, birds, mammals, nematodes and microorganisms such as bacteria and viruses. Pesticides may be classified as being biological or synthetic. Biological pesticides are derived from natural sources such as extracts from plants (e.g. pyrethrin insecticide from chrysanthemum plants and azadriachtin from neem trees). Majority of pesticides are synthetic as they are made through industrial processes. A pesticide may also be classified as broad-spectrum when used to control a wide range of species or as narrow-spectrum when used to control a small group of species. However, the most common classification of pesticides is based on the type of pest they are used to control. These include insecticides (control insects), herbicides (control weeds) and fungicides (control fungi). Pesticides are used in agriculture to maintain high production efficiency and there is a constant demand for stable crop production to support the growing human population. Therefore, use of pesticides is expected to increase in the near future [14]. However, their use is an environmental hazard and can affect non-targeted organisms, other than the targeted pests [15].

Pesticide pollution affects both aquatic and soil ecosystems. Factors that promote pesticide pollution include drainage patterns, properties of the pesticide, rainfall, microbial activity, treatment surface and rate of application. Pesticides are able to move from one ecosystem to another through processes such as transfer (mobility) and transformation (degradation). Transfer may occur through surface runoff, vapourization to atmosphere, sorption (adsorption/desorption), plant uptake or soil water fluxes. Transformation occurs through chemical, microbial and photo-degradation [16]. A risk to a water body by a particular pesticide is dictated by the unique properties of the pesticide. For example, half-life, mobility and solubility are three properties of pesticides which determine their specific effects.

Although pesticides are used on a local scale, their effects are ubiquitous and can be felt regionally and globally [17]. They are transported into aquatic systems through processes such as direct applications, surface runoffs, spray drifts, agricultural returns and groundwater intrusions; either as single chemicals or complex mixtures [18]. The transportation of pesticides to their final destination in the aquatic ecosystem may result in adverse health effects on the organisms found there. All members that form the different communities of an ecosystem, from the smallest invertebrates to birds and humans, are affected by pesticides. Most toxic pesticides in urban and agricultural settings are responsible for the deaths of many birds, fish and zooplanktons that fish depend on for food [19]. It has been reported that pesticides contaminate many breeding sites of amphibians and that some of them may persist in the environment for a very long time even at lower concentrations. Some effects of pesticides only become highlighted after long term exposure. For example, the survival patterns for early green frogs and late wood frogs are affected only after 24 days of exposure to atrazine [17].

2.2. Herbicides: weed control pesticides

Weeds are plants that grow in places people do not wish them to grow because they compete with “beneficial and desirable” plant species. Until the last century, much of the energy used in farming went into removing weeds to provide suitable conditions for efficient cropping. However, during the industrial revolution, more people moved to work in factories, thus creating a shortage of labour on farms and it became necessary to develop more efficient ways to control weeds [5]. Herbicides are chemical substances used to suppress or kill unwanted vegetation (weeds). They are only one of the many types of pesticides that include insecticides, fungicides, rodenticides and nematocides [5]. Herbicides may be classified based on the time of application: pre-plant herbicides are applied to the soil before the crop is planted; pre-emergence herbicides are applied to the soil after the crop is planted, but before the crop or weeds emerge; post-emergence herbicides are applied to both crop and weeds after they have germinated and emerged from the soil. Herbicides may also be classified by the way they kill or suppress plants. These include hormone inhibitors, cell division inhibitors, photosynthesis inhibitors, pigment synthesis inhibitors, lipid synthesis (cell membrane) inhibitors, or cell metabolism (e.g. amino acid biosynthesis) inhibitors [20].

All herbicide products have chemical properties that influence their ability to suppress growth or kill plants. While some of these properties are inherent in the chemical nature of the herbicides, others are added to enhance their efficacy. The following are some chemical properties of herbicides that influence their use:

- *Chemical structure*: The biologically active portion of a herbicide product is the *active ingredient*. It is the fundamental molecular composition and configuration of the herbicide.

The physical and chemical properties of a herbicide can also determine the method of application and use.

- *Water solubility and polarity*: Herbicides that are produced as salts dissolve quite well in water and are usually formulated to be applied in water, while non-polar herbicide sources are not. Water is the main substance used to disperse (spray) herbicides, and hence the water solubility of a herbicide influences the type of product that is formulated, how it is applied and the movement of the herbicide in the soil profile.
- *Volatility*: Herbicides with a high vapour pressure volatilise easily, while those with a low vapour pressure are relatively non-volatile. The volatility of a herbicide can determine the mode of action and the herbicide's fate in the environment.
- *Formulations*: Commercial herbicide products contain an active ingredient and "inert" ingredients. An "inert" ingredient could be a carrier that is used to dilute and disperse the herbicide (e.g. water, oil, certain types of clay, vermiculite, plant residues, starch polymers, certain dry fertilizers) or an adjuvant (e.g. activator, additive, dispersing agent, emulsifier, spreader, sticker, surfactant, thickener, wetting agent) that enhances the herbicide's performance, handling, or application [20]. In recent years, carriers and adjuvants have been implicated in adding to the toxicity of the active ingredients, and in some cases, have been even more toxic than the active ingredient alone [20].

Before herbicide products are registered for use, the registration authorities require experimental information on their toxicology, biology, chemistry, and biochemical degradation in addition to their effect on air and water quality, soil microorganisms, and wildlife. Although commercial herbicide products contain several different ingredients, toxicity tests are usually conducted only on the active ingredient, which is the component of the product believed to actually affect the target organism [20]. The criteria for assessing the possible effects of herbicides on the safety of humans, animals and the environment are the herbicide's toxicity (including carcinogenicity, mutagenicity, endocrine disruption, reproduction and developmental abnormalities), biomagnification, and persistence in the environment ([20].

Given the scarcity of water resources in South Africa, aquatic herbicides are of special interest. The potential of an aquatic herbicide to adversely affect aquatic organisms depends on its inherent toxicity to the specific organism and the organism's exposure to the compound in terms of concentration and duration [21]. The inherent toxicity of the pesticide, which is due to its mode of action, is a specific relationship between the organism and the chemical, whereas factors such as application rates and techniques, chemical and physical properties of the pesticide, and environmental conditions at the time of application can make exposures highly variable.

Herbicides now lead all other pesticide groups in terms of amount produced, total acreage treated, and total value from sale. Over the past decades, public awareness of the worldwide increase in the use of herbicides and their adverse effects on aquatic ecosystems has been growing [22]. Herbicides may reach water bodies directly by overhead spray of aquatic weeds, or indirectly through processes such as agricultural runoff, spray drift and leaching. Potential problems associated with herbicide-use include injury to non-target vegetation, injury to crops,

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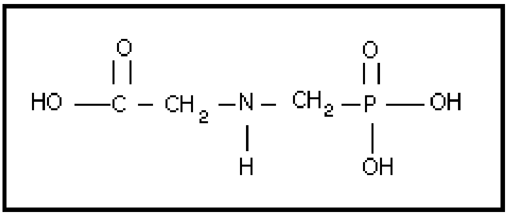

Figure 1. Molecular structure of N-(Phosphonomethyl) glycine

residue in soil or water, toxicity to non-target organisms, and concerns for human health and safety [20]. Herbicides can decrease environmental water quality and ecosystem functioning by reducing species diversity, changing community structure, modifying food chains, altering patterns of energy flow and nutrient recycling, and reducing resilience of ecosystems, among others [22].

3. Roundup®

3.1. Glyphosate-based herbicides

Glyphosate-based herbicides are the world's leading post-emergent, organophosphonate systemic, broad-spectrum and non-selective herbicides for the control of annual and perennial weeds [22]. Roundup® is the major glyphosate-based herbicide in which glyphosate (the active ingredient) is formulated as isopropylamine (IPA) salt, polyoxyethylene amine (POEA) (a surfactant), and water. Other formulations (e.g. Rodeo®) contain the IPA salt of glyphosate without POEA, and in some countries are primarily used for controlling aquatic weeds [23-24]. Other trade names of glyphosate-based herbicides include Roundup®, Roundup Ultra®, Roundup Pro®, Accord®, Honcho®, Pondmaster®, Protocol®, Rascal®, Expedite®, Ranger®, Bronco®, Campain®, Landmaster®, Fallow Master® and Aquamaster® by Monsanto; Glyphomax®, Glypro® and Rodeo® by Dow Agrosciences; Glyphosate herbicide by Du Pont; Silhouette® by Cenex/Land O'Lakes; Rattler® by Helena; MirageR® by Platte; JuryR® by Riverside/Terra; and Touchdown® by Zeneca [25].

Glyphosate is an aminophosphonic analogue of the natural amino acid glycine [22].The International Union of Pure and Applied Chemistry's (IUPAC) name for glyphosate is N-(phosphonomethyl) glycine and the Chemical Abstracts Service (CAS) registry number is 1071-83-6. The glyphosate molecule has several dissociable hydrogens, especially the first hydrogen of the phosphate group (Figure 1). Thus, a typical glyphosate molecule is an acid, and is often referred to as the technical grade glyphosate.

Technical-grade glyphosate has a relatively low solubility in water (12 g/L at 25° C and 60 g/L at 100° C), and is insoluble in other solvents because of strong intermolecular hydrogen bonds that stabilise the crystal lattice [26]. For this reason, commercial herbicide formulations contain glyphosate in the form of salt, which has much higher solubility but still maintains the herbicidal properties of the parent compound [22]. Formulations of glyphosate in salt form include monoammonium salt, diammonium salt, isopropylamine salt, potassium salt, sodium salt, and trimethylsulfonium or trimesium salt. Of these, the isopropylamine, sodium, and monoammonium salt forms are commonly used in formulated herbicide products [27].

The isopropylamine salt is the most commonly used in commercialised formulated products (e.g. Roundup®). The physical and chemical properties of glyphosate acid and two of its salt forms are listed in Table 1. The concentration of glyphosate is commonly expressed as mg a.i./L (active ingredient/Litre) or mg a.e./L (acid equivalents/Litre) [22]. Acid equivalent is the theoretical percent yield of parent acid from a pesticide active ingredient, which has been formulated as a derivative (usually esters, salts or amines) [28].

Active ingredient	Form	Vapour pressure	Henry's constant	Molecular weight	Solubility in water	Log K_{ow}	K_{oc}
Glyphosate acid	Odourless, white solid	1.31×10^{-2} mPa (25° C); 1.84×10^{-7} mmHg (45° C)	4.08×10^{-19} atm· m^3/mol	169.07 g/mol	pH 1.9: 10,500 mg/L; pH 7: 157,000 mg/L	< -3.2	300 - 20,100
Glyphosate Isopropylamine salt	Odourless, white solid	2.1×10^{-3} mPa (25° C); 1.58×10^{-8} mmHg (25° C)	6.27×10^{-27} atm· m^3/mol	228.19 g/mol	pH 4.1: 786 ,000 mg/L	-3.9 or -5.4	300 - 20,100
Glyphosate ammonium salt	Odourless, white solid	9×10^{-3} mPa (25° C); 6.75×10^{-8} mmHg (25° C)	1.5×10^{-13} atm· m^3/mol	186.11 g/mol	pH 3.2: 144,000 mg/L	-3.7 or -5.3	300 - 20,100

Table 1. Physical and chemical properties of glyphosate acid, glyphosate isopropylamine salt, and glyphosate ammonium salt [27]

3.2. Mode of action of glyphosate

As a systemic herbicide, glyphosate is readily translocated through the phloem to all parts of the plant. Glyphosate molecules are absorbed from the leaf surface into plant cells where they are symplastically translocated to the meristems of growing plants [22]. Glyphosate's phytotoxic symptoms usually start gradually, becoming visible within two to four days in most annual weeds, but may not occur until after seven days in most perennial weeds. Physical phytotoxic symptoms include progress from gradual wilting and chlorosis, to complete browning, total deterioration and finally, death [22]. The primary mode of action of glyphosate is confined to the shikimate pathway aromatic amino acid biosynthesis, a pathway that links primary and secondary metabolism.

Shikimate (shikimic acid) is an important biochemical intermediary in plants and microorganisms, such as bacteria and fungi. It is a precursor for the aromatic amino acids phenylalanine, tryptophan and tyrosine. Other precursors of the shikimate pathway are indole, indole derivatives (e.g. indole acetic acid), tannins, flavonoids, lignin, many alkaloids, and other aromatic metabolites. The biosynthesis of these essential substances is promoted by the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), the target enzyme of glyphosate (Figure 2). This enzyme is one of the seven enzymes that catalyse a series of reactions, which begins with the reaction between shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP). The shikimate pathway accounts for about 35 % of the plant mass in dry weight and therefore any interference in the pathway is highly detrimental to the plant. Glyphosate inhibits the activity of EPSPS, preventing the production of chorismate, the last common precursor in the biosynthesis of numerous aromatic compounds in bacteria, fungi and plants. This causes a deficiency in the production of the essential substances needed by the organisms to survive and propagate [22, 29]. The pathway is absent in animals, which may account for the low toxicity of glyphosate to animals.

However, acute effects in animals, following intraperitoneal administration of high glyphosate doses suggest altered mitochondrial activity, possibly due to uncoupling of oxidative phosphorylation during cellular respiration [26]. In summary, glyphosate ultimately interrupts various biochemical processes, including nucleic acid synthesis, protein synthesis, photosynthesis and respiration, which are essential life processes of living things.

3.3. Environmental fate of glyphosate

Glyphosate has a strong soil adsorption capacity, which limits its movement in the environment. The average half-life of glyphosate in soil is two months, but can range from weeks to years [25]. The presence of glyphosate in water systems may be due to runoff from vegetation surfaces, spray drift, and intentional or unintentional direct overspray, with an average half-life of two to ten weeks [25]. Glyphosate is susceptible to chemical and photo-degradation, although microbial degradation is the primary dissipation mechanism in soils. The rate of degradation in water is generally slower than in most soils because of fewer microorganisms in water than in soils [30]. When glyphosate degrades, it produces aminomethylphosphonic acid (AMPA) and carbon dioxide [31], both of which reduce pH when dissolved in water. However, pH is known to affect the stability of glyphosate in water. For instance, glyphosate did not undergo hydrolysis in buffered solution with a pH of 3, 6, or 9 at 35° C, while insignificant photodegradation has been recorded under natural light in a pH 5, 7, and 9 buffered solutions [27]. In natural water systems, glyphosate dissipates through degradation, dilution, and adsorption on organic substances, inorganic clays and the sediment (the major sink for glyphosate in water bodies) [25, 30]. With its long half-life and its ability to cause the death of organisms in aquatic systems, it is recommended that glyphosate should be used as an aquatic herbicide to treat only one-third to half a water body at any one time [25].

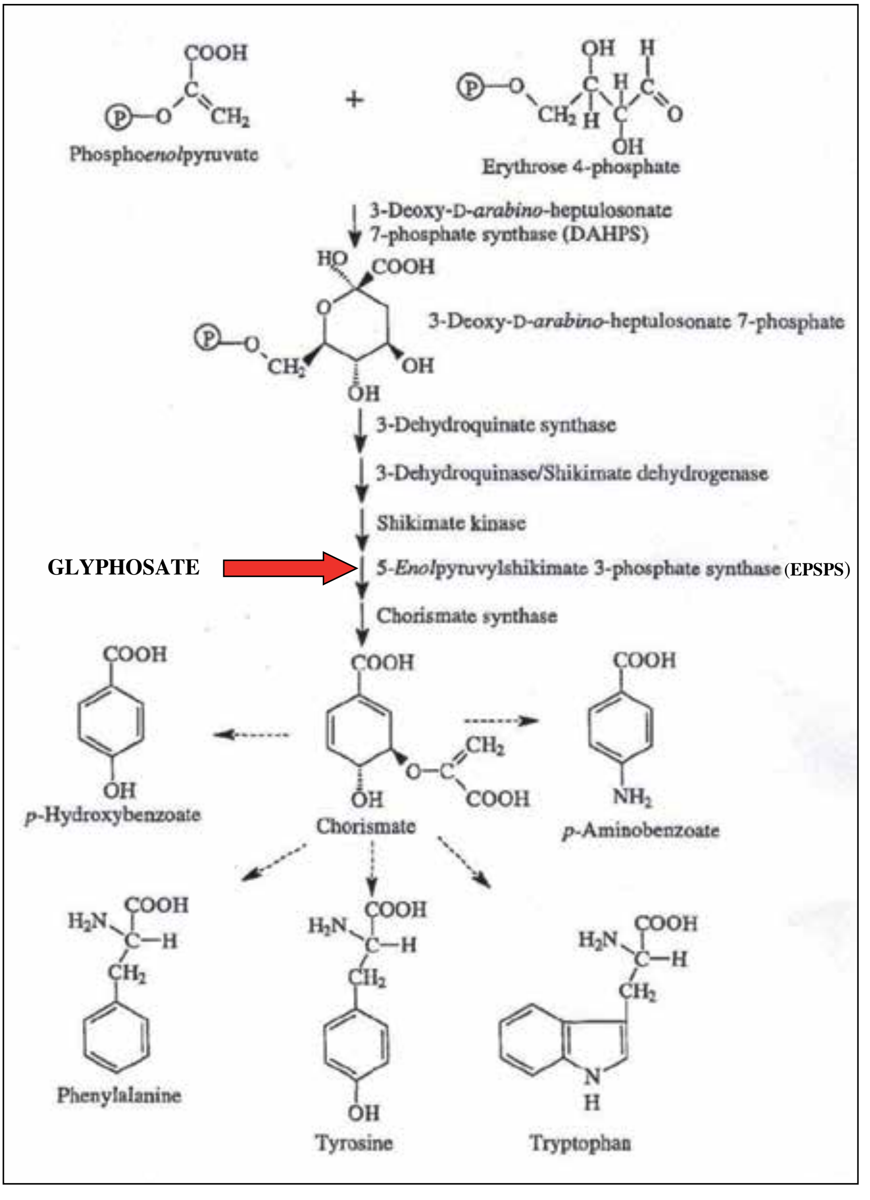

Figure 2. Glyphosate mode of action in plants with red arrow pointing to the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase (modified from [32])

3.4. Toxicology of glyphosate

Ecotoxicologists are greatly concerned about the exposure of non-target aquatic organisms to glyphosate formulations because of its high water solubility and the extensive use of glyphosate-based herbicides in the environment, especially in shallow water systems [23]. The surfactant polyoxyethylene amine (POEA) is thought to be responsible for the relatively high toxicity of Roundup® to several freshwater invertebrates and fishes, although isopropylamine (IPA) salt of glyphosate also contributes its share [23, 33]. Technical grade glyphosate is slightly to very slightly toxic, with reported LC50 values of greater than 55 mg/L and a 21 d NOEC value of 100 mg/L.

Conversely, formulations of glyphosate are moderately to very slightly toxic with 2 d EC50 values of 5.3-5600 mg/L and 21 d MATC values of 1.4-4.9 mg/L reported [26]. The LC50 values also determine which glyphosate formulation can be applied in aquatic systems. For example, Touchdown 4-LC® and Bronco® have low LC50s for aquatic species (<13 mg/L), and are not registered for aquatic use, while Rodeo® has relatively high LC50s (>900 mg/L) for aquatic species and is permitted for use in aquatic systems. In the same manner, Roundup® is not registered for use in aquatic systems in the United States because its 96-hour LC50 for *Daphnia* is 25.5 mg/L, while that of glyphosate alone is 962 mg/L [25].

3.5. Effects of glyphosate-based herbicides on aquatic animals

Glyphosate-based herbicides are used globally to control both aquatic and terrestrial weeds. In recent years, its use has increased tremendously and is likely to impact on non-target organisms in the environment. Even though it is generally regarded as having a low potential for contaminating surface waters due to its perceived rapid dissipation and strong adsorption to soils and sediments, it has been detected in surface waters long after being used to kill aquatic weeds [34]. In fact, its mode of action was designed to affect only plants [29], but various studies in recent years have reported adverse impact on non-target animals [23, 33, 35]. These impacts could be lethal or sublethal. Lethal effects are mainly mortality and immobility endpoint measures. However, there are several endpoint measures that can be used to assess sublethal effects. At the 'physical' level, measures of survival, growth, morphological changes, and behavioural changes exposed animals are used as endpoint indicators. Measures of reproductive performance that are often used to assess sublethal response include sexual maturity, time to first brood release, time required for egg development, fecundity, gonad histopathology, and alterations in reproductive characteristics. Biochemical measures used as possible endpoints to assess exposed animals include metabolic disruption, steroid metabolism, vitellogenin induction, lipid peroxidation, acetylcholinesterase activity, cytochrome P450enzymes and blood glucose levels.

4. Exposure effects

4.1. Classification of exposure effects

The effect caused by exposure to chemicals can be classified according to different exposure time (short-term or long-term) and exposure type (lethal or sublethal). Short-term exposure time is usually defined as not more than 96 h, while long-term exposure time defined as being more than 96 h (Table 2). There are different possibilities of effect to expect when animals are exposed to chemicals. Lethal exposure to stress can possibly cause a biological system to respond in short-term or long-term. Similarly, biological systems can experience sublethal responses to a stressor in short-term or long-term (Table 3). Lethal exposure will often result in mortality (i.e. immobility, decolouration and degeneration), whereas sublethal exposure normally results in a cellular, molecular or biochemical level response including growth (length, weight and moulting), reproduction (embryo and gonads) and biochemical (acetyl-cholinesterase and lipid peroxidation) (Table 3).

Exposure classification	Effect classification	Description
Exposure time	Short-term	≤ 96 h
	Long-term	≥ 96 h
Exposure type	Lethal	Mortality measure as endpoint
	Sublethal	Cellular/molecular/biochemical/physiological level measure as endpoint

Table 2. Exposure-effect classification of chemicals

Effect classification	Description
Short-term lethal	≤ 96 h and mortality measure as endpoint
Short-term sublethal	≤ 96 h and cellular/molecular/biochemical/physiological level measure as endpoint
Long-term lethal	≥ 96 h and mortality measure as endpoint
Long-term sublethal	≥ 96 h and cellular/molecular/biochemical/physiological level measure as endpoint

Table 3. Different possibilities of effect of animals exposed to chemicals

4.2. Effects of lethal exposure

Mortality is the most common endpoint measure when organisms are exposed to a lethal dose, although immobility is also considered as lethal effect of exposure. In [36] the relevance of using mortality and immobility as endpoints to reflect the toxicity of the organophosphorous insecticide chlorpyrifos in fourteen different freshwater arthropods was evaluated. Using dose response models and species sensitivity distributions (SSDs), they compared the differences in response dynamics during 96 h of exposure with these two endpoints across the different species. Their study suggests that freshwater arthropods vary less in their immobility response than in their mortality response. They suggested immobility as the relevant endpoint for SSDs and ERA (environmental risk assessment) because they found it was a more sensitive endpoint than mortality, with less variability across the tested species. Generally, effect concentrations for immobility and mortality will converge to the same value with time, but this does not occur with the same speed for all species [36]. However, a good match between effective (immobility) and lethal (mortality) concentrations can exist right from the start of a toxicity test where LC50/EC50 ratios equal one, approximately. For some species, the differences between LC50 and EC50 can remain relatively constant within the 96 h of testing. Furthermore, the extent to which LC50 and EC50 values differ for certain time points is species specific [36]. For example, exposure concentrations may not induce any significant incipient mortality in a particular species, but will induce immobility at very low concentrations in another species. This is due to differences in toxicokinetics and/or toxicodynamics between the species. For instance, differences in toxicokinetics may enable one species to decrease or regulate uptake and eliminate the test chemical, or detoxify it quickly, thereby significantly delaying incipient mortality. Toxicodynamic differences, such as differences in the interaction of the stressor and target enzyme, or in the ability to compensate or repair damage, may cause different species to respond differently to the test chemicals [36].

Mortality was also used as an endpoint response measure by [37] when they studied the acute mortality of adults and sub-lethal embryo responses of *Palaemonetes pugio* to endosulfan. Their findings suggest that the insecticide endosulfan may preferentially affect male grass shrimp, and exposed female grass shrimp may produce embryos with delayed hatching times. They suggested that the size difference between male and female grass shrimp might be the cause as mortality decreases by 25 % with a corresponding increase in size of 1 mm.

Some studies have reported correlation between lethal and sublethal effects. In [38], the correlation between 96 h mortality and 24 h acetylcholinesterase inhibition in three life stages of the grass shrimp (*Palaemonetes pugio*) after exposure to organophosphate pesticides was investigated. They found a strong positive relationship ($R^2 = 0.962$) between the ratio of the lowest observed effect concentration and 20 % effect concentration (LOEC/EC20). Therefore, they concluded that sublethal endpoints could be used as a predictor of 96 h mortality for the life stages of *P. pugio*.

4.3. Growth measures used as sub-lethal responses to exposure

Body weight and length are two direct measures of growth that may be used in the assessment of sub-lethal effects on arthropods. Simple dry weight can be determined by drying sampled animals at an average temperature of 60º C, and a mean drying time of 48 hours to constant weight [39]. However, for many invertebrates, ash-free dry weight (AFDW) is often used as the appropriate weight measurement because the method reduces any inaccuracies that might be introduced by inorganic constituents in the animal's body. Inorganic components may arise from processes such as the development of skeletal components, or from feeding (the ingestion of sediment) [39]. In small-sized crustaceans, such as caridean shrimps and mysids, the removal of ash from the dry weight measurement is unnecessary since it would have a negligible effect on the accuracy of the measurement [40]. Separate determinations should be made for male and female crustaceans because they might be different sizes [39]

Different body length dimensions of shrimp can be measured to determine growth. These may include the distance from the base of the eye-stalks to the tip of the telson or to the tip of the exopod; or from the tip of the carapace to the tip of the exopod along the midline of the body [39]. Sometimes, it is difficult to measure preserved animals because of the body curvature that results from the fixation process. Relaxing the animal and then determining length as the sum of a series of relatively straight-line measurements prior to fixation may reduce inaccuracy. Animals may be anaesthetized in soda water to relax them prior to length measurements [41-42].

Reduced growth may not be a particularly sensitive endpoint, but it is the most common response to sub-lethal exposure to toxicants [39]. Reduced growth is connected to reproductive success since the size of female crustaceans is directly related to fecundity [43]. The age of test animals and the toxicant concentration are related to the effect of toxicant exposure on growth. In general, young crustaceans are more sensitive than adults to toxicant exposure. However, effects of toxicants on juvenile survival do not always lead to reductions in population growth rate since survivors may compensate for the lost individuals by increasing their own reproduction [44]. Similarly, effects at the individual level may sometimes run opposite to the population level effects. This shows complex relationship between toxicant effects on individual performance versus population dynamics. [44] evaluated the effects of nonylphenol on two life-history traits (i.e. juvenile survival and fecundity) of the parthenogenetic springtail, *Folsomia candida,* in relation to population growth rate. They reported that the presence of nonylphenol stimulated fecundity and the body-growth rate of test organisms, but did not affect population growth rate. The authors found that the effect of the test chemical on fecundity was the main contributor of the observed effect on growth rate. However, since relative sensitivity of fecundity (elasticity) was very low, large changes in fecundity resulted in a minimal effect on population growth rate. Conversely, juvenile survival had higher elasticity, but was not affected by nonylphenol, and hence did not contribute to effects on population growth rate. The study by [44] revealed that increase in body size and fecundity after exposure to chemicals does not necessarily translate into increase in population growth rate. Their study also shows that effects of chemicals on individual life-history traits are attenuated at the population level and that population growth rate is an appropriate endpoint for ecotoxicological studies.

Moulting is an important physiological process in arthropods because it allows them to grow [45-46]. It is regulated by the interaction of moult stimulating hormones (MSHs, generally referred to as ecdysteroids), and nervous system secretions produced in the cephalothorax, and with moult-inhibiting hormones (MIHs) produced in the eyestalks [39]. In higher crustaceans such as the Malacostraca, paired cephalic endocrine organs called Y-organs (absent in lower crustaceans such as Entomostraca) secrete three different ecdysteroids, namely ecdysone (E), 25-deoxyecdysone (25dE), and 3-dehydroecdysone (3DE). Usually these organs produce either E + 25dE, or E + 3DE. Activities of the Y-organ are regulated mainly by the MIH, an inhibitory neuropeptide secreted from the X-organ-sinus gland complex [45-46]. Since hormones regulate moulting in crustaceans, moulting is a clear indicator of the adverse effects of endocrine disrupting chemicals, which include many pesticides. Hormonal regulation of moulting in crustaceans makes the process vulnerable to the adverse effect of endocrine disrupting chemicals (EDCs), including many pesticides [39]. Furthermore, since substantial growth in crustaceans can only occur as a result of moulting, any disruption in the moulting process could affect growth. Therefore, estimation of moulting frequency may be a useful endpoint.

Moult stage is a useful technique for measuring growth [39]. If moult stages are classified based on duration of different stages under normal laboratory conditions, then the environmental effects on relative duration of stages can be evaluated, using the moult-stage technique [47].

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However, moult-cycle chronology is a prerequisite for the use of moult staging in growth studies. The moult-stage technique was used to determine the main moult stages for juveniles and young adults of *Mysis mixta* and *Neomysis integer* under different temperature conditions and feeding. The technique was also used in the field to determine the moult cycle duration of *Mysis mixta* [47].

4.4. Reproductive measures used as sublethal responses to exposure

Embryotoxicity and gonad histopathology are two main reproductive measures used as sublethal responses to exposure. Embryo development time (or incubation period) in caridean shrimps is measured as the number of days between the first appearance of embryos in the brood pouch and the first release of neonates. In uncontaminated systems, incubation period is related to environmental temperature, salinity and an interaction between the two factors. However, the effect of most contaminants is to lengthen hatching time. In many embryotoxicity studies, either gravid females are placed in exposure containers, or fertilized eggs are removed from the female and placed in exposure containers where they develop to hatching. In [48], both gravid maternal and isolated embryos of *Daphnia magna* were exposed to the agricultural fungicide fenarimol to evaluate embryo development and susceptibility to the anti-ecdysteroidal properties of the fungicide. They reported that exposure of either gravid maternal animals or isolated embryos to the test toxicant resulted in embryo abnormalities which ranged from early partial developmental arrest to incomplete development of antennae and shell spines. They found that such developmental abnormalities were linked to suppressed ecdysone levels in the embryos and that the abnormalities could be prevented by co-exposure to 20-hydroxyecdysone. The results also showed how environmental anti-ecdysteroids, such as fenarimol, in many agro-chemicals disrupt the normal development of crustacean embryos.

Effective embryotoxicity investigations are based on identification of specific developmental features during embryogenesis and the susceptibility of such features to chemical exposure. Embryonic development of *C. nilotica* under laboratory conditions was investigated by [49] and identified stages in embryonic development which could be used as quantifiable experimental endpoints in toxicity tests. The author identified and described seven potential developmental stages that could be used in toxicity tests to study exposure-response relationships to stressors.

Histopathology is a technique that combines knowledge and experience of fundamental animal anatomy, physiology, endocrinology, pathology, and toxicology. It can enhance relevant biological information in sublethal exposure tests by allowing proper and more specific hazard identification, such as the organs targeted by toxic substances and mechanisms of action in aquatic ecotoxicological studies [50]. Histopathology is relevant to an ecological assessment of toxicants because it can detect critical adverse biological effects (e.g. reproductive abnormality) and is more sensitive than the classical toxicological testing, since histological effects are visible at lower exposure concentrations than they are at toxicological endpoints, such as mortality or behavioural changes [50]. The use of small crustaceans in practical histopathology makes it possible to embed the animals *in situ* for a quick overview of various relevant organs, making screening fast and comprehensive [50].

4.5. Biochemical measures used as sub-lethal responses to exposure

Acetylcholinesterase activity (AChE) and lipid peroxidation (LPx) are two biochemical measures often used as to assess sublethal responses to exposure. The main physiological function of the enzyme AChE is to hydrolyze acetylcholine (ACh), a neurotransmitter of cholinergic synapses during transduction of nerve impulses. Inhibition of AChE prevents the hydrolysis of ACh in nerve synapses and neuromuscular junctions, causing accumulation of excess ACh at these sites. This results in over-excitation of the synaptic and muscular tissues, which may lead to abnormal behaviours such as hyperactivity, asphyxia and death. AChE activity is therefore regarded as a good biomarker to detect a range of toxic compounds in aquatic animals, including insecticides, herbicides, surfactants and metals [51-52]. In a study to evaluate AChE activity in the oyster *Crassostrea corteziensis*, [53] exposed the organisms to the pesticide dichlorvos. The results of their study revealed that AChE activity was 65 % lower in oysters exposed to the pesticides than in control animals. Based on this outcome, they suggested using AChE activity in oysters as early biomarkers of effects and exposure to pesticides in aquatic environments. Similar observations and suggestions were made when the mosquito fish *Gambusia affinis* was exposed to the pesticide chlorpyrifos [54]. Although AChE is used as a classical biomarker in biomonitoring studies with regard to the exposure of a number of organophosphate and carbamate pesticides, recent studies have shown the existence of sublethal effects of glyphosate-based compounds on biomarkers of neurotoxicity including AChE [33, 55-56]

Lipid peroxidation is a recognised mechanism of cellular injury in plants and animals, and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides are unstable and decompose to form a complex series of compounds which include reactive carbonyl compounds. Polyunsaturated fatty acid peroxides decompose to produce malondialdehyde (MDA) and 4-hydroxyalkenals (HAE), and the measurement of MDA and HAE is used as an indicator of lipid peroxidation. Whether cells and tissues are susceptible to oxidative stress when exposed to pesticides reflects the balance between oxidative stress and the anti-oxidant defence capability. Since free radicals and hydroperoxides are potentially harmful, toxicants that stimulate lipid peroxidation and/or weaken anti-oxidant defence capability may cause or increase cellular susceptibility to oxidative damage. Animals exposed to pesticides may have their anti-oxidant defence capabilities directly or indirectly modified, rendering them susceptible to oxidative stress. Oxidative damage of cells and tissues of animals exposed to pesticides may be the result of insufficient anti-oxidant potential [57]. Developing biomarkers of oxidative stress as a pollution-mediated mechanism of toxicity requires knowledge of how anti-oxidant biochemical systems and target molecules are influenced by test toxicants [58].

Different toxicants may produce different anti-oxidant/pro-oxidant responses in organisms, depending on whether the organism can produce reactive oxygen species and anti-oxidant enzymes to detoxify them. Changes in juveniles of the freshwater crustacea *Daphnia magna* anti-oxidative processes in were assessed by [58] after exposure to paraquat and endosulfan in a 48 h sublethal toxicity test. They evaluated lipid peroxidation and activities of key anti-oxidant enzymes including catalase, superoxide dismutase, glutathione peroxidase and glutathione S-transferases. They found that increased lipid peroxidation produced low antioxidant enzyme activity for endosulfan, while decreased lipid peroxidation enhanced levels of anti-oxidant enzyme activities for paraquat. In [59], the authors suggested that glyphosate exposure and metabolism in the liver of animals can lead to excessive production of MDA and oxidative stress through unregulated generation of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, hydroxyl radical, peroxyl radicals and singlet oxygen. Excessive ROS in turn can be detrimental to cell structure through oxidative damage of lipids, proteins or DNA, and altered regulation of gene functions critical for development, differentiation, and aging.

5. Lethal and sublethal effects of *Caridina nilotica* exposure to Roundup®

5.1. *Caridina nilotica*: a decapod freshwater shrimp

The exposure of non-target aquatic organisms to glyphosate-based herbicides is of great concern because of the high water solubility of glyphosate and its extensive use in the environment. Thus, it is important to investigate the effects of these bioactive chemicals on aquatic organisms. *Caridina nilotica* (Decapoda: Atyidae) (Figure 3) is the most common of four indigenous freshwater caridean species found in South Africa. It has been used in ecotoxicological studies in South Africa since the early 1990s. Roundups® was selected as a representative of glyphosate-based herbicides by the virtue of it being the most popular and widely used herbicide in South Africa and most parts of the world [60-61]. In this section of this chapter, summary of findings of some exposure tests are given to demonstrate lethal and sublethal effects of Roundup® to *C. nilotica* at different biological scales. Mortality was the lethal effect investigated, whereas the sublethal effects studied were growth, acetylcholinesterase activity and lipid peroxidation. The tests were all aimed at demonstrating the use of *C. nilotica* as an early detection sensor system of pesticides pollution in South African aquatic ecosystems. Comprehensive reports of these tests are reported in [62-65].

5.2. Short-term lethal tests — Mortality

The toxicity of the herbicide Roundup® was assessed using three different life stages of *C. nilotica*. Neonate (<7 days post hatching (dph)), juvenile (>7 dph and <20 dph) and adult (>40 dph) shrimps were exposed to varying concentrations of the herbicide in 48 and 96 h short-term lethal tests in order to determine the most sensitive life-stage. Mortality was calculated at the end of each test period. Based on this, Roundup® 48 h and 96 h LC50 (median lethal concentration) values and the associated 95 % confidence limits were calculated for *C. nilotica* using the probit method. The results showed neonates to be more sensitive to Roundup® than both juveniles and adults. The estimated 96 h LC50 of neonates is much lower than the field application rate, though the application's impact will depend on the dilution rate of the applied concentration in the environment. This study shows that low levels of the Roundup® may adversely affect *C. nilotica* health and survival. Thus, the herbicide should be carefully managed to minimize any negative impact on non-target freshwater organisms.

Figure 3. Adult *Caridina nilotica*

5.3. Long-term sublethal tests — Growth

The possible use of growth measures in *C. nilotica* as biomarkers of Roundup® pollution as part of aquatic life in South Africa were evaluated. Using static-renewal methods in a 25-d growth toxicity test, 40 dph shrimps were exposed to different sublethal Roundup® concentrations. Shrimp total lengths and wet weights were measured every fifth day. These data were used to determine the shrimp's growth performance and feed utilization in terms of percent weight gain (PWG), percent length gain (PLG), specific growth rate (SGR), condition factor (CF), feed intake (FI), feed conversion ratio (FCR) and feed conversion efficiency (FCE). Moulting was observed for 14 d and the data used to determine the daily moult rate for each concentration. Results of growth performance and food utilization indices showed that growth was significantly impaired in all exposed groups compared to control. Moulting frequency was also higher in all exposed groups than in control. Although all the tested growth measures proved to be possible biomarkers of Roundup® pollution, moulting frequency gives a clearer indication of the sublethal effects of Roundup® toxicity to *C nilotica*.

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5.4. Short-term and long-term sublethal tests — Biochemical

The use of *C. nilotica* whole-body acetylcholinesterase (AChE) activity and lipid peroxidation as potential biomarkers of Roundup® pollution of aquatic ecosystems was investigated. Forty days post hatch (dph) shrimps were exposed to different concentrations of Roundup® in a 96 h short-term sublethal test and a 21 d long-term sublethal test. Shrimp whole-body AChE activities were determined at the end of the exposure periods by spectrophotometric assay of sample extract. Final AChE activities were expressed as nmol/min/mg proteins. Shrimp whole-body LPx was estimated by thiobarbituric acid reactive species (TBARS) assay, performed by a malondialdehyde (MDA) reaction with 2-thiobarbituric acid (TBA) measured spectrophotometrically. Final MDA concentrations were expressed as nmol MDA produced/mg protein. The results showed that AChE activity was concentration-dependent, with percent activity levels decreasing monotonically from control to the highest concentration. Conversely, LPx was significantly lower in control than in shrimps exposed to different Roundup® concentrations, increasing monotonically. The study provides ecotoxicological basis for the possible use of AChE activity and LPx in *C. nilotica* as possible biomarkers for monitoring effects of Roundup® pollution in freshwater systems.

6. Concluding remarks

In this chapter, the effects of rapid human population growth on aquatic ecosystems have been discussed. These effects are seen in such phenomena as climate change, nutrient enrichment of aquatic environments, and pollution by all types of chemicals including pesticides on local, regional and global scales. These anthropogenic disturbances adversely impact the normal functioning of organisms and are responsible for a number of developmental anomalies in a wide range of species; from invertebrates to higher mammals. It is expected that the use of pesticides, especially herbicides, will continue to increase and eventually becoming environmental hazard to non-target organisms at different biological scale levels unless proactive measures are taken. The case study, i.e. lethal and sublethal exposures of *C. nilotica* to varying environmentally relevant concentrations of Roundup®, showed that *C. nilotica* can be used as early detection system to assess glyphosate-based herbicides pollution effects on aquatic ecosystems.

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Chapter 8

Pesticides: Environmental Impacts and Management Strategies

Harsimran Kaur Gill and Harsh Garg

Additional information is available at the end of the chapter

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1. Introduction

Increase in food production is the prime-most objective of all countries, as world population is expected to grow to nearly 10 billion by 2050. Based on evidence, world population is increasing by an estimated 97 million per year (Saravi and Shokrzadeh, 2011). The Food and Agricultural Organization (FAO) of the United Nations has in-fact issued a sobering forecast that world food production needs to increase by 70%, in order to keep pace with the demand of growing population. However, increase in food production is faced with the ever-growing challenges especially the new area that can be increased for cultivation purposes is very limited (Saravi and Shokrzadeh, 2011). The increasing world population has therefore put a tremendous amount of pressure on the existing agricultural system so that food needs can be met from the same current resources like land, water etc. In the process of increasing crop production, herbicides, insecticides, fungicides, nematicides, fertilizers and soil amendments are now being used in higher quantities than in the past. These chemicals have mainly come into the picture since the introduction of synthetic insecticides in 1940, when organochlorine (OCl) insecticides were first used for pest management. Before this introduction, most weeds, pests, insects and diseases were controlled using sustainable practices such as cultural, mechanical, and physical control strategies.

Pesticides have now become an integral part of our modern life and are used to protect agricultural land, stored grain, flower gardens as well as to eradicate the pests transmitting dangerous infectious diseases. It has been estimated that globally nearly $38 billion are spent on pesticides each year (Pan-Germany, 2012). Manufacturers and researchers are designing new formulations of pesticides to meet the global demand. Ideally, the applied pesticides should only be toxic to the target organisms, should be biodegradable and eco-friendly to some extent (Rosell et al., 2008). Unfortunately, this is rarely the case as most of the pesticides are non-specific and may kill the organisms that are harmless or useful to the ecosystem. In general, it has been estimated that only about 0.1% of the pesticides reach the target organisms and the remaining bulk contaminates the surrounding environment (Carriger et al., 2006). The repeated use of persistent and non-biodegradable pesticides has polluted various components of water, air and soil ecosystem. Pesticides have also entered into the food chain and have bioaccumulated in the higher tropic level. More recently, several human acute and chronic illnesses have been associated with pesticides exposure (Mostafalou and Abdollahi, 2012). Below, we have detailed the effect of pesticides on target and non-target organisms including earthworms, predators, pollinators, humans, fishes, amphibians, and birds. Additionally, impact of pesticides on soil, water and air ecosystems is also discussed. Furthermore, an eco-friendly practice (Integrated Pest Management (IPM) approach) has been detailed as a strategy that could minimize the use of pesticides.

2. Effects of pesticides on target organisms

Over the past era there has been an increase in the development of pesticides to target a broad spectrum of pests. The increased quantity and frequency of pesticide applications have posed a major challenge to the targeted pests causing them to either disperse to new environment and/or adapt to the novel conditions (Meyers and Bull, 2002; Cothran et al., 2013). The adaptation of the pest to the new environment could be attributed to the several mechanisms such as gene mutation, change in population growth rates, and increase in number of generations etc. This has ultimately resulted in increased incidence of pest resurgence and appearance of pest species that are resistant to pesticides.

2.1. Pesticide resistance

"Resistance may be defined as a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species" (IRAC, 2013). Resistant individuals tend to be rare in a normal population, but indiscriminate use of chemicals can eliminate normal susceptible populations and thereby providing the resistant individuals a selective advantage in the presence of a pesticide. Resistant individuals continue to multiply in the absence of competition and eventually become the dominant portion of the population over generations. As majority of the individuals of a population are resistant, the insecticide is no longer effective thus causing the appearance or development of insecticide resistance.

Resistance is the most serious bottleneck in the successful use of pesticides these days. The intensive use of pesticides has led to the development of resistance in many targeted pest species around the globe (Tabashnik et al., 2009). Number of resistant insects and mite species had risen to 600 by the end of 1990, and increased to over 700 by the end of 2001. This trend is likely to be continued in 21^{st} century as well. Resistance has been found in different insecticides groups e.g., 291 species have developed cyclodiene resistance, followed by DDT (263 species), organophosphates (260 species), carbamates (85 species), pyrethroids (48 species), fumigants (12 species), and other (40 species) (Dhaliwal et al., 2006). Important crop pests, parasites of livestock, common urban pests and disease vectors in some cases have developed resistance to such an extent that their control has become exceedingly challenging (Van Leeuwen et al., 2010; Gondhalekar et al., 2011). However, many factors such as genetics, biology/ecology and control operations influence the development of pesticide resistance (Georghiou and Taylor, 1977).

Insecticide bioassays using whole insects continue to be one of the most widely used approaches for detecting resistance (Brown and Brogdon, 1987; Gondhalekar et al., 2013) despite some associated drawbacks. In the past two decades, however, several new methods employing advanced biochemical and molecular techniques, and combination of insecticide bioassays have been developed for detecting insecticide resistance (Symondson and Hemingway, 1997; Scharf et al., 1999; Zhou et al., 2002). Some examples of these techniques are enzyme electrophoresis, enzyme assays, immuno-assays, allele-specific polymerase chain reaction (PCR) etc.

2.2. Pest resurgence

Pest resurgence is defined as the rapid reappearance of a pest population in injurious numbers following pesticide application. Use of persistent and broad spectrum pesticides that kills the beneficial natural enemies is thought to be the leading cause of pest resurgence. However, resurgence is known to occur due to several reasons, for example, increase in feeding and reproductive rates of insect pests, due to application of sub-lethal doses of pesticides, and sometimes elimination of a primary pest provides favorable conditions for the secondary pests to become primary/key pests (Dhaliwal et al., 2006). There are many pesticide-induced pest outbreaks reported in walnut (*Juglans regia*) (Bartlett and Ewart, 1951), hemlock (*Conium maculatum*) (McClure, 1977), soybeans (*Glycine max*) (Shepard et al., 1977), and cotton (*Gossypium hirsutum*) (Bottrell and Rummel, 1978). Among these, brown plant hopper (BPH) (*Nilaparvata lugens* (Stal)) in rice (*Oryza sativa* L.) cultivation has gained a major importance in Asian countries (Chelliah and Heinrichs, 1984). In general, natural BPH populations were kept under check by natural enemies including mirid bugs, ladybird beetles, spiders and various pathogens. However, pesticides have not only destroyed the natural enemies (Fabellar and Heinrichs, 1986), but have influenced the fecundity of BPH females (Wang et al., 2010) further enhancing their resurgence. Additionally, the resurgence of bed bug, *Cimex lectularius* (Davies et al., 2012) and cotton bollworm *Helicoverpa armigera* (Mironidis et al., 2013) have been reported due to insecticide resistance and indiscriminate use of pesticides.

3. Effects of pesticides on non-target organisms

The effect of pesticides on non-target organisms has been a source of worldwide attention and concern for decades. Adverse effects of applied pesticides on non-target arthropods have been widely reported (Ware, 1980). Unfortunately, natural insect enemies e.g., parasitoids and predators are most susceptible to insecticides and are severely affected (Aveling, 1977; Vickerman, 1988). The destruction of natural enemies can exacerbate pest problems as they play an important role in regulating pest population levels. Usually, if natural enemies are absent, additional insecticide sprays are required to control the target pest. In some cases, natural enemies that normally keep minor pests under check are also affected and this can result in secondary pest outbreaks. Along with natural enemies, population of soil arthropods is also drastically disturbed because of indiscriminate pesticide application in agricultural systems. Soil invertebrates including nematodes, springtails, mites, micro-arthropods, earthworms, spiders, insects and other small organisms make up the soil food web and enable decomposition of organic compounds such as leaves, manure, plant residues etc. They are essential for the maintenance of soil structure, transformation and mineralization of organic matter. Pesticide effects on above mentioned soil arthropods therefore negatively impact several links in the food web. The following are the examples of non-target organisms that are adversely impacted by pesticides.

3.1. Earthworms

Earthworms represent the greatest proportion of terrestrial invertebrates (>80%) (Yasmin and D'Souza, 2010) and play a significant role in improving soil fertility by decomposing the organic matter into humus. Earthworms also play a major role in improving and maintaining soil structure, by creating channels in soil that enable the process of soil aeration and drainage. However, their diversity, density and biomass are strongly influenced by soil management. They are considered as an important indicator of soil quality in agricultural ecosystems (Paoletti, 1999). Earthworms are affected by various agricultural practices and indiscriminate use of pesticides is one of the leading practices affecting them (Pelosi et al., 2013).

Pesticide applications can cause decline in earthworm populations. For example, carbamate insecticides are very toxic to earthworms and some organophosphates have been shown to reduce earthworm populations (Edwards, 1987). Similarly, a field study conducted in South Africa has also reported that earthworms were influenced detrimentally due to chronic and intermittent exposures to chlorpyrifos and azinphos methyl, respectively (Reinecke and Reinecke, 2007). Various scientific studies reported that pesticides influence earthworm growth, reproduction (cocoon production, number of hatchlings per cocoon, and incubation period) in a dose-dependent manner (Yasmin and D'Souza, 2010). Earthworms exposed to different kind of pesticides showed rupturing of cuticle, oozing out of coelomic fluid, swelling, and paling of body that led to softening of body tissues (Solaimalai et al., 2004). Similarly a study carried out in France showed that the combination of insecticides and fungicides at different concentrations caused neurotoxic effects in earthworms (Schreck et al., 2008). Increased exposure period and higher dose of insecticides can also cause physiological damage (cellular dysfunction and protein catabolism) to earthworms (Schreck et al., 2008).

3.2. Predators

Predators are organisms that live by preying on other organisms and they play a very crucial role in keeping pest populations under control. Predators (beneficial organisms) are also an important part of the "biological control" approach which is one component of the integrated pest management strategy discussed later. In some of the examples cited below, pesticides were the main cause for decline in predator population:

- In brinjal (*Solanum melongena* L.) ecosystem, spraying with cypermethrin and imidacloprid caused higher mortality of coccinellids, braconid wasps and predatory spiders compared to when sprayed with bio-pesticides and neem (*Azadirachta indica*) based insecticides (Ghananand et al., 2011).
- Species diversity, richness and evenness of collembola, and numbers of spiders were found to be lower in chlorpyrifos treated plots compared with control, in grassland pastures in UK (Fountain et al., 2007).
- Studies were carried out to investigate the effects of chemicals on soil arthropods in agricultural area near Everglades National Park, USA. It was found that higher number of arthropods (including predators such as coccinelids and spiders) were present in non-sprayed fields compared to fields sprayed with insecticides and herbicides (Amalin et al., 2009).
- In foliar application, all the systemic neonicotinoids such as imidacloprid, clothianidin, admire, thiamethoxam and acetamiprid were found highly toxic to natural enemies in comparison with spirotetramat, buprofezin and fipronil (Kumar et al., 2012).

Additionally, pesticides can also affect predator behavior and their life-history parameters including growth rate, development time and other reproductive functions. For example, in the eastern USA, glyphosate-based herbicides affected behavior and survival of spiders and ground beetles, apart from affecting arthropod community dynamics that can also influence biological control in an agroecosystem (Evans et al., 2010). Similarly, dimethoate was shown to significantly decrease the body size, haemocyte counts and reduction of morphometric parameters on carabid beetle (*Pterostichus melas italicus*), in Calabria, Italy (Giglio et al., 2011).

3.3. Pollinators

Pollinators are biotic agents that play a very important role in pollination process. Some of the recognized pollinators are different species of bees, bumble bees (*Bombus* spp.), honey bees (*Apis* spp.), fruit flies, some beetles, and birds (e.g., hummingbirds, honeyeaters, and sunbirds etc.). Pollinators can be used as bioindicators of ecosystemic processes (process by which physical, chemical, biological events help connecting organisms with their environment) in many ways as their activities are affected by environmental stress caused by parasites, competitors, diseases, predators, pesticides and habitat modifications (Kevan, 1999). However, using pesticides causes direct loss of insect pollinators and indirect loss to crops because of the lack of adequate populations of pollinators (Fishel, 2011).

Pesticide application also affects various activities of pollinators including foraging behaviour, colony mortality and pollen collecting efficiency. Most of our current knowledge about effects of pesticides on change in pollinator behaviour has come from various bee studies as they comprise 80% of the insect pollinator population. For instance, many laboratory studies have demonstrated the lethal and sub-lethal effects of neonicotinoid insecticides (imidacloprid, acetamiprid, clothianidin, thiamethoxam, thiacloprid, dinotefuran and nitenpyram) on foraging behavior, learning and memory abilities of bees (Blacquie`re et al., 2012). Worker bee (female bees that lack full reproductive capacity and play many other roles in bee colony) mortality, decreased pollen collecting efficiency and eventually colony collapse occur due to pesticides (neonicotinoid and pyrethroid) application (Gill et al., 2012). In addition to this, non-lethal exposure of honey bees to neonicotinoid insecticide (thiamethoxam) causes high mortality due to homing failure at a level that could put a risk of colony collapse (Henry et al., 2012). Sub-lethal doses of imidacloprid (the most commonly used pesticide worldwide) affected longevity and foraging in honey bees (*A. mellifera*). *Nosema ceranae* (*Nosema* invades the intestinal tracts of adult bees causing colony collapse disorder (CCD) and nosema disease/ nosemosis, which consequently lead to decrease in honey production). Microsporidial infections increased significantly in gut of bees from imidacloprid treated hives. It has been anticipated that interactions between pathogens and imidacloprid pesticide could be a main reason for worldwide honey bee colony mortality, including CCD (Pettis et al., 2012; Wu et al., 2012). There are also reports that imidacloprid reduced brood production due to decline in the fecundity of bumble bees (*B. terrestris*) (Laycock et al., 2012; Whitehorn et al., 2012). On the other hand, little work has been done on the impact of pesticides on wild pollinators. For example, a field study carried out in Italy on an agricultural field found lower bumblebee and butterfly species richness associated with pesticide application. They also found that bees (insect pollinators) were at higher risk from pesticide use (Brittain et al., 2010).

3.4. Humans

The deleterious effects of pesticides on human health have started to grow due to their toxicity and persistence in environment and ability to enter into the food chain. Pesticides can enter the human body by direct contact with chemicals, through food especially fruits and vegetables, contaminated water or polluted air. Both acute and chronic diseases can result from pesticide exposure and these are summarized below:

3.4.1. Acute illness

Acute illness generally appears a short time after contact or exposure to the pesticide. Pesticide drift from agricultural fields, exposure to pesticides during application and intentional or unintentional poisoning generally leads to the acute illness in humans (Dawson et al., 2010; Lee et al., 2011b). Several symptoms such as headaches, body aches, skin rashes, poor concentration, nausea, dizziness, impaired vision, cramps, panic attacks and in severe cases coma and death could occur due to pesticide poisoning (Pan-Germany, 2012). The severity of these risks is normally associated with toxicity and quantity of the agents used, mode of action, mode of application, length and frequency of contact with pesticides and person that is exposed during application (Richter, 2002). About 3 million cases are reported worldwide every year that occur due to acute pesticides poisoning. Out of these 3 million pesticide poisoning cases, 2 million are suicide attempts and the rest of these are occupational or accidental poisoning cases (Singh and Mandal, 2013). Suicide attempts due to acute pesticide poisoning are mainly the result of widespread availability of pesticides in rural areas (Richter, 2002; Dawson et al., 2010). Several

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strategies have been proposed to reduce the incidences that occur due to acute pesticide poisoning such as restricting the availability of pesticides, substituting the pesticide with a less toxic but with an equally effective alternative and by promoting use of personal protection equipment (Murray and Taylor, 2000; Konradsen et al., 2003). Strict laws regulating pesticide sales along with preventive health programs and community development efforts are needed to enforce such strategies.

3.4.2. Chronic illness

Continued exposure to sub-lethal quantities of pesticides for a prolonged period of time (years to decades), results in chronic illness in humans (Pan-Germany, 2012). Symptoms are not immediately apparent and manifest at a later stage. Agricultural workers are at a higher risk to get affected, however general population is also affected especially due to contaminated food and water or pesticides drift from the fields (Pan-Germany, 2012). Incidences of chronic diseases have started to grow as pesticides have become an increasing part of our ecosystem. There is mounting evidence that establish a link between pesticides exposure and the incidences of human chronic diseases affecting nervous, reproductive, renal, cardiovascular, and respiratory systems (Mostafalou and Abdollahi, 2012). The list of chronic diseases that are linked to prolonged pesticide exposure by various studies is summarized in Table 1.

Diseases	References
Cancer (Childhood and adult brain cancer; Renal cell cancer; lymphocytic leukaemia (CLL); Prostate Cancer)	Lee et al., 2005; Shim et al., 2009; Heck et al., 2010; Xu et al., 2010; Band et al., 2011; Cocco et al., 2013
Neuro degenerative diseases including Parkinson disease, Alzheimer disease	Elbaz et al., 2009; Hayden et al., 2010;Tanner et al., 2011
Cardio-vascular disease including artery disease	Abdullah et al., 2011; Andersen et al., 2012
Diabetes (Type 2 Diabetes)	Son et al., 2010; Lee et al., 2011a
Reproductive disorders	Petrelli and Mantovani, 2002; Greenlee et al., 2003
Birth defects	Winchester et al., 2009; Mesnage et al., 2010
Hormonal imbalances including infertility and breast pain	Xavier et al., 2004
Respiratory diseases (Asthma, Chronic obstructive pulmonary disease (COPD))	Chakraborty et al., 2009; Hoppin et al., 2009

Table 1. The List of chronic diseases that are linked to the exposure to pesticides

Several mechanisms have been illustrated that link development of chronic diseases with pesticide exposure. Direct interaction of pesticides with genetic material resulting in DNA damages and chromosomal aberration is considered to be one of the primary mechanisms that lead to the chronic diseases such as cancer etc (Mostafalou and Abdollahi, 2012). In this context, several studies report an increase in frequency of chromosomal aberration, sister chromatid exchange, and breakage in DNA strand in pesticide applicators who worked in agricultural fields (Grover et al., 2003; Santovito et al., 2012). Similar to this, pesticides are also known to induce epigenetic changes (heritable changes without any alteration in DNA sequences) through DNA methylation, histone modifications and expression of non-coding RNAs. For example, neurotoxic pesticide paraquat has been implicated to induce the Parkinson's disease (PD) through epigenetic changes by promoting histone acetylation (Song et al., 2010). Pesticides may also induce oxidative stress by increasing reactive oxygen species (ROS) through altering levels of antioxidant enzymes such as superoxide dismutase, glutathione reductase and catalase (Agrawal and Sharma, 2010). Several health problems such as Parkinson disease, disruption of glucose homeostasis have been linked with pesticides induced oxidative stress (Mostafalou and Abdollahi, 2012).

4. Pesticides and soil environment

A major fraction of the pesticides that are used for agriculture and other purposes accumulates in the soil. The indiscriminate and repeated use of pesticides further aggravates this soil accumulation problem. Several factors such as soil properties and soil micro-flora determine the fate of applied pesticides, owing to which it undergoes a variety of degradation, transport, and adsorption/desorption processes (Weber et al., 2004; Laabs et al., 2007; Hussain et al., 2009). The degraded pesticides interact with the soil and with its indigenous microorganisms, thus altering its microbial diversity, biochemical reactions and enzymatic activity (Hussain et al., 2009; Munoz-Leoz et al., 2011). A summary of the effects of pesticides on its various components are given below:

1. Pesticides that reach the soil can alter the soil microbial diversity and microbial biomass. Any alteration in the activities of soil microorganisms due to applied pesticides eventually leads to the disturbance in soil ecosystem and loss of soil fertility (Handa et al., 1999). Numerous studies have been undertaken which highlight these adverse impacts of pesticides on soil microorganisms and soil respiration (Dutta et al., 2010; Sofo et al., 2012). In addition to this, exogenous applications of pesticides could also influence the function of beneficial root-colonizing microbes such as bacteria and arbuscular mycorrhiza (AM), fungi and algae in soil by influencing their growth, colonization and metabolic activities etc (Debenest et al., 2010; Menendez et al., 2010; Tien and Chen, 2012).

The pesticides that reach the soil can interact with soil microflora in several ways:

a. It can adversely affect the growth, microbial diversity or microbial biomass of the soil microflora. For example, sulfonylurea herbicides- metsulfuron methyl, chlorsulfuron and thifensulfuron methyl were reported to reduce the growth of the fluorescent bacteria *Pseudomonas* strains that were isolated from an agricultural soil (Boldt and Jacobson, 1998). The *Pseudomonas* spp. is known to play an important ecological role in the soil habitat (Boldt and Jacobson, 1998), and hence its reduction can adversely affect soil fertility. Similarly, benomyl, captan and chlorothalonil were reported to suppress the peak soil respiration (an indicator of microbial biomass) in an unamended soil by 30–50% (Chen et al., 2001b).

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b. Pesticide application may also inhibit or kill certain group of microorganisms and outnumber other groups by releasing them from the competition (Hussain et al., 2009). For example, increase in bacterial biomass by 76% was reported in response to endosulfan application and that reduced the fungal biomass by 47% (Xie et al., 2011).

c. Applied pesticide may also act as a source of energy to some of the microbial group which may lead to increase in their growth and disturbances in the soil ecosystem. For example, bacterial isolates collected from wastewater irrigated agricultural soil showed the capability to utilize chlorpyriphos as a carbon source for their growth (Bhagobaty and Malik, 2010).

d. Pesticides can alter and/or reduce the functional structure and functional diversity of microorganisms, but increase the microbial biomass (Lupwayi et al., 2009). In contrast, application of pesticides can also reduce the microbial biomass while increasing the functional diversity of microbial community. For example, methamidophos and urea decreased the microbial biomass and increased the functional diversity of soil as determined by microbial biomass and community level physiological profiles (Wang et al., 2006).

2. Pesticides may also adversely affect the soils vital biochemical reactions including nitrogen fixation, nitrification, and ammonification by activating/deactivating specific soil microorganisms and/or enzymes (Hussain et al., 2009; Munoz-Leoz et al., 2011). The synergistic and additive interactions between pesticides, micro-organisms and soil properties ultimately govern increase or decrease in rate of soil biochemical reactions. For example, populations of the *Azospirillum* spp. bacteria and the rate of ammonification was reported to increase at a particular pesticide concentration (i.e 2.5 to 5.0 kg ha^{-1}) in both laterite and vertisol soils planted to groundnut (*Arachis hypogaea* L.). But the tested pesticides exerted antagonistic interactions on the population of *Azospirillum* spp. and ammonification at higher concentrations (7.5 and 10.0 kg ha^{-1}) (Srinivasulu et al., 2012a).

3. Pesticides have also been reported to influence mineralization of soil organic matter, which is a key soil property that determines the soil quality and productivity. For example, a significant reduction in soil organic matter was found after the application of four herbicides (atrazine, primeextra, paraquat, and glyphosate) (Sebiomo et al., 2011). However, soil organic matter then increased after continuous application from the second to the sixth week of herbicide treatment.

4. Pesticides that reach the soil may also disturb local metabolism or can alter the soil enzymatic activity (Gonod et al., 2006; Floch et al., 2011). Soil in general contains an enzymatic pool which comprises of free enzymes, immobilized extracellular enzymes and enzymes excreted by (or within) microorganisms that are indicator of biological equilibrium including soil fertility and quality (Mayanglambam et al., 2005; Hussain et al., 2009). Degradation of both pesticides and natural substances in soil is catalyzed by this enzymatic pool (Floch et al., 2011; Kizilkaya et al., 2012). Due to this, measuring the change in enzymatic activity has now been classified as a biological indicator to identify the impact of chemical substances including pesticides on soil biological functions (Garcia et al., 1997; Romero et al., 2010). In fact, it has generally been assumed that measuring the change in enzyme activity is an earlier indicator of soil degradation as compared to the chemical or physical parameters (Dick et al., 1994). Several studies have already been undertaken which indicate both increase and decrease in activities of soil enzymes such as hydrolases, oxidoreductases, and dehydrogenase (Ismail et al., 1998; Megharaj et al., 1999). A description of pesticides interactions with soil enzymes has been summarized in Table 2.

Enzyme (Function in soil)	**Examples of the pesticides applied**	**Comments**
Nitrogenase (An enzyme used by organisms to fix atmospheric nitrogen gas).	Carbendazim, Imazetapir, Thiram, Captan, 2,4-D, Quinalphos, Monocrotophos, Endosulfan, γ-HCH, Butachlors	Pesticide reduced or inhibited the nitrogenase activity in laboratory or field conditions (Chalam et al., 1996; Martinez-Toledo et al., 1998; Niewiadomska, 2004; Niewiadomska and Klama, 2005; Prasad et al., 2011)/ Pesticides stimulated the nitrogenase activity (Patnaik et al., 1995)
Phosphatase (hydrolyzes organic P compounds to inorganic P)	2,4-D, Nitrapyrin, Monocrotophos, Chlorpyrifos, Mancozeb and Carbendazim	Inhibited (Tu, 1981); Activity increased, but higher concentration or increasing incubation period has inhibitory effects (Madhuri and Rangaswamy, 2002; Srinivasulu et al., 2012b)
Urease (catalyzes the hydrolysis of urea into CO_2 and NH_3 and is a key component in the nitrogen cycle in soils)	Isoproturon, Benomyl, Captan, Diazinon, Profenofos	Increase in urease activity (Chen et al., 2001a; Nowak et al., 2004), Pesticide reduced/inhibited urease activity (Abdel-Mallek et al., 1994; Ingram et al., 2005)
Dehydrogenase (DHA): (an oxidoreductase enzyme that catalyzes the removal of hydrogen)	Azadirachtin, Acetamiprid, Quinalphos,Glyphosate	Positive/stimulatory influence on the DHA (Singh and Kumar, 2008; Kizilkaya et al., 2012)/Initially inhibited but later on activity was restored (Andrea et al., 2000; Mayanglambam et al., 2005)
Invertase (hydrolyzes sucrose to fructose and glucose)	Atrazine, Carbaryl, Paraquat	Inhibited invertase activity (Gianfreda et al., 1995; Sannino and Gianfreda, 2001)
β-glucosidase (hydrolyzes disaccharides in soil to form β-glucose)	Metalaxyl, Ridomil gold plus copper	Enzyme activity increased and then decreased (Sukul, 2006) or inhibited (Demanou et al., 2004)
Cellulase (hydrolyzes cellulose to D-glucose)	Benlate, Captan, Brominal	Inhibited enzyme activity (Arinze and Yubedee, 2000; Omar and Abdel-Sater, 2001)

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Enzyme (Function in soil)	Examples of the pesticides applied	Comments
Arylsulphatase (an enzyme that hydrolyzes aryl sulfates)	Cinosulfuron, Prosulfuron, Thifensulfuron methyl, Triasulfuron	Decreased enzyme activity (Sofo et al., 2012)

Table 2. A summary of the effects of pesticides on different soil enzymes

Several environmental factors control the bioavailability, degradation and effect of pesticides on soil microorganisms in addition to the persistence, concentration and toxicity of the applied pesticides. These include soil texture, presence of organic matter, vegetation and cultural practices (Murage et al., 2007). For instance, a mixture of compost and straw was found to have the capability of bio-degrading different mixtures of fungicides that are usually applied in vineyards when tested under laboratory conditions (Coppola et al., 2011). Similarly, persistence of the herbicide imazapyr was reported to be different in three Argentinean soils (Tandil, Anguil, and Cerro Azul sites) and its half-life was negatively associated with soil pH, iron and aluminum content, and positively related with clay content (Gianelli et al., 2013). Additionally, level of soil moisture is also one of the most important factors that regulates pesticide bioavailability and degradation, as water acts as solvent for pesticide movement and diffusion, and is essential for microbial functioning (Pal and Tah, 2012). For example, degradation of herbicide saflufenacil was found to be faster at field capacity for Nada, Crowley and Gilbert soils as compared to the saturated soil conditions (Camargo et al., 2013).

It is important to monitor the response of soil microbial communities and various enzymatic activities to pesticide exposure in order to reduce their deleterious effects. A combination of both cultivation-dependent (e.g., community-level physiological profiling (CLPP), measuring overall rates of microbial activity) and cultivation-independent (e.g., DNA sequence information, proteomics of environmental samples) methods can be applied to measure and interpret the effects of pesticide exposure (Imfeld and Vuilleumier, 2012). With the advent of efficient new sequencing techniques and metagenomics, the scope of deploying cultivation independent methods for measuring bacterial diversity and function in soil ecosystem has been further increased. Metagenomics approach has been applied already to measure microbial diversity for a range of soil systems including contaminated sites (Ono et al., 2007) and land managed with different cultural practices (Souza et al., 2013). Such high-tech approaches hold the key for future methods to measure the mode of adaptation ecosystem to different pesticides and in development of new methods to better manage pesticide applications.

A careful screening of pesticide effects on soil microflora should be done in laboratory before their field applications. This is because pesticides tend to accumulate in soil due to repeated applications over time and can pose adverse effects on soil microflora even though they are applied at recommended doses (Ahemad et al., 2009). For instance, Ahemad and Khan (2011) reported the highest toxicity to plant growth promoting characteristics of the *Bradyrhi zobium* sp. when its strain MRM6 was grown with three times the recommended field rates of glyphosate, imidacloprid and hexaconazole. Similarly, Dunfield et al. (2000) assessed the effects of the fungicides captan and thiram at rates of 0.25-2 g a.i. kg^{-1} on the survival and phenotypic characteristics of bacteria *Rhizobium leguminosarum* bv. *viceae*, strain C1. They found that even though both captan and thiram significantly reduced the numbers of rhizobia recovered from seed and altered the FAME (fatty acid methyl ester) and biological profiles of recovered rhizobia, it was only the highest concentrations of captan that affected nodulation and plant growth. Similarly, herbicide mesotrione affected soil microbial communities, but the effects were only detected at doses far exceeding the recommended field rates (Crouzet et al., 2010). Overall, it is crucial to comprehend the role of pesticides in perturbing soil environment, so that the risk of pesticide contamination and its consequent adverse impacts on soil environment can be evaluated.

5. Pesticides in water and air ecosystem

Pesticide residues in water are a major concern as they pose a serious threat to biological communities including humans. There are different ways by which pesticides can get into water such as accidental spillage, industrial effluent, surface run off and transport from pesticide treated soils, washing of spray equipments after spray operation, drift into ponds, lakes, streams and river water, aerial spray to control water-inhibiting pests (Carter and Heather, 1995; Singh and Mandal, 2013). Pesticides generally move from fields to various water reservoirs by runoff or in drainage induced by rain or irrigation (Larson et al., 2010). Similarly, the presence of pesticides in air can be caused by number of factors including spray drift, volatilization from the treated surfaces, and aerial application of pesticides. Extent of drift depends on: droplet size and wind speed. The rate of volatilization is dependent on time after pesticide treatment, the surface on which the pesticide settles, the ambient temperature, humidity and wind speed and the vapor pressure of the ingredients (Kips, 1985). The volatility or semi-volatility nature of the pesticide compounds similarly constitutes an important risk of atmospheric pollution of large cities (Trajkovska et al., 2009). For instance, organophosphorus (OP) pesticides were identified from environmental samples of air and surface following agricultural spray applications in California and Washington (USA) (Armstrong et al., 2013). In Italian forests, indiscriminate use of pesticides and its active metabolites has led to the contamination of water bodies and ambient air, possibly affecting the health of aquatic biota fishes, amphibians and birds (Trevisan et al., 1993). The following section describes the effect of pesticides on fishes, amphibians and birds.

5.1. Fishes

Fishes are an important part of marine ecosystem as they interact closely with physical, biological and chemical environment. Fishes provide food source for other animals such as sea birds and marine mammals and thus fishes form an integral part of the marine food web. A lot of research has been carried out to examine the impact of pesticides on decline in fish population (Scholz et al., 2012). Pesticides have been directly linked to causing fish mortality worldwide. For example, 27 freshwater fish species are found to be affected by "plant protection products" (PPP) in Europe (Ibrahim et al., 2013). Another pesticide pentachlorophenol

(NaPCP) is reported to cause large numbers of fish mortality in the rice fields of Surinam (Vermeer et al., 1970). Pesticides not only impact the fish but also food webs related to them. The persistent pesticides (organochlorine pesticides and polychlorinated biphenyls) have already been found in the major Arctic Ocean food webs (Hargrave et al., 1992). A survey was conducted to examine the influence of pesticides on aquatic community in West Bengal, India. Many body tissues of the fish such as gills, alimentary canal, liver and brain of carp and catfish were found drastically damaged by pesticides. It was reported that such level of pesticides in fish could harm the fish consumers as well (Konar, 2011).

Several examples are available where pesticides impacted the vital fish organs and behavior. Organophosphate pesticide "Abate" has the potential to alter the vitellogenesis (the process whereby yolky eggs are produced) of catfish (*Heteropneustes fossilis* (Bloch.)), which can severely affect catfish farming (Kumari, 2012). Another major effect of toxic contaminants is on olfaction in fishes since it can affect activities such as mating, locating food, avoiding predators, discriminating kin and homing etc (Tierney et al., 2010). Simultaneous exposure of trematode parasite (*Telogaster opisthorchis*), freshwater fish (*Galaxias anomalus*) and snails to high glyphosate concentrations significantly reduced their survival and development. Within 24 hrs of exposure to higher glyphosate concentrations, 100% mortality of individuals was found (Kelly et al., 2010).

The impact of pesticides within an aquatic environment is influenced by their water solubility and uptake ability within an organism (Pereira et al., 2013). For example, Clomazone, a popular herbicide, is particularly water soluble; a property that increases its likelihood of contaminating surface and groundwater. The hydrophilic (water-loving) or lipophobic (fat-hating) nature of this pesticide makes it less available in the fatty tissues of an organism (Pereira et al., 2013). Further to this, the toxicity of chemical (e.g., endosulfan in this case) in juvenile rainbow trout (*Oncorhynchus mykiss*) was affected by alkalinity, temperature of water and size of the fish (Capkin et al., 2006).

Pesticides in natural water within the acceptable concentration range can still pose harmful effects. Kock-Schulmeyer et al. (2012) found that even if the pesticide levels found in Llobregat River basin of Spain were within the European Union Environmental Quality Standards, they still accounted for a low to high ecotoxicological risk for aquatic organisms, especially algae and macro-invertebrates. Proper measures should be taken while disposing of expired pesticides, so that their discharge into the water bodies does not danger the aquatic life. This is because the alteration in water pH by expired insecticides can lead to acute toxicity of different fish (Satyavani et al., 2011).

5.2. Amphibians

Amphibians are ectothermic, tetrapod vertebrates of class Amphibia. They inhabit a wide variety of habitats, with most species living within fossorial, arboreal, terrestrial, and freshwater aquatic ecosystems. The global decline in the amphibian population has become an environmental concern worldwide. Many amphibian species are on the brink of extinction with 7.4% listed as critically endangered, and at least 43.2% experiencing some sort of population decrease (Stuart et al., 2004). There could be multitude of reasons for decline in amphibian species diversity, but pesticides appear to be playing an important role. Global warming and climate change are leading to more variable and warmer temperatures which may have increased the impact of pesticides on amphibian populations (Relyea, 2003; Johnson et al., 2013).

Many studies showed that amphibians are susceptible to environmental contaminants due to their permeable skin, dual aquatic-terrestrial cycle and relatively rudimentary immune system (Kerby et al., 2010). Several studies showing the impact of pesticides on amphibians are being mentioned here. It has been reported that the world's most commonly used herbicide (Roundup (Glyphosate)) may have far reaching effects on non-target amphibians (Relyea, 2012). Roundup, a globally used herbicide caused high mortality of larval tadpoles (3 different species in North America) and juvenile frogs under natural conditions in an outdoor pond mesocosm (Relyea, 2005a). Most of the evidence supported the toxic effects of pesticides on juvenile European common frogs (*Rana temporaria*) in an agricultural field that was over sprayed. Mortality of frogs ranged from 100% after 1 hour to 40% after 7 days at the recommended concentrations of pesticides (Bruhl et al., 2013). It was found that population of the wood frog (*Lithobates sylyaticus*) near an agricultural area was more resistant to common insecticide (chlorpyrifos), but not to the common herbicide (Roundup). However, no evidence was reported that resistance carried a performance cost when facing competition and the fear of predation (Cothran et al., 2013).

Further to this, pesticides indirectly affect amphibian populations by influencing growth of aquatic communities such as fungi, zooplankton, and phytoplankton as they are one of their prime energy resources. Malathion is the most commonly used broad-spectrum insecticide in United States. It is legal to spray malathion over aquatic habitats to control mosquitoes (Family: Culicidae), that vector malaria and West Nile Virus. A study found that even low concentration of malathion caused direct and indirect effects on aquatic communities (Relyea, 2012). For example, indirect effect of malathion led to decrease in zooplankton diversity, that led to increase in phytoplankton, a decrease in periphyton, and finally decrease in growth of frog tadpoles (Relyea and Hoverman, 2008). Moreover, it was found that repeated applications of low doses had largest impacts than single high dose application of malathion on an aquatic system (Relyea and Diecks, 2008). A comprehensive study was conducted to examine the effect of globally used pesticides including insecticides (carbaryl, malathion, and herbicides (glyphosate, 2, 4-D)) on aquatic communities (algae, 25 animal species). Species richness reduced differentially, 15% with carbaryl, 30% with malathion, and 22% with roundup, whereas 0% with 2, 4-D. It was found that Roundup completely eliminated two species of tadpoles and led to 70% decline in tadpole species (Relyea, 2005b). Another study demonstrated that frogs (*Rana pipiens*) living in agricultural area, where they experienced higher exposure to chemicals were smaller in size and weight than frogs living in area exposed to low-levels of chemicals. It suggests that frogs living in agricultural areas might have more vulnerability to infections and diseases due to their smaller size and alternation in their immune system (decrease in number of splenocytes and phagocytic activity) (Christin et al., 2013).

5.3. Birds

Birds are a diverse group, and apart from their distinct songs and calls, showy displays and bright colors adding enjoyment to lives of humans, they play a very critical role in food chains and webs in our ecosystems. Birds are also called "aerial acrobats" consuming different kinds of insects such as mosquitoes, European corn borer moth (*Ostrinia nubilalis*), Japanese beetles (*Popillia japonica*), and many other insect species that are considered as some of the most serious agricultural and health pests. Birds are important biotic components of an ecosystem and help in maintaining a natural equilibrium of insect populations by predating on them. In absence of birds, outbreaks of insect pest populations would become more common, ultimately leading to increased pesticide use. Pesticides exposure by different means such as direct ingestion of pesticide granules and treated seeds, treated crops, direct exposure to sprays, contaminated water, or feeding on contaminated prey, and baits cause birds mortality (Fishel, 2011; Guerrero et al., 2012). In USA, almost 50 pesticides are known for killing song birds, game birds, seabirds, shorebirds, and raptors (BLI, 2004).

Pesticides have a potential to alter behavior and reproduction of birds. Some of the examples cited here, using different synthetic chemicals including carbamates, organochlorines, and organophosphates can cause a decline in the populations of raptorial birds by altering their feeding behavior and reproduction (Mitra et al., 2011). A large area in the world is under rice and therefore cultivation and volume of pesticides applied in rice field is quite significant. Many different kinds of organochlorines, cholinesterase-inhibiting insecticides including carbofuran, monocrotophos, phorate, diazinon, fenthion, phosphamidon, methyl parathion and azinphos-methyl along with fungicides, herbicides and molluscicides are being used in rice fields. Some of these chemicals are highly toxic to birds causing mortality and some chemicals even have the potential to affect their reproductive systems (Parsons et al., 2010). Indirect effects of pesticides, through food chain have been proposed as a possible factor in decline of farmland bird species. Insecticides applied in breeding season can affect breeding performance of corn bunting (*Miliaria calandra*) and yellowhammer (*Emberiza citrinella*) (Boatman et al., 2004).

Pesticides, especially insecticides such as carbamates and organophosphates have the potential to cause bird mortality due to their high toxicity (Hunter, 1995). Further to this, insecticides and fungicides pose a most prominent threat to ground-nesting farmland birds as compared to other agricultural practices. The decline of US grassland birds is attributed to acute pesticide toxicity and not agricultural intensification as previously thought (Mineau and Whiteside, 2013). An estimate suggests that 672 million birds are directly exposed to pesticides every year on farmlands, and 10% of these birds die due to acute toxic effects of pesticides (Williams, 1997). A study was conducted in rice fields of Surinam to examine the effects of pesticides, pentachlorophenol (NaPCP) on birds. NaPCP was sprayed for the purpose of killing *Pomacea* snails. Large numbers of dead sick/dead egrets, herons and jacana birds were found during the period of pesticide application. Pentachlorophenol and endrin levels in these birds suggested that ingestion of contaminated food was the probable cause of sickness and mortality (Vermeer et al., 1970).

6. Pesticides and biomagnification

The increase in concentration of pesticides due to its persistent and non-biodegradable nature in the tissues of organisms at each successive level of food chain is known as biomagnification. Due to this phenomenon, organisms at the higher levels of food chain experience greater harm as compared to those at lower levels. Several studies have been undertaken that demonstrate enhanced amount of toxic compounds with increase in trophic levels. For example, out of 36 species collected from three lakes of northeastern Louisiana (USA) that were found to contain residues of 13 organochlorines, tertiary consumers such as green-backed heron (*Butorides striatus*), and snakes etc., contained the highest residues as compared to secondary consumers (bluegill (*Lepomis macrochirus*), blacktail shiner (*Notopis venustus*)) (Niethammer et al., 1984). Similarly, significantly higher concentrations of dichlorodiphenyltrichloroethane (4,4′-DDE) were found in the top consumer fish in Lake Ziway, catflish (*Clarias gariepinus*) than in lower consumers, Nile tilapia (*Oreochromis niloticus*), tilapia (*Tilapia zillii*) and goldfish (*Carassius auratus*) (Deribe et al., 2013). Some of the adverse effects of pesticides on non-target organisms such as fish, amphibians and humans discussed in the above section have also occurred as a result of biomagnifications of the toxic compounds. For example, reproductive failure and population decline in the fish-eating birds (e.g., gulls, terns, herons etc.) was observed as a result of DDE induced eggshell thinning (Grasman et al., 1998). The extent of biomagnifications increases with increase in persistence and lipophilic (fat-loving) characteristics of the particular pesticide. As a result of this, organochlorines are known to have higher biomaginification rate and are more persistent in a wider range of organisms as compared to organophsphates (Favari et al., 2002). It is important to do the risk assessments associated with the pesticides on the basis of their bioaccumulation and biomagnifications before considering them for agricultural purposes.

7. Strategies for pesticide management

There are a relatively few pesticide resistance management tactics that have been proposed risk-free and have a reasonable chance of success under a variety of different circumstances. Headmost among these are: monitoring of pest population in field before any pesticide application, alteration of pesticides with different modes of action, restricting number of applications over time and space, creating or exploiting refugia, avoiding unnecessary persistence, targeting pesticide applications against the most vulnerable stages of pest life cycle, using synergists which can enhance the toxicity of given pesticides by inhibiting the detoxification mechanisms. The most difficult challenge in managing resistance is not the unavailability of appropriate methods but ensuring their adoption by growers and pest control operators (Denholm et al., 1998; Dhaliwal et al., 2006).

Pest resurgence is a dose-dependent process and there are ways to tackle this problem using correct dosage of effective and recommended pesticides. Resurgence problem occurs due to a number of reasons. One of them is due to farmers' tendency to apply low-dose insecticides due to economic constraints that lead to inadequate and ineffective control of pests. Pest resurgence also occurs due to reduced biological control (most common with insects), reduced competition (most common with weeds; monocots vs. dicots), direct stimulation of pest (due to sub-lethal dose), and improved crop growth.

In the current scenario, optimized use of pesticides is important to reduce environmental contamination while increasing their effectiveness against target pest. This way we can reduce pesticide resistance as well as pest resurgence problems. This has led to the consideration of rational use of pesticides, and the physiological and ecological selectivity of pesticides. Physiological selectivity is characterized by differential toxicity between taxa for a given insecticide. However, ecological selectivity refers to the modification of operational procedure in order to reduce unnecessary destruction to non-target organisms (Dent, 2000). Farmers should focus to use insecticides that are more toxic to target species than their natural enemies which could help to reduce resurgence to some extent (Dhaliwal et al., 2006).

One should consider adopting an Integrated Pest Management (IPM) approach for controlling pests, as these practices are designed to have minimal environment disturbance. The aim of IPM is not only to reduce indiscriminate pesticide use but also to substitute hazardous chemicals with safe chemistries. IPM is a process of achieving long-term, environmentally safe pest control using wide variety of technology and other potential pest management practices. According to National Academy of Science, "IPM refers to an ecological approach in pest management in which all available necessary techniques are consolidated in a unified program so that populations can be managed in such a manner that economic damage is avoided and adverse side effects are minimized" (NAS, 1969). In European arable systems, applied multi-disciplinary research and farmer incentives to encourage the adoption of innovative IPM strategies are essential for development of sustainable maize-based cropping systems. These IPM strategies can contribute immensely to address the European strategic commitment to the environmentally sustainable use of pesticides (Vasileiadis et al., 2011). The added cost and time to do an IPM approach is sometimes a difficult task for growers, but government and extension services can help in convincing and encouraging growers to go for IPM strategy for eco-friendly and long term pest control. We have already discussed earlier that continuous use of pesticides leads to pesticide resistance and pest resurgence problem. To avoid these issues we can always go for other potential management options that include cultural and physical control, host plant resistance, biocontrol, and the use of biopesticides etc.

7.1. Cultural control

Historically, cultural control methods were the farmer's most important tool of preventing crop losses. Cultural control for pest management has been adopted by growers throughout the world for a long time due to its environmentally friendly nature and minimal costs (Gill et al., 2013). Cultural control practices are regular farm operations, which are used to destroy the pests or to prevent them from causing plant damage. Several methods of cultural control have been practiced, such as crop rotation, sanitation, soil solarization, timed planting and harvest, use of resistant varieties, certified seeds, allelopathy, intercropping or "companion planting", use of farmyard manure, and living and organic mulches (Altieri et al., 1978; Dent, 2000; Dhaliwal et al., 2006). Soil solarization (McSorley and Gill, 2010; Gill and McSorley, 2011b) and organic mulches (Gill and McSorley, 2011a) alone and their integration (Gill and McSorley, 2010) were reported as economical and eco-friendly technique for controlling soil-surface arthropods (various insects, and nematodes) (Gill et al., 2010; Gill et al., 2011) and weeds (Gill et al., 2009; Gill and McSorley, 2011b). More effective cultural control can be achieved by synchronizing existing practices with life cycles of pests. This way the weakest link in their life cycle is subjected to adverse climatic conditions.

Large insect populations are killed automatically by farmers when they expose them to adverse climatic conditions through agricultural practices like weeding, ploughing, and hoeing. Ploughing of agricultural field allows turnover of the upper layer of soil while burying the weeds and residues from last year. For example, in South Africa, about 70% of overwintering populations of spotted stalk borer (*Chilo partellus*) and maize stalk borer (*Busseola fusca*) in grain sorghum (*Sorghum bicolor* L.) and maize (*Zea mays* L.) fields were destroyed by slashing the plants. Ploughing and discing of plant residues after slashing further destroyed 24% population on grain sorghum and 19% on maize (Kfir, 1990). Planting dates (Goyal and Kanta, 2005a), and barrier crops (teosinte (*Zea* spp.) and pearl millet (*Pennisetum glaucum* (L.)) (Goyal and Kanta, 2005b) were found to be effective against maize stem borer (*Chilo partellus*) in India. The brown seaweeds *Spatoglossum asperum* and *Sargassum swartzii* can be used as manure to protect plants (tomato (*Solanum lycopersicum* L.) in this case) from root rotting fungi, (*Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani*) and root-knot nematode (*Meloidogyne javanica*) and for providing necessary nutrients to plants (Sultana et al., 2012). In India, rodents are pests in agriculture, horticulture, forestry, animal husbandry as well as in human dwellings and rural and urban storage facilities. Cultural methods, such as clean cultivation, proper soil tillage and crop scheduling, barriers, repellents and proofing that reduce the rodent harbourage, food sources and immigration may have long lasting effects (Parshad, 1999).

7.2. Physical and mechanical control

Managing pest populations using devices which affect them physically or alter their physical environment is called physical control. Exposure to sun rays, steaming, moisture management especially for stored grain pests, and light traps for attracting various kinds of moths, beetles and other pests are different methods used in physical control. For example steaming woolen winter clothes help in eliminating population of the woolly bear moth, *Antherenus vorax* (waterhouse) (Dhaliwal et al., 2006). Hot water treatment of plant storage products like corns, and bulbs helps to kill many concealed pests such as eelworms and bulb flies. Superheating of empty grain storage godowns to a temperature of 50ºC for 10-12 hours helps killing hibernating stored grain pests. Exposure of cotton seeds to sun's heat in thin layers for 2-3 days during summer helps in killing diapausing larvae of pink bollworm (*Pectinophora gossypiella* Saunders) (Dhaliwal et al., 2006).

Mechanical control refers to suppression of pest population by manual devices. It includes various practices such as hand picking, trapping and suction devices, clipping, pruning and crushing of infested shoots and floral parts, and exclusion by screens and barriers to keep away house flies (*Musca domestica*), mosquitoes and other pests. In south-eastern Australia, the common starling (*Sturnus vulgaris*) is an established invasive avian pest that is now making incursions into Western Australia which is currently free of this species. Trapping with live-lure birds is suggested to be the most cost-effective and widely implemented starling control technique (Campbell et al., 2012). Numerous wildlife species such as coyotes (*Canis latrans* Say), squirrels (Sciuridae family), and birds are known pests of California agriculture in the United States. For these pests, different non-lethal control options including habitat modification, exclusionary devices, and baiting are generally preferred (Baldwin et al., 2013). Mechanical weed control is mainly associated with tillage practices which are performed with special tools such as harrows, hoes, and brushes in growing crops. Increased knowledge about side effects of herbicides has further driven the interest in adoption of mechanical weed control thus increasing the prevalence of organic farming (Rueda-Ayala et al., 2010; Jat et al., 2011). Trapping using yellow colored sticky traps is an effective way for controlling tephritid flies (Dhaliwal et al., 2006).

7.3. Host plant resistance

Host plant resistance (HPR) is the genetic ability of the plant to improve its survival and reproduction by a range of adaptations as compared to the other cultivars when exposed to the same level of pest infestation. HPR offers the most effective, economical and eco-friendly method of pest control (Sharma and Ortiz, 2002), and is considered to be a key element of the IPM strategy. Due to this, identifying and developing HPR has always been a major thrust area of plant breeding, and a number of breeding programs aiming to develop pest resistant crops have been deployed in almost all the cultivated crop species. For example, identification and/or development of resistant varieties in maize against European corn borer (*Ostrinia nubilalis* (Hubner)) (Dhaliwal et al., 2006) , brassica against cabbage butterfly (*Pieris brassicae* Linn.) (Chahil and Kular, 2013), wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) against *Fusarium* diseases (Miedaner, 1997) *Brassica* sp. against Sclerotinia disease (Garg et al., 2008), and in rice against bacterial blight (Khush et al., 1989). Additionally, availability and access to various germplasm collections have increased the scope of widening the gene-pool of cultivated crops and further identifying and developing HPR. Wild species are especially known to possess a rich repository of genes against various defense traits as they have evolved under different geographic locations. Considerable progress has been made where identification and/or transfer of resistance gene from wild to cultivated species against various pest species has been achieved such as in potato (*Solanum tuberosum* L.) against late blight (*Phytophthora infestans*) from ten wild *Solanum sp.* (Colon and Budding, 1988), wheat against powdery mildew (*Erysiphe graminis*) from wild emmer wheat (*Triticum dicoccoides*) (Reader and Miller, 1991) and mustard (*Brassica juncea*) against *Sclerotinia sclerotiorum* from *Erucastrum cardaminoides* (Garg et al., 2010).

7.3.1. Use of biotechnology and molecular approaches for developing resistant genotype

The advent of new biotechnological and molecular approaches has opened the way to develop resistant genotype that could not only reduce the pesticides application, but it also has a potential to be a part of IPM. Development of resistant genotypes in classical breeding is met with several challenges such as it is time consuming, desired traits are linked with the undesirable traits (linkage drag) and most importantly lack of resistant genotypes in the gene pool. On the other hand, use of biotechnology in crop improvement ensures the development of pest-resistant genotypes in a comparatively short period of time and minimizes the effects of linkage drag. One of the classic examples where biotechnology was successfully deployed to develop resistant genotype is by the synthesis of transgenic plants which involves modifying plant traits by inserting foreign DNA from a different species (De la Pena et al., 1987). A number of different crops including cotton, rice, mustard, and maize have been modified up to now to engineer the genotypes against various biotic stresses (Ahmad et al., 2012). One of the most successful examples of synthesis of transgenic genotype against pest resistance is in cotton where the gene coding for Bt toxin from the bacterium *Bacillus thuringiensis* (Bt) was inserted leading cotton genotypes to produce Bt toxin in its tissue (Pray et al., 2002; Wu et al., 2008). The lepidopteran larvae that fed on the transgenic plants were killed due to Bt toxin eventually decreasing the amount of pesticide applied to the field. Examples of transgenic crops that have been developed with a potential to reduce pesticides use are abound and few of them include potato lines against potato tuber moth (*Phthorimaea operculella*) expressing Cry1Ab (Kumar et al., 2010), rice against yellow stem borer (*Scirpophaga incertulas*) expressing potato proteinase inhibitor 2 (Bhutani et al., 2006) and oilseed rape lines resistant to various fungal attack over-expressing tomato chitinase gene (Grison et al., 1996).

Another strategy where biotechnology and molecular approaches have been deployed to combat biotic stresses involves the use of RNA interference (RNAi) technique. This technique primarily uses transgenic plants expressing double stranded RNA (dsRNA) and that reduces the messenger RNA (mRNA) levels (with a high specificity and fidelity) of a crucial gene in the target pest upon feeding (Price and Gatehouse, 2008; Kos et al., 2009). This ultimately interferes with the development and survival of the target pest. RNAi has emerged as a powerful functional genomics approach and it has been used to engineer several crops against number of insect-pests. For example, RNAi technique was used in tobacco genotype that targeted the gene "integrase splicing factor" in root knot nematode, *Meloidogyne incognita* nematode eventually leading to the decrease in the number of nematodes 6-7 weeks post inoculation (Yadav et al., 2006). When such an advanced and effective approach is combined with IPM, it has a great potential to decrease chemical use in agricultural and other ecosystems.

7.4. Biological control

The process of using natural enemies of particular pests to reduce their populations to such a level where economic losses are either eliminated or suppressed is called biological control. Traditionally the most important biocontrol agents are parasitoids, predators and pathogens. Biological control involves three major techniques, *viz.,* introduction, conservation, and augmentation of natural enemies. Biocontrol agents include vertebrates, nemathelminthes (flatworms, and roundworms), arthropods (spiders, mites, and insects), pathogens like viruses, bacteria, protozoa, fungi and rickettsiae all of which play a dynamic role in natural regulation of insect and mite populations (Dhaliwal et al., 2006). In 1762, the Indian Mynah, *Acridotheres tristis* (Linnaeus), was introduced to control red locust in Mauritius. First significant success in controlling a pest was achieved on the suggestion of C. V. Riley of California (USA) in 1888. The Vedalia beetle (*Rodolia cardinalis* (Mulsant)), was introduced from Australia into California (USA) for the control of cottony-cushion scale (*Icerya purchasi* maskell) on citrus plants. This scale insect had been accidentally introduced earlier from Australia (Dhaliwal et al., 2006).

Biological control of weeds has been very successful worldwide. There are about 41 species of weeds which have been successfully controlled using insects and pathogens as biocontrol agents. Also, 3 weed species have been controlled using native fungi as mycoherbicides (Mcfadyen, 2000). A total of 12 insects were released in Australia against prickly pear (*Opuntia stricta*), out of these, *Dactylopius opuntiae* and *Cactoblastis cactorum* were responsible for the successful control of prickly pear weed (Julien and Griffiths, 1998). In the past decade, Australia has released 43 species of arthropods and pathogens in 19 different projects for successful biological control of many exotic weeds. Effective biological control was achieved in several projects and outstanding success was achieved in the control of rubber vine (*Cryptostegia grandiflora*), and bridal creeper (*Asparagus asparagoides*) (Palmer et al., 2010).

Examples of biological control are available for other organisms like helminthes, nematodes, fungi, bacteria etc. A nematophagous fungus (*Monacrosporium thaumasium*) was found to be effective in controlling cyathostomin, one of the most important helminthes in tropical region of southeastern Brazil (Tavela et al., 2011). *Trichoderma* species are free-living fungi that have been used to control a broad range of plant pathogenic fungi, viruses, bacteria and nematodes especially root-knot nematodes (*Meloidogyne javanica* and *M. incognita*) (Sharon et al., 2011).

7.4.1. Biorational pesticides

Biorational pesticides/ biopesticides are considered as third-generation pesticides that are rapidly gaining popularity. The word biorational is derived from two words, "biological" and "rational", which means pesticides of natural origin that have limited or no adverse effects on the environment or beneficial organisms. Biopesticides encompass a broad array of microbial pesticides, plant pesticides and biochemical pesticides which are derived from micro-organisms and other natural sources, and processes involving the genetic incorporation of DNA into agricultural commodities. The most commonly used biopesticides include biofungicides (e.g., *Trichoderma* spp.), bioherbicides (*Phytopthora* spp.), bioinsecticides (spore forming bacteria, *Bacillus thuringiensis*, and *B. popilliae*, Actinomycetes), naturally occurring fungi (*Beauveria bassiana*), microscopic roundworms (Entomopathogenic nematodes), Spinosad, insect hormones and insect growth regulators (Gupta and Dikshit, 2010; Singh et al., 2013).

Applications of microbial insecticide, *Chromobacterium subtsugae* for suppression of pecan weevil (*Curculio caryae* (Horn)), and combination of eucalyptus extract and microbial insecticide, *Isaria fumosorosea* (Wize) for control of black pecan aphid (*Melanocallis caryaefoliae* (Davis)) were found promising as alternative insecticides (Shapiro-Ilan et al., 2013). Entomopathogenic nematodes (EPNs) belonging to the families Heterorhabditidae and Steinernematidae are potentially used in South Africa as biocontrol agents against vine mealybug (*Planococcus ficus* (Signoret)) (le Vieux and Malan, 2013). Spinosad was found effective in controlling Colorado potato beetle (*Leptinotarsa decemlineata*) in Iran, and is recommended for use in IPM program for Colorado potato beetle (Soltani and Agricultural, 2011). In China, entomopathogenic fungus (*Beauveria bassiana*) has shown great potential for the management of some bark beetle species including red turpentine beetle (RTB) (*Dendroctonus valens* LeConte), a destructive invasive pest (Zhang et al., 2011).

The allelopathic properties of plants can be exploited successfully as a tool for weed and pathogen reduction. In a rice field, application of allelopathic plant material @ 1-2 tonne/ha reduced weed diversity by 70% and increased yield by 20%. Numerous growth inhibitors identified from these allelopathic plants are responsible for their allelopathic properties and may be a useful source for the future development of bio-herbicides and pesticides (Xuan et al., 2005). A combination of coleopteran-active toxin, *Bacillus thuringiensis* Cry3Aa protoxin and protease inhibitors, especially a potato carboxypeptidase inhibitor, have efficiency in preventing damage to stored products and grains by stored grain coleopteran pests (Oppert et al., 2011).

7.5. Chemical control

Sometimes cultural and other agro-technical practices are not sufficient to keep pest population below economic injury level (lowest pest population density that will cause economic crop damage). Therefore, the chemical control agents are resorted to both as preventive and curative measures to minimize the insect pest damage. A good pesticide should be potent against pests, should not endanger the health of humans and non-target organisms, and should ultimately break down into harmless compounds so that it does not persist in environment. Both relative and specific toxicities of the pesticide need to be estimated in order to determine its potency.

It is very important to know spray droplet size and density chemical dosage, application timing, which can provide adequate pest control. There is also a need for research into the development of suitable packaging and disposal procedures, as well as refining of the application equipment. All of these shall rationalize the use of pesticides, so that they can be used in an acceptable way.

Very strict laws should be enacted to protect wildlife and other non-target organisms. Following directions on the pesticide label can prevent injury to non-target organisms. However, when these directions are not followed, benefits from pesticides can be outweighed by the harm and risk associated with pesticides (Fishel, 2011). During pesticide application, things that need to be considered are timing of insecticide application, dosage and persistence, and selective placement of insecticides as discussed below.

7.5.1. Timing of pesticide application

The timing of pesticide application is an important factor to consider before doing any pesticide application. Appropriate application time can ensure not only maximum impact on the target organisms but also least impact on beneficial organisms. Pesticide application timing mainly depends on availability of weather window, time at which pests can be best controlled, and when least damage will be caused to non-target organisms and environment. Flowering period in crops and middle of the day are the times when bees are more prone to insecticides. Hence, insecticide application should not happen at those times to avoid decline in bee populations. Time of insecticide application should coincide with the most vulnerable stage of insect life cycle. Monitoring of insects in the field is thus extremely important for knowing the stage of insect pest in the field. Monitoring systems are available for most of the insect pests, but spray regime or experiments need to be carried out to determine the most appropriate time for insecticide application for insects for which monitoring systems are not available (Hull and Starner, 1983; Richter and Fuxa, 1984).

Time of the day and season of the year are also important to consider when making pesticide applications. The early morning and evening hours are often the best times for pesticide application because windy conditions are more likely to occur around midday when the temperature warms near the ground level. This causes hot air to rise quickly and mix rapidly with the cooler air above it, favoring drift. During stable conditions, a layer of warm air can stay overhead and not promote mixing with colder air that stays below and closer to the ground. Inversions tend to dissipate during the middle of the day when wind currents mix the air layers. It is very important that applicators recognize thermal inversions and do not spray under those conditions. A temperature or thermal inversion is a condition that occurs naturally and exists when the air at ground level is cooler than the temperature of the air above it. Wind speed is the most important weather factor influencing drift. High wind speeds will move droplets downwind and deposit them off the target. On the other hand, dead calm conditions are never recommended due to likelihood of temperature inversions (Fishel and Ferrell, 2013). Drifting of pesticides increases the possibility of injury to pollinators, humans, domestic animals and wildlife. It is recommended not to spray in wind speed above 2.5 miles/second which otherwise can cause excessive drift and eventually contamination of adjacent areas (Matthews, 1981). Pesticide application should not be made just before rain because pesticides can be washed off by the rain without any impact on the target pest.

7.5.2. Dosage and persistence

Pesticide dose should be sufficient but no greater than the level required for best results. The pesticide manufacturer sets the dose to ensure an acceptable level of control, producing acceptable residue levels, and maximizing returns per unit of formulated insecticide. Persistent pesticides have their benefit of longer persistence on the target and therefore requires less frequent spraying compared to non- persistent pesticides. But care should be taken while using persistent pesticides since these might diminish benefits from natural enemies even at lower doses. If an insecticide is persistent in nature, chances of insecticide residues being harmful to natural enemies are greatly increased (Dent, 2000).

7.5.3. Selective placement

Distribution of pesticides in the field should be such that maximum target cover is achieved. Usually only about 1% of the applied pesticides is able to reach its target, while a large amount of it is wasted. Understanding the pest biology and behavior is critical as it can provide information on pest's habitat, fecundity, feeding etc., which can be important considerations before applying pesticides. Most of the pesticides are applied in liquid form and thus the droplet size is very important in determining their effectiveness. Small droplets provide better coverage and greater likelihood of coming in contact with the target compared to larger droplets that can bounce off the plant surface very easily. The disadvantage with smaller and bigger droplets is the increased chance of drift and therefore a balance has to be considered between smaller droplets to obtain the maximum effectiveness and reduced drift.

In situations where crops are grown on beds covered with plastic mulch, pesticides should be injected into soil at the time the plastic is laid or injected afterward through drip irrigation system to achieve maximum pesticide effectiveness. For termite (Order: Isoptera) treatments, sometimes perimeter application of insecticides is required around structures/buildings. Additionally, liquids that form foams following injections can be injected into small spaces that are or might be inhabited by termites or other small creatures.

8. Conclusion

Although, pesticides were used initially to benefit human life through increase in agricultural productivity and by controlling infectious disease, their adverse effects have overweighed the benefits associated with their use. The above discussion clearly highlights the severe consequences of indiscriminate pesticide use on different environmental components. Some of the adverse effects associated with pesticide application have emerged in the form of increase in resistant pest population, decline in on beneficial organisms such as predators, pollinators and earthworms, change in soil microbial diversity, and contamination of water and air ecosystem. The persistent nature of pesticides has impacted our ecosystem to such an extent that pesticides have entered into various food chains and into the higher trophic levels such as that of humans and other large mammals. Some of the acute and chronic human illnesses have now emerged as a consequence of intake of polluted water, air or food.

This is the time that necessitates the proper use of pesticides to protect our environment and eventually health hazards associated with it. Alternative pest control strategies such as IPM that deploys a combination of different control measures such as cultural control, use of resistant genotype, physical and mechanical control, and rational use of pesticide could reduce the number and amount of pesticide applications. Further, advanced approaches such as biotechnology and nanotechnology could facilitate in developing resistant genotype or pesticides with fewer adverse effects. Community development and various extension programs that could educate and encourage farmers to adopt the innovative IPM strategies hold the key to reduce the deleterious impact of pesticides on our environment.

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The edited book Pesticides - Toxic Aspects contains an overview of attractive researchers of pesticide toxicology that covers the hazardous effects of common chemical pesticide agents employed every day in our agricultural practices. The combination of experimental and theoretical pesticide investigations of current interest will make this book of significance to researchers, scientists, engineers, and graduate students who make use of those different investigations to understand the toxic aspects of pesticides. We hope that this book will continue to meet the expectations and needs of all interested in different aspects of pesticide toxicity.

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