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Occupational exposure to pesticides as a possible risk factor for the development of chronic diseases in humans (Review)

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Abstract. It is well known that pesticides are widely used compounds. In fact, their use in agriculture, forestry, fishery and the food industry has granted a huge improvement in terms of productive efficiency. However, a great number of epidemiological surveys have demonstrated that these toxic compounds can interact and exert negative effects not only with their targets (pests, herbs and fungi), but also with the rest of the environment, including humans. This is particularly relevant in the case of workers involved in the production, transportation, preparation and application of these toxicants. Accordingly, a growing body of evidence has demonstrated the correlation between occupational exposure to pesticides and the development of a wide spectrum of pathologies, ranging from eczema to neurological diseases and cancer. Pesticide exposure is often quite difficult to establish, as many currently used modules do not take into account all of the many variables that can occur in a diverse environment, such as the agricultural sector, and the assessment of the real risk for every single worker is problematic. Indeed, the use of personal protection equipment is necessary while handling these toxic compounds, but education of workers can be even more important: personal contamination with pesticides may occur even in apparently harmless situations. This review summarises the most recent findings describing the association between pesticide occupational exposure and the development of chronic diseases.

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1. Introduction

Different environmental exposures during work activities may affect the health conditions of workers, although they use the personal protective equipment in agreement with the current regulations (1-3). Several examples of the adverse effects linked with such occupational exposures have been observed in our previous studies (4-14). All of these effects may cause biomolecular alterations, resulting in the development of specific diseases (15-20). An example of occupational environmental exposure is that of the agricultural setting where pesticides are commonly used (21-23). Many components included in pesticides are apparently not toxic to humans (24,25); however, studies have demonstrated that chronic exposure to pesticides may be a risk factor for microenvironment perturbation and for the development of chronic diseases (26,27).

Humans are exposed to pesticides though occupational or environmental exposure. In the first case, rural workers, greenhouse workers, workers in pesticide manufacture, mixing or application, bystanders, are considered the group that receive the greatest exposure, by mixing, loading, transporting and applying pesticides. Furthermore, many workers, particularly in developing countries, are not adequately informed about the risks associated with the use of pesticides, and the lack of training and equipment to safely handle pesticides increases the health risk (28).

As regards environmental exposure, this mainly affects the general population through the consumption of polluted food and drinking contaminated water or using pesticides in the home or living close to sprayed fields. Thus, risk assessment and prevention from pesticide exposure is not a simple process, particularly when we consider several limitations, such as differences in the time and the levels of exposure, the class of pesticides (as regards chemical structure and toxicity), mixtures or cocktails used, and the geographical and climate features of the areas where pesticides are applied.

For these reasons, it has been suggested that pesticide safety should be tested before and after marketing, in order to evaluate whether the estimated or predicted individual exposure on a given task is suitable (29).

Furthermore, the use of human epidemiological studies can improve the efficacy and strength of the risk assessment process, as epidemiological data represent the most direct evidence of an association between health effects and pesticide exposure (30).

On this ground, this review first summarises the risk assessment and management for workers exposed to pesticides and then focuses on the association between occupational exposure to pesticides and the occurrence of chronic diseases (Table I).

2. Pesticide risk assessment

Due to the potential toxic effects of pesticide exposure, both acute and chronic, it is important to determine the amount of active principle to which a worker is exposed during his/her occupational activities. However, this is not always simple (31), either due to the fact that in rural areas, the separation between the working environment and the living one is not well-defined, as fields can be very close to farmers' houses, or due to other confounding factors.

The use of personal protective equipment has always been considered fundamental for the safety of workers, as firstly suggested by Libich *et al* (32), who reported a significant reduction in the urinary levels of herbicide metabolite in pesticide applicators, following an educational path on the hazards of dermal contact with pesticides and the implementation of personal protection equipment (PPE) in their working routine.

Interestingly, Arbuckle *et al* (33), in a study conducted on a population of farm workers in Ontario, did not highlight any significant correlation between the use of pesticides and the implementation of PPE in individual working routines, with the exception for the use of gloves or face shields during the application of 2,4-dichlorophenoxyacetic acid (2,4-D).

Environmental monitoring is often not reliable for the determination of pesticide exposure due to certain, such as climate changes and variables in the use of these substances by the operators. In this regard, a study performed in northern Italy (34) provided some interesting evidence. The study was conducted on 11 rice and 13 maize farms in Lombardy (Italy), which had a small number of workers (3 or 4), and participated in the study with one worker only, who was in charge for pesticides application. Since the main route of pesticide absorption is cutaneous (35-37), the exposure was assessed by using pads (skin exposure) and by washing workers' hands at the end of the working shift with 200 ml of iso-propanol. The hand wash liquid was collected for analysis (hand skin exposure) according to the Organization for Economic Cooperation and Development (OECD) guidelines (38). A 24-h urine collection was performed for biomonitoring. The study results showed a non-linear relationship between parameters, such as the amount of pesticides used and the amount of pesticides found on the workers' clothes, between the amount of pesticides on the clothes and the amount of pesticides on workers' skin, and, finally, between the amount of pesticides excreted in the urine and the amount found on the skin. Furthermore, authors demonstrated an inverse correlation between the size of the estate and the levels of workers' exposure, similar to the results obtained by Baldi *et al* (39).

As suggested by the above-mentioned previous study (34), real values of exposure to pesticides are not always easily predictable. A recent study conducted in France, the PESTEXPO study (40), highlighted workers' specific tasks as another important determinant regarding exposure to pesticides. Occupational exposure to pesticides, in fact, is usually associated only with the mixing of these substances, transportation, application to crops and cleaning of the equipment. Actually, exposure to pesticides can occur in other situations, such as the so-called re-entry operations, such as cutting branches and the raising of wires, when operators manipulate plants that can still be contaminated with toxicants. The PESTEXPO study involved vineyard workers of the area of Bordeaux, which is particularly rich in vineyards, and exposure was assessed by the application of patches over the clothes and directly on the skin, changed after every working switch. Hand skin exposure was evaluated by washing the hands with bottled water and collecting the wastewater, similarly to the method by Rubino *et al* (34). The study examined exposure levels during various re-entry activities and during the harvest, finding that contamination with pesticides is maximal when raising wires and cutting branches (among re-entry activities) and when picking grapes during the harvest. A lower level of exposure was registered when re-entry activities followed pesticide application by more than one week, even if no statistically significant correlation was found between workers' contamination and time since treatment. Furthermore, gender appeared to be the only worker's characteristic associated with different contamination, with the female median contamination much higher than that of the male one, and other elements such as humidity, previous rainfalls, and the characteristics of the crops and the estate appeared to be significant. This study demonstrated the importance of taking into account other activities such as harvesting and re-entry operations for a proper estimate of worker's exposure, adding complexity to the estimate of risk.

Over time, studies such as the PESTEXPO study and others have proposed new models to forecast workers' specific risk (41), even if it is still difficult to determine the effectiveness of the estimation of exposure, convenience and costs.

3. Use of job-exposure matrices and quantitative algorithms

A great number of environmental and biological monitoring methods have been improved to estimate human exposure to pesticides.

Over the past decade, it has become clear that it is necessary to develop new approaches for evaluating the safety of a pesticide before its introduction to the market (pre-marketing risk assessment and management) and during its use in industry and agriculture (post-marketing risk assessment and management) (29).

The pre-marketing risk assessment of pesticides is based on toxicological studies in order to evaluate the predicted exposure of the operator, taking into account the typical conditions of pesticide application. However, authors have revealed some limitations: the majority of toxicological studies are based on tests performed on animals which were exposed to high levels of a single pesticide at a time; the examined conditions of application are not absolutely representative of the variability of scenarios, cultures and exposure conditions of all geographical areas. As regards the post-marketing assessment, it involves occupational exposure measurements in industry and in agriculture and public health. In the industrial setting, it is easy to evaluate such exposure due to the limited number of pesticides involved, whereas, the exposure conditions, the use of mixtures of pesticides, the variety of operator tasks, the environmental conditions and the individual characteristics (use of PPE, personal hygienic behavior, professional training) make the assessment difficult in agriculture and public health (29).

Data from epidemiological studies are not currently used for human risk assessment in a systematic consistent way due to several limitations, such as inadequate exposure characterization, lack of quantitative data, potential confounding factors and any biological plausibility. As epidemiological data represent the most direct evidence of an association between health effects and pesticide exposure, several authors suggest the opportunity of an integrated approach of *in vivo*, *in vitro* and *in silico* data, together with well-designed epidemiological studies (30,41-43).

Another tool in the assessment of the risk due to pesticide exposure is represented by the application of indirect measurements, such as expert's judgment, job questionnaires, calendars and job-exposure matrices (JEMs) (44-46). Even in this case, care must be given to avoid worker's misclassification due to the said great difference in the degree of exposure depending on the worker's task. Due to the different cultural levels among workers and to the wide amount of pesticides used and the different jobs that a single worker may have done in his/her life, self-reported information is not always reliable and requires the judgment of an expert or the consultation of registers and work calendars. The use of JEMs is a valuable resource, particularly when it is difficult to reconstruct an accurate work history regarding exposure to pesticides, as might be the case for retrospective studies. A JEM is compiled taking into account the work done and different exposure parameters, such as intensity and duration, as well as various details about the temporal variations of exposure.

A systematic review (47) summarised various models of JEMs that have been developed over time to assess pesticide exposure. The authors differentiated between JEMs studied specifically for agricultural cohorts, and others that have been produced to assess exposure in the general population.

The comparison between different JEMs (47) led the authors to the conclusion that these tools can be particularly useful to pesticide toxicology, particularly towards the study of the impact of these toxicants in the development of chronic diseases. Even if the results obtained with the use of JEMs look promising, the literature lacks comparisons between these results and those obtained with other exposure assessment methods.

Dosemeci *et al* (48), in a cohort study conducted on more than 58,000 pesticide applicators of North Carolina and Iowa, suggested a quantitative method, based on two algorithms (a 'general' algorithm and a 'detailed' algorithm), in order to evaluate long-term chemical-specific pesticide exposure. Occupational data were obtained using an enrolment questionnaire and a detailed take-home questionnaire, to estimate possible selection bias for the subcohort in terms of differences in pesticide exposure levels. The weighting factors applied in both algorithms were calculated using measurement data from the published pesticide exposure literature and professional judgement. The distribution patterns of all basic exposure indices (i.e., intensity, duration and cumulative exposure to 2,4-D and chlorpyrifos) by state, gender, age and applicator type were almost equal in two study populations, demonstrating that the take-home questionnaire subcohort of applicators is characteristic of the entire cohort in terms of exposure. Despite the use of different variables for each algorithm, high concordance was detected between the 'general' algorithm and the 'detailed' one, as regards percentage agreement and correlation. The results of that study suggest that this quantitative method can be very helpful to evaluate the risk of developing several chronic diseases in subjects exposed to pesticides (48).

Negatu *et al* (49) developed two semi-quantitative exposure algorithms to assess cumulative exposure to pesticides in a group of applicators and re-entry workers of different farming systems in Ethiopia. These algorithms were based on detailed exposure factors, including application methods, farm layout (open or closed), pesticide mixing circumstances, the cleaning of spraying equipment, the amount of pesticide application per day, the utilization of PPE, personal hygienic behavior, the annual frequency of application, and the length of employment at the farm. The algorithms assessed the daily, annual and cumulative lifetime exposure for applicators and re-entry workers by farming system, gender and age group; these can be helpful mainly for evaluating acute and chronic health effects in workers exposed to pesticides, particularly in low- and middle-income countries (49).

Nevertheless, establishing the correct level of exposure of an individual is not sufficient for a proper assessment of risk. Individual characteristics may, in turn, determine different levels of susceptibility (genetic or epigenetic) to a chemical compound between different individuals and even in the same individual. For example, cutaneous lesions can greatly increase the percentage dose of pesticides absorbed, and chronic skin pathologies, such as psoriasis can exert a similar effect. In addition, chronic liver diseases can slow down the metabolism of pesticides, resulting in a longer plasmatic half-life and, consequently, in an enhanced toxicity.

4. Epigenetics

The term 'epigenetics' refers to alterations in genomic expression that do not involve alterations in DNA. Even if the DNA sequence is not altered, epigenetic alterations can be persistent through several cell division cycles, and can even be inheritable.

Epigenetics has been linked to the development of cancer, and a recent study indicated that epigenetic alterations may be involved in the pathogenesis of diseases as a result of chronic toxic chemical exposure (50).

Table I. Studies focusing on the assessment of pesticide exposure.

Year	Authors/(Refs.)	Article type	Title	Subject
1984	Libich *et a*l (32)	Research	Occupational exposure of herbicide applicators to herbicides used along electric power transmission line right-of-way	Pesticide exposure routes
2002	Aprea *et a*l (31)	Review	Biological monitoring of pesticide exposure: a review of analytical methods	Analytical methods for pesticide biological monitoring
2002	Dosemeci *et a*l (48)	Research	A quantitative approach for estimating exposure to pesticides in the Agricultural Health Study	A prospective cohort study on more than 58,000 pesticide applicators aimed to develop a quantitative method to estimate long term chemical-specific pesticide exposure
2005	Arbuckle *et a*l (33)	Research	Biomonitoring of herbicides in Ontario farm applicators	Biomonitoring of pesticide metabolites in the urine of farm applicators
2005	Aprea *et a*l (35)	Research	Evaluation of skin and respiratory doses and urinary excretion of alkylphosphates in workers exposed to dimethoate during treatment of olive trees	Pesticide exposure routes
2006	Baldi *et a*l (39)	Research	Pesticide contamination of workers in vineyards in France	Exposure assessment in farm workers with different tasks
2006	Maroni *et a*l (29)	Research	Risk assessment and management of. occupational exposure to pesticides in agriculture	A study on the pre-marketing and post-marketing assessment and management of occupational exposure to pesticides as the base for improving health surveillance of exposed workers
2008	Flack *et a*l (36)	Research	Development and application of quantitative methods for monitoring dermal and inhalation exposure to propiconazole	Pesticide exposure routes
2009	Vitali *et a*l (37)	Research	Operative modalities and exposure to pesticides during open field treatments among a group of agricultural subcontractors	Pesticide exposure routes
2012	Colosio *et a*l (41)	Research	Integration of biological monitoring, environmental monitoring and computational modelling into the interpretation of pesticide exposure data: Introduction to a proposed approach	Combined approach to estimate worker's pesticide exposure
2012	Rubino *et a*l (34)	Research	Farmers' exposure to herbicides in North Italy: Assessment under real-life conditions in small-size rice and corn farms	Herbicide exposure assessment in a population of farmers
2014	Baldi *et a*l (40)	Research	Levels and determinants of pesticide exposure in re-entryworkers in vineyards: Results of the PESTEXPO study	Exposure assessment in vineyard workers after re-entry tasks
2016	Peters *et a*l (44)	Research	A Quantitative Job-Exposure Matrix for Five Lung Carcinogens	Development of a JEM for lung carcinogens
2016	Alif *et a*l (45)	Review	Occupational exposure and risk of chronic obstructive pulmonary disease: A systematic review and meta-analysis	Assessment of risk for COPD through the use of JEMs
2016	Milner *et a*l (46)	Research	Validity of a Job-Exposure Matrix for Survey Psychosocial Job Stressors: Results from the Household Income and Labour Dynamics in Australia Survey	Development of a JEM
2016	Carles *et a*l (47)	Review	Use of job-exposure matrices to estimate occupational exposure to pesticides: A review	Systematic review of JEMs related to pesticide exposure

Table I. Continued.

Year	Authors/(Refs.)	Article type	Title	Subject
2016	Negatu *et a*l (49)	Research	A Method for Semi-quantitative Assessment of Exposure to Pesticides of Applicators and Re-entry Workers: An Application in Three Farming Systems in Ethiopia	A cross-sectional study on farmers and farm workers, including applicators and re-entry farmers, aimed to identify an easily adaptable semi-quantitative method to assess pesticide exposure and farm workers in low- and middle-income countries
2016	Hernández Jerez *et al* (30)	Congress Abstract	Integrating epidemiological, mechanistic and experimental toxicology data for pesticide risk assessment	Authors suggest an integrated approach of *in vivo*, *in vitro* and *in silico* data, together with well-designed epidemiological studies in order to improve hazard and exposure assessments of pesticides

JEM, job-exposure matrice; COPD, chronic obstructive pulmonary disease.

The most typical epigenetic mechanism is DNA methylation, which consists of the addition of a methyl group on a cytosine nucleotide 5th carbon, when the cytosine is followed by a guanine nucleotide in the DNA sequence (CpG island). The hypermetilation of promoters usually produces an inactivation of the following gene transcription (51,52). Other mechanisms include post-translational histone modification, which is thought to alter chromosome structure and function (53), and miRNAs, namely non-coding RNA sequences with regulatory functions towards gene expression (54).

In vitro, animal and human studies have recognised several classes of pesticides that can alter epigenetic markers (55) (Table II).

Collotta *et al* highlighted how DNA methylation can cause the skewing of an allele, resulting in the expression of only one of the two (paternal or maternal), with possible implication in the metabolism of toxic substances (55). The administration of methoxyclor, an organochlorine used as an insecticide, disrupts the female offspring reproductive capacity by remodelling the expression of sex hormones (56). As regards the regulatory role of epigenetics in pesticide toxicology, Holland *et al* (57) investigated the role of paraoxonase 1 (PON1), a multifunctional enzyme particularly implicated in the metabolism of organophosphates (58,59). PON1 activity varies significantly among individuals, and several molecular variants of this enzyme are recognised. Pesticide exposure and the consequent oxidative stress both have an impact on PON1 gene polymorphisms (60), and research has indicated that an epigenetic mechanism may be responsible for these alterations (61,62).

In a recent case-control study, Howard *et al* demonstrated an increase in DNAmethylation at 36 CpG sites in a group of Latino farmworkers compared to a sample of 60 Latino non-farmworkers. The authors for the first time analysed gene-specific, genome-wide DNA methylation in subjects occupationally exposed to pesticides, and their results suggest that there is a single epigenetic profile in farmworkers over the growing season, compared to non-farmworkers. A variety of 72 genes may be involved, supporting the hypothesis that alterations in DNA methylation may affect several biological-pathways and potential disease development (63).

5. Diabetes mellitus

Diabetes mellitus (DM) is a disease characterised by chronic hyperglycaemia due to absolute or relative insulin deficiency, resistance, or both. Two patterns of diabetes are commonly recognised: DM type 1, characterised by early onset in life and a likely autoimmune pathogenesis, and DM type 2, by far the most common form, with a later onset in life and a multifactorial pathogenesis.

Nowadays, DM type 2 is one of the most common chronic diseases in first world countries with 415 million individuals affected, and its incidence is expected to increase in the following years (64). Even though individuals affected by diabetes can lead a normal life due to pharmacological therapy, long-term complications are still very common and include cardiovascular, neurological and renal diseases. Obviously, the health-related costs of diabetes are extremely high.

Epidemiological studies have suggested that the pathogenesis of DM is multifactorial, involving both genetic and lifestyle risk factors: a poor unhealthy diet and obesity, a sedentary lifestyle and cigarette smoking can contribute to the pathogenesis of the disease (65).

Over the past years, pesticides, as well as other environmental factors, have been indicated as possible co-factors in the pathogenesis of DM (66-68) (Table III). A systematic review analysed the link between pesticides, DM, and diabetes-related pathologies (69), based on the increasing prevalence of both DM2 and the use of pesticides in Asia. The results, even if often suggestive of a potential link, were nevertheless inconclusive due to the lack of a proper assessment of exposure or other confounding factors.

Another systematic review (70) pointed out a very likely association between certain types of pesticides, in particular organochlorines and organophosphates, and the development of DM. Even if suggestive, the results of the reviewed studies

Table II. Studies focusing on the interaction between epigenetics and pesticides.

Year	Authors/(Refs.)	Article type	Title	Subject
2002	Orphanides and Reinberg (51)	Review	A unified theory of gene expression	New hypothesis about gene expression
2003	Grewal and Moazed (52)	Review	Heterochromatin and epigenetic	Regulation of gene expression control of gene expression
2003	Lee *et al* (58)	Research	Association between human paraoxonase gene polymorphism and chronic symptoms in pesticide-exposed workers	Paraoxonase polymorphism in pesticide-exposed workers
2005	Robertson (61)	Review	DNA methylation and human disease	The epigenetic role of DNA methylation
2007	Kouzarides *et a*l (53)	Review	Chromatin modifications and their function in DNA expression	The role of chromatin alterations
2007	Jackson and Standart (54)	Review	How do microRNAs regulate	The role of microRNAs in gene expression? gene expression
2011	Stouder and Paoloni-Giacobino (56)	Research	Specific transgenerational imprinting effects of the endocrine disruptor methoxychlor on male gametes	Endocrine-disrupting effects of pesticides
2011	Matthews *et a*l (59)	Review	Serum paraoxonase 1 (PON 1) genotype and exposure to organophosphorous insectides - is there a high-risk population?	Interaction between POn-1 enzyme and organophosphates
2013	Collotta *et a*l (55)	Review	Epigenetics and pesticides modifications	Pesticide-induced epigenetic
2013	Tammen *et a*l (62)	Review	Epigenetics: The link between nature and nurture	Interactions between environment and epigenetic modifications
2015	Holland *et a*l (57)	Review	Recent progress in the genetics and epigenetics of paraoxonase: Why it is relevant to children's environmental health	Implication of pareoxonase in pesticide-mediated chronic effects
2015	Costa *et a*l (60)	Research	Oxidative stress biomarkers and paraoxonase 1 polymorphism frequency in farmers occupationally exposed to pesticides	Evaluation of PON-1 polymorphism in a group of farmers exposed to pesticides
2016	Howard *et a*l (63)	Research	Changes in DNA methylation over the growing season difference between North Carolina farmworkers and non-farmworkers	A case-control study on the effects on DNA methylation of pesticide exposure in a group of Latino farmworkers

were criticised by the authors due to the multifactorial pathogenesis of diabetes, which is a confounding factor that is not always considered during experimental studies.

An interesting aspect pointed out by Leso *et al* was that many of the studies examined the retrospectively addressed mortality rates for diabetes, while only a few studies assessed early disturbances, such as impaired glucose tolerance, which would be of particular interest as regards prevention (70).

6. Neurological diseases

Even if there is not a consensus about the causal role of pesticides in the development of neurodegenerative disorders, increasing evidences has indicated an association between occupational and environmental exposure to these toxicants and the development of neurological diseases (71) (Table IV). Amongst these diseases, Parkinson's disease (PD), Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) seem to be the most likely to be associated with the neurotoxic effects of pesticides. Discovered by James Parkinson in 1817, PD is clinically characterised by the onset of progressive tremors, rigidity and akinesia, the severity of which gradually increases over the years. AD is the most common form of dementia, of which the most important features are a slow onset with a progressive loss of the ability to learn, retain and process information, apraxia, agnosia, the progressive loss of executive function and behavioural changes. ALS is also a progressive disease, which leads to spastic tetraparesis or paraparesis with the addition of lower motoneurone signs (72-74).

A recent meta-analysis (75) confronted several systematic reviews, which correlated the exposure to pesticides with neurodevelopmental disorders in children or major clinical outcomes, such as PD and AD in adults, aiming to establish a better assessment of risk for individuals exposed to these

Table III. Studies focusing on pesticide exposure and the incidence of diabetes.

Year	Authors/(Refs.)	Article type	Title	Subject
2008	Montgomery *et al* (67)	Research	Incident diabetes and pesticide exposure among licensed pesticide applicators: Agricultural Health Study, 1993-2003	Incidence of diabetes in a population of agricultural workers
2011	Hu (65)	Review	Globalization of diabetes: The role of diet, lifestyle, and genes	Role of different factors in the increase of diabetes type 2
2013	Malekirad *et al* (66)	Research	Neurocognitive, mental health, and glucose disorders in farmers exposed to organophosphorus pesticides	Cross-sectional study on workers exposed to pesticides
2014	Starling *et al* (68)	Research	Pesticide use and incident diabetes among wives of farmers in the Agricultural Health Study	Diabetes incidence among wives of farmers, based on a previous study in Iowa
2015	Jaacks and Staimez (69)	Review	Association of persistent organic pollutants and non-persistent pesticides with diabetes and diabetes-related health outcomes in Asia: A systematic review	Systematic review on the role of pesticides and pollutants in diabetes incidence
2016	Leso *et al* (70)	Review	Occupational chemical exposure and diabetes mellitus risk	Systematic review about the role of occupational exposure to chemicals and diabetes incidence

toxic compounds. As regards neurodegenerative disorders, the authors found a correlation between pesticide exposure and AD, particularly in men, probably due to the fact that mens' working activities usually involve a greater exposure to pesticides compared to those of women. The strongest correlation was shown between pesticides (in general) and the development of PD. Nevertheless, even if the association appears clear, it is difficult to narrow the field to a selected group of pesticides as responsible for this disease (75).

A recent study conducted in California (76) evaluated the association between pesticides and PD, with particular attention being paid to avoiding bias, such as ensuring the adequate representativeness of the source population (cases and controls were selected from the same region) and exposure assessment, which considered sources of contamination from the working environment, but also the household and the general environment. The authors found a positive correlation between pesticide exposure and PD and, according to several studies (77-79), noted the importance of individual susceptibility in the assessment of risk when exposed to toxicants. They also confirmed that the PON1 polymorphysms increase organophosphate susceptibility in slow metabolizers, as well as nitric oxide synthase 1 (NOS1) gene variants that could be responsible for an enhanced nitrosative stress pathway. Finally, they indicated that multidrug resistance protein 1 (ABCB1) gene variants may be responsible for organochlorine-induced PD, and that pyrethroid insecticides can alter immune response in HLA-DR variant carriers.

Recently, the use of adverse outcome (AO) pathways (AOPs) represents a potential tool in toxicology and human risk assessment (80). An AOP is defined as the progression from a molecular initiating event (MIE; a direct interaction of a chemical with its molecular target) through subsequent responses at cellular, tissue, organ and individual organism levels, leading to an AO that is important to risk assessment and regulatory decision-making (81). The European Food Safety Authority (EFSA) suggest that the AOPs share several pragmatic characteristics which make them useful to better identify a possible relationship of cause and effect between exposure to pesticides and PD. In PD, many different AOPs can be identified; but for several specific characteristics, the 'parkinsonian motor symptoms' were selected as the AO (82). In this regard, the overall weight of evidence indicates a significant correlation between the MIE and the AO in PD. A better understanding of key events and key event relationships will make it possible for researchers to use all available data in order to assess the risk and recognise possible causal relationship between exposure to specific pesticides and PD (71).

A meta-analysis in 2012 (83) examined the correlation between pesticide exposure and the development of ALS, and the results showed a positive link with exposure to pesticides as a group, particularly with organochlorines. Another meta-analysis (84) tried to evaluate the correlation between ALS and rural areas, with particular attention being paid to assessing pesticide exposure. The authors noted an increased risk of ALS with pesticide exposure and farmer occupation, but not with rural living. Furthermore, the risk related to the use of pesticides had a significant positive association in men.

As regards neurodevelopmental disorders, Hernandez *et al* (75) found a solid correlation between prenatal exposure to organophosphates and the alteration of mental or psychomotor development, or disorders like pervasive developmental disorder or attention deficit and hyperactivity disorder in preschool and school children (85,86). Other studies correlated maternal exposure to organochlorines and the development of motor or cognitive disorders in children, or pre- and post-natal

Table IV. Studies focusing on the link between pesticide exposure and neurological diseases.

Year	Authors/(Refs.)	Article type	Title	Subject
2012	Freire and Koifman (78)	Review	Pesticide exposure and Parkinson's disease: epidemiological evidence of association	Systematic review about the relation between pesticides and Parkinson's disease
2012	Kamel *et al* (83)	Review	Pesticide exposure and amyotrophic lateral sclerosis	Meta-analysis about the impact of pesticides exposure in the development of ALS
2013	Muñoz-Quezada *et al* (86)	Review	Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review	Systematic review on the effects of pesticides exposure in children
2013	Dardiotis *et al* (79)	Review	The interplay between environmental and genetic factors in Parkinson's disease susceptibility: The evidence forpesticides	Review about the relation between pesticides and Parkinson's disease
2014	Gonzalez *et al* (85)	Review	A systematic review of neurodevelopmental effects of prenatal and postnatal organophosphate pesticide exposure	Systematic review on the effects of prenatal and postnatal pesticides exposure in children
2014	Kang *et al* (84)	Review	Amyotrophic lateral sclerosis and agricultural environments: A systematic review	Systematic review about the impact of pesticides exposure in the development of ALS
2014	Wang *et al* (77)	Research	The association between ambient exposure to organophosphates and Parkinson's disease risk	Case-control study about the relation between OP exposure and Parkinson's disease
2016	Elbaz and Moisan (72)	Review	The scientific bases to consider Parkinson's disease an occupational disease in agriculture professionals exposed to pesticides in France	Occupational exposure to pesticides and the incidence of Parkinson's disease
2016	EFSA (82)	Research	Investigation into experimental toxicological properties of plant protection products having a potential link to Parkinson's disease and childhood leukaemia	A study based on the adverse outcome pathways (AOPs) in order to investigate the plausible involvement of pesticide exposure as a risk factor for Parkinson's disease
2016	Hernandez *et al* (75)	Review	Systematic reviews on neurodegenerative disorders neurodevelopmental and linked to pesticide exposure: Methodological features and impact on risk assessment	Systematic review on occupational exposure to pesticides and incidence of neurological diseases
2016	Ritz *et al* (77)	Review	Of Pesticides and Men: A California Story of Genes and Environment in Parkinson's Disease	Pathways involved in pesticide-induced Parkinson's disease
2016	Wilks (71)	Congress Abstract	Pesticides and Parkinson's disease - What is the evidence from epidemiological and experimental studies?	The author deals with the use of the OECD Adverse Outcome Pathways as a tool for integration of heterogenous data from different sources (human, animal *in vitro*) to better define a possible causality between exposure to pesticides and Parkinson's disease

exposure to pesticides and the development of autism spectrum disorders; however, factors such as design of the studies did not allow the authors of the meta-analysis to draw any definitive conclusions.

Table V. Studies focusing on the association between pesticide exposure and reproductive disorders.

Year	Authors/(Refs.)	Article type	Title	Subject
2001	Arbuckle *et al* (90)	Research	An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population	The impact of pesticide exposure on spontaneous abortion in a population of women
2004	Hanke and Jurewicz (88)	Review	The risk of adverse reproductive and developmental disorders due to occupational pesticide exposure: an overview of current epidemiological evidence	The risk of pathological alterations on offspring due to parent's pesticide exposure
2004	Kumar (89)	Review	Occupational exposure associated with reproductive dysfunction	The harmful effects of pesticide exposure on the reproductive system
2007	Jurewicz and (92) Hanke	Review	Risk of reproductive disorders in greenhouse workers	A literature review about the association of greenhouse work and reproductive disorders
2008	Jurewicz *et al* (91)	Research	Low risk of reproductive disorders among female greenhouse workers-safe work conditions or health selection for the light work?	Impact of hard work and pesticide exposure on pathological pregnancy in a population of greenhouse women workers
2012	Reynolds and Caton (87)	Review	Role of the pre- and postnatal environment in developmental programming of health and productivity	The role of developmental insults on offspring
2014	Neghab *et al* (96)	Research	The effects of exposure to pesticides on the fecundity status of farm workers resident in a rural region of Fars province, southern Iran	A study regarding the prevalence of reproductive disorders in a population of male and female subjects in Iran
2015	Miranda-Contreras *et al* (93)	Research	Effects of occupational exposure to pesticides on semen quality of workers in an agricultural community of Merida state, Venezuela	A case-control study aimed at investigating the association between occupational exposure to pesticides and semen quality in a group of farmers stratified by age
2015	Radwan *et al* (94)	Research	The association between environmental exposure to pyrethroids and sperm aneuploidy	A study aimed at evaluating whether the environmental exposure to pyrethroids is associated with male sperm chromosomal disomy
2015	Jamal *et al* (95)	Research	The influence of organophosphate and carbamate on sperm chromatin and and reproductive hormones among pesticide sprayers	A study aimed at evaluating the association between occupational exposure to organophosphate and carbamate pesticides and semen quality, as well as the levels of reproductive and thyroid hormones in a group of pesticide sprayers in Malihabad

7. Reproductive disorders

There is strong evidence to suggest that certain environmental conditions can have a long-term impact on human health, particularly on the male and female reproductive systems, before birth or during infancy (87).

The link between occupational and environmental pesticide exposure has been widely evaluated and studies have demonstrated that exposure to pesticides can impair fertility in both male and female subjects (88,89) (Table V).

A study conducted in Ontario examined the correlation between pesticide exposure in farms and spontaneous abortion. The authors observed a moderate risk of early abortions for preconception exposures to phenoxyacetic acid herbicides, triazines and any herbicide. They also noted that pre-conceptional exposure to a miscellaneous class of

Table VI. Studies focusing on the link between pesticide exposure and cancer.

Year	Authors/(Refs.)	Article type	Title	Subject
2013	Costa *et al* (108)	Research	Cytokine patterns in greenhouse workers occupationally exposed to α-cypermethrin: An observational study	The authors support the hypothesis that pyrethroid exposure may reduce host defenses against infection and cancer, particularly in subjects with impaired immune capacity
2014	Doolan *et al* (102)	Review	An update on occupation and prostate cancer	The authors review occupational exposures and related factors associated with the risk of prostate cancer and they supported the involvement of polychlorinated biphenyls (PCBs) in prostate cancer
2014	Fenga *et al* (107)	Research	IL-17 and IL- 22 serum levels in greenhouse workers exposed to pesticides	The authors investigate IL-17 and IL-22 alterations in serum of subjects exposed to pesticides, supporting the hypothesis that exposure to pesticides may reduce host defenses against infections and cancer
2014	Schinasi and Leon (100)	Research	Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis	This research describes results from a systematic review and a series of meta analyses of nearly three decades worth of epidemiological research on the association between non-Hodgkin's lymphoma (NHL) and occupational exposure to agricultural pesticides
2015	Del Pup *et al* (99)	Review	Endocrine disruptors and female cancer: Informing the patients (Review)	The review investigates the effects of endocrine disruptors, including pesticides, on female neoplasms (breast, ovarian and endometrial cancer)
2016	Lewis-Mikhael *et al* (101)	Review	Occupational exposure to pesticides and prostate cancer: A systematic review and meta-analysis	A systematic review to assess the potential association between different degrees of exposure to pesticides and the risk of prostate cancer
2016	Gangemi *et al* (27)	Review	Occupational and environmental exposure to pesticides and cytokine pathways in chronic diseases (Review)	The review focuses on the immunotoxicity of pesticides and the development of several chronic diseases, including cancer
2016	Hernández and Menéndez (106)	Review	Linking Pesticide Exposure with Pediatric Leukemia: Potential Underlying Mechanisms	The authors review the available experimental and epidemiological evidence linking pesticide exposure to infant and childhood leukemia and provide a mechanistic basis to support the association
2016	Fenga (14)	Review	Occupational exposure and risk of breast cancer	A review investigating occupational exposure to several risk factors, including pesticides, and the risk of breast cancer

pesticides, thiocarbamates and glyphosate, was linked with a high-risk of late abortion (90). By contrast, a study performed on a population of female greenhouse workers did not reveal any significant association between exposure to pesticides and the occurrence of pre-term delivery, spontaneous abortion and birth defects (91).

As regards male subjects, exposure to pesticides can affect semen quality, sperm parameters (concentration, morphology, motility), sex hormone blood levels or the structure of the testes.

Jurewicz and Hanke showed a mean decrease in sperm count in men who were employed in greenhouses for more than ten years (92).

A comparative study performed in Venezuela by Miranda-Contreras *et al* analysed the sperm quality in farmers exposed to pesticides, showing significant variations of main semen characteristics in exposed subjects, such as a decrease in sperm concentration, reduced motility and alterations in the sperm membrane (93). Pyrethroids may cause sperm aneuploidy (94). Organophosphates and carbamates can act on semen quality and sex hormones, increasing thyroid-stimulating hormone (TSH) and luteinizing hormone (LH) blood levels (95).

A recent study compared a population of randomly selected couples, in which the man was employed on a farm, and the rest of the local population. The results revealed a higher prevalence of primary infertility, along with more frequent stillbirths and spontaneous abortion in farm working couples (96).

8. Cancer

The correlation between human pesticide exposure and the development of cancer has been a subject of great interest to the scientific community during the last half of the century, including the International Community for Research on Cancer (IARC) (Table VI).

Cancer is a multifactorial disease resulting from the exposure to several risk factors, including lifestyle habits (97). Even if neoplasms at different sites have been related to pesticide exposure, it is difficult to identify the specific role of a single pesticide in the development of these pathologies, particularly at low doses of exposure.

A review published in 2008 discussed the link between environmental and occupational exposure to harmful stimuli and cancer, observing an increased risk of leukaemia in subjects exposed to organochlorines (98). A correlation was also noted between pesticide exposure and lung cancer, multiple myeloma, non-Hodgkin's lymphoma, ovarian cancer, prostate cancer, rectal cancer, testicular cancer and skin cancer (27). Other studies have highlighted the role of environmental endocrine disruptors in female cancer (99), occupational exposure and breast cancer (14), non-Hodgkin's lymphoma (100) and prostate cancer (101,102).

These environmental stimuli may induce the involvement of the p53, Raf/MEK/ERK and PI3K/AKT pathways in the majority of these cancers, providing giving further insight into the mechanisms of malignant transformation (103-104).

Chronic low-dose exposure, in particular, is thought to be responsible for pre-natal and early post-natal alterations in children. A recent study (106) suggested a possible role of pesticides in the development of infant and pediatric leukaemia, the most common type of cancer in children, through interaction with DNA enzymes such as topoisomerase II and oxidative stress-induced DNA damage.

Among the potential mechanisms of cancerogenesis induced by pesticide exposure, it has been reported that pesticides can alter the immune system, leading to chronic inflammation and the increased production of inflammatory chemokines and cytokines (22).

In support of this suggestion, a cross-sectional study investigated the levels of IL-17 and IL-22 in a cohort of greenhouse workers occupationally exposed to several pesticides (neonicotinoids, pyrethroids and ogranophosphates). The result revealed a significant increase in IL-22 values, while IL-17 did not show significant alterations (107). These findings suggest a possible role of immune deregulation in pesticide-induced pathologies and cancer development. Another study by the same authors noted the potential role of altered immunity in the development of chronic diseases for workers exposed to pesticides (108).

9. Conclusions

The aim of this review was to evaluate the impact of pesticides on human health, particularly as regards the development of chronic diseases. Some pesticide-related diseases are extremely common and represent a heavy burden for public health expenditure.

Even though there are many clues pointing towards the association between pesticides, as a group, and the incidence of diseases such as PD, DM or cancer, experimental studies and systematic reviews on the subject are not always conclusive.

The main confounding factor, in our opinion, remains an accurate exposure assessment in epidemiological studies. It has been demonstrated that exposure can vary greatly in the same apparent conditions, even between individuals in the same workplace. Experimental studies and reviews have shown that the most important determinant for personal exposure is a worker's specific task, which can determine consistent deviations from the forecasted exposure values based on environmental data. This, along with personal susceptibility, indicates that some of the values obtained from previous studies based on questionnaires or disease/death registers could be considered not completely reliable.

A proper exposure assessment should, then, be a priority for future studies, with an aim to shed light on the true links between environmental and occupational exposure to pesticides and disease, particularly highly prevalent ones.

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