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Epigenetics and pesticides

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ABSTRACT

Pesticides, a wide class of environmental contaminants, may cause both acute and delayed health effects in exposed subjects. These effects can range from simple irritation of the skin and eyes to more severe effects such as affecting the nervous system, the reproductive system and cancer. The molecular mechanisms underlying such effects are still under investigation.

Epigenetics is the study of heritable changes in gene expression that occur without a change in the DNA sequence. Several epigenetic mechanisms, including DNA methylation, histone modifications and microRNA expression, can be triggered by environmental factors. We review current evidences indicating that epigenetic modifications may mediate pesticide effects on human health.

In vitro, animal, and human investigations have identified several classes of pesticides that modify epigenetic marks, including endocrine disruptors, persistent organic pollutants, arsenic, several herbicides and insecticides.

Several investigations have examined the effects of environmental exposures and epigenetic markers, and identified toxicants that modify epigenetic states. These modifications are similar to the ones found in pathological tissue samples. In spite of the current limitations, available evidence supports the concept that epigenetics holds substantial potential for furthering our understanding of the molecular mechanisms of pesticides health effects, as well as for predicting health-related risks due to conditions of environmental exposure and individual susceptibility.

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1. Introduction

The noxious effects that pesticides have on human health have been widely studied in the last century. Observational studies on workers exposed to pesticide (Damalas and Eleftherohorinos, 2011), along with animal models of pesticides toxicity (Vandegheuchte and Janssen, 2011) showed how these chemicals can be responsible for detrimental effects on health.

Recently, a new approach aimed at evaluating different mechanisms by which pesticides could impact on human health, altering gene regulation has been developed. Among these new approaches, epigenetics seems a promising tool. Thus, understanding the molecular mechanisms able to mediate the effects of environment is of great importance.

Epigenetics is the study of heritable changes in gene expression that occur without a change in the DNA sequence. Interestingly, epigenetic changes can be triggered by environmental factors. Environmental exposure to metals, persistent organic pollutants or endocrine disrupting chemicals has been shown to modulate

epigenetic marks (Baccarelli and Bollati, 2009). There is a growing interest in evaluating the alterations that environmental exposures may produce on epigenetic states, and whether such changes might activate pathways leading to detrimental effects on human health (Baccarelli and Bollati, 2009).

Several epigenetic mechanisms, including DNA methylation, histone modifications, and microRNA (miRNA) expression, can change genome function under exogenous influence, such as environmental pollutants. Epigenetic changes may mediate specific mechanisms of toxicity and responses to certain chemicals. Furthermore such modifications might persist even in the absence of the factors that established them (Anway et al., 2006; Dolinoy, 2008).

Here, we review current evidence indicating that epigenetic alterations mediate toxicity from pesticides (Table 1).

2. Pesticides: uses and health effects

Pesticides are chemicals used to control noxious or unwanted living species (Baxter et al., 2010). Therefore, they find use in agriculture, in public health for controlling vector borne diseases, in industry to protect machineries and products from biological degradation and in “do it yourself” activities, such as gardening.

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Table 1
Epigenetic modifications induced by pesticides.

Class	Exposure	Modification	Type	Tissue	Reference
Endocrine Disruptors	Methoxychlor	DNA methylation	Rat	Sperm, tail, liver, skeletal muscle, and ovaries	Stouder and Paoloni-Giacobino (2011) and Zama and Uzumcu (2009)
Endocrine disruptors	Vinclozoin	DNA methylation	Mouse embryo	Placenta, yolk sac, amnion, head, body, heart, liver, lung, stomach, and intestines	Hypothalamus Kang et al. (2011)
Persistent organic pollutants (POPs)	Dichlorodiphenyl-trichloroethane (DDT)	DNA methylation	Rat	Hypothalamus	Shutoh et al. (2009)
Persistent organic pollutants (POPs)	Organochlorine pesticides	DNA methylation	Human	Blood	Kim et al. (2010)
Persistent organic pollutants (POPs)	DDT, DDE, β -BHC, oxychlordane, α -chlordane, mirex, PCBs	DNA methylation	Human	Blood	Rusiecki et al. (2008)
Metals	Arsenic	DNA methylation	In vitro	Rat liver epithelial cells	Zhao et al. (1997)
Metals	Arsenic	DNA methylation	In vitro	Mouse liver	Chen et al. (2004)
Metals	Arsenic	DNA methylation	In vitro	V79-C13 Chinese hamster cells; ASO cells	Sciandrello et al. (2004)
Metals	Arsenic	DNA methylation	Human	Blood	Chanda et al. (2006) and Pilsner et al. (2007, 2009)
Metals	Arsenic	microRNA expression	Human	Human lymphoblastoid cells	Marsit et al. (2006)
Herbicides	Paraquat	Histone modifications	In vitro	Immortalized rat mesencephalic dopaminergic cells (N27 cells)	Song et al. (2010) and Song et al. (2011)
Herbicides	Dieldrin	Histone modifications	In vitro	Mesencephalic dopaminergic neuronal cells	Song et al. (2010)
Insecticides	Propoxur	Histone modifications	In vitro	Gastric cells	Kuo et al. (2008)
Insecticides	Dichlorvos	microRNA expression	In vitro	Porcine kidney epithelial cells	Li et al. (2011)
Insecticides	Fipronil, triazophos	microRNA expression	Zebrafish	Whole body homogenate	Wang et al. (2010)
Fungicides	Triadimefon, propiconazole, myclobutanil	microRNA expression	Mouse	Liver	Ross et al. (2010)

Pesticides can be classified based on their chemical structure (for example, carbamates, organophosphates, organochlorines, and pyrethroids), their target (for example, insecticides, herbicides, fungicides, rodenticides, molluscicides, nematocides and acaricides), their mode of action (for example, acetylcholinesterase inhibitors, calcium channels inhibitors). Further classification of pesticides is based on their toxicity: for example, the classes of toxicity defined by the World Health Organization, based on the LD50 levels and the International Agency for Research on Cancer (IARC) classification based on evidences of carcinogenicity.

Pesticides exposure may cause acute and delayed health effects, ranging from simple irritation of the skin and eyes to general malaise and chronic and long term severe effects on the nervous system including mild cognitive dysfunction (e.g. mood changes, neurobehavioral alterations), cognitive and psychomotor dysfunction, minor psychiatric morbidity, depression and death from mental disorders, neurodegenerative (e.g. Parkinson's and Alzheimer's diseases) and neurodevelopmental effects (Kanthasamy et al., 2012; Kwok, 2010; Migliore and Coppede, 2009; Sanborn et al., 2007).

Reproductive functions can also be affected, with birth defects, impaired fecundability, infertility and altered growth (Jurewicz and Hanke, 2008; Sanborn et al., 2007).

Although hundreds of papers on pesticides and cancer have been published so far (Ferri et al., 2007; Johnson et al., 1990; Keller-Byrne et al., 1995, 1997; Khuder et al., 1998; Turner et al., 2010; Van Maele-Fabry and Willems, 2003; Vinson et al., 2011), to date the results of epidemiological studies have been inconsistent (Alavanja et al., 2004). As for agricultural workers, supposed

to be more exposed to pesticides than other workers subgroups, current evidence is of a cancer risk lower than expected (Blair et al., 1992); in particular the mortality is lower for esophagus, lung, bladder and colon cancer. However, in this scenario of lower cancer risk some specific cancers show an incidence higher than expected. Soft tissue sarcoma, Hodgkin's and non-Hodgkin's lymphoma, leukemia, multiple myeloma, stomach, brain, prostate, pancreatic, breast and ovarian cancer have been associated with various degrees of consistency to pesticides exposure (Bassil et al., 2007; Blair et al., 1992; Dich et al., 1997). The strongest epidemiological associations reported, are those concerning hematological malignancies and pesticides exposure (Bassil et al., 2007; Chiu and Blair, 2009).

While acute toxic effects of pesticides are well known, uncertainties still remain regarding chronic and long term effects. For some pesticides, mechanisms such as the endocrine disruption (De Coster and van Larebeke, 2012) have been hypothesized. Moreover, it has been speculated that health effects observed in agricultural population may be related to the mutagenic effect of solar radiation (Nordby et al., 2004). To date, however, the specific molecular mechanisms linking exposure to health effects are still lacking.

It is also necessary taking into account that pesticide market is quickly changing in the so-called "developed countries", also as a consequence of new and more stringent legislation regarding authorization procedures, and organophosphates and carbamates are being replaced by the less toxic pyrethroids and the more efficient, selective and more expensive new compounds. Conversely in the developing countries, the old generation compounds are still largely used. The complexity of the field, makes extremely

difficult to formulate a unifying theory, able to explain at what level pesticides exert their toxic function.

Recently some environmental factors have been linked to aberrant changes in epigenetic pathways both in experimental and epidemiological studies (Baccarelli and Bollati, 2009). In addition, epigenetic mechanisms may mediate specific mechanisms of toxicity and responses to certain chemicals (Marsit et al., 2006). In this context, we will review the current evidences which seem to indicate epigenetics as a possible link between pesticides exposure and health effects.

3. Epigenetic mechanisms

Epigenetic modifications include DNA methylation, histone modifications, and microRNAs (Chuang and Jones, 2007).

DNA methylation is a covalent modification, involved in regulating many cellular processes including chromatin structure and remodeling, X-chromosome inactivation, genomic imprinting, chromosome stability, and gene transcription (Grewal and Moazed, 2003; Reik et al., 2001). DNA methylation is heritable by somatic cells after cell division. The 5-methyl-cytosine (5MeC) represents 2–5% of all cytosines in mammalian genomes and is found primarily on CpG dinucleotides (Millar et al., 2003). Generally, gene promoter hypermethylation is associated with decreased expression of the gene (Orphanides and Reinberg, 2002). On the other hand, a hypomethylation of non-coding region has been linked to chromosome instability (Watanabe and Maekawa, 2010). Genomic imprinting, a genetic phenomenon by which certain genes are expressed in a parent-of-origin-specific manner, involves the methylation of the unexpressed allele (Eggermann et al., 2011).

Post-translational modifications of histone tails, have been shown to be important in altering chromatin structure and therefore DNA accessibility (Kouzarides, 2007). The functional effects of such modifications depend on the specific amino acid that is modified and on the specific covalently attached group: e.g. acetylation results in the loosening of chromatin and lends itself to replication and transcription, whereas methylated histones tight DNA and restrict access to various enzymes. Histones modifications can regulate gene expression, chromatin remodeling, cell survival and cell death (Kouzarides, 2007).

microRNAs (miRNA) are single-stranded RNAs of about 21–23 nucleotides in length that are transcribed from DNA but not translated into proteins (non-coding RNAs). Their functional role is gene expression regulation mediated by a control of messenger RNA (mRNA) stability or translation. Mature miRNAs can be totally complementary to the mRNA: the pairing between the miRNA and the mRNA leads to the mRNA degradation, therefore impairing gene expression. Otherwise miRNA can be only partially complementary to mRNA molecules: their regulatory function is thus mediated by a block in mRNA translation (Jackson and Standart, 2007; Pillai et al., 2007). One single miRNA regulates the expression of hundreds of different target genes, vice versa one gene can be regulated by hundreds of miRNA. MicroRNAs play a key role in diverse biological processes, including development, cell proliferation, differentiation, and apoptosis.

4. DNA methylation

4.1. Pesticide exposure and DNA methylation

Emerging evidence indicates that epigenetic changes are important cellular and molecular correlates of neurodegenerative diseases resulting from chronic neurotoxic chemical exposure. Kwok et al. recognized the role of DNA methylation following environmental chemical exposure in the pathogenesis of

neurodegenerative diseases. DNA methylation causes an allelic skewing in a significant proportion of genes, that is, one allele can be transcribed or expressed at a higher level than the other allele, differentiating between the maternal and paternal origin allele. This phenomenon may determine how an individual's genotype can alter the effect an environmental factor has on their risk of developing neurodegeneration (Kanthasamy et al., 2012).

Exposure to dichlorodiphenyltrichloroethane (DDT) alters the methylation pattern in the hypothalamus of young male rats: the experiment conducted by Shutoh et al. (2009) showed that 6 CpG islands (in Sst, Gal, Arf1, Ttr, Msx1 and Grifin genes) were significantly hypomethylated compared with controls. The DNA methylation machinery malfunctions under low levels of oxidative stress, thereby leading to incomplete methylation of specific gene regions.

The DNA methylation system can be affected by exposure to high doses of organochlorine pesticides, methylmercury chloride or polychlorinated biphenyls. Zama et Uzumcu reported an altered methylation pattern in livers collected from rats treated in utero and postnatally with these chemicals. Pyrosequencing methylation analysis revealed that the high-dose groups generally decreased the methylation of CpG sites in the promoter of the tumor suppressor gene p16(INK4a) (Desaulniers et al., 2009).

4.2. Endocrine disruptors and DNA methylation

Some pesticides belong to the environmental endocrine disruptors (EDs) family, synthetic chemicals that resemble natural hormones and are known to cause epigenetic perturbations (McLachlan et al., 2006).

Among them methoxychlor (MXC), an organochlorine insecticide, has been reported to affect the male reproductive system (Stouder and Paoloni-Giacobino, 2011).

Gestational exposure to MXC disrupts the female offspring reproductive system in adulthood, re-programming the expression of a suite of hypothalamic genes that control reproductive function. Rats treated with MXC had a different methylation pattern of two paternally imprinted (H19 and Meg3 (Gtl2)) and three maternally imprinted (Mest (Peg1), Snrpn, and Peg3) genes (Stouder and Paoloni-Giacobino, 2011). Previous studies showed that fetal/neonatal exposure to MXC caused adult ovarian dysfunction due to altered expression of key ovarian genes including estrogen receptor (ER)-beta, which was down-regulated, whereas ER-alpha was unaffected (Zama and Uzumcu, 2009). Thus, early life exposure to endocrine disruptors has lifelong effects on neuroendocrine gene expression and DNA methylation, together with causing the reproductive dysfunctions.

The research conducted by Stouder and Paoloni-Giacobino (2011) evaluates the possible deleterious effects of MXC on imprinted genes. MXC treatment of pregnant mice altered the methylation pattern of all the imprinted genes tested. MXC effects were transgenerational but disappeared gradually from F1 to F3. MXC did not affect imprinting in the somatic cells, suggesting that its effects are restricted to gamete development. Further investigations must be carried out in order to understand if other epigenetic modifications can explain the transgenerational effects of MXC (Stouder and Paoloni-Giacobino, 2011).

Another chemical belonging to the EDs family is vinclozolin, a dicarboximide fungicides, which has been implicated in causing imprinting alterations in mouse embryos (Kang et al., 2011). To screen for possible epigenetic perturbations caused by EDs at imprinted loci, Kang et al. treated pregnant mice with di-(2-ethylhexyl)-phthalate (DEHP), bisphenol A (BPA), vinclozolin (VZ), or control oil vehicle. After isolating RNA from the placenta, yolk sac, amnion, head, body, heart, liver, lung, stomach, and intestines of embryos they measured the allele-specific expression of 38

imprinted transcripts. Data suggested that the maintenance of monoallelic expression of imprinted genes is slightly sensitive to EDs in the embryo and extra-embryonic organs (Kang et al., 2011).

4.3. Persistent organic pollutants (POPs) and DNA methylation

Persistent organic pollutants (POPs) are organic compounds that are resistant to environmental degradation through chemical, biological, and photolytic processes. Many pesticides can be considered as POPs.

Global DNA methylation levels have been reported to be inversely associated with blood levels of persistent organic pollutants (POPs), xenobiotics that accumulate in adipose tissue. Kim et al. found that low-dose exposure to POPs, in particular organochlorine pesticides, was associated with global DNA hypomethylation, estimated by the percent 5-methyl-cytosine (%5-mC) in Alu and LINE-1 assays, in healthy Koreans (Kim et al., 2010).

The same relationship between plasma POP concentrations and blood global DNA methylation, estimated in Alu repeated elements, was evaluated in 70 Greenlandic Inuit, a population presenting some of the highest reported levels of POPs worldwide. In this work, a significant inverse linear relationship was found for DDT, DDE, β -BHC, oxychlorane, α -chlordane, mirex, several PCBs, and sum of all POPs (Rusiecki et al., 2008). The levels found in this Arctic population, although extremely high, are comparable to those found in other regions. For example, an environmental assessment conducted in a Lacandon Maya community in the Southeast part of Mexico (Perez-Maldonado et al., 2006) showed levels of exposure to DDT comparable to those reported by Rusiecki et al. (2008).

4.4. Arsenic and DNA methylation

Arsenic and its compounds, especially the trioxide, have been widely used in the past in the production of biocides for wood conservative treatments, herbicides, and insecticides, however arsenical pesticides are still used in some countries and are still present in several wood products. Arsenic is a non-mutagenic human carcinogen that induces tumors through unknown mechanisms. A growing body of evidence suggests that its carcinogenicity may result from epigenetic changes, particularly in DNA methylation. Changes in oncogenes or tumor suppressor genes methylation can lead to long-term changes in the activity of genes controlling cell transformation (Laird, 2005).

In arsenic-treated cells, arsenic exposure was associated with the global hypomethylation (Chen et al., 2004; Sciandrello et al., 2004; Zhao et al., 1997).

Arsenic is metabolized through repeated reduction and oxidative methylation. In the presence of high arsenic exposure, this detoxification process can compete with DNA methylation for methyl donors, thus causing hypomethylation (Mass and Wang, 1997).

Inorganic arsenic is enzymatically methylated for detoxification, using up S-adenosyl-methionine (SAM) in the process. The observation that DNA methyltransferases also require SAM as their methyl donor suggested a role for DNA methylation in arsenic carcinogenesis and other arsenic-related effects. In rat-liver epithelial cell lines treated with chronic low arsenic doses, Zhao et al. showed malignant transformation associated with depressed SAM levels and global DNA hypomethylation (Zhao et al., 1997). An in vitro study on mammalian cells directly demonstrated that arsenic induces DNA hypomethylation that was associated with chromosomal instability (Sciandrello et al., 2004). In addition, arsenite has been shown to increase both the levels of the repressive histone mark dimethylated H3K9 and the activating mark trimethylated H3K4, and decreases the repressive mark trimethylated H3K27 in human lung carcinoma A549 cells (Zhou et al., 2008).

An unexpected finding was recently reported in vivo, as a global dose-dependent hypermethylation of blood DNA was observed in Bangladeshi adults with chronic arsenic exposure (Pilsner et al., 2007). This effect was modified by folate, suggesting that arsenic-induced increases in DNA methylation were dependent from methyl availability (Pilsner et al., 2007). The same group, however, reported that lower blood DNA methylation was a risk factor for arsenic-induced skin lesions in a related Bangladeshi population (Pilsner et al., 2009).

In a human study from India, significant DNA hypermethylation of p53 and p16 promoter regions was observed in blood DNA of subjects exposed to toxic level of arsenic compared to controls (Chanda et al., 2006). In this study, hypermethylation showed a dose-response relationship with arsenic measured in drinking water.

5. Histones modifications

Environmental factors can alter gene expression by epigenetic mechanisms and lead to late-onset neurodegenerative diseases. Exposure to environmental neurotoxic metals, pesticides and other chemicals is increasingly recognized as a key risk factor in the pathogenesis of chronic neurodegenerative disorders such as Parkinson's and Alzheimer's diseases (Kanthasamy et al., 2012; Kwok, 2010; Migliore and Coppede, 2009).

Kanthasamy et al. (2012) described the role of acetylation of histones and non-histone proteins in neurotoxicant-induced neurodegenerative processes in the nigral dopaminergic neuronal system.

Paraquat, a widely used herbicide, and the organochlorine insecticide Dieldrin, are among the environmental chemicals potentially linked with Parkinson's disease. Histone acetylation may represent the key epigenetic change in dopaminergic neuronal cells during neurotoxic insults. Experimental evidence comes from the research conducted by Song et al. on N27 dopaminergic cells. Exposure to Paraquat induced histone H3 acetylation in a time-dependent manner and decreased total histone deacetylase (HDAC) activity (Song et al., 2010, 2011). In mesencephalic dopaminergic neuronal cells, Dieldrin lead to a time-dependent increase in the acetylation of core histones H3 and H4 by a Dieldrin-induced proteasomal dysfunction, resulting in accumulation of a key histone acetyltransferase (HAT). Furthermore, prolonged exposure to dieldrin in mouse models induced histone hyperacetylation in the striatum and substantia nigra (Song et al., 2010).

According to recent researches conducted by Maloney et al. (2012), latent early-life associated regulation (LEARn) can be the link between epigenetics and Alzheimer disease. The LEARn are apparently temporary changes, induced by environmental agents, which become latent and present themselves once again at maturity or senescence causing diseases such as Alzheimer. The epigenetic changes caused by environmental agents such as pesticides can increase the production of amyloid b protein and cause Alzheimer disease (Maloney et al., 2012).

Beyond the concausal role that pesticides can have onto the pathogenesis of neurodegenerative diseases by epigenetic alterations, recent evidences suggest that pesticide toxicity can be mediated by changes in histone structure.

Propoxur, a member of the N-methylcarbamate insecticide group, is among the most popular insect control agents in subtropical countries. Due to the fact that the stomach has been identified as its major target, the investigation conducted by Kuo et al. (2008) used a human gastric cell line in order to achieve a better understanding of the adverse effects of this compound on human health. Assays for the expression of phosphorylated histone H2AX confirmed the N-nitroso Propoxur-induced cellular damage.

Exposure to tetrachloromethane and chlorophos leads to a damage to chromatin structure, which can be prevented by the injection of BTK-8L, a phytosteroid preparation. This preparation interacts with chromatin binding to histone proteins and changes the nucleoprotein complex structure as a result of which the chromatin fraction components become less accessible to the damaging action of tetrachloromethane and chlorophos. The protective role of BTK-8L indirectly confirms the epigenetic mechanism of action of these pesticides (Levitkii et al., 1996).

6. miRNAs

The effects on the epigenome caused by pesticides can be attributed also to a change in the miRNA expression profile, thus leading to changes in gene regulation which can explain the noxious effects that these chemicals have on human health.

Li et al. (Cerri et al., 2011) evaluated the epigenetic effects of dichlorvos (DIC), an organophosphorus insecticide, in a porcine kidney epithelial cell line (PK15) in order to achieve a better understanding of its non-neuronal cytotoxicity. Microarray analyses showed an altered miRNA and mRNA expression profile, thus demonstrating that the epigenetic mechanisms involving miRNA expression modifications play a pivotal role in DIC cytotoxicity.

Wang et al. (2010) evaluated the effect of Fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-[(trifluoromethyl) sulfinyl]-1H-pyrazole-3-carbonitrile) and Triazophos (3-(O,O-diethyl)-1-phenyl thiophosphoryl-1,2,4-triazol) and their mixture on miRNA expression in zebrafish. miRNA expression profiles in zebrafish were altered after treatment with these chemicals, suggesting their role in the toxicity mechanisms of these compounds and representing a possible novel toxicological biomarker.

Triadimefon, propiconazole, and myclobutanil are conazoles, an important class of agricultural fungicides. Triadimefon and propiconazole are mouse liver tumorigens, while myclobutanil is not. Ross et al. (2010) treated mice with conazoles (triadimefon, propiconazole, and myclobutanil) to understand the molecular determinants of its tumorigenicity. MicroRNA was isolated from livers and analyzed: the tumorigenic conazoles induced many more changes in miRNA expression than the nontumorigenic conazoles.

Arsenic toxicity has been recently related to changes in miRNA expression. Marsit et al. showed alterations in miRNA profiles of human lymphoblastoid cells grown under sodium arsenite treatment. Interestingly, Arsenic altered expression of specific miRNAs that were involved in one-carbon metabolism (Marsit et al., 2006).

7. Pesticides, NHL and DNA methylation

Use of synthetic organic pesticides became widespread during the second half of the 20th century and the incidence of non-Hodgkin's lymphomas (NHL) also increased during this time (Wheeler, 2002). Some pesticides have demonstrated tumor initiating and/or promoting effects in animals (Selkirk and Soward, 1993). Results from these previous studies suggested a number of pesticides as potential risk factors for NHL.

According to EPA's evaluation, almost all pesticides on the US market have been shown not to be directly genotoxic. Because pesticides do not increase cancer risks via a directly genotoxic mechanism, we hypothesize that they may operate through a mode of action involving epigenetic mechanisms.

Exposure to a variety of environmental factors can alter DNA methylation patterns, inducing destabilizing changes in gene expression patterns potentially leading to cell transformation and tumorigenesis. Pesticides (e.g. arsenic, trichloroacetic acid, and daminozide) may cause NHL via DNA

methylation alterations which may be specific to each of the different NHL subtypes (Zhang et al., 2012).

Alteration of DNA methylation patterns such as global genome hypomethylation and promoter hypermethylation of cytosine-guanine dinucleotide (CpG) islands of specific genes, have been increasingly found in different types of tumors, including hematological malignancies (Das and Singal, 2004; Laird, 2005).

Other possible mechanisms involved in tumorigenesis are oxidative stress-induced ROS generation (Sesti et al., 2012), endocrine disruption (Sesti et al., 2012), DNA damages (Sesti et al., 2012), disruption of methyltransferases activity (Lin et al., 2010) and reduction of S-adenosyl-methionine (SAM) availability (Selhub, 2002).

Oxidative stress has been associated not only with global hypomethylation, but also with increased dense methylation of specific genes (Franco et al., 2008). Even if it is possible to hypothesize that oxidative stress may exert its own effects by interfering with epigenetic regulation mechanisms, oxidative stress itself may be responsible for pesticide-induced health effects (Hernandez et al., 2012).

Certain organophosphate methyl esters in organophosphate compounds allow promutagenic alkylation damage to DNA, which in turn can produce methylation of DNA (Ray and Richards, 2001).

In addition, pesticides exposure can also interact with other methylation-related factors, for example, methyl-donor-related dietary factors and genetic predispositions, to confer increased NHL risk.

8. Conclusions

Epigenetic modifications are relative stable over time and may be influenced by the environment. Exposure to pesticides may lead to epigenome modifications. Experimental, clinical, and epidemiological studies of epigenetic changes caused by pesticides exposure have increased our understanding of the mechanisms of action by which they can modify gene expression.

Most of the studies conducted so far have been centered on DNA methylation, whereas only a few recent investigations have studied the effects on histone modifications and miRNAs. Many questions remain open, for example if the observed effects may be the result of the exposure either to a single pesticide compound or to a complex mixture of different chemicals.

Far from being conclusive, the reported evidences suggest that epigenetic modifications may be one of the mechanism by which pesticides can have noxious effects on human health. Further studies are warranted to evaluate if epigenetic modifications may act as a causal link between pesticide exposure and health effects, or rather be a sensitive marker of exposure.

Conflict of interest

The authors state that they have no conflict of interest.

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