Genotoxicity and Cytotoxicity Exerted by Pesticides in Different Biotic Matrices-An Overview of More Than a Decade of Experimental Evaluation

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Abstract

Agrochemicals represent one of the most important sources of environmental pollution. Although attempts to reduce agrochemical use through organic agricultural practices and the use of other technologies to control pests continue, the problem is still unsolved. Recent technological advances in molecular biology and analytical science have allowed the development of rapid, robust, and sensitive diagnostic tests (biomarkers) that can be used to monitor exposure to, and the effects of pollution. One of the major goals of our research laboratory is to evaluate comparatively the genotoxic and cytotoxic effects exerted by several pure agrochemicals and their technical formulations commonly used in Argentina on vertebrate cells in vitro and in vivo employing several end-points for geno and cytotoxicity. Among them are listed the herbicides dicamba and fluorochloridone, the fungicide zineb, the insecticides pirimicarb and imidacloprid. Overall, the results clearly demonstrated that the damage induced by the commercial formulations is in general greater than that produced by the pure pesticides, suggesting the presence of deleterious components in the excipients with either a putative intrinsic toxic effect or with the capacity of exacerbating the toxicity of the pure agrochemicals, or both. Accordingly, the results highlight that: 1) A complete knowledge of the toxic effect/s of the active ingredient is not enough in biomonitoring studies; 2) Pesticide/s toxic effect/s should be evaluated assaying to the commercial formulation available in market; 3) The deleterious effect/s of the excipient/s present within the commercial formulation should not be either discarded nor underestimated, and 4) A single bioassay is not enough to characterize the toxicity of a agrochemical under study.

Keywords: Agrochemicals; Pesticides; Commercial formulations; in vitro; In vivo; Biomarkers

Abbreviations

CA, chromosomal aberrations; CCP, cell-cycle progression; CHO-K1, Chinese hamster ovary cells; IARC, International Agency for Research on Cancer; MN, micronucleus/micronuclei; MI, mitotic index; NR, neutral red; OECD, Organization for Economic Cooperation and Development; SCE, sister chromatid exchange; SCGE, single cell gel electrophoresis assay; US, United States; US EPA, United States Environmental Protection Agency; WHO, World Health Organization

Problem framework

Nowadays, it is worldwide accepted that the survival of humans as a species is intimately linked to the well-being of ecosystems and the resources they can provide. However, it is also well assume that the well-being of ecosystems depends, in turn, on minimizing the damaging impacts of anthropogenic activities. Irrespective of the kinds of habitats we choose to protect or restore, we need to understand how ecosystems, and the organisms that inhabit them, respond to chemicals exposure, among other detrimental factors. Recent technological advances in molecular biology and analytical science have allowed the development of rapid, robust, and sensitive diagnostic tests (biomarkers) to monitor both exposure and the effects of pollutants. For the first time, we are able to make health assessments of individual organisms in much the same way that we evaluate human health.

It is estimated that approximately 1.8 billion people worldwide engage in agriculture and most use pesticides to protect the food and commercial products that they produce. Others use pesticides occupationally for public health programs, and in commercial applications, while many others use pesticides for lawn and garden applications and in and around the home [1,2]. Pesticides are defined as “chemical substance or mixture of substances used to prevent, destroy, repel or mitigate any pest ranging from insects (i.e., insecticides), rodents (i.e., rodenticides), and weeds (i.e., herbicides) to microorganisms (i.e., algicides, fungicides, and bactericides)” [1,3,4]. Definition of pesticide varied with times and countries. Nevertheless, the essence of pesticide has remained and remains basically constant, i.e., it is a (mixed) substance that is poisonous and efficient to target organisms and is safe to non-target organisms and environments.

Years ago, it has been reported that more than 2,000,000 million tn of pesticides are used only in the US each year whereas approximately over 11,000,000 million tn are used worldwide [1]. However, it is very well known that in many developing countries programs to control exposures are limited or even non-existent. Therefore, it has been estimated that among living species worldwide, only as many as 25 million agricultural workers experience unintentional pesticide poisonings each year [5]. According to the WHO [6] unintentional poisonings kill an estimated 355,000 people globally each year. In developing countries, where two thirds of these deaths occur, such poisonings are associated strongly with excessive exposure to, and inappropriate use of, toxic chemicals. Furthermore, the OECD has estimated that by the year 2020, nearly one third of the world’s chemical

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production will take place in non-OECD countries and that global output will be 85% higher than it was in 1995. Therefore, the chemical shift of production from developed countries to poor countries could cause an increase in both the risks of environmental health in the second category of countries [7].

Although attempts to reduce pesticide use through organic agricultural practices and the use of other technologies to control pests continue, exposure to pesticides occupationally, through home and garden use, through termite control or indirectly through spray drifts and through residues in household dust, and in food and water are common [8-14]. The US Department of Agriculture has estimated that 50 million people in the US obtain their drinking water from groundwater that is potentially contaminated by pesticides and other agricultural chemicals [9,15-26]. Children from 3-6 years old received most of their dermal and non-dietary oral doses from playing with and while playing on carpets which contributed the largest portion of their exposure [22-27].

In epidemiological and in experimental biology studies, the existence of an increasing interest in biomonitoring markers to achieve both a measurement and an estimation of biologically active/passive exposure to genotoxic pollutants, is nowadays a real fact. Significant contributions to the advancement of pesticide toxicology came and continue to come from many sources, e.g., academic, governmental/regulatory, and industrial. Regulatory agencies, private sector, and academia worldwide combine expertise to assess pesticide safety and risk potential demanding adequate data of high quality to serve as the basis for establishing safe exposure levels. The extent of testing was and is often determined by the depth of the science, as well as the chemical and physical properties of the agent and the extent of exposure. The importance of pesticide toxicology has evolved from listing poisons to protecting the public from the adverse effects of chemicals, from simply identifying effects (qualitative toxicology), to identifying and quantifying human risks from exposure (quantitative toxicology), and from observing phenomena to experimenting and determining mechanisms of action of pesticide agents and rational management for intoxication. Humans and living species may, therefore, be exposed to a number of different chemicals through dietary and other routes of exposure.

Pesticides are ubiquitous on the planet and they are employed to control or eliminate a variety of agricultural and household pests that can damage crops and livestock and to enhance the productivity. Despite the many benefits of the use of pesticides in crops field and its significant contribution to the lifestyles we have come to expect, pesticides can also be hazardous if not used appropriately and many of them may represent potential hazards due to the contamination of food, water, and air, which can result in severe health problems not only for humans but also for ecosystems [28]. The actual number of pesticide-related illnesses is unknown, since many poisonings go unreported. It has been estimated that at least three million cases of pesticide poisoning occur worldwide each year (www.who.int). Yet, cases of pesticide intoxication may be the result of various causes in different regions of the world. In emerging countries, where there is insufficient regulation, lack of surveillance systems, less enforcement, lack of training, inadequate or reduced access to information systems, poorly maintained or nonexistent personal protective equipment, and larger agriculturally based populations, the incidences are expected, then, to be higher [31]. Despite the magnitude of the problem of pesticide poisoning, there have been very few detailed studies around the world to identify the risk factors involved with their use. The use of pesticides banned in industrialized countries, in particular, highly toxic pesticides as classified by WHO, US EPA, and IARC, obsolete stockpiles and improper storage techniques may provide unique risks in the developing world, where 25% of the global pesticide production is consumed [28]. Particularly, the impact of increased deregulation of agrochemicals in Latin America threatens to increase the incidence of pesticide poisoning, which has already been termed a serious public health problem throughout the continent by the WHO. Many of the pesticides used in Latin America are US exports and the companies can make a number of changes to ensure the "safe" use of their products. However, the social, economic and cultural conditions under which they are used, pesticides acutely poison hundreds of thousands each year, including many children.

There is an aspect related with use and misuse of pesticides that should be commenter further. The continuous subtoxic exposures of these agrochemicals raises the concern about which is the behavior, environmental fate and the potential adverse effects on both target and non target organisms once incorporated into the environment. The different chemical products used in agriculture could be distributed within the environment by means of drift, surface runoff, and drainage [32,33] and, thus, can be found far away from the point of application. The mobility of pesticides in soil and hence their transfer to other environmental compartments, depends on a variety of complex dynamic physical, chemical and biological processes, including sorption–desorption, volatilization, chemical and/or biological degradation, uptake, runoff, and leaching, among other factors [34-37]. In addition, many pesticides can persist for long periods in the ecosystem. Furthermore, once a persistent pesticide has entered the food chain, it can undergo "biomagnification", i.e., accumulation in the body tissues of organisms, where it may reach concentrations many times higher than in the surrounding environment and directly compromising the health of organisms, including humans [38-40].

In the majority of Latin American countries, poisoning registries are so inadequate that most acute poisoning cases never get recorded. Meanwhile, health effects of chronic or long-term pesticide exposures such as cancer or birth defects are not available, omissions that serve to hide the epidemic proportion of pesticide-related illness in the region. In Argentina, e.g., available official data revealed that 79% of the intoxications due to pesticides are related with the use of herbicides followed by insecticides and fungicides (www.msal.gov.ar), values that correlate with the evolution of the phytosanitary market demonstrating that herbicides accounted for the largest portion of total use (69%), followed by insecticides (13%), and fungicides (11%) (www.casafe.org). Consequently, Argentina a larger producer of cereals, including soy, is actually the world eight-largest agrochemical market. The country has seen an explosion in genetically modified soybean production with soy exports topping $16.5 billion in 2008 (www.casafe.org). The fertile South American nation is now the world’s third largest producer of soy, trailing behind the United States and Brazil.

Furthermore, there is an aspect that should be further considered.
It is well known that in agriculture, pesticides are usually applied in their formulated forms, where the active ingredient is combined with organic solvents and emulsifying and wetting agents, which affect the pesticide penetration and performance [41]. The additives may synergize or antagonize the toxicity of the active ingredient. However, additive compounds frequently make up part of a commercial pesticide formulation, they are not usually included in any discussion of the effects on living organisms, and their adverse effects may exceed those of the active ingredient. Although pesticides are developed through very strict regulation processes to function with reasonable certainty and minimal impact on human health and the environment, serious concerns have been raised about health risks resulting from occupational exposure and from residues in food and drinking water [41]. Several investigations have demonstrated that the additive compounds present in pesticide commercial formulations have the ability to induce cellular toxicity, including genotoxicity and genotoxicity by themselves, separate from the active ingredient [42-51]. Accordingly, risk assessment must also consider additional toxic effects caused by the excipient(s). Thus, both the workers as well as non-target organisms are exposed to the simultaneous action of the active ingredient and a variety of other chemicals contained in the formulated product.

Since more than a decade, one of the major goals of our research group has been to evaluate comparatively the genotoxic and cytotoxic effects exerted by several pure pesticides Pestanal® analytical standards (Riedel-de Haën, Germany) and their technical formulations commonly used in Argentina on eukaryotic cells employing several biotic matrices both in vitro and in vivo. Among them are included the herbicides dicamba and the 57.7% dicamba-based formulation Banvel® (Syngenta Agro S.A., Buenos Aires, Argentina) and fluoroacetone and the 25.0% fluoroacetone-based formulations Twin Pack Gold® (Magan Argentina S.A., Buenos Aires, Argentina) and Rainbow® (Syngenta Agro S.A., Buenos Aires, Argentina), the fungicide zineb and the 70.0% zineb-based formulation Azzuro® (Chemiplant, Buenos Aires, Argentina), and the insecticides pirimicarb and the 50.0% pirimicarb-based formulations Aficida® (Syngenta Agro S.A., Buenos Aires, Argentina) and Patton Flow® (Gleba S.A., Buenos Aires, Argentina). For the particular case of the insecticide imidacloprid, the 35.0% imidacloprid-based formulation Glacoxan imida® (Punch Quimica S.A., Buenos Aires, Argentina) was assayed. The sister chromatid exchange (SCE), cell-cycle progression (CCP), structural chromosome aberrations (CA), single cell gel electrophoresis assay (SCGE), spindle disturbances, micronuclei (MI), mitotic index (MI), MTT, and neutral red (NR) bioassays were used as end-points for geno and cytotoxicity in several cell systems including in vitro non-transformed and transformed mammalian cells, and in vivo Allium cepa meristematic root cells as well as circulating blood cells from Rhinella arenarum (Anura, Bufonidae) and Hypsibous pulchellus (Anura, Hylidae) tadpoles. The aforementioned agrochemicals were chosen because they represent one of the most employed pesticides used for pest control not only in Argentina but also worldwide scale. A simple search within the Farm Chemical International database clearly reveals this concept (www.farmchemicalsinternational.com). So far, whereas available information indicates the existence of 34 basic producers and eight formulators for dicamba, six basic producers and at least two formulators worldwide are related with the manufacture and marketing of the herbicide fluoroacetone. For the fungicide zineb, it has been reported the existence of 21 and at least seven basic producers and formulators, respectively. Finally, at global scale, the existence of 19 basic producers and at least four formulators as well as 117 basic producers and at least 49 formulators are related with the manufacture and marketing of the insecticides pirimicarb and imidacloprid.

Dicamba. Genotoxicity and Cytotoxicity Profiles

Dicamba (3,6-dichloro-2-methoxybenzoic acid; CASRN: 1918-00-9) is a selective systemic herbicide, absorbed by the leaves and roots, acts as an auxin-like growth regulator causing uncontrolled growth [52]. It is used to control annual and perennial broad-leaved weeds and bush species, e.g. cereals, maize, sorghum, sugarcane, asparagus, perennial seed grasses, turf, pastures, rangeland, and non-crop land [52]. Based on its acute toxicity, dicamba has been classified as a class II member (moderately hazardous) by WHO (http://www.who.int/ipcs/publications/pesticides_hazard/en/) and slightly to moderately toxic (category II-III) by US EPA [52]. Genotoxicity and cytotoxicity investigations have been conducted with this auxinic member using several end-points on different cellular systems. When mutagenic activity was assessed in bacterial systems with the Salmonella typhimurium Ames test either positive or negative results have been reported [53-55]. Furthermore, similar situation were observed in Escherichia coli and Bacillus subtilis when the reverse mutation assay was applied [53,56,57]. Whereas the herbicide was unable to induce mitotic recombination on Saccharomyces cerevisiae [58], negative and positive results were obtained for the induction of unscheduled DNA synthesis in human primary fibroblasts regardless of the presence or absence of S9 mix [53,59], Sorensen et al. [60,61] found positive results on dicamba-treated CHO-K1 cells cultured in the presence of reduced-clay smectites but not when the clay system were not included within the culture protocol. Perocco et al. [59] demonstrated the ability of the herbicide to induce SCEs in CHO-K1 cells and human lymphocytes in vitro with and without S9 fraction, respectively. It has been reported the ability of the herbicide to give positive results by using the gene mutation and recombination assays when Arabidopsis thaliana was used as experimental model [62]. However, both negative and inconclusive results were reported for the sex-linked recessive lethal mutation end-point in dicamba-exposed Drosophila melanogaster [57,63]. Perocco and co-workers [59] reported an increased frequency of DNA unwinding rate in rat hepatocytes. It has been also reported that the herbicide is able to enhance the frequency of CA in the root- and hoot-tip cells of barley and in rat bone marrow cells [64]. Finally, Mohamed and Ma [65] reported the MN induction in Tradescantia sp.

In our laboratory, we have studied the genotoxicity and cytotoxicity in vitro of the herbicide dicamba and the dicamba-containing commercial formulation Banvel® in human lymphocytes as well as in CHO-K1 cells (Figure 1). We were able to demonstrate that dicamba is a DNA-damaging agent since enhancement of the frequency of SCEs (Figure 1A), MN (Figure 1C), and single DNA strand breaks (Figure 1B) in mammalian in vitro cells [66,67]. Similarly, we demonstrated the induction of alterations in the CCP (Figure 1E), reduction of the MI status (Figure 1D), and cell viability after in vitro dicamba and Banvel® exposure [68-66].

Flurochloridone. Genotoxicity and Cytotoxicity Profiles

Flurochloridone (3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl)phenyl]-2-pyrrolodinone; CASRN: 89286-81-7) is a pre-emergence herbicide used to control a range of weeds in umbelliferous, cereal, sunflower, and potato crops, among others [69]. Toxicological information for flurochloridone has been poorly documented. So far, it has been reported that the herbicide does not reveal genotoxic, carcinogenic, or neurotoxic potential in rodents [69].
its also observed after flurochloridone exposure [70]. Chromosome breaks, bridges, fragments, sister union, and MN were observed abnormalities were c-metaphases, multipolarity, polyploidy, and chromosome lagging. In addition, chromosomal stickiness, system alterations [69]. Accessible information on the genotoxic properties of flurochloridone is scarce. To the best of our knowledge, a single report has been reported so far. When root meristematic cells were exposed to the herbicide, abnormal CCP and cellular mitodepressive activity were found [70]. The most frequently observed abnormalities were c-metaphases, multipolarity, polyploidy, and chromosome lagging. In addition, chromosomal stickiness, chromosome breaks, bridges, fragments, sister union, and MN were also observed after flurochloridone exposure [70].

Recently, we demonstrated that both flurochloridone and its formulations Twin Pack Gold® and Rainbow® are DNA-damaging agents (Figure 2), since an enhancement of the frequency of SCEs (Figure 2A), alterations in lysosomal (Figure 2G) and mitochondrial activities (Figure 2F), a delay in the CCP (Figure 2E) as well as a decrease of the MI (Figure 2D) were observed to occur in vitro treated mammalian CHO-K1 cells [48]. Furthermore, by using the same in vitro cellular system, we recently demonstrated the ability of flurochloridone to induce DNA single-strand breaks (Figure 2B) and MN frequency (Figure 2C) [47]. Similarly, both flurochloridone and the flurochloridone-based formulation were able to exert the same genotoxic and cytotoxic pattern on HepG2 cells in vitro (Figures 2B,C), hepatocellular carcinoma cell line maintaining phase I and II enzymes [71]. Finally, when the MN induction (Figure 2C) and DNA strand breaks (Figure 2B) estimation by the SCGE assay were employed as in vivo end-points, positive results were reported in erythrocytes of Twin Pack Gold®- and Rainbow®-exposed R. arenarum tadpoles by Nikoloff and collaborators [72].

Zineb. Genotoxicity and Cytotoxicity Profiles

Zineb (ethylene bis(dithiocarbamate) zinc; CASRN: 12122-67-7) is a widely employed foliar fungicide with prime agricultural and industrial applications [73]. Although zineb has been mainly registered to be used on a large number of fruits, vegetables, field crops, ornamental plants, and for the treatment of seeds, it has also been registered to be used as a fungicide in paints and for mold control on fabrics, leather, linen, painted surfaces, surfaces to be painted, and on paper, plastic, and wood surfaces [73]. It has been classified as a compound practically nontoxic (class IV) by US EPA [73] based on its potency by the oral and inhalation exposure routes. The available data on the deleterious effects of zineb do not allow a definitive evaluation of its carcinogenic potential and it has been not classified as to its carcinogenicity to humans (category III) by IARC [74]. This fungicide alters thyroid hormone levels and/or weights. The reproductive system is generally unaffected after zineb exposure [73].

Genotoxicity and cytotoxicity studies have been conducted with this dithiocarbamate member using several end-points on different cellular matrices. Zineb have been generally recognized as non-mutagenic...
in bacteria, yeast and fungi as well as in mammalian cells [73]. Plate incorporation assay with *S. typhimurium* demonstrated a direct non-mutagenic effect of the fungicide whereas mitotic chromosome malsegregation, gene conversion and point mutation assays with *S. cerevisiae* and *B. subtilis* gave positive results [75, 76]. Tripathy et al. [77] reported zineb as positive genotoxic agent to somatic and germ cells in *Drosophila* sp. While Chernov and Khitsenko [78] observed an increased incidence of lung tumors after its oral administration to C57BL mice, negative results have been also reported to occur either in other mouse strains [79] or in rats [80]. A variety of sarcomas were observed after subcutaneous administration in mice and rats [81]. Also, Enninga and coworkers [82] showed that zineb induced structural CA in CHO cells both with and without S9. In contrast to these studies, it was reported that the fungicide did not induce MN in bone marrow cells of Wistar male rats after oral administration [83]. In humans, haemolytic alterations have been reported after zineb contact [84]. Finally, an increase in the frequency of CA was observed in the lymphocytes of persons occupationally exposed to zineb [85]. Several assays have been developed to assess the ability of zineb to cause cytotoxic effects on different cellular systems. Zineb exerted a high dose-related cytotoxicity in BALB/c 3T3 mouse cells *in vitro* but only in the absence of an exogenous metabolizing system [86]. However, Whalen and coworkers [87] reported negative results when human natural killer cells were exposed to zineb. However, alterations in the mitochondrial transmembrane potential and cardiolipin content were reported to occur after zineb administration in rats [88].

We evaluated comparatively the genotoxic and cytotoxicity *in vitro* effects induced *in vitro* by the pure fungicide and its commercial formulation Azzurro® on CHO-K1 cells, human non-transformed fibroblast and circulating lymphocytes as well as on *in vivo* A. *cepa* meristematic root cells (Figure 3). Our observations revealed the ability of both zineb and the zineb-based formulation to induce CA in human lymphocytes (Figure 3D) [89,90]. Similarly, the fungicide increased the frequency of SCEs (Figure 3A) and modified the CCP (Figure 3F) and the MI status (Figure 3E) on human lymphocytes and CHO-K1 cells [89,90]. We have also demonstrated that both zineb and Azzurro® were not only able to induce MN in human lymphocytes *in vitro*, but also that such induction was restricted to B CD20+ and T suppressor/cytotoxic CD8+ cell subsets [91]. Furthermore, when assessing DNA damage and repair kinetics analyzed using the SCGE assay on zineb-and Azzurro®-CHO-K1 exposed cells, we observed that single strand breaks introduced into the DNA molecule likely reflect those induced by alkylating agents rather than those produced by active oxygen species (Figure 3B) [92]. Finally, we have also observed using a β-tubulin immunodetection assay that the exposure to Azzurro® interferes with normal assembly of microtubule structures during the mitosis of *A. c*epa meristematic root cells [93] and in mammalian transformed and non-transformed expressed cell lines [94].

**Pirimicarb. Genotoxicity and Cytotoxicity Profiles**

Pirimicarb (2-dimethylamino-5,6-dimethylpyrimidine-4-yldimethylcarbamate; CASRN: 23103-98-2) is a derivative of carbamic acid insecticide member with both contact and systemic activity. Based on its acute toxicity, pirimicarb has been classified as a moderately hazardous compound (class II) by WHO [95] and slightly to moderately toxic (category II-III) by US EPA [96]. Pirimicarb is registered as a fast-acting selective aphicide mostly used in a broad range of crops, including cereals, sugar beet, potatoes, fruit, and vegetables, and is relatively non-toxic to beneficial predators, parasites, and bees [28,97]. Its mode of action is inhibiting acetylcholinesterase activity [28,97].

Available information on the genotoxic and cytotoxic properties of pirimicarb is limited and inconsistent. Only few data are available in the literature [28,97]. Genotoxicity and cytotoxicity studies have been conducted with this carbamate using several end-points on different cellular systems. Pirimicarb has been generally recognized as non-genotoxic in bacteria, yeast and fungi as well as in mammalian cells [28,97]. It has been reported to be non-mutagenic in bacteria systems [98,99]. Negative and positive results were obtained for the induction of mutagenicity in mouse lymphoma L5178Y cells regardless of the presence or absence of S9 mix [100]. Furthermore, evaluation of the induction of DNA single strand breaks revealed positive results in human lymphocytes exposed *in vitro* [101]. It has been reported the ability of the insecticide to give positive results by using the eye mosaic system white/white* (w/w+) somatic mutation and recombination test (SMART) when *D. melanogaster* was employed [102]. However, others authors reported negative results when mutation bioassays was performed in rats [103,104]. At the chromosomal level, pirimicarb did not induce CA in bone marrow cells of rats after oral administration [105,106]. Contrarily, Pilinskaia [107] observed a significant increase of CA in the peripheral blood lymphocytes from occupational workers after pirimicarb exposure.

We evaluated comparatively the genotoxic and cytotoxicity *in vitro* effects induced by the pure insecticide and its commercial formulation Aficida® *on* *vitro* CHO-K1 cells (Figure 4) as well as on *in vivo* biotic matrices including the fish *C. decemmaculatus* and amphibian *R. arenarum* tadpoles (Figure 5). Our observations revealed positive
results for both compounds results when the either the CA (Figure 4B) and the SCE (Figure 4A) assays were performed in CHO-K1 cells [51]. Furthermore, the induction of alterations in the CCP (Figure 4D) and MI status (Figure 4C) on CHO-K1 cells was reported to occur after in vitro exposure to pirimicarb [51]. Finally, when the MN induction (Figure 5A), alterations in the erythrocytes:erythroblasts ratios, and SCGE end-points (Figure 5B) were employed after in vivo exposure to the pirimicarb-based formulations Aficida® and Patton Flow®, positive results were reported by Vera Candioti and collaborators in C. decemmaculatus [108,109] and R. arenarum tadpoles exposed under laboratory conditions [110].

Imidacloprid. Genotoxicity and Cytotoxicity Profiles

Imidacloprid, (2E)-1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine; CASRN: 138261-41-3), is a nicotine-derived systemic insecticide belonging to the neonicotinoids pesticide group. These insecticides act as an insect neurotoxin and belongs to a class of chemicals, chloronicotinyl nitroguanidine chemical family, which affect the central nervous system of insects [111,112]. It is effective on contact and via stomach action (http://extoxnet.orst.edu/pips/imidaclo.htm). Because imidacloprid binds much more strongly to insect nicotinic neuron receptors than that of mammal neurons, this insecticide results selectively more toxic to insects than mammals [112,113]. Imidacloprid has been ranked as a class II chemical (moderately hazardous) by the WHO [114] whereas the US EPA [115] has included the insecticide into the Group E of compounds with no evidence of carcinogenicity.

Imidacloprid decreases the reproduction rates in Caenorhabditis elegans and Eisenia fetida [116]. After S9 metabolic activation in vitro, imidacloprid produces calf thymus DNA adducts [117], increases the frequency of spermatogenic abnormalities in E. fetida [118], and is mutagenic in S. typhimurium strains, with or without S9 fraction [119]. The insecticide also induces significant increases in the frequency of SCE and MN formation in human peripheral blood lymphocytes [120,121], mice and rat bone-marrow cells [119,122], peripheral blood erythrocytes from Rana N-Hallowell tadpoles [123], and Vicia faba root cells [118]. Furthermore, imidacloprid causes DNA strand breaks in the colomocytes of E. fetida [118], erythrocytes from Rana N-Hallowell anuran tadpoles [123], human peripheral blood lymphocytes [120], and leukocytes in vitro [121]. However, it does not cause DNA strand breaks in V. faba root cells [123].
In our laboratory, we have recently studied the in vivo genotoxic effects induced by the imidacloprid-based commercial formulation Glacoxan imida® on H. pulchellus tadpoles exposed under laboratory conditions (Figure 6). Our observations demonstrated that the insecticide is able to exert DNA and chromosomal damage evaluated by the MN (Figure 6A) and SCGE (Figure 6B) bioassays [124].

Final Remarks

Overall, a comparative analysis of results revealed, depending upon the end-point employed, that the damage induced by the commercial formulations of the pesticides is, in general and regardless of the type of the active ingredient, greater than that produced by the pure compounds by themselves. Unfortunately, the identity of the components present within the excipient formulations was not made available by the manufacturer. These final remarks are in accord with previous observations not only reported by us but also by other research groups indicating the presence of xenobiotics within the composition of the commercial formulations with genotoxic and cytotoxic effects as previously mentioned [44,46,51,66-68,89,90,125-130]. Hence, risk assessment must also consider additional genotoxic effects caused by the excipient/s. Thus, both the workers as well as non-target organisms are exposed to the simultaneous action of the active ingredient and a variety of other chemical/s contained in the formulated product.

Finally, the results highlight that a whole knowledge of the toxic effect/s of the active ingredient of a pesticide is not enough in biomonitoring studies as well as that agrochemical/s toxic effect/s should be evaluated according to the commercial formulation available in market. Furthermore, the deleterious effect/s of the excipient/s present within the commercial formulation should be neither discarded nor underestimated. The importance of further studies on this type of pesticide in order to achieve a complete knowledge on its genetic toxicology seems to be, then, more than evident.

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