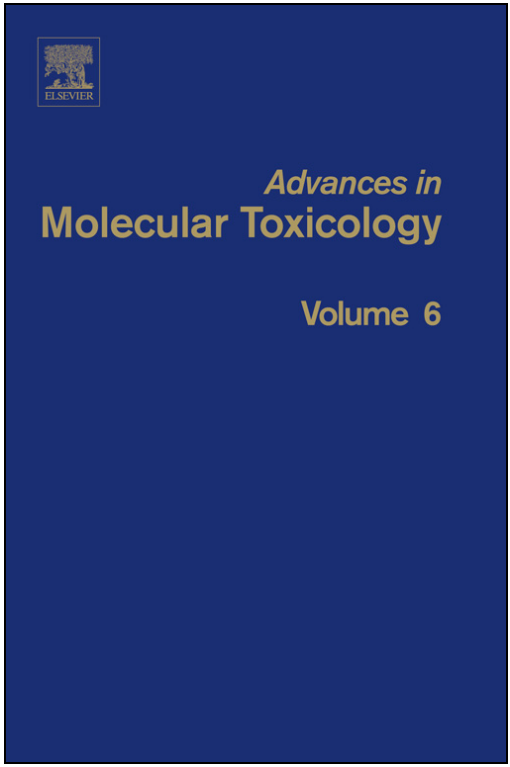

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CHAPTER TWO

Pesticides Used in South American GMO-Based Agriculture: A Review of Their Effects on Humans and Animal Models

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Abstract

In South America, the incorporation of genetically modified organisms (GMO) engineered to be resistant to pesticides changed the agricultural model into one dependent on the massive use of agrochemicals. Different pesticides are used in response to the demands of the global consuming market to control weeds, herbivorous arthropods, and crop diseases. Here, we review their effects on humans and animal models, in terms of genotoxicity, teratogenicity, and cell damage. We also stress the importance of biomarkers for medical surveillance of populations at risk and propose the use of biosensors as sensitive resources to detect undesirable effects of new molecules and environmental pollutants. The compatibility of glyphosate, the most intensively used herbicide associated to GMO crops, with an integrated pest management for soybean crops, is also discussed.

1. Introduction

The horticultural productivity in the subtropical regions of the world is severely limited by the pests and diseases affecting crops, and therefore, the quality of the products, which has become a priority worldwide in response to the demands of the consuming market. The use of agrochemicals is the most common strategy for fertilizing soils, control weeds, herbivorous arthropods, and crop diseases, but it also constitutes a major factor affecting natural resources as well as the health of the rural workers and potential consumers.

In South America, different agrochemicals are massively used, with a preponderance of the broad-spectrum glyphosate-based herbicides (GBHs), which utterly depend on genetically modified organisms (GMO) engineered to be glyphosate-resistant, such as soy crops.

In Argentina, the extension of soil devoted to transgenic soy reached 20 millions of hectares. 200 millions of liters of GBH are used for a production of 50 millions tons of soy beans per year [1,2]. Paraguay currently presents a great soybean culture expansion, with GMO seeds introduced illegally in the country and the infrastructure mounted and controlled by big corporations [3–5]. In the 2006/2007 harvest, the acreage reached 2,426,000 ha, almost 400,000 ha more than the preceding harvest (2005/2006). Of the herbicides imported in 2002, 75% was destined to soybean culture; of the imported pesticides, 68% was used in the same area; and of the fungicides, 65% had a similar destination [6].

The extensive agricultural model based in the GMO technological package is currently applied in South America (mainly in Argentina, Bolivia, Brazil, Paraguay, and Uruguay) without critical evaluation, rigorous regulations and adequate information about the impact of sublethal doses on human health and environment, leading to a conflictive situation. Studies of the possible impacts are absolutely necessary, since there is a paucity of data regarding chronic exposure to agrochemicals.

2. Cellular and Biochemical Effects of GBHs

GBHs are considered endocrine disruptors because of their ability to impair the synthesis of steroid hormones [7]. The GBH Roundup disrupts the activity of aromatase, a member of the cytochrome P450 family crucial for sex steroid hormone synthesis. In cultures of JEG3 placental cells, the GBH decreases the mRNA levels of the enzyme CYP19 (an essential component of cytochrome p450 aromatase, which is responsible for the irreversible conversion of androgens into estrogens). Importantly, the active principle glyphosate interacts with the active site of the purified enzyme. The effects of glyphosate on cell cultures and microsomes are facilitated by other components in the Roundup formulation that presumably increase the bioavailability of the active principle [8]. Glyphosate penetration through the cell membrane and its subsequent intracellular action is greatly facilitated by adjuvants such as surfactants [9,10]. Moreover, results from cell cultures indicate that the adjuvants *per se* may pose adverse effects [11].

In addition, both glyphosate and the commercial herbicide severely affect embryonic and placental cells, producing mitochondrial damage, necrosis and programmed cell death by activation of caspases 3/7 in cell culture within 24 h, with doses far below those used in the agricultural practice. Other effects observed include cytotoxicity and genotoxicity, endocrine disruption of the androgen and estrogen receptors, and DNA damage in cell lines [11,12].

Another line of evidence supporting adverse effects of glyphosate was provided by the sea urchin embryo, suggesting that glyphosate and its

principal metabolite, AMPA (amino-methylphosphonic acid), alter cell-cycle checkpoints by interfering with the physiological DNA repair machinery. Several GBHs were assayed and they induced cell-cycle dysfunction from the first cell division in these embryos [13,14]. The threshold concentration for this effect is 500–4000-fold lower than that sprayed on crops in the field. At a concentration of 8 mM, glyphosate induces a delay in the kinetics of the first cell cleavage of sea urchin embryos, altering the entry into S-phase by interfering with the activation of the CDK1/cyclin B complex [10,15]. Failure of cell-cycle checkpoints is known to lead to genomic instability and the potential development of cancer.

3. Agrochemicals, Biomarkers, Genotoxicity, and Congenital Malformations in Humans

Human biomonitoring is a useful tool to estimate risk posed by an integrated exposure to complex mixtures of chemicals. It depends on the use of biomarkers, defined as quantitative indicators of molecular and cellular events in biological systems, relevant to human health, development, and aging. Biomarkers are measured in biological material collected from volunteer subjects in observational or intervention studies [16].

Genotoxicity is the process by which an agent produces a deleterious effect on DNA and other cellular targets that control the integrity of genetic material [17]. Genotoxic agents are those that cause structural alterations in DNA, causing changes or rearrangements in the genes, and therefore inducing mutations. Once produced, these changes are permanent and therefore heritable to other cells during mitosis in the case of somatic mutations, or from parent to offspring when mutations involve germ cells (gametes). The relationship between the appearance of neoplastic processes and the accumulation of these mutations in mammalian cells was already shown [18].

Many methods have been developed to assess genotoxicity. These tests can be developed using *in vitro* or *in vivo* models and were designed to detect substances that might cause genetic damage directly or indirectly by a number of mechanisms.

The sensitive assay of chromosomal aberrations (CA) is widely used for detection of genotoxic agents. It provides information related to the possible deleterious effects produced at the chromosomal level, both in structure (damage to chromosomes or chromatids) and in number (aneugenesis).

Another useful assay for the detection of potentially genotoxic substances is the micronuclei test (MN). The latter is used to detect damage at the level of the chromosomes or the mitotic apparatus (clastogenesis and aneugenesis, respectively) in immature erythrocytes from bone marrow or

peripheral blood lymphocyte cultures [19]. Micronuclei are small particles formed by fragments or whole chromosomes after cell division which are not included in the nucleus of the daughter cells [20].

The comet assay or gel electrophoresis of individual cells has been introduced in recent years to identify genotoxic chemicals for regulatory purposes. This assay can be performed both *in vivo* and *in vitro*. Increased migration in the comet assay can be attributed to strand breaks, alkali-labile sites, and incomplete excision repair sites, while decreased DNA migration could be attributed to cross-links, DNA–DNA, or DNA–protein interactions. When the core is subjected to electrophoresis, DNA fragments migrate giving the appearance of a comet [21,22]. It has the advantage over other methods that it can be performed on all tissues regardless of their mitotic activity [23]. The comet assay has been used to determine the extent of DNA damage in leukocytes from rural workers occupationally exposed to a variety of pesticides [24–26].

The toxicity and genotoxicity evaluation carried out more than 10 years ago classified glyphosate as a low-risk herbicide for animal and human health [27–29]. Williams *et al.* conclude their review by stating that "under the conditions of present and expected use, there is no possibility that glyphosate poses a risk to human health" [29]. This review devotes many pages to dismiss genotoxicity and other toxicology data on glyphosate published by other researchers. Although up to 13.29% of the research discussed by Williams are unpublished reports of research conducted by or for Monsanto, this review has become one of the most cited papers of glyphosate toxicology by the scientific community worldwide.

The comet assay provided evidence that glyphosate produces DNA damage both *in vitro* and *in vivo*. A series of tests was conducted to determine the genotoxic potential of glyphosate and its main degradation product, AMPA. A statistically significant increase in levels of chromosome aberrations with a concentration of 200 μg/ml of AMPA was found by the CA test in human peripheral blood cells [30]. Only two previous and contradictory works assessing CA in human peripheral blood exposed to glyphosate were reported. Van de Waart obtained negative results from lymphocyte cultures exposed to concentrations between 0.33 and 0.56 mg/ml of glyphosate [31]. Although this research was not published in a peer reviewed scientific journal, it is cited by Williams *et al.* in the review [29] as an unpublished report. On the other hand, Lioi *et al.* obtained positive results even after working with a much lower concentration of glyphosate (0.0014 mg/ml) [32].

Moreover, the comet assay showed evidence of DNA damage in different human cell lines for glyphosate and AMPA [30,33,34] and in liver and kidney of mice intraperitoneally injected with 300 mg/kg of glyphosate [35]. A statistically significant increase in the index of DNA damage was obtained in peripheral blood from Balb C mice exposed to 400 mg/kg of

glyphosate in the same way [36]. Similarly, there was a rise in the number of micronucleated cells in bone marrow of animals exposed to glyphosate and AMPA [30,34,36]. Therefore, genotoxic effects of glyphosate and AMPA in mice are found both as DNA damage and in more complex structures such as chromosomes or the mitotic apparatus.

Genotoxicity studies in populations exposed to an agent suspected of producing effects on the hereditary material complement epidemiological studies with the aim of disease prevention by identifying the environmental agent that causes the disease. Experimental data reveal that several components of chemicals produce genetic damage and induce mutations [37,38]. Chemicals used in agriculture could be responsible for the high incidence of cancer in farm workers such as cancers of the lip, stomach, brain, prostate, connective tissue, lymphatic, and hematopoietic system. In the few studies about exposure of women to pesticides, ovarian and breast cancer, multiple myeloma, and non-Hodgkin lymphoma were associated with exposure to triazine-based herbicides, insecticides, and various chemical compounds [39,40].

Studies of populations exposed to pesticides, mostly in European applicators, reported a positive association between exposure to a complex mixture of chemicals and increased genetic damage, as quantified by CA, sister chromatid exchange (SCE), MN and comet assay [35,41,42]. In the past 10 years, several countries in Latin America have initiated studies about the environmental consequences of the use of herbicides and pesticides. In México, a study involving 30 workers found significantly higher genotoxicity in the exposed group in relation to the unexposed group, when evaluated by SCE and MN tests [43]. In Bolivia, agricultural workers exposed to mixtures of chemicals without protection or security measures have experienced genotoxic risk, as revealed by a high frequency of SCE, MN, CA, and parameters of the comet assay [44]. In Ecuador, a highly significant increase in the DNA damage values measured by comet assay and CA was observed in 45 applicators in the Colombia-Ecuador border [45].

3.1. Genotoxicity in agricultural regions in the province of Córdoba, Argentina

A high percentage of the population of the province of Córdoba, Argentina, lives in rural areas devoted to agriculture, where large amounts of chemicals are used. Genetic studies in humans exposed to pesticides were conducted in this province. The first report presented a cytogenetic monitoring of rural workers (sprayers). The CA test revealed that the number of CA in peripheral blood was significantly higher in the exposed group in comparison to the reference group [46].

In a more recent study in the same province, genotoxicity was monitored by the CA, MN, and comet assay in a group of 80 exposed people in

relation to a reference group. Ninety-five percent applicators used more than one pesticide and the rest used only one. The pesticides used were glyphosate, cypermethrin (CY), 2–4D, endosulfan, atrazine, and chlorpyrifos. The analysis of health status showed that 50% of the participants of the exposed group reported persistent symptomatology associated with respiratory (sneezing, coughing, bronchospasm, etc.), dermatological and/or mucocutaneous (skin and eye itching, tearing, pigmentation, etc.), digestive (vomiting), and neurological problems (headache and dizziness). The indicators of genetic damage observed in the exposed group were all significantly increased in comparison to the reference group in the three tests used (Mañas, F. *et al.*, unpublished results; see Figure 1).

The results indicate that genetic damage could be attributed to exposure to various agrochemicals. It is important to remark that the comet assay detects ruptures at the level of DNA strands (single and double) with a high sensitivity. Therefore, high levels of DNA damage quantified by this assay without an increase in the values of CA and/or micronuclei may be an early indicator of a recent molecular insult that have not yet affected more complex structures such as chromosomes and/or the mitotic apparatus. The CA test is probably the most important in terms of information, since chromatid or chromosome breaks, duplications in the number of chromosomes, recombination, and other chromosomal rearrangements are characteristic of certain neoplastic diseases. This means that at the time of the study, individuals exposed to pesticides have a higher probability of irreversible genetic damage that could result in the development of cancer due to the saturation of DNA repair systems. The MN test detects damage at the level of the chromosome and/or the mitotic apparatus. An increase in the number of micronucleated cells is suggestive of genomic instability, increased susceptibility to breakage, and alterations in chromosomes, including risks of aneuploidy (loss of the normal number of

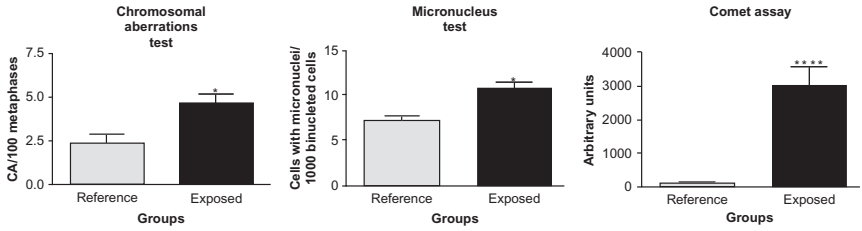

Figure 1 Genetic damage quantified by chromosomal aberrations, micronuclei, and comet assay. The indicators of genetic damage observed in the reference group and the group exposed to pesticides were, respectively, 2.36 ± 1.74 and 4.68 ± 3.55 AC per 100 metaphases analyzed; 7.25 ± 1.48 and 10.81 ± 5.21 MN per 1000 binucleated cells analyzed; and 115.1 ± 71.11 and 3037 ± 3731 arbitrary units of DNA damage (tail moment of the comet). * $p < 0.05$, **** $p < 0.0001$, Mann–Whitney test.

chromosomes), suggesting a relationship between the increase in the number of micronucleated cells and an increased risk of cancer.

3.2. A survey of biomarkers in agricultural regions in the province of Santa Fe, Argentina

Genotoxic and oxidative damage was studied in a group of horticultural workers in Santa Fe, Argentina [47,48]. DNA damage (evaluated through comet assay), modifications in oxidative balance (catalase (CAT) activity and lipid peroxidation), and exposure biomarkers (cholinesterase enzymes) were evaluated in groups of individuals occupationally exposed to mixtures of agrochemicals. The study involved 105 farm workers (nonpesticide applicators or indirectly exposed) and pesticide applicators (or directly exposed) from the horticultural belt of Santa Fe, and 112 donors from the same area, without current or previous exposure to pesticides in their workplace, as a control group. In the exposed group, the great majority of the subjects were in contact with many pesticides, including captan, copper, mancozeb, chlorpyrifos, carbofuran, CY, dimethoate, endosulfan, imidacloprid, malathion, methamidophos, parathion, permethrin, and glyphosate.

Blood samples were assayed for butyrylcholinesterase (BChE), acetylcholinesterase (AChE), and CAT activity. Malondialdehyde (MDA) was used as a marker for lipid peroxidation in erythrocytes and was determined by measuring the production of the color generated during the reaction of thiobarbituric acid (TBA) with MDA (TBARS assay). Damage Index Comet Assay (DICA) was calculated for each sample. The results of this study are shown in detail in Figure 2 and clearly indicate that, under the conditions of their work, subjects directly and indirectly exposed to pesticides have enzymatic alterations, modifications in oxidative balance, and genotoxic damage when compared to controls. The influence of confounding factors, such as age, gender, smoking, and alcohol consumption, on all biomarkers used was investigated and no significant differences were observed ($p > 0.05$).

The invaluable role of AChE monitoring in rural workers at high risk of exposure to organophosphorus and methyl carbamate pesticides has been previously recognized in different investigations [49]. The results obtained from the horticultural workers in Santa Fe are in agreement with other reports [50,51]. AChE showed a significant decrease in workers directly and indirectly exposed to pesticides.

Oxidative damage is thought to be an important mechanism of action of several pesticides [52,53]. Different pesticides have been reported to induce oxidative stress as shown by enhancement of MDA production [53–59]. The results of the Santa Fe's study indicate that CAT activity decreased significantly in both pesticide applicators and nonpesticide applicator workers, and TBARS was significantly increased in pesticide applicators. Also,

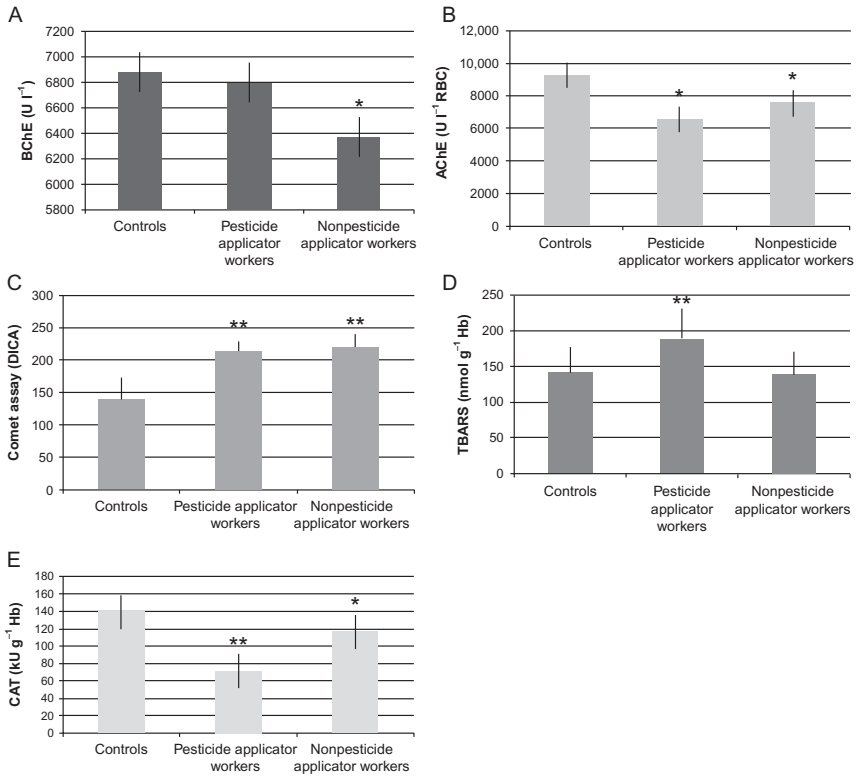

Figure 2 Statistical evaluations of the two exposed groups (pesticide applicator, $n = 59$, and nonpesticide applicator workers, $n = 53$) were contrasted in all cases with the control population ($n = 112$). (A) Butyrylcholinesterase (BChE), (B) acetylcholinesterase (AChE), (C) comet assay (DICA), (D) assay of thiobarbituric acid reactive substances (TBARS), and (E) catalase (CAT) activity. Values are presented as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$ (Mann–Whitney test).

the correlation between TBARS and AChE activity found in the Santa Fe study is similar to that obtained by other authors [50,51,60].

Several different pathways by which oxidative DNA damage occurs have been proposed. These include chemical modification of nucleotides [61], direct action of reactive oxygen species on DNA, or indirect lipid peroxidation degradation products [62]. The comet assay results from the Santa Fe study showed that pesticide-spraying workers and farmers presented a significant increase in DICA when compared to controls.

New, more selective and efficient pesticides, possibly "safer" for nontarget organisms, have been produced in the past few years. So far, they have coexisted in the farming practice with agents in which one or more active

principles have been found to be genotoxic and cytotoxic to various systems [63]. The agricultural workers included in the Santa Fe study were also exposed to a great number of pesticides (all of the subjects were exposed to more than two different pesticides), some of which are classified as being carcinogenic by the U.S. Environmental Protection Agency (US-EPA) and hazardous by the World Health Organization (WHO), although not yet listed by the IARC. Considering the chemicals used, it is important to note that some of these, such as methamidophos, have been banned in other countries because of their high toxicity, while in developing countries such a prohibition is limited [64].

3.3. Congenital malformations and genotoxicity in populations exposed to pesticides in Paraguay

During the 2005/2006 harvest, the Itapúa department in Paraguay occupied the second place in the estimation of the soybean acreage with the consequent mass and intensive use of pesticides. From March 2006 to February 2007, a case–control study was carried out regarding congenital malformations associated with pesticides in the Regional Hospital of Encarnación (Itapúa Department). This research identified living near pesticide-fumigated soy fields, in dwellings located less than 1 km from pesticide-fumigated fields, as significantly associated risk factors for congenital malformations (OR = 2.46 (IC 1.09–5.57) $p < 0.02$; OR = 2.66 (IC 1.19–5.97) $p < 0.008$, respectively). It was also striking that during the research period, besides the registered cases, there were 32 stillborns with no obvious malformations and that did not enter into the research protocol, as it was only included as cases when stillbirth was accompanied with obvious malformations. In this research, only two stillborns with multiple malformations were included [65].

This research did not discriminate against what pesticide the studied population was exposed to. However, the zone is mainly agricultural, its main resource is the soybean culture and it currently occupies the second place in the 2010/2011 harvest compared to other departments. Several studies from other regions found an increased risk of congenital malformations associated with occupational exposure, especially when this took place in the first months of gestation [66–69]. In particular, the epidemiological study of Winchester and Huskins [69] related the amount of agrochemicals measured in the water surface with the congenital malformations rates during 1996 and 2002. The births were grouped according to the months of conception, considering the last menstruation period (LMP) as reference. The increase in the levels of pesticides in the water in the spring season coincided with a higher rate of congenital malformations in children whose mothers had their LMP in the last spring months. The correlation was statistically significant.

In June 2003, a factory dedicated to the chemical formulation and synthesis of pesticides, fungicides, herbicides, and other agricultural products was installed in "Los Naranjos" neighborhood of Nemby, Central department, Paraguay. This situation was defined as a potential risk factor for the health according to the conclusions of the technical inspection report made by the Ministry of Public Health and Welfare, the Ministry of Environment and the PAHO/WHO. This technical team concluded: "The existence of emissions and the eventual exposure to the community, pose a risk to the population and the workers' health" [70]. The fact of going to a school located 50 m from the factory was considered as a potential risk of pesticide exposure for school children. Therefore, damage in the genetic material was investigated in a child population through the measurement of the micronucleus frequency in epithelial cells of the oral mucosa. Two groups of school children were compared. One group attended the school located 50 m from the agrochemicals' factory (exposed group). The control group included children that attended another school located 5.5 km from the factory. In the exposed group, a higher frequency of micronucleus and binucleated cells was found with a highly significantly difference (t-test, $p < 0.0001$). The frequency of karyorrhexis and pyknosis also showed a significant increase in the exposed group [71].

4. Agrochemicals, Biomarkers, Genotoxicity, and Teratogenesis in Animal Models

Toxicological studies of pesticides have focused on the evaluation of exposures to single compounds, but animals and humans are often exposed to different pesticides or pesticide mixtures, either simultaneously or in series. Assessment of the associations with individual pesticide exposure is very difficult because most of the agricultural practice involves the regular use of a large number of different pesticides, together with other chemicals such as coformulants, which vary greatly in their potential toxicities and potencies. Furthermore, measurements of systemic exposure to pesticides were not taken and therefore correlations between increased genotoxicity and biomarker variations with the degree of exposure were not possible to obtain [72].

When considering risks from a health perspective, it is necessary to assess whether the chemicals in a mixture interact to cause either an increased or a different overall response as compared with the sum of the responses of the individual chemicals present in the mixture, or whether the overall effect is simply a summation of the expected effect of each chemical [73].

In this context, controlled experiments in the laboratory with different *in vitro* and *in vivo* models regarding genotoxicity, teratogenicity, biochemical, physiological, reproductive, and behavioral changes are certainly

necessary to evaluate potential harms of individual pesticides or of their mixtures on human health and biodiversity. In this section, we review the effects of pesticides on different vertebrate models.

4.1. Amphibian ecotoxicology in mid-east Argentina

The main application period of agrochemicals in this region extends from November to March [74], generally coinciding with the reproductive period of amphibians [75]. In these months, short but heavy rainfalls are very common and can cause intensive pesticide runoff to nontarget compartments such as aquatic ecosystems. Indeed, CY, endosulfan, chlorpyrifos, and glyphosate were detected in sediments, suspended particles, and water of some intensively cultivated land in Argentina [76,77].

Field studies demonstrated that agricultural runoff has serious consequences on amphibian's survival and health [78]. Indeed, agricultural activities not only deprive some species from healthy environments but also produce biochemical negative responses, hematological disturbances, testicular damage, and morphological abnormalities [79–86].

Investigating how pesticides affect the survival and different biology traits of anuran amphibians is especially important when one considers the meaning of amphibians in the food webs of diverse ecosystem communities and as biological indicators of environmental health. Here, we review the existing ecotoxicological data from the mid-eastern region of Argentina about the influence of the most common pesticides on anuran tadpoles.

4.1.1. Cypermethrin

CY [(RS)-alpha-cyano-3-phenoxybenzyl (1RS)-*cis*,*trans*-3-(2,2,- dichlorovinyl)-2,2-dimethylcyclopropane carboxylate] is a highly active synthetic pyrethroid insecticide. It was demonstrated that treatments with CY caused apoptotic cell death in the telencephalon of *Physalaemus biligonigerus* larvae and in immature cells of the central nervous system in *Rhinella arenarum* tadpoles [87–89]. These contributions postulated a new neurotoxic mechanism by which CY induces apoptosis in cells of the central nervous system in vertebrates. Although CY is not mutagenic in *in vitro* assays [90], *in vivo* assays showed that the commercial formulation of CY significantly increased the frequency of MN in anuran larvae of *Odontophrynus americanus*, demonstrating genotoxic effects [91].

Although the presence of aquatic plants such as the fern *Salvinia herzogii* reduced the mortality of amphibian tadpoles of *P. biligonigerus* exposed to CY, these experiments corroborated that the sublethal doses of this pyrethroid induced signs equivalent to the toxic phases I and II (intents of escape, swimming to top of bowls, spiral while swimming, laying on the side or back, and lateral curve in tail) [92]. In addition, morphological analysis in *P. biligonigerus* and *R. arenarum* tadpoles revealed that exposure

to CY affected the development of the body axis, reducing the head and body size and significantly altering other morphometric parameters in relation to control tadpoles [89].

4.1.2. Endosulfan

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3 benzodiox-athiepine 3-oxide) is a broad-spectrum nonsystemic contact and stomach insecticide, effective for control of sucking, chewing, and boring insects and mites on a very wide range of crops. An international treaty signed in 2001 by the Stockholm Convention agreed to gradually eliminate or restrict the production and use of 12 persistent organic pollutants, including endosulfan [93].

Several studies from Argentina found endosulfan in milk and derivatives produced in Santa Fe and Entre Ríos provinces [94,95]. Endosulfan residues were also found in amphibian fauna in an average of 12.5 ng/g tissue in *Leptodactylus latrans*, *L. chaquensis*, *Hypsiboas pulchellus*, and *Rhinella schneider* [96,97]. Moreover, in raptor birds (*Parabuteo unicintus*), endosulfan residues were found at higher levels than those found in their potential preys, revealing a biomagnification phenomenon [97]. In the same region, another carnivorous at the top of the food web, *Caiman latirostris*, also has the potential to accumulate high concentrations of endosulfan residues [98].

During the seeding period of soybean in Entre Ríos province, endosulfan sulfate and endosulfan II were detected in agricultural ponds in higher concentrations (25.9 ng of total endosulfan/L) [78] than the value recommended by the National Guide of Quality of Environmental Water in Argentina to preserve aquatic fauna (0.8 ng/L) in Argentinean water bodies [99]. Moreover, a commercial formulation of endosulfan produced genetic damage in erythrocytes of *H. pulchellus* tadpoles, as revealed by the MN test [100].

4.1.3. Glyphosate

Glyphosate (*N*-[phosphonomethyl] glycine) is a weak organic acid that inhibits the shikimic acid biosynthesis pathway in plants. Numerous studies have shown that amphibians are one of the most sensitive vertebrate groups to the toxicological effects of GBH [101].

A commercial GBH produced malformations in 75% of treated tadpoles of the South American amphibian *Scinax nasicus*. Malformations appeared even at low concentrations of GBH (1.47 mg a.e./L after 96 h of exposure) and included craniofacial and mouth deformities, eye abnormalities, and bent or curved tails [102]. The toxicity of GBH formulations varied according to the type of exposure. Specifically, exposed tadpoles did not die after 24 h when exposure to GBH was not continuous [103].

In another study with *R. arenarum* tadpoles, four different GBH commercial formulations were evaluated for their toxicity during the first 6 h of exposure. AChE, BChE, carboxylesterase, and glutathione-*S*-transferase

activities were evaluated as bioindicators of GBH toxicity. The results showed the difficulty of formulating environmental regulations to legislate the CF-GLY, taking into account that different commercial formulations can produce widely different toxicities, considering both lethality and variation of the biomarkers [104]. The disparities likely occur because of the inclusion of unspecified surfactants, which are often referred to as "inert" or which remain proprietary information (i.e., trade secrets). For example, it has been documented how a derivative of polyoxyethyleneamine (POEA) used as a surfactant component of GBHs, is the major contributor of acute tadpole toxicity [105]. Therefore, it would be highly recommendable that those "trade secrets" should be made of public domain to properly evaluate their toxic effects to the wild fauna.

4.2. Teratogenesis by GBH and other pesticides. Relationship with the retinoic acid pathway

A recent study using a commercial formulation of GBH showed that treatments with a 1/5000 dilution (equivalent to 430 μM of glyphosate) were sufficient to induce reproducible malformations in embryos of the South African clawed frog *Xenopus laevis*, a widely used vertebrate model for embryological studies [106]. The phenotypes observed include shortening of the trunk, cephalic reduction, microphthalmy, cyclopia, reduction of the neural crest territory at neurula stages and craniofacial malformations at tadpole stages. GBH inhibits the anterior expression domain of the morphogen sonic hedgehog (*shh)*, reduces the domain of the cephalic marker *otx2*, prevents the subdivision of the eye field, and impairs craniofacial development. Moreover, in recent experiments with another commercial formulation of GBH, the malformations observed before were reproduced in a dose-dependent manner, even at dilutions of 1/500,000, which produced developmental abnormalities in 17% of the embryos, without lethality (unpublished results).

It is known that glyphosate penetration through the cell membrane and subsequent intracellular action is greatly facilitated by adjuvants such as surfactants [9,10]. For this reason, the active principle was also tested by injecting frog embryos with glyphosate alone (between 8 and 12 μM per injected cell). The calculated intracellular concentration for glyphosate injected into embryos was 60 times lower than the glyphosate concentration present in the 1/5000 dilution of the GBH which was used to culture whole embryos. Notwithstanding this, both produced similar phenotypes and changes in gene expression, suggesting that the effects are attributable to the active principle of the herbicide.

It is very well known that acute or chronic increase of retinoic acid (RA) levels leads to teratogenic effects during human pregnancy and in experimental models. The characteristic features displayed by RA embryopathy in

humans include brain abnormalities such as microcephaly, microphtalmia, and impairment of hindbrain development; abnormal external and middle ears (microtia or anotia), mandibular and midfacial underdevelopment, and cleft palate. Many craniofacial malformations can be attributed to defects in cranial neural crest cells. An excessive cell death in regions where apoptosis normally takes place may underlie a general mechanism for craniofacial malformations associated to teratogens [107,108].

In fact, an excess of RA signaling is able to downregulate the expression of *shh* in the embryonic dorsal midline in *Xenopus* [109,110]. *Shh* deficiency is associated to the holoprosencephaly syndrome (HPE), a CNS malformation with a frequency of 1/250 of pregnancies and 1/10,000 of live births. The HPE is a defect generated by the deficiency of the embryonic dorsal midline which results in a failure in the division of the brain hemispheres, leading to different grades of craniofacial malformations that range from unilobar brain and cyclopia in the most severe cases to hypotelorism (abnormally decreased distance between the eyes). Other defects include failure in the closure of the dorsal neural tube, microcephaly, and proboscis [111,112]. Moreover, Shh signaling is also necessary for the development of the cranial neural crest derivatives. In mouse, specific removal of the Shh responsiveness in the neural crest cells that give rise to skeleton and connective tissue in the head, increases apoptosis and decreases proliferation in the branchial arches, leading to facial truncations [113]. Shh signaling from the ventral midline is necessary, as an antiapoptotic agent, for the survival of the neural epithelium, and it is also essential for the rapid and extensive expansion of the early vesicles of the developing midbrain and forebrain [114–116].

An excess of RA signaling also downregulates *otx2* expression in *Xenopus*, chicken, and mouse embryos [108]. Knockout mice for *otx2* lack all the brain structures anterior to rhombomere 3. Interestingly, heterozygous mutants showed craniofacial malformations including loss of the eyes and lower jaw (agnathia). These phenotypes are reminiscent of otocephaly reported in humans and other animals and suggest that *otx2* plays an essential role in the development of cranial skeletons of mesencephalic neural crest origin [117–119].

All this evidence indicates that RA, *otx2*, and *shh* are part of a genetic cascade critical for the development of the brain and craniofacial skeleton of neural crest origin. Glyphosate inhibits the anterior expression of *shh*, reduces the domain of *otx2*, prevents the subdivision of the eye field, and impairs craniofacial development, resembling aspects of the holoprensecephalic and otocephalic syndromes [120,121]. Indeed, assays using a RA-dependent gene reporter revealed that GBH treatment increases the endogenous RA activity in *Xenopus* embryos. Moreover, an antagonist of RA rescued the morphological phenotype produced by GBH. This leads to the conclusion that at least some of the teratogenic effects of GBH were mediated by increased endogenous RA activity in the embryos [106]. This

is consistent with the very well-known syndrome produced by excess of RA, as described by the epidemiological study of Lammer *et al.* in humans [122] and in vertebrate embryos [107,108,123–127].

In *Xenopus* embryos, the endogenous activity of retinoids gradually increases during early embryogenesis and is finely regulated in space. At late gastrula, a rostral–caudal gradient from 0.01 to 0.16 μM RA is established, with highest levels at the posterior end of the embryo. The gradient persists at the early neurula stage. Synthesis and degradation of RA seem to be the mechanisms that lead to this uneven distribution [128]. This gradient explains why low doses of applied RA affect primarily the cephalic region and increasing the doses begins to affect the trunk [123,124]. Moreover, maintaining a normal endogenous distribution of RA is important for axial patterning and organogenesis in vertebrates [125,128–136].

The spatial distribution of retinoid activity is regulated by degradation of RA by the CYP26 enzymes, which are members of the cytochrome P450 family and are present in all vertebrates from early stages of embryogenesis. Transcription of CYP26 is developmentally and spatially regulated. Deficiencies of this enzyme produce serious malformations in different vertebrate models consistent with an important increase in RA signaling. These phenotypes include cephalic defects, abnormalities of the eye and the forebrain, agnathia, and caudal truncations [137–144]. In this context, it will be interesting to elucidate in the future whether the increase of RA signaling induced by GBH could be a consequence of inhibiting the activity of CYP26 enzymes.

Other authors observed craniofacial ossification defects and loss of caudal vertebrae in rats orally treated with sublethal doses of GBH. These alterations were statistically significant in comparison with the control group, and importantly, they were dose-dependent, indicating a specific effect [145]. Although these authors did not address the molecular basis of the teratogenic effects, an altered retinoid signaling pathway is a major candidate to be considered. Normal craniofacial morphology is the result of complex interactions between embryonic tissues and requires precise regulation of cell movements, growth, patterning, and differentiation. Mutations or missregulation of genes that influence any of these processes would cause craniofacial abnormalities, such as facial clefting and craniosynostosis. Among the critical genes involved in craniofacial development is the Msx family of homeodomain transcription factors [146]. *Msx* genes contribute to maintaining the balance between proliferation and differentiation during pre- and postnatal skull morphogenesis. Mutant mice for *msx2* show incomplete or delayed ossification of the calvarial bones (i.e., those that constitute the upper part of the cranium and surround the cranial cavity), while the double mutants for *msx1* and *msx2* are deficient in calvarial ossification, thus resembling in the "skull, general incomplete ossification" observed in GBH-exposed embryos by

Dallegrave *et al.* [145]. Regulation of the *msx* genes by retinoids is supported by (a) the identification of a RA-responsive element in the 5′ flanking region of human *MSX1* gene and (b) functional *in vivo* evidence that indicates endogenous retinoid signaling controls the spatial expression of this gene by inhibition [146]. Therefore, it is conceivable that an increase in retinoid signaling upon exposure to GBH might inhibit *msx* expression, thus impairing the ossification of the cranial bones.

The other significant, dose-dependent effect of GBH exposure in rodent embryos described by Dallegrave *et al.* is "caudal vertebrae: absent" [145]. It is known that exposure of mouse embryos to RA at a similar period of development produces agenesis of caudal vertebrae, which is caused by the downregulation of posterior *hox* genes [147].

A recent report showed that limb and craniofacial malformations were found more frequently in different wild amphibian species living in agricultural sites in the mid-eastern region of Argentina [86]. These results suggest that pesticides used extensively may underlie developmental disturbancies in contaminated fields. Noteworthy, those malformations are similar to the ones resulting from an increase of endogenous RA activity.

The epidemiological study carried out by Benitez-Leite *et al.* in Paraguay identified 52 cases of malformations in the offspring of women exposed during pregnancy to agrochemicals. The congenital malformations observed include anencephaly, microcephaly, facial defects, myelomeningocele, cleft palate, ear malformations, polydactily, syndactily [65]. These defects are indeed consistent with the well-known and expected syndrome caused by misregulation of the RA pathway. In addition, a prevalence study in seven geographic regions of Argentina encompassing births between 1994 and 2007 showed that out of the 27 congenital anomalies analyzed, 14 showed a frequency significatively higher in one or more regions [148]. It is worthwiling to note that the province of Córdoba, which represents the geographic region with the most intensive practice of GMO crops-based agriculture in Argentina, revealed one of the largest spectrums of birth defects with a frequency significatively higher than in the other regions. This spectrum consisted of spina bifida, microtia, cleft lip with cleft palate, polycystic kidney, postaxial polydactyly, and Down syndrome. Most of these defects are consistent with an RA excess syndrome and/or with genotoxicity effects.

These conclusions should be taken into account together with the incidence of malformations and cancer in Chaco, an Argentine province with soybean harvest and massive use of glyphosate. Official records reveal a threefold increase in developmental malformations in the province and a fourfold increase of cancer in the locality of La Leonesa in the past decade [149]. GBHs are also used for the eradication of coca plantations in Colombia. Suggestively, an epidemiological surveillance conducted between December 2004 and April 2008 in Cali and Valle del Cauca revealed that cyclopia is an

endemic event with a 14–43-fold higher prevalence than that reported in the literature [150].

All this information is extremely worrying because the risk of environmental-induced disruptions in human development is highest during the critical period of gestation (2–8 weeks) [151]. Moreover, the mature human placenta has been shown to be permeable to glyphosate. After 2.5 h of perfusion, 15% of administered glyphosate is transferred to the fetal compartment [152]. Indeed, a two-compartment model study suggested that a considerable diffusion of glyphosate into the tissue is reached after intravenous administration in rats. These authors conclude that direct blood concentration is only an average indicator of the presence of the chemical and does not provide evidence about its tissue distribution [153]. It is necessary to consider the possibility that very low concentrations (pg/cell and not necessarily evenly distributed to all cells) may be sufficient to cause embryonic lethality (which is consistent with increased frequency of embryonic death and spontaneous abortions) or to modify normal embryonic pattern formation.

4.3. Pesticide-induced genotoxicity in caimans in Argentina

In Argentina, one of the main problems affecting natural populations of Broad-snouted caiman (*C. latirostris)* is habitat loss and exposure to massive amounts of pesticides used due to agriculture expansion [154]. Caimans embryos and hatchlings are particularly vulnerable to pesticides because the incubation period and hatching take place during the same months of maximal pesticide application (from December to April) [155]. An *in vivo* toxicological evaluation of pesticides was carried out on embryos and hatchlings, through different ways of exposure. This evaluation included laboratory controlled conditions and field-like studies.

Genotoxicity was evaluated through the MN test and the comet assay in erythrocytes of the hatchlings after *in ovo* exposure of *C. latirostris* embryos to pesticides. Both the formulation Roundup and its active principle glyphosate significantly induced genotoxicity in *C. latirostris* embryos and hatchlings under laboratory controlled conditions and field-like experiments that take into account the degradation curve for glyphosate in water. Genotoxicity occurred even at concentrations commonly applied in the field and through different types of exposure [155–157].

In order to simulate the exposure that a caiman nest may receive in habitats surrounded by croplands, the genotoxic effects of Roundup were analyzed under field-like exposure conditions in combination with endosulfan and cypermethrin formulations. Three experimental groups of three artificial caiman nests each in a field-like area were constructed. A total of 81 eggs were distributed into the three experimental groups and treated with 3% of Roundup (3 l/100 l water/ha) alone or in combination with 0.85% of

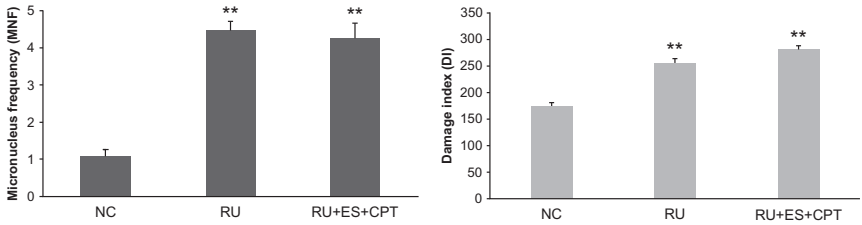

Figure 3 Micronucleus frequency MNF (A) and damage index (DI) from comet assay (B) observed in *C. latirostris* hatchlings after exposure to pesticides under field-like conditions. NC, negative control; RU, Roundup; ES, endosulfan; CPT, cypermethrin. All values are expressed as mean ± SE. $^{**}p < 0.001$ compared to control.

endosulfan formulation (0.85 l/100 l water/ha) and 0.12% of cypermethrin formulation (0.12 l/100 l water/ha). The results obtained from genotoxic endpoints demonstrated that both Roundup alone and the mixture of the three formulations induced a significant and similar increase in DNA damage when compared to the control group (Figure 3) [158]. The final fate of these alterations is uncertain, but they could affect the normal function of physiological processes, with consequences at cellular, individual, and population levels. In relation to this, alterations in the activity of different enzymatic and biochemical parameters were observed, as well as lower growth during the first month of life in caimans exposed to pesticides *in ovo* or *in vivo* (hatchlings) [155,158].

5. Is an Integrated Pest Management for Soybean Compatible with Glyphosate?

The occurrence of pests and weeds is an ecological process intimately related to the disturbance of ecosystems by man. Pickett and White describe disturbance as "any relatively discrete event in time that disrupts ecosystem, community, or population structure and changes resources, substrate availability, or the physical environment" [159]. From an ecological point of view, one of the greatest disturbances is caused by the widespread use of pesticides.

Since the decade of 1970, researchers began to pay more attention to the harmful effects of human activities on ecosystem processes and nontarget organisms. Some of the pioneers to identify pests as an ecological problem and to warn about the dangers of pesticides were De Bach and Rosen [160]. The traditional reliance on chemicals to control injurious organisms started to be considered a very reductionist approach to the problem. From the second half of the twentieth century to the present, the ecological theory has made a

great contribution to the management of agricultural systems, shifting the focus to a holistic mode of viewing the world, integrating the different components and processes of the ecosystem that affect their sustainability.

A new paradigm has emerged, the Integrated Pest Management (IPM). This is an effective strategy of pest control based on the biological and ecological knowledge of pests, an appropriate monitoring, and the combination of different control methods (biological, cultural, chemical), using selective chemicals only when absolutely necessary, to avoid risks to man and environment. According to Levins, this ecological model of pest management represents "an intermediate step along a path from a high-intervention, single-goal, industrial model of agriculture toward a gentle, ecologically and humanly rational production system" [161].

The great expansion of transgenic soybean (Roundup Ready, RR) since 1996 led Argentina to become the third world's major soybean producer and the first exporter of oil and meal soybean. This resulted in the intensive use of glyphosate, along with several pesticides such as cypermethrin, chlorpyrifos, endosulfan, and spinosad.

Despite the great spatial and temporal simplification of landscape due to monoculture, soybean crop is inhabited by a number of arthropods (parasitoids and predators) that play a relevant role in reducing populations of many herbivore pests, giving place to a fairly complex food web.

Field results from an ecological research program conducted on soybean pests in the Buenos Aires province (Argentina) indicated that a number of species usually considered pests in the 1970s and 1980s only reached levels of economic damage in certain local areas in some years of the 1990s. A complex of 30 species of larval parasitoids and generalist predators caused significant mortality of these pests [162–164]. Moreover, it was found that the population of the green bug *Nezara viridula* was regulated by predation of young nymphal instars and the parasitism by *Trichopoda giacomelli* (Diptera: Tachinidae) and *Trissolcus basalis* (Hymenoptera: Scelionidae), and has persisted at low population densities for the past 10–15 years [165,166]. This knowledge made it possible to think of the potential to develop successful alternatives capitalizing on natural pest mortality, such as biological control, which alone or in combination with an integrated management, could help to replace and/or reduce the use of pesticides.

One of the cornerstones of IPM is the biological control of pests through the use of natural enemies. Pest control in soybean agroecosystem is strongly based on chemical pesticides, which also affects natural enemies of pests, mainly pest predators, since they are more exposed to pesticides because of their behavior. If the herbicide glyphosate, in addition to killing weeds, affects the survival and/or behavior of nontarget arthropods, the food web could be seriously altered in an unintentional way.

Currently, there is debate about the toxicological effect of glyphosate on nontarget organisms. While there are studies that report the lack of toxicity

of this herbicide for other organisms that are not weeds, several studies have shown the contrary. This has created many uncertainties when planning an integrated management of soybean pests, since the compatibility of agrochemicals (pesticides and herbicides) with natural enemies is a crucial purpose in IPM.

Usually, the impact of pesticides on nontarget organisms such as natural enemies of pests and pollinators has been determined in the short term, by assessing the toxicity through the median lethal dose (LD_{50}). Though sublethal effects have been less investigated, they are equally important since they may affect fertility, behavior, and the duration of the life cycle, among other important traits affecting population performance.

The results of laboratory tests in the same research program showed sublethal effects on two abundant predators of soybean pests, *Chrysoperla externa* (Neuroptera: Chrysopidae) and *Alpaida veniliae* (Araneae, Araneidae). In both cases, commercial Glyfoglex 48 (48% glyphosate, Gleba S.A., Buenos Aires, Argentina) was used in toxicity tests in a 192 mg/l a.i. solution (maximum field registered nominal concentration). The exposure route was by ingestion through the treated prey and short- and long-term toxicity was analyzed. Individuals of *C. externa* in third larval instar were fed daily with freshly glyphosate-treated prey during 48 h and adult females of *A. veniliae* during 4 days, subject to previous starvation of 1 week.

The commercial GBH formulation had no negative direct lethal effects in either of the two species. Nevertheless, several long-term harmful effects were observed [167,168]. In *C. externa*, the herbicide shortened development time of immature stages as well as adult longevity, while it increased the prereproductive period. Both fecundity and fertility were negatively affected. The intrinsic rate of increase (r) and the net reproductive rate (R_o) of the population were significantly reduced. Moreover, several malformations and abnormalities were observed in most eggs laid by treated females. Adults, mainly females, developed tumors in the abdominal region at 20 days after emergence.

Side effects were also registered in the spider *A. veniliae*. GBH decreased prey consumption, increased the length of developmental time of the progeny, and interfered with the web building, which was started by the females 17 days later than in controls. Moreover, only 20% of the exposed females wove a normal web. Since this spider uses its web to capture prey for feeding, the negative effect on web building would adversely affect the efficiency of females to intercept and capture prey under field conditions, impacting negatively on their feeding capacity. Moreover, GBH induced abnormal ovaries with scarce development of oocytes and fatty granules around them. Like *C. externa*, fecundity and fertility were negatively affected.

The reported data provide insights on the side effects of GBH on two species of arthropod predators that are natural enemies of soybean pests. Our

findings contribute to the growing body of work demonstrating that glyphosate affects development and demography of nontarget arthropods in laboratory.

Although it is difficult to predict its effect on the field, it seems reasonable to expect that populations under continuous use of GBH would be exposed at greater detrimental effects, decreasing their performance and threatening their persistence in the long term, negatively affecting the local biodiversity.

If man seeks a more rational and sustainable management of agroecosystems, with regard to pest management, IPM seems to be a promising and viable strategy. However, it is essential to address new approaches in agricultural research that take into account relevant ecological processes such as crop–pest–natural enemy interactions. The search for an alternative agriculture model requires more creativity and research efforts than the simple application of a pesticide protocol. For example, the agroecosystem design can be manipulated to provide necessary resources (alternative food and refugee) to preserve natural enemies [169]. In spite of the fact that the potential of biological control of pests is recognized worldwide, there are not enough research efforts in relation to chemical control. In a similar way, the many studies that report negative sublethal effects of glyphosate on nontarget organisms, including humans, should alert us about its use and the need of encouraging more research on this topic. The available data so far allow us to infer that glyphosate is not compatible with the implementation of an IPM that includes the use of natural enemies. Conservation of biodiversity seems to be a sound alternative to the sustainability of agricultural systems. However, the intensive and continuous use of glyphosate over large areas threatens the maintenance of species diversity in natural and agricultural landscapes.

6. Concluding Remarks and Forthcoming Implications

6.1. Importance of biomarkers and biosensors

The findings so far in areas exposed to agrochemicals are indicative of the importance of the tests used for early detection of an increased risk of developing various diseases such as cancers, reproductive problems, and birth defects. The early detection of genetic damage would allow us to take steps to reduce or eliminate exposure to deleterious agents when damage is still reversible, thus decreasing the risk of developing diseases. Genotoxicity screening should be considered as an indispensable tool in the implementation of a comprehensive medical surveillance in people potentially exposed to various environmental pollutants.

It has been reported that triadimefon, a systemic fungicide with teratogenic effects in rodent models, produces craniofacial malformations in

X. laevis by altering endogenous RA signaling [170]. Arsenic, another endocrine disruptor, also increases RA signaling at low, noncytotoxic doses, in human embryonic NT2 cells [171]. In addition, atrazine produces teratogenic effects and decreases the levels of *cyp26* transcripts in *Xenopus* tadpoles, suggesting that this herbicide also disrupts the RA signaling pathway [172,173]. RA signaling is one of the finest pathways to tune gene regulation during development, and all this evidence raises the possibility that disturbances in RA distribution may be a more general mechanism underlying the teratogenic effects of xenobiotics in vertebrates. Since mechanisms of development are highly conserved in evolution among vertebrates [174], we would like to stress that they could be useful as very sensitive biosensors to detect undesirable effects of new molecules.

The evidence that links GBH (and potentially other chemicals) to increased activity of the RA signaling pathway might explain the higher incidence of embryonic malformations and spontaneous abortions observed in populations exposed to pesticides.

6.2. Is food containing GMO derivatives safe?

Given the harmful effects that pesticides produce on human health and animal models, a new concern arises about the safety of our food. A very recent study evaluated the presence of pesticides (and its metabolites) associated with genetically modified foods in blood from pregnant and nonpregnant women living in an urban area of Quebec, Canada. The subjects had a typical diet of a middle class population of Western industrialized countries and were not in direct or indirect contact with pesticides. The pesticides considered were the herbicides glyphosate and gluphosinate and its metabolites, and the bacterial insecticide CryAb1 toxin from Bacillus thuringiensis (Bt). Serum glyphosate and gluphosinate were detected in nonpregnant women. The CryAb1 toxin and gluphosinate's metabolite 3-methylphosphinicopropionic acid were both detected in pregnant women, their fetuses, and nonpregnant women. These results raise concerns about the exposure to environmental pollutants from nutritional sources [175]. In this sense, it is worrying that rats fed with glyphosate-resistant genetically modified corn showed functional alterations in heart, the hematopoietic system, and in two detoxifying organs: kidney and liver [176].

6.3. The precautionary principle

The reports in South America based on direct observations from physicians and health workers about the effects of agrochemicals on human health (including findings of teratogenesis and neoplasia) were an important warning about the environmental consequences of the intensive use of pesticides. This led to a vigorous debate about the safety of agrochemicals,

resulting in a push to initiate epidemiological studies and to apply the precautionary principle. For this principle, the 1992 Earth Summit of the Río Conference states the following definition: "In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation."

So far, all the evidence reported in the scientific literature and the clinical observations in the field were not sufficient to activate in Latin America the precautionary principle contemplated by the international environmental agreements.

6.4. A need for critical and independent science

Multinational corporations handle virtually most of the seed and chemical products market in the world. It cannot be inferred that research performed or supported by such companies is completely objective. Dismissal of research from independent groups harkens back to the ongoing debate about bisphenol A, where no single industry funded study has ever found adverse consequences linked with bisphenol A exposure, whereas 90% ($n > 100$) of non-industry funded studies show significant adverse consequences of bisphenol A exposure [177,178].

Several malformations were found in rabbits and rats according to the industry's own teratogenicity studies submitted for the 2002 EU approval of the active ingredient glyphosate. The original industry studies are claimed to be commercially confidential. However, the said industry data were compiled from the 1998 draft assessment report (DAR) by the German government, since Germany has been the rapporteur member state for glyphosate and will remain in this role for the next review of glyphosate in 2015. Malformations include extra ribs, distortions affecting thoracic ribs, heart malformations, kidney agenesia, unossified sternebrae, reduced ossification of cranial centers and sacrocaudal vertebral arches, and also skeletal variations and major visceral malformations, which were unspecified in the DAR [179].

It is indispensable to change the direction of scientific research, leaving behind the reductionism and pragmatism that dominated agriculture in the past decades. It will not be possible to devise a sustainable agriculture that satisfies social needs if man does not begin to prioritize policies that enhance environmental and food security over the interests of private agrochemical industries and markets. The authors of this chapter appeal to the scientific community to be aware of the hazards involved on a local and a global scale, anticipating the problems before they surprise us.

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