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Review

Does exposure to glyphosate lead to an increase in the micronuclei frequency? A systematic and meta-analytic review

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Highlights

- Systematic meta-analytical review correlating glyphosate exposure and micronuclei.
- Groups exposed to glyphosate formulations have increased formation of micronuclei.
- Significant difference among glyphosate (GLY) and its commercial formulations.
- Difference in MN formation among different exposure routes of GLY.
- Difference in MN formation among different groups of vertebrates.

Abstract

Glyphosate-based herbicides are among the most used pesticides worldwide. Reviews on the safety of glyphosate have been conducted by several regulatory agencies and researches centers, many times with contradictory results. This study is a systematic meta-analytical review of experimental studies on the relationship between exposure to the glyphosate (GLY) and its formulations with the formation of micronuclei (MN) to establish a quantitative estimate of the environmental risks. The natural logarithm (ln) of the estimated response ratio was calculated from 81 experiments. A meta-analysis was performed on the complete data set, and individual meta-analyses were conducted after stratification by test system, class of vertebrate, exposure route, gender, endpoints, type of literature, formulation, GLY dose and exposure time. A forest plot showed an overall positive association between GLY exposure and its formulations and MN, corroborated by the cumulative effects size. Different

responses were observed on mammalian and non-mammalian. Interesting results was noticed in exposure route where oral administration of GLY presented no significance. Exposure by intraperitoneal injection presented the highest MN formation. Pure GLY caused fewer effects than to commercial mixtures, but both presented mutagenic effects. The studies with males presented significant responses, while studies with females were not significant. The cumulative effects size was not clearly related to GLY dose, and was negatively related to exposure time. It can be attributed to different test systems, exposure routes and protocols analyzed. In conclusion, our results support the hypothesis that exposure to GLY and its formulations increases the frequency of MN formation.

Introduction

Glyphosate [N-(phosphonomethyl) glycine] (GLY) is one of the main pesticides that have been discovered to date and is the most globally commercialized pesticide for the non-selective control of weeds (Baylis, 2000, Monsanto, 2005). This systemic herbicide inhibits the growth of plants by interfering in the production of the aromatic amino acids phenylalanine, tyrosine and tryptophan, which causes a reduction in protein synthesis (Faus et al., 2015).

Current agricultural activities are highly dependent on the use of glyphosate-based commercial formulations, and this has become even more true in recent years because more than 75% of genetically modified plants have been formulated to tolerate high levels of glyphosate (Vera-Candioti et al., 2013). The formulations of glyphosate-based herbicides are complex and variable mixtures – adjuvants and surfactants are added to the active ingredient (GLY) with the objective of increasing its absorption and effectiveness (Baylis, 2000). Unfortunately, surfactants can present toxicity many times greater than GLY, making the formulated product much more toxic than the isolated active ingredient (Vera-Candioti et al., 2013). The specific original Roundup® (RU) formulation was composed by 41% isopropylamine glyphosate salt and surfactant (15.4% a polyethoxylated tallowamine). Nowadays, it is no longer sold in many markets, and other glyphosate formulations with different compositions are sold under the Roundup® brand name, with different glyphosate forms, concentrations and surfactant systems (Kier and Kirkland, 2013a). Despite the great number of benefits of the use of pesticides in agriculture, such as GLY, these agrochemicals can be dangerous if not used appropriately, and many of them pose a potential risk due to their contamination of foods, water and air (WHO, 1994). The great use and ubiquity of this GLY-based products increases the need for toxicological studies that determine the level of environmental risks of these products and their effects on non-target organisms (Borggaard and Gimsing, 2008). In this regard, numerous studies have been performed in recent years with different test systems to evaluate the harmful effects of GLY, both alone and in its commercial formulations, but the results of these studies are highly conflicting.

On the one hand, glyphosate-based herbicides are very effective in the control of undesired vegetation and are described by their manufacturers as having low toxicity and good environmental compatibility (Cox, 1998), and they are believed to be less toxic than other pesticides. Nonetheless, other studies have shown that GLY is moderately persistent in water under low light conditions and it is also highly persistent in the dark (Mercurio et al., 2014). It can potentially contaminate rivers, surface waters and soil, in which the detection levels of the herbicide is increased proportionally to the dosage of applications. Likewise, the flow increased by rain causes the transport of the herbicide from the direct area of influence to downstream sites (Peruzzo et al., 2008). A recent study shows that GLY can induce the growth of human breast cancer cells via estrogen receptors, and also tumor promoting activity in mice (George et al., 2010, Thongprakaisang et al., 2013).

Pesticide and its residues are subjected to chemical reactions with environmental reagents from the very beginning. The main reactions in the environment include oxidation, reduction, and nucleophilic displacements in biomolecules such as DNA (Crosby, 1982), and for this reason the genotoxicity of pesticides is a worldwide concern. The genotoxic and mutagenic effects of

GLY and RU have been studied in different manners (Grisolia, 2002, Li and Long, 1988, Mañas et al., 2009, Poletta et al., 2009, Seiler, 1977 among many others), and these studies have generated some contradictory results. According to Williams et al. (2000), there is no *in vitro* or *in vivo* evidence that RU causes direct damage to DNA, indicating that it and its components do not present risks in regard to somatic or heritable mutations in humans. Similar results were obtained in a genetic mutation test with *Salmonella typhimurium* and in a mammalian cell culture study (Wildeman, 1982). Additionally, Li and Long (1988) performed an *in vitro* DNA synthesis test in rat hepatocytes to examine the genotoxicity of GLY and reported no DNA damage; they also reported that GLY did not cause DNA damage in the bone marrow of rats using a chromosome aberration test. In the same manner, other studies have found that neither GLY nor RU caused an increase in the frequency of micronuclei and chromosomal aberrations in rats after *in vivo* exposure to these pesticides (Dimitrov et al., 2006, Rank et al., 1993). Many interesting results from several databases were compiled in the recent review paper from Kier and Kirkland (2013a). As pointed by authors, negative results for *in vitro* gene mutation and a majority of negative results for chromosomal effect assays in mammalian cells have provided evidences that glyphosate is not typically genotoxic for these endpoints in mammalian systems. Mixed results were observed for micronucleus assays of GLY-based formulations in non-mammalian systems. Reports of positive results for DNA damage endpoints indicate that glyphosate and its formulations tend to elicit DNA damage effects at high or toxic dose levels, but this can be due to cytotoxicity or to the surfactants present in complex commercial mixtures (Kier and Kirkland, 2013a).

The individual study by Bolognesi et al. (1997) observed that both pure GLY and RU had DNA-damaging activity in the forms of DNA single-strand breaks and a significant increase in chromosomal alterations *in vivo* and *in vitro*. In the same study, weak genotoxic activity was evident for RU (Bolognesi et al., 1997). Positive results for *in vivo* DNA adducts in rats and chromosomal aberrations in the onion *Allium cepa* have also been demonstrated for RU, but not for GLY (Peluso et al., 1998, Rank et al., 1993). Other studies have shown that RU induces an increase in micronuclei (MN) and DNA damage in goldfish (Çavas and Könen, 2007) and *Tilapia rendalli*, but not in rats (Grisolia, 2002). A more recent study indicated that RU can be significantly harmful to the DNA of fish, even with exposure to extremely low realistic levels (parts per billion – μg/L) for short period of time (Ghisi and Cestari, 2013).

The micronucleus (MN) test is one of the most well-established and commonly used methods for evaluating the mutagenic effects of a wide spectrum of compounds. The MN test shows great potential because it can be executed rapidly, is relatively inexpensive and is a good indicator of chemical contamination in organisms. Micronuclei are small masses of chromatin that are found outside the main nucleus of cells, and they originate from chromosome breaks or malfunction of the mitotic fuse during nuclear division (Fenech, 2007). During cell division, entire chromosomes or partial chromosomes that were not incorporated into the main nucleus of the daughter cell, appear as small, round, dark structures, with the same appearance and refraction as the nuclear material (Fenech, 2007). Although there is a basal level of spontaneous formation of micronuclei in most of the species (Mañas et al., 2009), the exposure of organisms to clastogenic substances, such as some pesticides, have been shown to increase the frequency of micronuclei formation in the laboratory and in field studies (Bombail et al., 2001, Grisolia and Starling, 2001, Guilherme et al., 2010).

This study evaluated the relationship between exposure to glyphosate (in different formulations) and micronuclei formation frequency through a systematic review of the literature. Using these data in a meta-analytic study, we aimed to furnish a quantitative estimate of the environmental risk of GLY pesticides. Our hypotheses were the following: (i) The damage rate is expected to be higher in the exposed experimental groups than in the control groups, independent of the chemical formulations of GLY; (ii) GLY will present less mutagenicity than the complex commercial mixture; (iii) different test systems and class of vertebrates will present different responses in MN formation after GLY exposure and its formulations; (iv) there are differences

among genders; (v) different responses are expected according to the exposure route; (vi) different responses are observed in counting of all erythrocytes or only polychromatic cells; (vii) The damage rate is expected to increase with exposure time; (vii) The damage rate is expected to increase with dose; and (ix) Publication bias is not expected.

Section snippets

Identification and selection of studies

A search of the electronic databases in "ISI Web of Knowledge®" (http://apps.webofknowledge.com/↗) and "Science Direct" (http://www.sciencedirect.com/↗) was conducted. The search was limited to references from 1975 (when the micronucleus test to evaluate genetic damage caused by chemical substances was first described) to June 1st, 2014 and used combinations of the following words: micronucleus, micronucleus test, glyphosate and Roundup®. The reference lists of relevant publications were reviewed

General view of the literature: selection of the references and characteristics of the study

A total relevant number of studies were obtained from the "ISI Web of Knowledge" and "Science Direct" databases and from databases of theses and dissertations. Repeated studies were excluded from the sampling. In addition, a large number of studies were excluded for the following reasons: (i) defined dose of exposure was not presented; (ii) micronucleus test was not performed and (iii) insufficient data for the meta-analyses.

After a thorough scanning, 41 references were selected, 24 of these

Discussion

In recent years, a large number of articles have reported the evaluation of damaging effects caused by glyphosate in various organisms using different test systems. However, to the best of our knowledge, this study is the first meta-analysis that combined data about micronuclei formation frequency with the exposure of different organisms to the herbicide glyphosate and glyphosate formulations. We believe that systemic reviews of the literature and meta-analyses of data in the literature (as

Conclusion

The present study provides support for the hypothesis that exposure to the pesticide glyphosate and its formulations increases micronucleus formation. In several categorizations, we can see different responses according to the test system, the group of animals tested, and the type of cells analyzed (polychromatic erythrocytes or all erythrocytes). For all categories above, the results were significant to MN formation. In segregation by gender, we found that males are more responsive than

Conflict of interest statement

The author's affiliation is as shown on the cover page. The authors are solely responsible for the analyses and preparation of this manuscript; the opinions and conclusions are those of the authors and are not necessarily those of the sponsoring entity. The

authors declare that there are no conflicts of interest. Universidade Estadual de Maringá (UEM) and Universidade Tecnológica Federal do Paraná (UTFPR) are Brazilian universities dedicated to teaching, research and extension of knowledge.

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References (76)

L.N. Abaid *et al.*

Reducing publication bias of prospective clinical trials through trial registration

Contraception (2007)

V. Bombail *et al.*

Application of the comet and micronucleus assays to butter fish (Pholis gunnellus) erythrocytes from the Firth of Forth

Scotl. Mol. Toxicol. (2001)

D.G.S.M. Cavalcante *et al.*

Genotoxic effects of Roundup on the fish Prochilodus lineatus

Mutat. Res. (2008)

J. George *et al.*

Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach

J. Proteomics (2010)

C.K. Grisolia

A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides

Mutat. Res. (2002)

C.K. Grisolia *et al.*

Micronuclei monitoring of fishes from Lake Paranoá, under influence of sewage treatment plant discharges

Mutat. Res. (2001)

A.P. Li *et al.*

An evaluation of the genotoxic potential of glyphosate

Fundam. Appl. Toxicol. (1988)

F. Mañas *et al.*

Genotoxicity of glyphosate assessed by the comet assay and cytogenetic tests

Environ. Toxicol. Pharmacol. (2009)

J.L.R. Martin *et al.*

Is grey literature essential for a better control of publication bias in psychiatry? an example from three meta-analyses of schizophrenia

Eur. Psychiatry (2005)

P. Mercurio *et al.*

Glyphosate persistence in seawater

Mar. Pollut. Bull. (2014)

View more references

Cited by (44)

Meta-analytical review of antioxidant mechanisms responses in animals exposed to herbicide 2,4-D herbicide

2024, Science of the Total Environment

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Toxicity of glyphosate to animals: A meta-analytical approach

2024, Environmental Pollution

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Mapping the key characteristics of carcinogens for glyphosate and its formulations: A systematic review

2023, Chemosphere

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The role of funding on research and science: The impact of glyphosate herbicides on health and the environment

2023, Journal of Policy Modeling

Show abstract

Does exposure to environmental 2,4-dichlorophenoxyacetic acid concentrations increase mortality rate in animals? A meta-analytic review

2022, Environmental Pollution

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Evaluation of the genotoxic, mutagenic, and histopathological hepatic effects of polyoxyethylene amine (POEA) and glyphosate on Dendropsophus minutus tadpoles

2021, Environmental Pollution

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