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Corresponding Author:	Alfredo Santovito Universita degli Studi di Torino Dipartimento di Scienze della Vita e Biologia dei Sistemi Turin, ITALY
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Universita degli Studi di Torino Dipartimento di Scienze della Vita e Biologia dei Sistemi
Corresponding Author's Secondary Institution:	
First Author:	Alfredo Santovito
First Author Secondary Information:	
Order of Authors:	Alfredo Santovito
	Stefano Ruberto
	Claudio Gendusa
	Piero Cervella
Order of Authors Secondary Information:	
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Abstract:	Glyphosate is an important broad-spectrum herbicide used in agriculture and residential areas for weed and vegetation control, respectively. In our study, we analysed the in vitro clastogenic and/or aneugenic effects of glyphosate by chromosomal aberrations and micronuclei assays. Human lymphocytes were exposed to five glyphosate concentrations: 0.500, 0.100, 0.050, 0.025, and 0.0125 µg/mL, where 0.500 µg/mL represents the established ADI value, and the other concentrations were tested in order to establish the genotoxicity threshold for this compound. We observed CAs and MNi frequencies significant increased at all tested concentrations, with exception of 0.0125 µg/mL. Vice versa, no effect has been observed on the frequencies of nuclear buds and nucleoplasmic bridges, with the only exception of 0.500 µg/mL of glyphosate that was found to increase in a significant manner the frequency of nucleoplasmic bridges. Finally, the cytokinesis-block proliferation index and the mitotic index were not significantly reduced, indicating that glyphosate does not produce effects on the proliferation/mitotic index at the tested concentrations. Although the limitations due to the small sample size, results obtained in the present paper point to the necessity of further investigations in order to establish the real genotoxic potential of glyphosate.
Suggested Reviewers:	Nora Gorla Universidad Nacional de Río Cuarto (UNRC), Ruta 36, KM 601, 5800, Río Cuarto, Argentina ngorla@ayv.unrc.edu.ar This author has experience in genotoxicological studies and cytogenetic techniques. In particular, she published papers reporting data about genotoxicity of glyphosate.

	Arnoldo Quero Universidad Juan Augustin Maza aamartinquero@gmail.com This author has experience in genotoxicological studies and cytogenetic techniques.
	Raimunda Nonata Fortes Carvalho Neta Universidade Estadual do Maranhao raimundafortes@yahoo.com.br
Opposed Reviewers:	
Additional Information:	
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Title: *In vitro* evaluation of genomic damage induced by Glyphosate on human lymphocytes

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Authors: Alfredo SANTOVITO[a*], Stefano RUBERTO[a], Claudio GENDUSA[a] and Piero CERVELLA[a]

[a] University of Turin, Department of Life Sciences and Systems Biology, Via Accademia Albertina n. 13, 10123 Torino (Italy)

*Corresponding Author:

Alfredo SANTOVITO, University of Turin,

Department of Life Sciences and Systems Biology

Via Accademia Albertina n. 13, 10123 – Torino (Italy)

Tel.: +39-0116704554; Fax: +39-0116704508;

E-mail: alfredo.santovito@unito.it

Abstract

Glyphosate is an important broad-spectrum herbicide used in agriculture and residential areas for weed and vegetation control, respectively. In our study, we analysed the *in vitro* clastogenic and/or aneugenic effects of glyphosate by chromosomal aberrations and micronuclei assays. Human lymphocytes were exposed to five glyphosate concentrations: 0.500, 0.100, 0.050, 0.025, and 0.0125 µg/mL, where 0.500 µg/mL represents the established ADI value, and the other concentrations were tested in order to establish the genotoxicity threshold for this compound. We observed CAs and MNi frequencies significant increased at all tested concentrations, with exception of 0.0125 µg/mL. *Vice versa*, no effect has been observed on the frequencies of nuclear buds and nucleoplasmic bridges, with the only exception of 0.500 µg/mL of glyphosate that was found to increase in a significant manner the frequency of nucleoplasmic bridges. Finally, the cytokinesis-block proliferation index and the mitotic index were not significantly reduced, indicating that glyphosate does not produce effects on the proliferation/mitotic index at the tested concentrations. Although the limitations due to the small sample size, results obtained in the present paper point to the necessity of further investigations in order to establish the real genotoxic potential of glyphosate.

Keywords

Human biomonitoring; herbicides; genotoxicology; chromosomal aberrations; micronuclei; lymphocytes

Abbreviations

ABBREVIATIONS

Ab.C = Aberrant Cells

ADI = Acceptable Daily Intake

AF = Acentric Fragments;

B' = Chromatid Breaks

B'' = Chromosome Breaks

BNCs = Binucleated Cells

CAs = Chromosomal Aberrations

CBPI = Cytokinesis-block Proliferation Index

DC = Dicentric;

DMSO = Dimethyl Sulfoxide

EFSA = European Food Safety Authority

IARC = International Agency for Research on Cancer

JMPR = Joint FAO/WHO Meeting on Pesticide Residues

MI = Mitotic Index

MMC = Mitomycin-C

MNC = micronucleated cell

MNi = Micronuclei

MRL = Maximum Residue Limits

NBUDs = Nuclear Buds

NPBs = Nucleoplasmic Bridges

R = Rings;

RfD = Reference Dose

S.E. = Standard Error;

TR = Tri-Tetraradials;

US EPA = United States Environmental Protection Agency

Introduction

Glyphosate is the most commonly used herbicide, employed in agriculture for weed control, in urban areas for vegetation control, and during harvesting for crop desiccant (Duke 2017). Because of its massive use, glyphosate is routinely detected in foodstuffs (EFSA 2014), air, water and rain (Majewski et al. 2014), food (Ferrer et al. 2011), and, consequently, in human biological samples (Hoppe et al. 2017). From ecological point of view, glyphosate was found able to reduce the earthworm biomass and the soil microbial diversity (Bai and Ogbourne 2016) and, at concentrations over 400 μg/L, it resulted potentially toxic for some aquatic species, including amphibians and fish (King and Wagner 2010; Braz-Mota et al. 2015). Glyphosate was also suggested to have endocrine interference properties and, in humans, it was associated with various disorders such as diabetes, obesity, asthma, Alzheimer's and Parkinson's diseases (Romano et al. 2012; Kwiatkowska et al. 2016).

At genomic and cellular levels, glyphosate showed a genotoxic potential in *in vitro* cultured lymphocytes (Lioi et al., 1998; Mladinic et al., 2009), as well as it was found to affect the cell cycle regulation (Marc et al., 2004). However, other authors reported contradictory results or, in some cases, no clastogenic effects for this compound (Šiviková and Dianovský, 2006; Piesova, 2005).

Maximum residue limits (MRL) of glyphosate have been reviewed in 2015 by the European Food Safety Authority (EFSA), and generally ranged from 0.025 to 2 mg/kg in different food sources (EFSA 2015).

Data about carcinogenicity and mutagenicity of glyphosate are discordant. In 2015, the EFSA established that, for this compound, there are no strong evidences of cytotoxicity and genotoxicity (EFSA 2015). On the contrary, in the same year, the International Agency for Research on Cancer (IARC), citing sufficient evidences of carcinogenicity in experimental animals and in *in vitro* systems, classified glyphosate as probably carcinogenic to humans (IARC 2015). However, in 2016, the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) established that glyphosate is not carcinogenic in rats, and carcinogenic in mice at very high doses, excluding the same risks to humans from exposure to glyphosate through the diet (FAO/WHO 2016). Finally, in 2017, the European Union recently extended the glyphosate use from the 15 December 2017 for another 5 years (European Commission 2017).

Based on available data about carcinogenicity, genotoxicity and cytotoxicity, the US EPA established for glyphosate a reference dose (RfD) of 1.75 mg/kg body mass/day (US EPA 2012). *Vice versa*, the Acceptable Daily Intake (ADI) established by JMPR/WHO and FAO/WHO was 1 mg/Kg body mass/day (FAO/WHO 2014; FAO/WHO 2016), whereas the EFSA established the more precautionary ADI value of 0.5 mg/kg body mass/day (EFSA 2015).

Most of the published works were focused on the *in vitro* effects on human cells of high glyphosate concentrations (Mañas et al., 2009; Mladinic et al., 2009; Šiviková and Dianovský, 2006; Koller et al., 2012), whereas few data were reported about the effects of small doses of this compound (Kašuba et al., 2017). For this reason, the aim of the present study was to evaluate the *in vitro* effects on human lymphocytes of low concentrations of glyphosate. We decided to test glyphosate concentrations corresponding to 0.5 µg/mL (EFSA ADI value) and its submultiples, by chromosomal aberrations (CAs) and micronuclei (MNi) assays, that allow the evaluation of the clastogenic and/or aneugenic properties of a single compound or a mixture of different compounds.

Materials and methods

Study population

Peripheral venous blood was collected from 6 healthy Italian subjects (2 males and 4 females, mean age±S.D., 27.50±12.55), non-smoking, not alcoholics, not under drug therapy, and with no recent history of exposure to mutagens. All subjects signed the Informed Consent. The study was approved by the University of Turin ethics committee and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Blood Sample Collection and Lymphocyte cultures

Blood samples were obtained by venipuncture, collected in heparinised tubes, cooled (4°C) and processed within 2 h after collection. Lymphocyte cultures, fixation and staining procedures were performed according to Santovito et al. (2018). After 24 h of incubation, 8.6 µL of glyphosate stock solution at concentration of 0.5 mg/mL were added to the lymphocyte culture in order to reach a final glyphosate concentration of 0.500 µg/mL. Similarly, 8.6 µL of glyphosate stock solution diluted 5, 10, 20, and 40 times with dimethyl sulfoxide (DMSO) were added to the lymphocyte cultures in order to reach the final glyphosate concentrations of 0.100 µg/mL, 0.050 µg/mL, 0.025 µg/mL and 0.0125 µg/mL, respectively. In particular, 0.500 µg/mL represents the ADI concentration established by EFSA for this compound, whereas 0.100, 0.050, 0.025 and 0.0125 µg/mL concentrations were tested in order to evaluate the genotoxicity threshold for this compound. Three control cultures were assessed: 1) positive control, by adding only MMC (final concentration 0.1 µg/mL culture); 2) 0.1% DMSO solvent control, obtained by adding 8.6 µL of DMSO to the lymphocyte culture; 3) negative control culture without both glyphosate and DMSO, obtained adding 8.6 µL of RPMI medium to the lymphocyte culture. Only for MNi assay, after 44 h of incubation, cytochalasin-B was added to the cultures at a concentration of 6 µg/mL to block cytokinesis. Similarly, only for CAs assay, to arrest cells in mitosis, colchicine was added at the concentration of 0.06 µg/mL during the last 2 h of culture.

Cytokinesis-Block Micronucleus Assays

Microscope analysis was performed at 40X magnification on a light microscope (Dialux 20, Leica, Germany). MNi, nucleoplasmic bridges (NPB) and nuclear buds (NBUD) were scored in 2000 binucleated lymphocytes with well-preserved cytoplasm per subject (total 12,000 binucleated cells per concentration). Cells containing one of more MNi were scored as "micronucleated cell" (MNC). A total of 2000 lymphocytes per donor per concentration were scored to evaluate the cytokinesis-block proliferation index (CBPI), according to the following formula:

[1 × N1] + [2 × N2] + [3 × (N3 + N4)]/N, where N1-N4 represents the number of cells with 1-4 nuclei, respectively, and N is the total number of cells scored.

Chromosomal Aberration Assay

Microscope analysis was performed at 100X magnification on a light microscope (Dialux 20, Leica, Germany). For each subject and glyphosate concentration, 200 first-division complete metaphases (for a total of 1200 metaphases for each dose) were analysed. Cells containing one of more types of CAs were scored as "aberrant cell" (Ab.C). In order to determine cytotoxicity, the mitotic index (MI) was calculated from the number of metaphases in 1000 cells analysed per subject per concentration (a total of 6000 cells per concentration).

Statistical analysis

Comparison of mean values of the percentage of cells with MNi, MNC, CBPI, NPBs, NBUDs, CAs, Ab.C and MI between exposure levels and controls was assessed by the non-parametric Mann-Whitney test. Statistical calculations were carried out using the SPSS software package program (version 24.0, Inc., Chicago, IL, USA). All *P* values were two tailed, and *P* values of 5% or less were considered statistically significant for all tests carried out.

Results

Effect of glyphosate on CAs formation

Glyphosate was found to induce the following structural CAs: gaps, chromatid and chromosome breaks, dicentric chromosomes, rings, tri-tetra radials, and acentric fragments. This last, together to chromatid breaks, represent the most frequent observed aberrations (Table 1). Because of the conflicting opinions about the possibility to consider gaps as indicators of genomic damage, we decided to exclude gaps from statistical analysis.

Glyphosate was found to significantly (P = 0.004) increase the CAs and Ab.C frequencies at all tested concentrations when compared with the solvent control, including the concentration of 0.025 μg/ml (P = 0.006), but with the exception of 0.0125 μg/mL (P = 0.181). A dose-effect was also observed, since the regression analysis revealed a significant correlation between glyphosate concentrations and the CAs and Ab.C frequencies (Table 2). *Vice versa*, no significant differences (P>0.05) were found between the DMSO solvent-control and the negative control, whereas the cultures treated with the MMC showed a significant increase of the cytogenetic damage with respect to all concentrations of glyphosate. Finally, no significant differences were found in the MI values between solvent control and all tested concentrations of glyphosate, although at 0.500 μg/mL the P-value resulted to be borderline (P = 0.058).

Effect of glyphosate on MNi formation

Similarly to what we already observed with the CAs assay, our results indicated that glyphosate significantly increased (P = 0.004) the MNi frequency at all tested concentrations, with exception of 0.0125 μg/mL (P = 0.360) (Table 3). *Vice versa*, no effect has been observed on the frequencies of NBUDs and NPBs, with the only exception of 0.500 μg/mL of glyphosate that was found to increase in a significant manner the frequency of NPBs with respect to the solvent control (P = 0.004). Also in this case a relationship between the frequency of MNi and the concentrations of glyphosate was observed (Table 2), as well as the DMSO solvent-control cultures did not show significant differences (P = 0.071) with respect to the negative controls. MMC was found to significantly increase the MNi, NPBs and NBUDs formation compared with the negative control solvent controls and all tested concentrations of glyphosate (P <0.001), with exception of 0.500 μg/mL (0.373). After 48-h exposure, a significant reduction of the CBPI value in cultures treated with glyphosate was not observed (P = 0.522 for 0.500 μg/mL and P = 0.336 for all other concentrations of glyphosate), indicating that, at the tested concentrations, glyphosate does not seem to produce effects on the proliferation index. Finally, at 0.500 μg/mL, glyphosate significantly (P = 0.004) induced the NPBs formation, whereas no differences were found in the frequency of NBUDs between DMSO solvent control and all glyphosate concentrations.

Discussion

Glyphosate is an active ingredient of most widely used herbicides. Although it is believed to be less toxic than other herbicides, data about its possible genotoxicity are controversy and IARC classified this compound as probably carcinogenic to human (IARC 2015).

The genotoxic effects of high concentrations of glyphosate have been documented, although with contradictory results, in a great number of scientific papers (for a review see Kier and Kirkland, 2013), as well as in evaluation reports of different international agencies (EFSA, 2015; FAO/WHO, 2016; IARC, 2015). On the other hand, the effects of low concentrations of this compound, likely to be encountered in everyday life, were poorly investigated (Kašuba et al., 2017).

Results of our study provided information about *in vitro* clastogenic and/or aneugenic effects of glyphosate on human lymphocytes at the low ADI concentration of 0.500 µg/mL and its submultiples. Based on the obtained data, it can be concluded that glyphosate significantly increase the CAs and MNi levels in human lymphocytes at the ADI concentration of 0.500 µg/mL established by EFSA and at its submultiple concentrations, up to 0.025 µg/mL. The mechanisms underlying genotoxic potential of glyphosate alone or in complex with other compounds are unknown, although the exposure to glyphosate was found to trigger oxidative processes involved in the increase of the genomic damage (Marques et al. 2014).

Also other authors analysed *in vitro* the genotoxic potential of glyphosate in lymphocytes, but at exposure levels of higher magnitude orders (Kier and Kirkland, 2013). For example, Mladinic et al. (2009), in human lymphocytes cultured without S9 and in presence of glyphosate at concentrations of 3.5, 92.8, and 580 µg/mL, observed a slight increased frequency of MNi and a significant tail length increase after a comet assay. Other authors evaluated the induction of CAs and MNi in blood cells of other animal models. Lioi et al. (1998) reported positive clastogenic and genotoxic effects of glyphosate on bovine peripheral lymphocytes cultured *in vitro* with herbicide concentrations ranging from 17 µM (2.874 µg/mL) to 170 µM (28.740 µg/mL), whereas Šiviková and Dianovský (2006) reported no CAs effect of glyphosate at concentrations ranging from 28 (4.734 µg/mL) to 1120 µM (189 µg/mL). Contradictory results were obtained by Piesova (2005), who observed, after 48 h of treatment without S9, a statistically significant increase in MNi frequency at 280 µM (47.34 µg/mL) but not at 560 µM (94.68 µg/mL) of glyphosate in one donor, and the opposite in a second donor (positive at 560 µM but not at 280 µM). Finally, Alvarez-Moya et al. (2014), in *in vitro* experiments based on comet assay, showed that 7 mM of glyphosate (1183 µg/mL) caused DNA damage in b0lood cells of Nile Tilapia (*Oreochromis niloticus*).

Concentrations of glyphosate similar to those evaluated in the present paper were tested by Kašuba et al. (2017) in HepG2 cells by the MNi assay. Similarly to what we observed in human lymphocytes, these authors found a significantly higher number of MNi at the ADI value of 0.500 µg/mL, as well as at the residential exposure level of 2.91 µg/mL, after 4 h of treatment. *Vive versa*, negative results on Hep-2 cells were obtained by Mañas et al. (2009) with CAs assay at glyphosate concentrations of 0.20 mM (33,8 µg/mL), 1.20 mM (203 µg/mL) and 6.00 mM (1014 µg/mL).

Significant levels of DNA damage were also observed in human buccal epithelial cells exposed to glyphosate concentrations ranging between 10 and 20 mg/L (Koller et al. 2012), whereas Kwiatkowska et al. (2017), showed that, in peripheral blood mononuclear cells, glyphosate induce DNA damage in the concentrations range from 0.5 mM (84.54 µg/mL) to 10 mM (1690 µg/mL), and a significant decrease of global DNA methylation at concentration of 0.25

mM (42.27 μg/mL). Interestingly, the same authors also observed a significantly increased methylation of p53 promoter at concentrations of 0.25 mM and 0.5 mM (42.27 and 84.54 μg/mL). This hyper-methylation was found to be able to down-regulate the p53 gene expression and to activate proto-oncogenes, with consequent genomic alterations and cancer risk. The possibility of glyphosate causing cancer promotion in skin cells and proliferation in breast cells has been also observed *in vivo* and *in vitro* studies by mouse and human models, respectively (George et al. 2010; Thongprakaisang et al. 2013). In this scenario, the results obtained in the present study require attention. Indeed, increased CAs and MNi frequencies in peripheral blood lymphocytes have been positively associated with increased cancer risk and early events in carcinogenesis, respectively (Bonassi et al. 2004; 2011).

Moreover, it should be emphasized that, beyond the cases of intoxication where glyphosate content in blood was found to range from 0.6 to 150 μg/mL (Zouaoui et al. 2013), in subjects who were indirectly exposed to this substance, glyphosate was found in blood at concentrations of 0.074± 0.028 μg/mL (Aris and Leblanc 2011), a value about seven times lower with respect to the established ADI value (EFSA 2015), but in the range of concentrations we tested (from 0.5 to 0.0125 μg/mL).

At the same time, the genotoxicity of a compound should not be evaluated only after single administrations in *in vitro* or *in vivo* systems, but also, and especially, after chronic administration of the same compound, even at lower quantities than those established by the competent agencies. In this sense, the clastogenicity and aneugenicity we observed at concentrations of 0.100, 0.050 and 0.025 μg/mL represent an important signal, especially in view of a chronic exposure to these glyphosate concentration levels.

Finally, no significant differences in CBPI and MI values were found between all tested concentrations and the solvent control, indicating that, at these concentrations, glyphosate do not influence in a significant manner the replicative capacity of the cells. These data differ from those found by Šiviková and Dianovský (2006) who observed a reduction of mitotic and proliferation indices in bovine lymphocytes, but at higher glyphosate concentrations (94.68 μg/mL and 189 μg/mL). Similarly, other authors described increased levels of MI for other herbicides or insecticides, also in this case, at concetrations much higher than those tested in the present work (Kocaman et al., 2014; Yüzbaşioğlu et al., 2006).

Conclusions

In the present work we provided evidences for clastogenic and/or aneugenic effects of glyphosate on cultured human lymphocytes. Despite the limitations of an *in vitro* study due to the reduced sample size, it is our opinion that the increased cytogenetic damage observed by our group at glyphosate concentrations equal and lower than the established ADI value requires further investigations in order to establish the effective genotoxicity threshold of this extensively used compound. Indeed, the glyphosate concentrations tested in the present work represent more realistic concentrations, likely to be encountered in everyday life, with respect to the higher doses evaluated in other published papers. In this scenario, in order to draw conclusions about the effects associated to the chronic exposure to low doses, *in vitro* studies are useful tools to investigate the dose response effects, the molecular mechanisms of action of different environmental xenobiotics and their genotoxicity. This last, compared to other types of toxicity, may result in severe consequences that can be also inherited after long periods following exposure. The same DNA damage that occurs in a single cell, caused by low but chronic exposure to genotoxic compounds, can cause unexpected severe consequences in the long run.

Disclosure of interest

The authors report no conflicts of interest.

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ORCID

Alfredo Santovito https://orcid.org/0000-0001-5292-5206

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Table 1

Table 1 – Induction of chromosomal aberrations by Glyphosate in human lymphocytes *in vitro*.
Number of scored metaphases for each concentration = 1200

Test substance (µg/ml)	**Structural CAs**							**Total CAs**	**Total CAs + Gaps**	**Total Ab.C**	**Total Ab.C + Gaps**	**(%) CAs/Cell ± S.E.**	**(%) Ab.C/Cell ± S.E.**	**(%) MI ± S.E.**
	Gaps	**B’**	**B’’**	**DC**	**R**	**TR**	**AF**							
NC	8	8	2	0	0	0	7	17	25	17	25	1.417±0.154	1.417±0.154	5.567±0.042
0.1% DMSO	10	9	4	1	0	6	7	27	37	27	37	2.250±0.335	2.250±0.250	5.433±0.056
MMC (0.100)	41	46	30	9	10	14	36	145	186	127	168	12.083±0.300 [a]	10.583±0.473 [a]	4.200±0.058 [a]
Gly (0.500)	17	41	12	12	3	0	28	96	113	95	112	8.000±0.428 [a]	7.917±0.375 [a]	5.300±0.026
Gly (0.100)	23	31	10	7	2	2	23	75	98	75	98	6.250±0.359 [a]	6.250±0.359 [a]	5.333±0.080
Gly (0.050)	9	21	6	7	0	0	16	50	59	50	59	4.167±0.167 [a]	4.167±0.167 [a]	5.367±0.095
Gly (0.025)	10	15	4	4	3	0	20	46	56	46	56	3.833±0.211 [b]	3.833±0.211 [b]	5.383±0.040
Gly (0.0125)	8	14	5	1	0	0	14	34	42	34	42	2.833±0.211	2.833±0.211	5.400±0.037

CAs = chromosomal aberrations; Ab.C = aberrant cells (cells with 1 ore more aberrations); MI = Mitotic Index; NC = Negative Control; MMC = Mitomycin-C; B’: chromatid break; B’’: chromosome break; DC: dicentric; R: ring; TR = tri-tetraradials; AF = acentric fragments; S.E. = standard error; Gly = Gliphosate

[a] $P = 0.004$; [b] $P < 0.006$ (significantly differs from the DMSO solvent control, Mann-Whitney test).

Table 2

Table 2. Multiple regression analysis between Glyphosate concentrations

Biomarker	**β-co**	**95% CI** (Lower) – (Upper)	***P*-value**
CAs	0.914	(2.112) – (2.988)	<0.001
Cells with CAs	0.919	(2.099) – (2.935)	<0.001
MI	-0.275	(-0.666) – (0.099)	0.141
MNi	0.908	(4.025) – (5.075)	<0.001
Cells with MNi	0.935	(3.639) – (4.527)	<0.001
CBPI	0.269	(-28.171) – (4.571)	0.151
NPBs	0.674	(0.268) – (0.665)	<0.001
NBUDs	0.395	(0.023) – (0.444)	0.031

CAs = Chromosomal Aberrations; MI = Mitotic Index; MNi = Micronuclei; CBPI = Cytokinesis-block Proliferation Index; NPBs = nucleoplasmic bridges NBUDs = nuclear buds

Table 3

Table 3 – Induction of micronuclei by Glyphosate in human lymphocytes in vitro.
Number of scored binucleated cells for each concentration of the test substance = 12,000

Test substance (µg/ml)	Distribution of BNCs according to the number of MNi				MNi	MNC	MNi/BNCs ± S.E. (%)	MNC/BNCs ± S.E. (%)	CBPI ± S.E	Frequency of BNCs with NPBs (‰)	Frequency of BNCs with NBUDs (‰)
	1	2	3	4							
NC	27	0	0	0	27	27	0.225±0.021	0.225±0.021	1.713±0.003	0.417±0.083	0.8333±0.105
0.1% DMSO	33	1	0	0	35	34	0.292±0.024	0.283±0.025	1.589±0.076	0.500±0.129	1.083±0.154
MMC (0.100)	129	9	3	2	164	143	1.367±0.067 [a]	1.192±0.015 [a]	1.366±0.019	2.333±0.357 [a]	3.083±0.473 [a]
Gly (0.500)	132	6	2	1	154	141	1.283±0.017 [a]	1.175±0.021 [a]	1.545±0.054	1.667±0.211 [a]	1.6657±0.247
Gly (0.100)	107	7	0	0	121	114	1.008±0.030 [a]	0.950±0.029 [a]	1.556±0.017	0.883±0.105	1.333±0.167
Gly (0.050)	93	6	1	0	108	100	0.900±0.053 [a]	0.833±0.046 [a]	1.576±0.015	0.750±0.111	1.250±0.1112
Gly (0.025)	68	5	0	0	78	73	0.650±0.048 [a]	0.608±0.035 [a]	1.585±0.010	0.667±0.105	1.167±0.105
Gly (0.0125)	39	0	0	0	39	39	0.325±0.021	0.325±0.021	1.589±0.008	0.583±0.083	1.167±0.167

BNCs = Binucleated cells; MNi = micronuclei; MNC = cells with 1 or more micronuclei; CBPI = Cytokinesis-Block Proliferation Index; NPBs = nucleoplasmic bridges; NBUDs = nuclear buds; S.E. = Standard Error; NC = Negative Control; MMC = Mitomycin-C; Gly = Gliphosate.
[a]$P = 0.004$ (significantly differs from the DMSO solvent control, Mann-Whitney test)

Figure 1

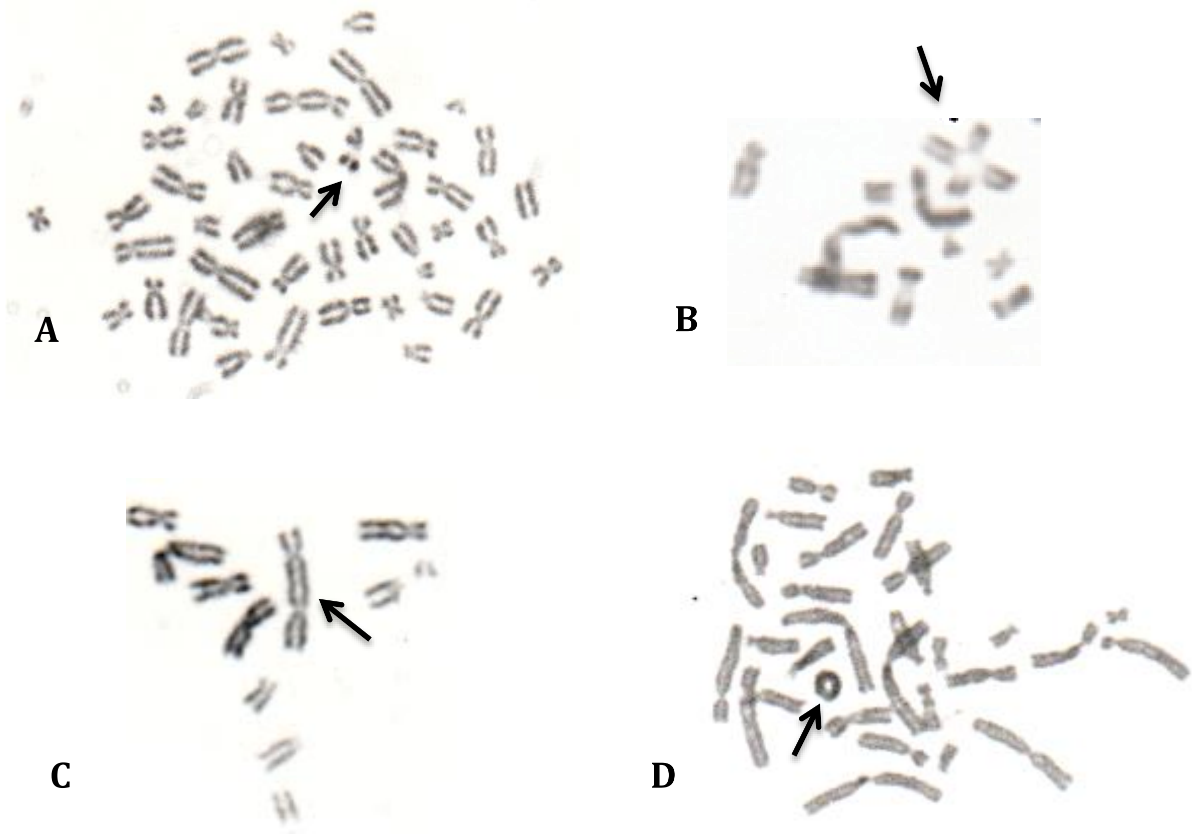

Figure 1 – Example of complete metaphase carrying an acentric fragment (A) and other four different metaphases details showing some examples of observed chromosomal aberrations. The arrows indicate, respectively: acentric fragment (Figure A), tetraradial (Figure B), dicentric chromosome (C), and ring (Figure D). All these aberrations were observed at 0.500 μg/mL concentration of glyphosate, with exception of the tetraradial aberration observed at 0.1 μg/mL concentration.

Figure 2

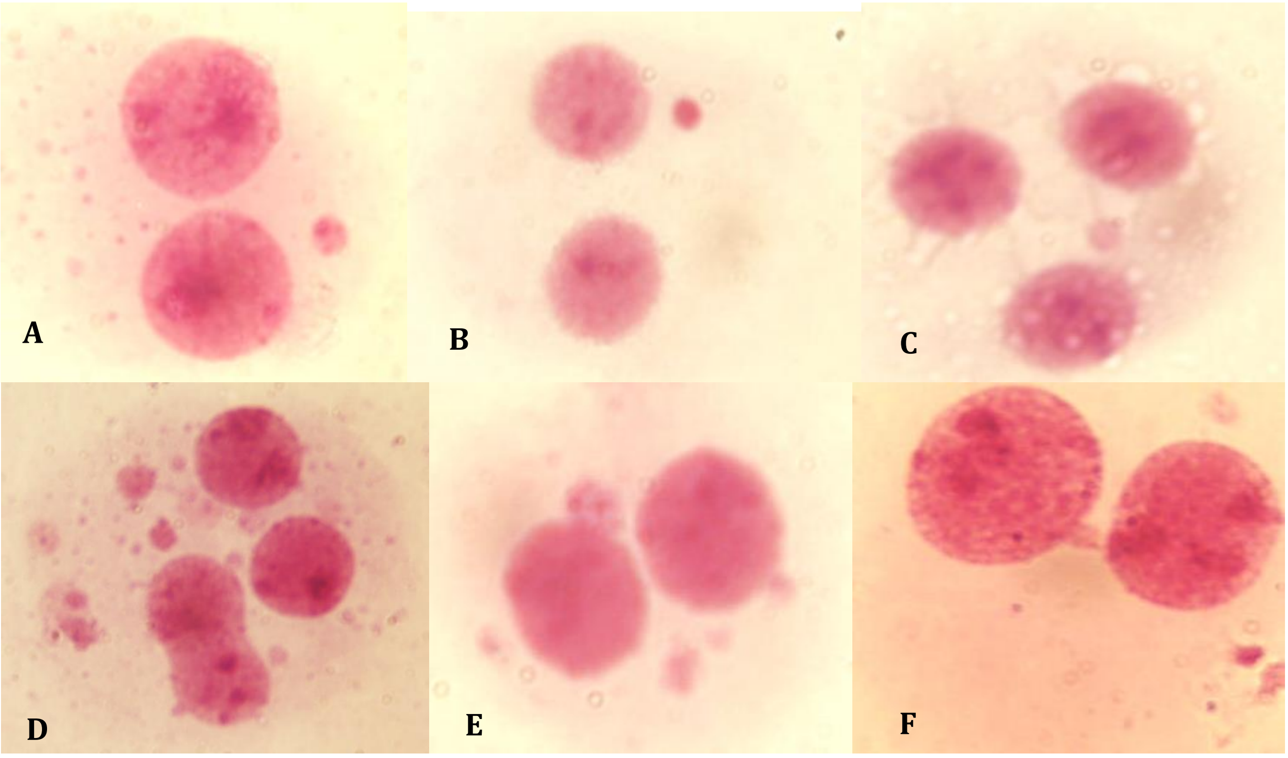

Figure 2 – Examples of observed micronuclei in bi-nucleated cells (A and B), tri-nucleated cell with a micronucleus (C) and tetra-nucleated cell with micronuclei (D). According to standardized procedures, micronuclei of tri- and tetra-nucleated cells were not scored in the evaluation of the total genomic damage. All these cells were observed at 0.500 μg/mL concentration of glyphosate. Examples of bi-nucleated cells with nuclear buds (E) and bi-nucleated cell with nucleoplasmic bridge are also reported (F).