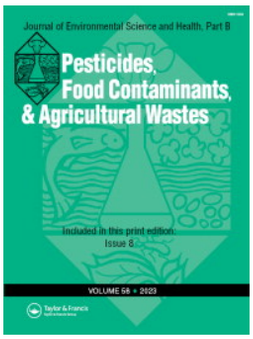

Taylor & Francis
Taylor & Francis Group

Journal of Environmental Science and Health, Part B
Pesticides, Food Contaminants, and Agricultural Wastes

ISSN: 0360-1234 (Print) 1532-4109 (Online) Journal homepage: www.tandfonline.com/journals/lesb20

Evaluation of the cytotoxicity and genotoxicity of glufosinate-ammonium at technical and commercial grades in HepG2 cells

Ezeidy Denisse Gallardo-Valle, Dayanne Carbajal-Nogueda, Ma. Elena Moreno-Godínez, Eugenia Flores-Alfaro, Isela Parra-Rojas, Gerardo Huerta-Beristain, Teresa Domínguez-Reyes & Marco Antonio Ramírez-Vargas

To cite this article: Ezeidy Denisse Gallardo-Valle, Dayanne Carbajal-Nogueda, Ma. Elena Moreno-Godínez, Eugenia Flores-Alfaro, Isela Parra-Rojas, Gerardo Huerta-Beristain, Teresa Domínguez-Reyes & Marco Antonio Ramírez-Vargas (2023) Evaluation of the cytotoxicity and genotoxicity of glufosinate-ammonium at technical and commercial grades in HepG2 cells, Journal of Environmental Science and Health, Part B, 58:8, 577-582, DOI: 10.1080/03601234.2023.2241322

To link to this article: https://doi.org/10.1080/03601234.2023.2241322

JOURNAL OF ENVIRONMENTAL SCIENCE AND HEALTH, PART B
2023, VOL. 58, NO. 8, 577–582
https://doi.org/10.1080/03601234.2023.2241322

Evaluation of the cytotoxicity and genotoxicity of glufosinate-ammonium at technical and commercial grades in HepG2 cells

Ezeidy Denisse Gallardo-Valle[a], Dayanne Carbajal-Nogueda[a], Ma. Elena Moreno-Godínez[a], Eugenia Flores-Alfaro[b], Isela Parra-Rojas[c], Gerardo Huerta-Beristain[a], Teresa Domínguez-Reyes[c], and Marco Antonio Ramírez-Vargas[a]

[a]Laboratorio de Toxicología y Salud Ambiental, Facultad De Ciencias Químico-Biológicas, Universidad Autónoma De Guerrero, Guerrero, México; [b]Laboratorio de Epidemiologia Clínica y Molecular, Facultad De Ciencias Químico-Biológicas, Universidad Autónoma De Guerrero, Guerrero, México; [c]Laboratorio de Investigación en Obesidad y Diabetes, Facultad De Ciencias Químico-Biológicas, Universidad Autónoma De Guerrero, Guerrero, México

ABSTRACT

Exposure to genotoxic agents is associated with the development of cancer and related diseases. For this reason, assessing the genotoxicity of chemical compounds is necessary. In this line, information about the genotoxic effect of glufosinate-ammonium (GLA) has been reported only for the technical grade. However, humans are frequently exposed to commercial formulations of pesticides. Commercial formulations are characterized by using inner agents that increase toxicity compared to pesticides in technical grade. This study aimed to determine the cytotoxic and genotoxic effects of GLA on HepG2 cells. MTT and comet assays were performed to evaluate cell viability and DNA damage, respectively. HepG2 cells were exposed for 24 h to different concentrations of GLA (at 0.01 µg/mL; 0.04 µg/mL; 0.1 µg/mL; 0.24 µg/mL; 0.52 µg/mL; 1.25 µg/mL; 2.62 µg/mL and 13.12 µg/mL) in commercial- (Finale Ultra®) or technical-grade (GLA_T). The results indicated that only Finale Ultra® induced a reduction in cell viability at 13.12 µg/mL. Furthermore, exposure to Finale Ultra® or GLA_T was associated with increased DNA damage at concentrations from 0.52–13.12- µg/mL. This study shows the genotoxic effect of GLA on HepG2 cells.

ARTICLE HISTORY
Received 5 March 2023
Accepted 16 July 2023

KEYWORDS
Finale Ultra; commercial formulations; comet assay; tail length; tail intensity

Introduction

Pesticides are widely used to control several vector-borne diseases and increase agricultural and livestock production.[1] Phosphinates and amino phosphonates are the most commonly used herbicides in the world.[2] The molecular mechanisms through which phosphinates and amino phosphonates cause toxicity vary according to the chemical composition. However, inhibiting enzymes related to synthesizing essential amino acids is a critical molecular mechanism of its toxicity. Moreover, the induction of oxidative stress and DNA damage is frequently observed in nontarget and target organisms exposed to phosphinates.[3,4] Pesticide-induced DNA damage is a risk factor for developing cancer and related diseases.[5,6]

Glufosinate-ammonium (GLA; 2-amino-4-[hydroxy (methyl)phosphoryl]butanoic acid; azane) is a nonselective herbicide with a broad spectrum of weed eradication. GLA contains a phosphorus-carbon bond from methyl phosphonic acid. For this reason, GLA is classified as an organophosphorus pesticide.[7] The World Health Organization (WHO) states that GLA can be moderately hazardous.[8] Weed eradication due to GLA exposure is related to the inhibition of glutamine synthetase (GS). GLA is used to eradicate glyphosate-resistant weeds and is considered a "safe alternative" to glyphosate use.[9]

Several methods have been developed to assess the genotoxicity associated with chemical compounds. The single comet assay is considered a sensitive method for measuring transient lesions that result in irreversible, stable, and hereditary DNA damage. The alkaline comet assay shows superior accuracy and repeatability compared to the neutral version. Moreover, the alkaline comet assay detects single-strand breaks, double-strand breaks, and apurinic/apyrimidinic sites. This assay is based on damaged/fragmented DNA migrating faster than undamaged/intact DNA through an agarose matrix in electrophoresis.[10]

The evaluation of the genotoxic effect induced by a pesticide is frequently performed using pesticides of technical grade (purity ≥ 95.5%).[11] However, occupational, paraoccupational, and environmental exposure is frequently related to commercial formulations of pesticides. These formulations are characterized by a mixture of pesticide and inert ingredients, resulting in a higher toxic effect than the active ingredient alone.[12] In this line, the evidence of the genotoxic effect

CONTACT Marco Antonio Ramírez-Vargas marvarant@gmail.com; marco-qbp@homail.com Laboratorio De Toxicología y Salud Ambiental, Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Guerrero, Av. Lázaro Cárdenas s/n, 39089 Chilpancingo, GRO, México.

(evaluated by chromosomal aberrations assay) of GLA_T shows negative results in human lymphocytes exposed to 1,000–4,640 µg/mL or 2,150–10,000 µg/mL (added with S9 fraction) for 24 h.[13] Nevertheless, the genotoxic effect is characterized by a nonlinear response. Thedose–response relationship between DNA damage and genotoxic exposure shows a linear response and a threshold of nonlinear response.[14,15] For this reason, the genotoxic effect of GLA could be analyzed using lower concentrations than previously reported. Furthermore, the type of cell line used to assess genotoxicity is crucial. For example, nongenotoxic effects have been observed in human lymphocytes exposed to OPs. However, when the assay is performed using human hepatic cell lines, the OPs induce genotoxic effects.[16,17] This study evaluated whether GLA_T and Finale Ultra® can induce a genotoxic effect at concentrations lower than 1 µg/mL in HepG2 cells.

Materials and methods

Cell culture

The HepG2 cell line (hepatocellular carcinoma) was purchased from ATCC (Manassas, VA, USA). The cells were cultured in William's Medium E (Sigma–Aldrich, St. Louis, MO, USA) supplemented with 10% FBS (fetal bovine serum; Gibco by Life Technologies, Grand Island, NY, USA) and 1% antibiotic/antimycotic (100 penicillin units, streptomycin 0.1 mg, 0.25 µg amphotericin B; Invitrogen, Inc., Grand Island, NY, USA). The cell cultures were incubated in a 5% CO_2 atmosphere at 37 °C.

Pesticides and treatment conditions

GLA_T (purity 98.8%; CAS#: 77182-82-2) was obtained from Sigma–Aldrich (Seelze, GER). Finale Ultra® (Finale Ultra®; BASF, Mex, registry number: RSCO-HEDE-0231-310-375-025) composed of 24.5% active ingredient (GLA) and 75.5% inert ingredients (including antifreeze, spreader, defoamer, dye, solvent, impurities, and related compounds) was purchased from a local distributor. Technical GLA or Finale Ultra® were dissolved in HPLC-Pure-water, such that the final volume of treatment was <1% in the final volume from cell cultures.

The ADI (acceptable daily intake) and ARfD (acute reference dose) from GLA were established at 0.01 mg/kg/day by FAO/WHO in 2012.[13] Moreover, in 2015, the EFSA (European Food Safety Authority) established the ADI and ARfD at 0.021 mg/kg/day and the AOEL (acceptable operator level) dose at 0.0021 mg/kg/day.[18] The concentrations in the present study were estimated using the abovementioned toxicological limits. The extrapolation from ADI, ARfD, or AOEL to *in vitro* concentration for treatment was performed considering the total body fluid (42 L) for a male of 70 kg of body weight.[19] We considered the distribution of a one-compartment model.[20] The 0.01 µg/mL concentration represents the AOEL, the EFSA-established ADI, and ARfD corresponds to 0.1 µg/mL, and the concentrations of 0.52 µg/mL represent the WHO-established ADI and ARfD. The other concentrations employed were estimated for an increase of 5-fold, 25-fold, or 125-fold.

Cytotoxicity assay

The cytotoxic effect of GLA_T or Finale Ultra® on HepG2 cells was determined using an MTT assay (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide).[21] In brief, HepG2 cells (at 4×10^5cells/cm^2) were exposed to GLA (0.01 µg/mL; 0.04 µg/mL; 0.1 µg/mL; 0.24 µg/mL; 0.52 µg/mL; 0.25 µg/mL; 2.62 µg/mL; 13.12 µg/mL) for 24 h. Untreated cells were used as the basal control (0 mM). Then, HepG2 cells were washed with 200 µL of PBS (phosphate-buffered saline; pH 7.4, NaCl 0.138 M, KCl 0.003 M) three times. Next, 50 µL of MTT (at 0.5 mg/mL in DMEM without phenol red) was added, and the cells were incubated for four hours. Finally, 150 µL of DMSO was added to each well, and the plate was shaken for 30 min. The absorbance was measured at 545 nm using an Epoch 2 microplate reader (Palm City, FL, USA). All experiments were performed in triplicate in two independent experiments.

Genotoxicity test

DNA damage was determined by employing the alkaline comet assay according to the protocol described by Singh *et al.* (1988).[22] In brief, the slides were pre-coated with 150 µL of 0.5% (w/v) standard melting agarose. After 24 h of treatment, the HepG2 cells (1×10^5cells/cm^2) were trypsinized (using 0.25% porcine trypsin and 0.2% EDTA (ethylenediaminetetraacetic acid) dissolved in Hanks' Balanced Salt Solution) for 5 min. We used unexposed cells as a control group. The trypsinized cells were mixed with 150 µL of low melting point agarose (LMPA; the final concentration of LMPA after the cell was added was 0.5% w/v). Next, 85 µL from the mixture was placed onto the base precoated slides, and a coverslip was placed on it. The gelling agarose gently slid off the coverslip, and a third LMPA layer (0.5% w/v at final concentration) was added. The slides were placed in lysis solution at a pH of 10 (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris-HCl, 0.5% (v/v) Triton X-100, and 5% (v/v) DMSO) for 12 h at 4 °C. Before electrophoresis, the slides were incubated for 20 min in electrophoretic buffer (200 mM Na_2EDTA, 10 M NaOH, pH 13 at 4 °C). Then, electrophoresis was performed at 0.73 v/cm, ~300 mA for 20 min at 4 °C, pH 13. The slides were neutralized with Tris-HCl (0.4 M) at pH 7.4. Then, the slides were dehydrated with ethanol (96%) and stained with 30 µL of ethidium bromide (20 µg/mL). Blinding scoring was performed. The DNA damage was measured as tail length (TL) and tail intensity (TI) based on 50 cells by gel. Three gels were scored in two independent experiments. The mean from comets was analyzed. The analysis was performed employing the Comet Assay IV, version 4.3.1 (Perspective Instruments, Suffolk, UK).

Statistical analyses

The normal distribution of the cell viability or genotoxicity results was assessed using Shapiro–Wilk tests. Then, a one-way analysis of variance (ANOVA) followed by Dunnett *post hoc* tests was performed. P values <0.05 were considered

statistically significant. The analyses were performed using GraphPad Prism 8.01.

Results

Cytotoxicity of Glufosinate-ammonium

The evaluation of cell viability using the MTT assay showed that GLA_T does not induce a cytotoxic effect on HepG2 cells (Figure 1a). However, exposure to 13.12 µg/mL Finale Ultra® decreased cell viability by more than 20% (cytotoxic effect) compared with the basal control ($P < 0.05$) (Figure 1b).

DNA damage by exposure to Glufosinate-ammonium

The changes in TI or TL were measured as markers for assessing the genotoxic effect induced by GLA. The results showed that exposure to GLA_T or Finale Ultra® induced DNA damage in HepG2 cells. Compared with the control, exposure to GLA_T increased the TL by 3.04-fold or 3.88-fold ($P < 0.05$) for concentrations of 2.62 µg/mL; 13.12 µg/mL, respectively (Figure 2a). Furthermore, GLA_T increased the TI at concentrations ≥ 0.52 µg/mL (Figure 3a). In contrast, exposure to 0.24 µg/mL; 0.52 µg/mL; 1.25 µg/mL; 2.62 µg/mL, or 13.12 µg/mL of Finale Ultra® induced an increase in TL of 1.62-fold, 2.59-fold, 2.79-fold, 3.04-fold, or 3.88-fold ($P < 0.05$), respectively (Figure 2b). Similar results were observed for TI induced by Finale Ultra® exposure at concentrations ≥ 0.24 µg/mL (Figure 3b).

Discussion

Genotoxicity is considered the capacity of toxins to induce DNA damage in a cell as a result of two main mechanisms: (1) alteration in the DNA structure (single/double-strand break; bulky adducts, intercalating agents, interstrand cross-link); and (2) disruption of DNA metabolism (that includes alterations in replication resulting in mismatch and/or a reduction in the functionality of DNA repair systems).[23] Exposure to pesticides has been associated with DNA damage.[24] The present study assessed whether GLA induces DNA damage in HepG2 cells. The results indicated that exposure to technical- or commercial-grade GLA increases the levels of TL and TI in HepG2 cells.

The loss of cellular homeostasis could result in cell death. The MTT assay was developed to assess cell viability based on the activity of mitochondrial oxidoreductase.[25] In the present work, only Finale Ultra® induced a cytotoxic effect on HepG2 cells. Human spermatozoids exposed to Liberty® (a commercial formulation of GLA composed of 24.5% active ingredient manufactured by BASF) for 1 h at 1,000 nM (0.2 µg/mL) showed reduced mitochondrial metabolic activity.[26]

The cytotoxic effect of GLA_T has been reported in cellular models exposed to concentrations ≥ 198.2 µg/mL. For example, exposure to 198.2 µg/mL of GLA_T for 24 h induced a cytotoxic effect on murine embryoid cells.[27] Cell death of prostatic adenocarcinoma cells was induced at 990.8 µg/mL (5 mM) GLA_T for 24 h.[28] It is widely accepted that commercial formulations of pesticides are frequently more toxic than technical-grade pesticides.[12] This increase in toxicity is

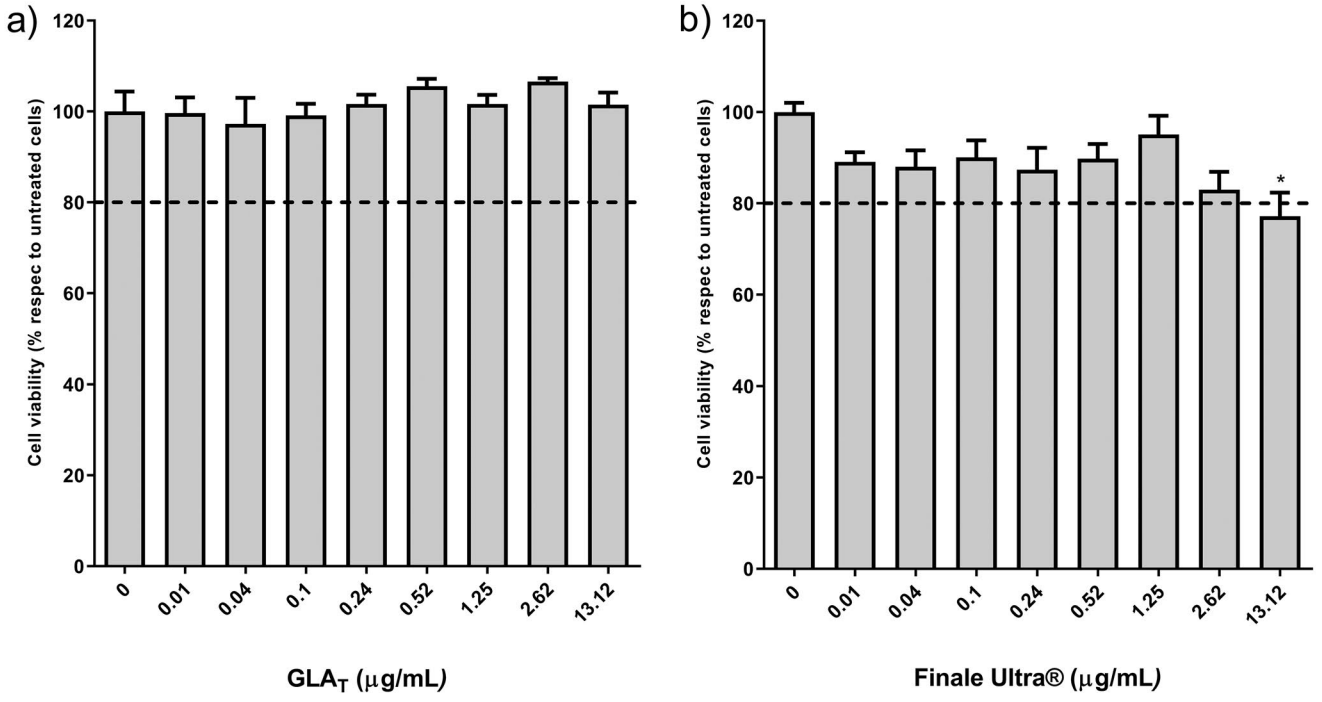

Figure 1. Effect of GLA on HepG2 viability. HepG2 cells were incubated with various concentrations of GLA for 24 h, and the cell viability (%) was determined using the MTT assay. a) HepG2 cells exposed to GLA_T; b) HepG2 exposed to Finale Ultra®. *$p < 0.05$ compared to untreated cells. The dotted line indicates a reduction of 20% in cell viability (cytotoxic effect). The data represent the mean and standard error of three independent experiments.

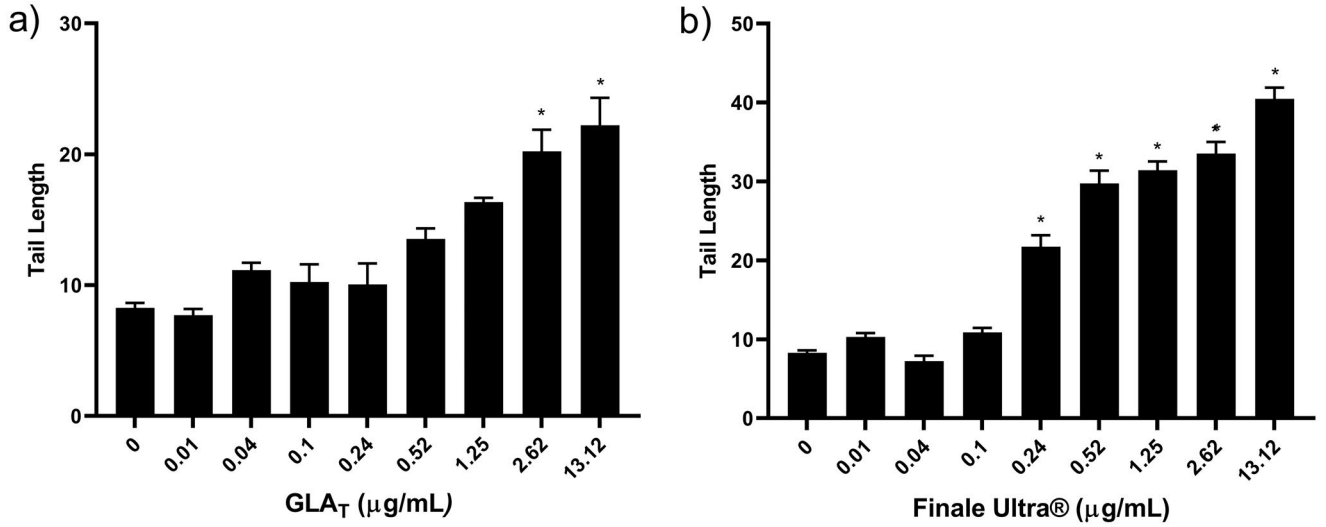

Figure 2. Effect of GLA on TL parameter. HepG2 cells were exposed to various concentrations of (a) GLA$_T$ or (b) Finale Ultra® for 24 h. After, an alkaline comet assay was performed. *$p < 0.05$ compared to untreated cells. The data represent the mean and standard error from 50 cells scored for three slides in two independent experiments.

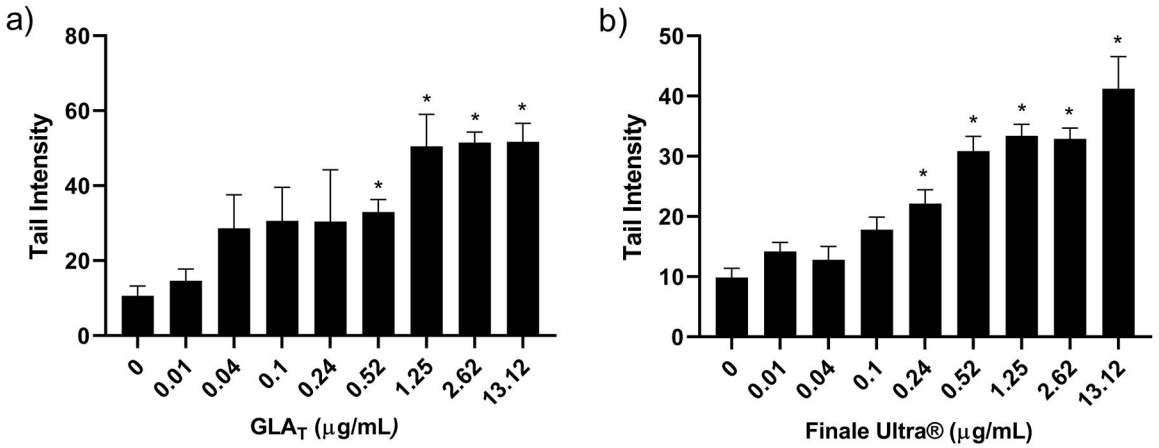

Figure 3. Effect of GLA on TI parameter. HepG2 cells were exposed to various concentrations of (a) GLA$_T$ or (b) Finale Ultra® for 24 h. After, an alkaline comet assay was performed. *$p < 0.05$ compared to untreated cells. The data represent the mean and standard error from 50 cells scored for tree slides in two independent experiments.

associated with an inert adjuvant composition that increases the stability and permeability of active ingredients, resulting in a synergistic effect.[12] Furthermore, early reports about the toxic effect of acute and chronic exposure to GLA in mammals indicate that GLA inhibits the activity of glutamine synthetase primarily in the liver with subsequent depletion of glutamine levels.[29] In this line, the reduction in glutamine levels leads to the induction of apoptosis in hepatocarcinoma cells.[30] More studies are necessary to evaluate the role of glutamine synthetase inhibition in GLA-induced apoptosis.

This study shows that exposure to GLA$_T$ or Finale Ultra® is related to the induction of a genotoxic effect on HepG2 cells. According to data collected by FAO (Food and Agriculture Organization of the United Nations)/WHO (up to 2012), there is no evidence of GLA$_T$-induced genotoxic effects in several *in vitro* models. Negative results for the chromosomal aberration assay were observed in human lymphocytes exposed for 24 h to GLA$_T$ (1,000–4,640 μg/mL or 2,150–10,000 μg/mL coculture with S9 hepatic fraction). Mouse lymphoma cells exposed for 24 h to GLA$_T$ (50–5,000 μg/mL or 300–5,000 μg/mL coculture with liver S9 fraction) do not show gene mutations. Exposure to GLA$_T$ (625–10,000 μg/mL or 625–8,000 μg/mL coculture with liver S9 fraction) does not induce gene mutations in hamster lung fibroblast cells. (4) In rat-isolated primary hepatocytes, exposure to GLA$_T$ (26.2–5,240 μg/mL) did not increase global genomic nucleotide excision repair.[13] The contradictory results between previous reports and the present study could be explained by the following facts: (1) HepG2 cells are considered cells capable of performing xenobiotic metabolism, including the bioactivation process.[31] Using nonmetabolically competent cells (for example, fibroblasts, lymphocytes, and nonhepatic cell lines in general) can result in low detection of genotoxic effects. Moreover, using the liver S9 fraction could not be the best option for increasing the sensitivity

for assessing genotoxicity compared to human hepatic cell models.[32] Furthermore, it is highly recommended that the *in vitro* assays employed concentrations must not be greater than 10 mM for testing the genotoxic effect. Concentrations >10 mM could change the osmotic concentration of the culture medium and decrease cell proliferation.[33]

In this study, HepG2 cells exposed to Finale Ultra® showed a genotoxic effect. This result concurs with previous reports that showed an increase in the DNA damage index estimated by comet assay in amphibian tadpoles chronically exposed to 2.77 µg/mL of Liberty® (for 45 days).[34] Moreover, in *Rhinella arenarum*, exposure for 48 h or 96 h at 3.96 µg/mL, 7.53 µg/mL, or 15.06 µg/mL of Liberty® increases the frequency of micronuclei and nuclear abnormalities from erythrocytes.[35] In zebrafish embryos, exposure to 9.91×10^{-3} µg/mL of GLA_T was associated with increased malonaldehyde, superoxide dismutase, and catalase. The authors suggested that GLA_T can induce oxidative stress.[36] Similar results were observed for Timón® (a commercial formulation of GLA with 20% active ingredient), which decreased the activity of antioxidant enzymes such as catalase.[34] The induction of oxidative stress is recognized as a toxic mechanism of herbicides.[3] The increase in hydroxyl radicals induces oxidative damage in DNA, and this event is related to the increase in the TI or TL parameter from the comet assay.[37] More studies are necessary to evaluate the role of oxidative stress in the GLA-induced genotoxic effect on HepG2 cells.

Conclusions

The data in this study show that exposure to GLA_T or Finale Ultra® causes increases in the TL and TI in HepG2 cells. Moreover, there is the first evidence of the genotoxic effect attributed to GLA_T. Biologically relevant concentrations of GLA induce DNA damage in HepG2 cells.

Disclosure statement

The authors report there are no competing interests to declare.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data availability statement

The data supporting this study's findings are available from the corresponding author, [MARV], upon reasonable request.

References

[1] Carvalho, F. P. Pesticides, Environment, and Food Safety. *Food Energy Secur* **2017**, *6*, 48–60. DOI: 10.1002/fes3.108.

[2] Benbrook, C. M.; Benbrook, R. 2 - A Minimum Data Set for Tracking Changes in Pesticide Use. In *Herbicides*; Mesnage, R., Zaller, J. G., Eds. Elsevier: Huston, TX, **2021**, 21–39.

[3] Székács, A. 3 - Herbicide Mode of Action. In *Herbicides*; Mesnage, R., Zaller, J. G., Eds. Elsevier: Huston, TX, **2021**; 41–86

[4] Mesnage, R.; Antoniou, M. 6 - Mammalian Toxicity of Herbicides Used in Intensive GM Crop Farming. In *Herbicides* ; Mesnage, R., Zaller, J. G., Eds. Elsevier: Huston, TX, **2021**;pp 143–180

[5] Pluth, T. B.; Zanini, L. A. G.; Battisti, I. D. E. Pesticide Exposure and Cancer: An Integrative Literature Review. *Saúde Debate* **2019**, *43*, 906–924. DOI: 10.1590/0103-1104201912220.

[6] Yang, K. J.; Lee, J.; Park, H. L. Organophosphate Pesticide Exposure and Breast Cancer Risk: A Rapid Review of Human, Animal, and Cell-Based Studies. *Int. J. Environ. Res. Public Health*. **2020**, *17*, 5030. DOI: 10.3390/ijerph17145030.

[7] Hoerlein, G. Glufosinate (Phosphinothricin), a Natural Amino Acid with Unexpected Herbicidal Properties. In *Reviews of Environmental Contamination and Toxicology*; Ware, G.W., Ed. Springer New York: New York, NY, **1994**; 138, 73–145

[8] Organization, W. H. The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 2019. *World Health Organization:* **2020**, *1,* 86–87.

[9] Takano, H. K.; Dayan, F. E. Glufosinate-Ammonium: A Review of the Current State of Knowledge. *Pest Manag Sci* **2020**, *76*, 3911–3925. DOI: 10.1002/ps.5965.

[10] Bonassi, S.; Ceppi, M.; Møller, P.; Azqueta, A.; Milić, M.; Neri, M.; Brunborg, G.; Godschalk, R.; Koppen, G.; Langie, S. A. et al. DNA Damage in Circulating Leukocytes Measured with the Comet Assay May Predict the Risk of Death. *Sci Rep* **2021**, *11*, 16793. DOI: 10.1038/s41598-021-95976-7.

[11] Dearfield, K. L.; Cimino, M. C.; McCarroll, N. E.; Mauer, I.; Valcovic, L. R. Genotoxicity Risk Assessment: A Proposed Classification Strategy. *Mutat Res* **2002**, *521*, 121–135. DOI: 10.1016/s1383-5718(02)00236-x.

[12] Nagy, K.; Duca, R. C.; Lovas, S.; Creta, M.; Scheepers, P. T. J.; Godderis, L.; Ádám, B. Systematic Review of Comparative Studies Assessing the Toxicity of Pesticide Active Ingredients and Their Product Formulations. *Environ Res* **2020**, *181*, 108926. DOI: 10.1016/j.envres.2019.108926.

[13] FAO Panel of Experts on Pesticide Residues in Food and the Environment; WHO Core Assessment Group on Pesticides Residues; Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues, R., Italy, 11-20 September 2012. Pesticide Residues in Food: 2012: Toxicological Evaluations. *World Health Organization*: Rome, It, **2013**; 847pp.

[14] Calabrese, E. J.; Stanek, E. J., III, Nascarella, M. A. Evidence for Hormesis in Mutagenicity Dose–Response Relationships. *Mutat Res* **2011**, *726*, 91–97. DOI: 10.1016/j.mrgentox.2011.04.006.

[15] Jenkins, G. J.; Zaïr, Z.; Johnson, G. E.; Doak, S. H. Genotoxic Thresholds, DNA Repair, and Susceptibility in Human Populations. *Toxicology* **2010**, *278*, 305–310. DOI: 10.1016/j.tox.2009.11.016.

[16] Hernández-Toledano, D. S.; Estrada-Muñiz, E.; Vega, L. Genotoxicity of the Organophosphate Pesticide Malathion and Its Metabolite Dimethylthiophosphate in Human Cells in Vitro. *Mutat Res Genet Toxicol Environ Mutagen* **2020**, *856–857*, 503233. DOI: 10.1016/j.mrgentox.2020.503233.

[17] Benitez-Trinidad, A. B.; Herrera-Moreno, J. F.; Vázquez-Estrada, G.; Verdín-Betancourt, F. A.; Sordo, M.; Ostrosky-Wegman, P.; Bernal-Hernández, Y. Y.; Medina-Díaz, I. M.; Barrón-Vivanco, B. S.; Robledo-Marenco, M. L.; et al. Cytostatic and Genotoxic Effect of Temephos in Human Lymphocytes and HepG2 Cells. *Toxicol in Vitro* **2015**, *29*, 779–786. DOI: 10.1016/j.tiv.2015.02.008.

[18] Authority, E. F. S. Reasoned Opinion on the Review of the Existing Maximum Residue Levels (MRLs) for Glufosinate according to Article 12 of Regulation (EC) No 396/2005. *EFSA J.* **2015**, *13*, 3950.

[19] Hall, J. E.; Hall, M. E. Guyton and Hall Textbook of Medical Physiology e-Book. *Elsevier Health Sciences* **2020**, *1*, 305–320.

[20] Klaassen, C. D.; Watkins, J. B. *Casarett & Doull's Essentials of Toxicology*. McGraw Hill Professional: New York, NY; **2021**.

[21] Mosmann, T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *J Immunol Methods* **1983**, *65*, 55–63. DOI: 10.1016/0022-1759(83)90303-4.

[22] Singh, N. P.; McCoy, M. T.; Tice, R. R.; Schneider, E. L. A Simple Technique for Quantitation of Low Levels of DNA Damage in Individual Cells. *Exp Cell Res* **1988**, *175*, 184–191. DOI: 10.1016/0014-4827(88)90265-0.

[23] Klaunig, J. E.; Wang, Z. *Chemical Carcinogenesis. In Casarett and Doull's TOXICOLOGY the Basic Science of Poisons*; McGraw-Hill: USA, **2019**.

[24] Ogut, S. Genotoxic Effects of Pesticides. *J. Environ. Prot. Ecol.* **2019**, *20*, 224–229.

[25] Kamiloglu, S.; Sari, G.; Ozdal, T.; Capanoglu, E. Guidelines for Cell Viability Assays. *Food Frontiers* **2020**, *1*, 332–349. DOI: 10.1002/fft2.44.

[26] Ferramosca, A.; Lorenzetti, S.; Di Giacomo, M.; Murrieri, F.; Coppola, L.; Zara, V. Herbicides Glyphosate and Glufosinate Ammonium Negatively Affect Human Sperm Mitochondria Respiration Efficiency. *Reprod Toxicol* **2021**, *99*, 48–55. DOI: 10.1016/j.reprotox.2020.11.011.

[27] Watanabe, T.; Iwase, T. Developmental and Dysmorphogenic Effects of Glufosinate Ammonium on Mouse Embryos in Culture. *Teratog. Carcinog. Mutagen* **1996**, *16*, 287–299. DOI: 10.1002/(SICI)1520-6866(1996)16:6<287::AID-TCM1>3.0.CO;2-E.

[28] Lorenzetti, S.; Marcoccia, D.; Narciso, L.; Mantovani, A. Cell Viability and PSA Secretion Assays in LNCaP Cells: A Tiered in Vitro Approach to Screen Chemicals with a Prostate-Mediated Effect on Male Reproduction within the ReProTect Project. *Reprod Toxicol* **2010**, *30*, 25–35. DOI: 10.1016/j.reprotox.2010.03.008.

[29] Hack, R.; Ebert, E.; Ehling, G.; Leist, K.-H. Glufosinate Ammonium—Some Aspects of Its Mode of Action in Mammals. *Food Chem Toxicol* **1994**, *32*, 461–470. DOI: 10.1016/0278-6915(94)90043-4.

[30] Fuchs, B. C.; Perez, J. C.; Suetterlin, J. E.; Chaudhry, S. B.; Bode, B. P. Inducible Antisense RNA Targeting Amino Acid Transporter ATB0/ASCT2 Elicits Apoptosis in Human Hepatoma Cells. *Am J Physiol Gastrointest Liver Physiol* **2004**, *286*, G467–G478. DOI: 10.1152/ajpgi.00344.2003.

[31] Hewitt, N. J.; Hewitt, P. Phase I and II Enzyme Characterization of Two Sources of HepG2 Cell Lines. *Xenobiotica* **2004**, *34*, 243–256. DOI: 10.1080/00498250310001657568.

[32] Guo, X.; Seo, J.-E.; Li, X.; Mei, N. Genetic Toxicity Assessment Using Liver Cell Models: Past, Present, and Future. *J Toxicol Environ Health B Crit Rev* **2020**, *23*, 27–50. DOI: 10.1080/10937404.2019.1692744.

[33] OECD, *Test No. 487: In Vitro Mammalian Cell Micronucleus Test, OECD Guidelines for the Testing of Chemicals, Section 4*, OECD Publishing, Paris. **2016**.

[34] Cuzziol Boccioni, A. P.; Lener, G.; Peluso, J.; Peltzer, P. M.; Attademo, A. M.; Aronzon, C.; Simoniello, M. F.; Demonte, L. D.; Repetti, M. R.; Lajmanovich, R. C. Comparative Assessment of Individual and Mixture Chronic Toxicity of Glyphosate and Glufosinate Ammonium on Amphibian Tadpoles: A Multibiomarker Approach. *Chemosphere* **2022**, *309*, 136554. DOI: 10.1016/j.chemosphere.2022.136554.

[35] Lajmanovich, R. C.; Cabagna-Zenklusen, M. C.; Attademo, A. M.; Junges, C. M.; Peltzer, P. M.; Bassó, A.; Lorenzatti, E. Induction of Micronuclei and Nuclear Abnormalities in Tadpoles of the Common Toad (Rhinella Arenarum) Treated with the Herbicides Liberty® and Glufosinate-Ammonium. *Mutat Res Genet Toxicol Environ Mutagen* **2014**, *769*, 7–12. DOI: 10.1016/j.mrgentox.2014.04.009.

[36] Xiong, G.; Deng, Y.; Li, J.; Cao, Z.; Liao, X.; Liu, Y.; Lu, H. Immunotoxicity and Transcriptome Analysis of Zebrafish Embryos in Response to Glufosinate-Ammonium Exposure. *Chemosphere* **2019**, *236*, 124423. DOI: 10.1016/j.chemosphere.2019.124423.

[37] Cordelli, E.; Bignami, M.; Pacchierotti, F. Comet Assay: A Versatile but Complex Tool in Genotoxicity Testing. *Toxicol Res (Camb)* **2021**, *10*, 68–78. DOI: 10.1093/toxres/tfaa093.