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Article

Glyphosate Levels in American Food Products Meet Government Safety Levels Yet Exceed Concentrations Associated with Negative Biological Effects

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SUMMARY

Glyphosate, the active ingredient in the weed killer Roundup, is the most popular herbicide in the agricultural industry, with 6.1 billion kilograms sprayed worldwide in the past decade (1). Residue findings in breakfast foods and alcoholic beverages have prompted quantitative human and vertebrate studies yielding contradictory results (2). This study tested the hypothesis that glyphosate concentrations in frequently consumed food products exceed the levels legally allowed in Australia, the European Union, and the United States. The enzyme-linked immunosorbent assay (ELISA) method was utilized to measure glyphosate concentrations in cereal, gluten-free white and yellow corn tortillas, regular and whole grain pasta, white and whole wheat bread, and soy chocolate milk. The results showed that mean glyphosate residues were the highest in cereal (1903 parts per billion [ppb]) and significantly higher in whole grain pasta (45 ppb) than regular pasta (19 ppb), in gluten-free yellow corn tortilla (35 ppb) than gluten-free white corn tortilla (none detected), and in whole wheat bread (888 ppb) than white bread (56 ppb). Mean glyphosate concentrations in 25% of samples exceeded the threshold (500 ppb) at which negative effects were observed in previous studies. However, glyphosate concentrations in none of the samples exceeded government-imposed limits. These results raise concerns about whether government-imposed limits are sufficient to protect human health. Future research should quantify glyphosate residues in a wider variety of foods and conduct epidemiological studies to reassess the sufficiency of current safety limits.

INTRODUCTION

N-(phosphonomethyl)glycine, more commonly known as glyphosate, is a non-selective herbicide that prevents plant growth by disrupting the shikimic acid pathway, a metabolic pathway responsible for aromatic amino acid synthesis in plants (3). In the early 1970s, American scientist John E. Franz discovered glyphosate's application as a broad-spectrum herbicide while working under the agrochemical company Monsanto, which patented the product as "Roundup®" (4). Glyphosate remains the most frequently used chemical in the agricultural industry, raising concerns about the possible health effects of glyphosate exposure on humans (5).

A previous study which exposed glyphosate-based herbicides (GBH) to human liver cells found androgen receptor endocrine disruption in cells exposed to 500 ppb GBH, transcription inhibition of estrogen receptor genes in cells exposed to 2,000 ppb GBH, and DNA damage in cells exposed to 5,000 ppb GBH (6).

Another study administered 3.8 mg glyphosate/L (Monsanto's recommended maximum dosage of Roundup®) to a pond community and reported the loss of two species and a 70% reduction in total amphibian biodiversity after 13 days (7). It has also been found that African clawed frog (*Xenopus laevis*) embryos injected with 500 pg glyphosate experienced developmental changes in the head and neural crest regions, and chicken embryos injected with 4.4x10-3 µL GBH experienced microcephaly and optic vesicle reduction (8). Brown trout exposed to 10 ppb glyphosate exhibited up-regulation of the tumor-suppressor protein p53 (TP53), which stimulates cell cycle inhibitors that can inhibit cancer, and hypoxia up-regulated protein 1 (HYOU1), which promotes hypoxia-induced cellular perturbation. Brown trout exposed to 50 ppb Roundup® exhibited significant up-regulation of mitochondrion-associated apoptosis-inducing factor (AIFM2) (9-11). Another study on peppered catfish (*Corydoras paleatus*) blood and hepatic cells found increased DNA damage in the treatment group exposed to 3.2 ppb glyphosate for three days (13). Other studies on fish have reported decreased 17β-estradiol in female South American catfish (*Rhamdia quelen*) exposed to 3,600 ppb glyphosate and decreased aggressive behavior and distance traveled in adult zebrafish (*Danio rerio*) exposed to 500 ppb glyphosate (13,14). Increased testosterone levels, estradiol levels, and sperm production in male rats have been observed with 50 mg/kg (50,000 ppb) Roundup® exposure (15).

A population analysis reported that in an Argentinian farming town where crops were grown using GBH, birth defect and miscarriage rates were two and three times greater than the national average, respectively (16). Another study on herbicide and chlorophenol exposure demonstrated an association between glyphosate exposure and non-Hodgkin lymphoma development in adults. Subjects exposed to glyphosate were over two times more likely to have non-Hodgkin lymphoma (17).

Other research, however, has yielded inconsistent results. For example, the Agricultural Health Study (a collaboration between the National Cancer Institute, National Institute of Environmental Health Sciences, Environmental Protection Agency (EPA), and National Institute for Occupational

Safety and Health) on private and commercial licensed glyphosate applicators in Iowa and North Carolina found that the association between glyphosate exposure and cancer was not statistically significant (2). Another study on human umbilical, placental, and embryonic cells reported glyphosate toxicity beginning at a concentration of 1% (10,000,000 ppb) and cell death occurring within 24 hours of exposure to 105 times dilutions (100,00,000 ppb) of Roundup® formulations, levels significantly higher than those found to be detrimental in other studies (18).

Contradictory data have prompted governmental and non-governmental organizations to adopt differing stances on glyphosate safety. The European Food Safety Authority and United States EPA have both determined that glyphosate is unlikely to be carcinogenic (19,20). However, the State of California listed it as a "chemical known to the state of California to cause cancer" under Proposition 65, and the World Health Organization concluded that the herbicide was "probably carcinogenic" after the International Agency for Research on Cancer's Assessment (21,22).

At the time of writing, the EPA limits the amount of glyphosate that should be applied to raw agricultural commodities to 5,000 ppb for corn, 20,000 ppb for soybean seeds, and 30,000 ppb for Group 15 grains, including wheat, oats, barley, and rice (23). Residue limits vary by food because exposure changes with each product's growing environment and processing method. These tolerances are set by evaluating the dietary risk assessments, exposure, and toxicity of contaminants (24). The EPA limits glyphosate's Maximum Contaminant Level (MCL), the maximum concentration of a contaminant permitted in drinking water, to 700 ppb (25). MCLs are determined by the level at which a chemical may be present in drinking water without known risk to health. This level is determined by evaluating the concentration associated with non-carcinogenic effects while accounting for body weight, daily water consumption, and drinking water exposure (26).

The European Union's maximum residue level (MRL) for glyphosate is 1,000 ppb for corn, 20,000 ppb for soybeans, 10,000 ppb for wheat, and 20,000 ppb for oats (27). These MRLs are determined by factors such as application, anticipated residues, and toxicology of each herbicide (28).

Glyphosate's acceptable daily intake (ADI), the recommended amount that can be safely consumed on a daily basis for an extended period of time, in the European Union is 0.5 mg per kg of body weight per day (27). The Australian Government's Pesticides and Veterinary Medicines Authority defines the ADI for glyphosate as 0.3 mg per kg of body weight per day. This level was determined through identifying the concentration at which studies found no observed adverse effects and dividing it by a factor to account for variation in data (29). The limits enforced by the United States, European Union, and Australia are significantly higher than concentrations associated with adverse side effects in previously mentioned studies.

Food	EPA Glyphosate Tolerance Level (ppb)	European Commission Glyphosate MRL (ppb)
Oats	30,000	20,000
Corn	5,000	1,000
Wheat	30,000	10,000
Soybean	20,000	20,000

Table 1. Government enforced glyphosate limits. EPA tolerance levels (ppb) and European Commission MRLs (ppb) for glyphosate and their corresponding foods (23,27).

RESULTS

Three trials of ELISA were run to measure glyphosate residues in Cheerios Gluten-Free Toasted Whole Grain Oats Cereal, Mission Gluten-Free Yellow Corn Tortilla, Mission Gluten-Free White Corn Tortilla, Kroger Vermicelli Enriched Macaroni Product, Kroger 100% Whole Grain Thin Spaghetti, Van de Kamp's Vegan White Enriched Bread, Van de Kamp's 100% Whole Wheat Bread, and Silk Chocolate Soymilk.

The mean glyphosate concentration was 1903 ± 6.93 ppb in the cereal sample, 888 ± 45.40 ppb in the whole wheat bread sample, 56 ± 4.36 ppb in the white bread sample, 45 ± 6.08 ppb in the whole grain pasta sample, 35 ± 6.08 ppb in the yellow corn tortilla sample, and 19 ± 5.29 ppb in the regular pasta sample. No glyphosate was detected in the gluten-free white corn tortilla or soy chocolate milk samples.

The recorded amount of glyphosate was significantly increased ($p < 0.017$) in whole grain pasta as compared with regular pasta, and in whole wheat bread as compared with white bread. Moreover, the recorded amount of glyphosate was significantly increased ($p < 0.017$) in gluten-free yellow corn tortilla as compared to gluten-free white corn tortilla. The residues of all food samples were significantly ($p < 6.25 \times 10^{-3}$) within permitted government levels deemed safe for human consumption, and European Commission MRLs (**Figure 1**).

DISCUSSION

Our data did not support our hypothesis that the samples' glyphosate residues were greater than the ADI, MRLs, and tolerance levels outlined by the Australian Pesticides and Veterinary Medicines Authority, European Commission, and the EPA (**Table 1**). Lower-tailed, Bonferroni corrected t-tests indicated statistically significant evidence at the $\alpha = 6.25 \times 10^{-3}$ level that the glyphosate concentrations of the foods tested were lower than their corresponding EPA tolerance levels and European Commission MRLs (**Figure 1**). However, glyphosate levels measured in the cereal and whole wheat bread samples were significantly higher than the threshold at which negative effects were observed in previous studies (500 ppb) by as much as three times. The whole grain pasta and whole wheat bread samples may have had higher glyphosate concentrations than the regular pasta and white bread samples, respectively, because unlike refined grain products, whole grain products contain both bran and germ.

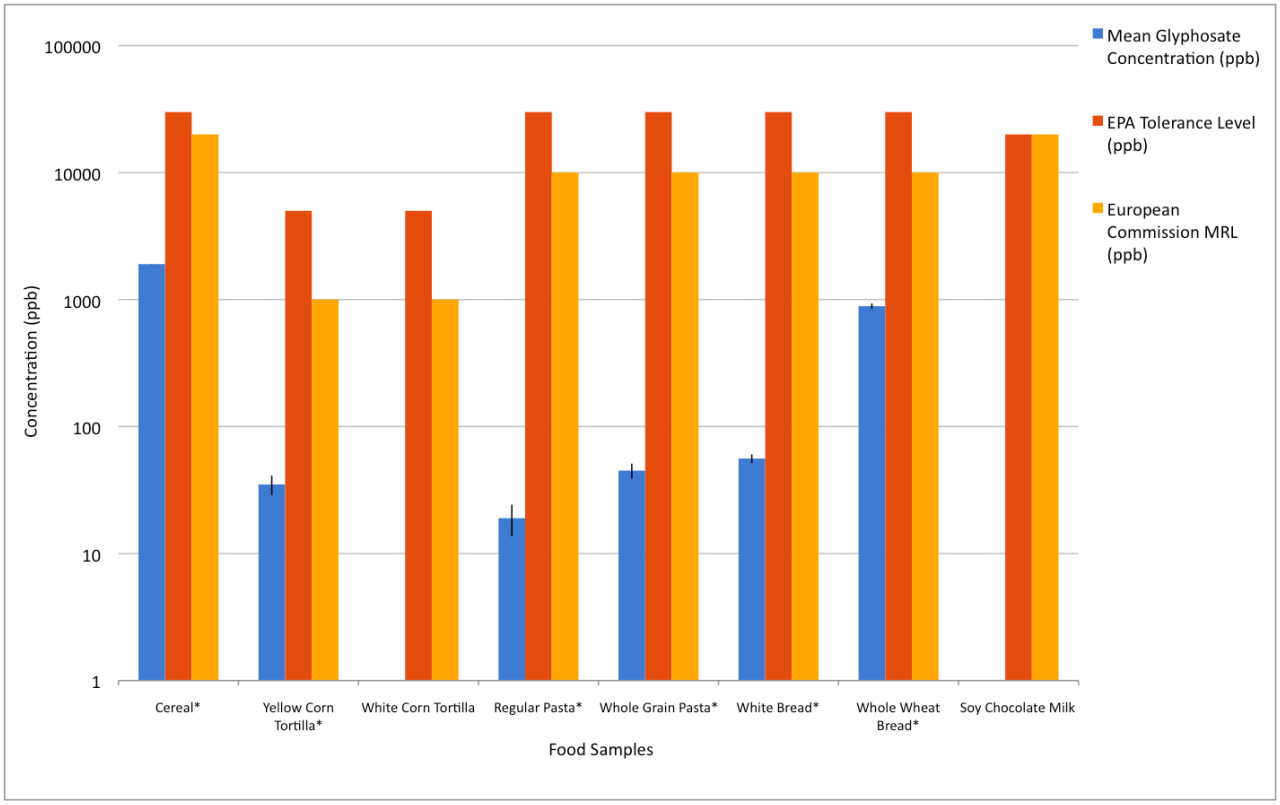

Figure 1. ELISA results reveal glyphosate levels in common household foods are within EPA regulations but may still exceed safe ingestion limits. Three independent trials of competitive ELISA were run to measure glyphosate concentrations in each food. Bars represent the average glyphosate concentrations (ppb) of the three independent trials, error bars represent the standard deviation, * indicates $p < 6.25 \times 10^{-3}$ by *t*-test. The Bonferroni correction, a conservative test that protects from Type I error, was applied to *t*-tests by dividing the initial critical *p*-value ($\alpha = 0.05$) by the number of foods test (n = 8)

The bran, the protective outer layer, and germ are removed from the kernel during processing.

In order to exceed daily regulatory guidelines placed by government agencies, a person would need to consume an overwhelming amount of these foods. To surpass the Australian ADI of 0.3 mg per kg of body weight per day, the average American adult male would have to consume approximately 110 servings of cereal, 2,995 servings of yellow corn tortilla, 5,518 servings of regular pasta, 2,330 servings of whole grain pasta, 3,744 servings of white bread, or 116 servings of whole wheat bread a day. If the average American 2-year-old, 10-year-old, adult male, or adult female fulfilled their USDA grain serving recommendation by only consuming cereal, the food with the greatest glyphosate concentration, their daily glyphosate consumption would still not exceed the Australian or European ADI (30-32).

This research studied a small number of food products and their glyphosate residues. More research is needed to test a wider variety of food products for glyphosate. These experiments were performed under controlled laboratory settings, and three trials were conducted to verify results.

It is interesting to note that while gluten-free and non-GMO labels often prompt consumers to assume certain products have increased health benefits across the board, this is not always the case. For instance, the cereal tested in this experiment was labeled as non-GMO, but it contained the greatest glyphosate concentration among the eight foods tested with an average glyphosate concentration of 1903 ppb (33). United States Department of Agriculture Organic Certification labels are not adequate for consumer awareness concerning pesticide contamination, because glyphosate residues may come from adjacent crops grown with Roundup® or other GBH. If food products containing glyphosate concentrations similar to these samples are consumed on a regular basis, individuals may unknowingly ingest unsafe levels of glyphosate due to lack of awareness and insufficient governmental regulation.

Despite the fact that glyphosate residues in all foods tested were lower than government limits, two were still significantly higher than concentrations associated with negative effects in humans and fish. The United States Food and Drug Administration, along with other governmental organizations, should consider lowering glyphosate tolerance levels to reflect this threshold.

METHODS

Sample Preparation

Cereal, gluten-free yellow corn tortilla, gluten-free white corn tortilla, regular pasta, whole grain pasta, white bread, and wheat bread samples: According to the protocol included

in the Glyphosate Microtiter Plate ELISA Kit (PN500086) purchased from Abraxis Inc., Warminster PA, USA, a 0.5 g aliquot of sample was transferred to a beaker containing 10.0 mL of boiling deionized water. Samples were vortexed, put on a shaker, and allowed to rest. A 2.0 mL aliquot of supernatant was centrifuged. An 800 µL aliquot of glyphosate sample diluent from the ELISA Kit and a 200 µL aliquot of the supernatant were added to a beaker and vortexed.

Soy chocolate milk sample: According to Abraxis procedure, a 0.5 g aliquot of sample was added to 0.5 mL of 1.0N hydrochloric acid and vortexed in a microcentrifuge tube. 40.0 µL of this solution was vortexed in a glass vial containing 3.96 mL of glyphosate diluent.

Sample Derivatization

According to Abraxis procedure, a 1.0 mL aliquot of glyphosate assay buffer was dispensed into test tubes containing 250 µL aliquots of the control, standards 0-5 (containing 0, 0.075, 0.20, 0.5, 1.0, 4.0 ppb glyphosate), and each sample, and vortexed. The derivatization reagent was diluted with a 3.5 mL aliquot of derivatization diluent and 100 µL of this solution was dispensed into each test tube and vortexed. Each test tube was incubated for 10 minutes before ELISA analysis.

Plate Procedure and ELISA Analysis

According to Abraxis procedure, the 100 mL of 5X wash buffer was diluted with 400 mL of deionized water to make a 1X solution. 50.0 µL of the derivatized standard solutions, control, and samples were transferred into the wells containing goat anti-rabbit antibody. New pipette tips were used for every sample to avoid possible contamination. Each pipette tip was conditioned by aspirating and discharging the sample into and from the tip before drawing the correct volume. A 50.0 µL aliquot of the anti-glyphosate antibody solution was added into the wells. The wells were covered with parafilm, mixed, and incubated for 30 minutes at room temperature. A 50.0 µL aliquot of enzyme conjugate was added to the wells that were mixed and incubated for 60 minutes at room temperature. Glyphosate residues in samples competed with enzyme-labeled glyphosate, glyphosate enzyme conjugate horseradish peroxidase, for antigen-binding sites in the microtiter wells. The covering of the wells was removed, and contents washed three times using the 1X wash solution. A 150 µL aliquot of "color solution", composed of hydrogen peroxide and 3,3'5,5'-tetramethylbenzidine, was added to each well and allowed the enzyme-labeled glyphosate in antigen-binding sites to catalyze the production of color, which was inversely proportional to the samples' glyphosate residue concentrations. The wells were covered, and the strips were incubated for 30 minutes at room temperature without light exposure. A 100 µL aliquot of "stop solution", or diluted acid, was added to the wells to terminate the chemical reaction.

Evaluation

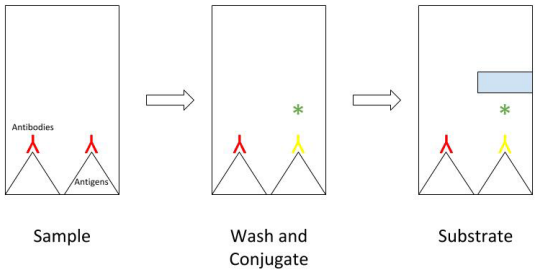

Figure 2. Competitive ELISA process. Competitive ELISA was utilized to determine the foods' glyphosate concentrations. Sample antibodies (red) were added to microtiter plate wells coated with antigens and competed with enzyme-labeled antibodies (yellow) for antigen-binding sites; the introduction of enzyme substrate (green) allowed the sample remaining after wash to catalyze the production of color (blue), which was inversely proportional to the concentration of sample.

Spectrophotometric analysis was used to quantify the color with a microplate ELISA reader measuring absorbance at 450 nm. A commercially available ELISA data solver (Abraxis Inc.) determined the foods' glyphosate concentrations in ppb using the standard curve run with the ELISA. The glyphosate concentration of each food sample was determined in ppb using the standard curve run with the ELISA and was inversely proportional to the resulting color (**Figure 2**). Samples with lower absorbances than a standard had greater glyphosate concentrations than the standard. Sample handling and preparation, such as the addition of boiling water, was taken into account when calculating glyphosate concentrations in ppb.

Statistical Analysis

Lower-tailed *t*-tests were conducted to verify that the foods' glyphosate concentrations were significantly lower than their corresponding government tolerance levels. Two p-values were calculated for each food, one with the null (Ho) and alternative hypotheses (Ha) based on the corresponding EPA tolerance level, and one with Ho and Ha based on the corresponding European Commission MRL.

The critical *p*-values were calculated using the Bonferroni correction, which divided the initial critical p-value ($\alpha = 0.05$) by the number of foods tested ($n = 8$) for comparisons to corresponding government tolerance levels, and by the number of ELISA trials ($n = 3$) for comparisons to other foods. The Bonferroni correction is a conservative test that was applied to protect from Type I error by constricting the critical *p*-value.

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