

# Survey of Glyphosate in Domestic and Imported Beer and Wine

Fernando Rubio, Thomas Glaze, John Lance, Zakary Hutchinson

## Objectives

Analyze domestic and imported beer and wine samples for the presence of glyphosate residues.

## Introduction

Glyphosate or N-(phosphonomethyl)glycine (Fig. 1) is the world's most widely used broad-spectrum herbicide and crop desiccant, accounting for about 25% of the global herbicide market. It is an organophosphorous compound (phosphonate). It was first discovered to be a herbicide by Monsanto<sup>1</sup> and introduced into the market in 1974 under the trade name "Roundup". Due to its high usage, glyphosate tends to be ubiquitous in the environment and our food supply.

Glyphosate is usually sprayed on weeds as a solution. It works by blocking a metabolic pathway involved in the synthesis of three aromatic amino acids (tyrosine, tryptophan, phenylalanine) which are essential for the plant's growth<sup>2</sup>.

Contradictory findings on carcinogenic risks have thrust glyphosate into the center of dispute between EU and U.S. politicians, regulators and researchers. In March 2015 the WHO International Agency for Research on Cancer classified glyphosate as "probably carcinogenic in humans" (category 2A) based on epidemiological, animal and *in vitro* studies<sup>3</sup>. In November 2015, EFSA, published a report, concluding that "glyphosate was unlikely to be genotoxic or pose a carcinogenic threat to humans"<sup>4</sup>. On June 2016, the European Commission could not agree to re-register glyphosate for another 15 years and instead granted a temporary license extension for 18 months pending further scientific studies.

In water, the USEPA has a MCL for glyphosate of 700 ppb. The EU water regulatory limit is 0.1 ppb. The EPA (40 CFR Part 180) has established tolerances for various commodities: grapes have a tolerance of 0.2 ppm. Tolerance for wheat and barley is 30 ppm, and hops 7 ppm.

In vineyards, glyphosate is commonly used to control in-row, under-vine weed growth. A high proportion of vineyards are sprayed with glyphosate two or more times during the year. Glyphosate is applied pre-harvest on barley and wheat to aid during harvesting to speed up harvesting operations.

On February 2016, the Munich Environmental Institute<sup>5</sup>, reported glyphosate residues in all German beers tested. This report shocked the public, since Germany takes pride in their 500 year old beer purity laws. The technique used for the analysis of the samples was ELISA and later confirmed by I.C.

One hundred and twelve (112) domestic and imported beers and thirty (30) wine bottles were purchased around the Philadelphia area and analyzed by ELISA to determine the concentration of glyphosate residues in the samples. The beer samples consisted of ales, lagers, pilsners, mai bocks, IPAs, stouts, and porters. The wine samples consisted of whites such as chardonnays, pinot grigios and reds such as cabernets, merlots, tempranillos, and others.

## Sample preparation

### Glyphosate in Beer

- Pour 2-5 mL of beer into a tube with cap and vortex or invert for 2 min, stopping every 30 sec to unscrew the cap to allow carbonation to escape.
- Dilute 0.2 mL of beer sample with 0.8 mL of Sample Diluent in a test tube, vortex to mix.
- Derivatize sample and test according to ELISA kit instructions (Fig. 2).

### Glyphosate in Wine

- Add 2 mL of wine sample to a micro-centrifuge tube containing 50 mg of "Clean-Up Reagent" and vortex for 30 sec.
- Centrifuge sample at 8000 X g for 5 min.
- Add 0.2 mL of the supernatant to a test tube and 0.8 mL of Sample Diluent.
- Derivatize sample and test according to ELISA kit instructions (Fig. 2).

## Methods

### Glyphosate Plate, Concise ELISA Procedure

**1. Addition of Standards, Samples**  
Add 50 µL of the derivatized standard solutions, control, or samples.

**2. Addition of Antibody Solution**  
Add 50 µL of the anti-Glyphosate Antibody Solution. Cover and mix for 30 seconds. Incubate for 30 minutes at room temperature.

**3. Addition of Enzyme Conjugate**  
Add 50 µL of the enzyme conjugate. Cover and mix for 30 seconds. Incubate for 60 minutes at room temperature.

**4. Washing of Plates**  
Wash the plates three times with 250 µL of 1X washing buffer.

**5. Addition of Substrate/Color Solution**  
Add 150 µL of substrate/color solution. Incubate the strips for 20-30 minutes at room temperature and away from direct sunlight.

**6. Addition of Stopping Solution**  
Add 100 µL of stop solution.

**7. Measurement of Color**  
Read the absorbance at 450nm using a microplate ELISA reader. Calculate the results.

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ABRAXIS, INC.  
124 Railroad Drive, Warminster, PA 18974  
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Glyphosate Plate Kit Part # 500086

Figure 2: Glyphosate ELISA procedure

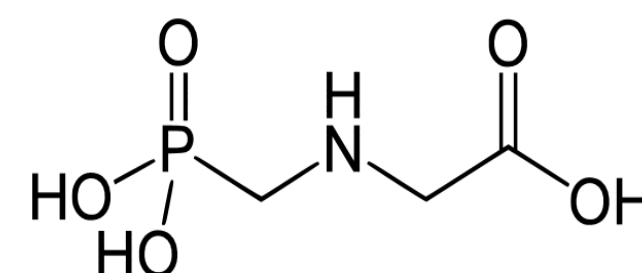


Figure 1: Glyphosate Structure



## Results & Discussion

• The Glyphosate ELISA is very sensitive (Fig. 3) with a quantitation range in water on a direct sample of 0.075 - 5 ppb; LOD = 0.05 ppb. The limit of quantitation on beer and wine samples is 0.38 - 25 ppb (samples with higher concentration are diluted in buffer and re-analyzed). Results obtained in beer and wine samples are shown in Fig. 4, and 5, respectively.

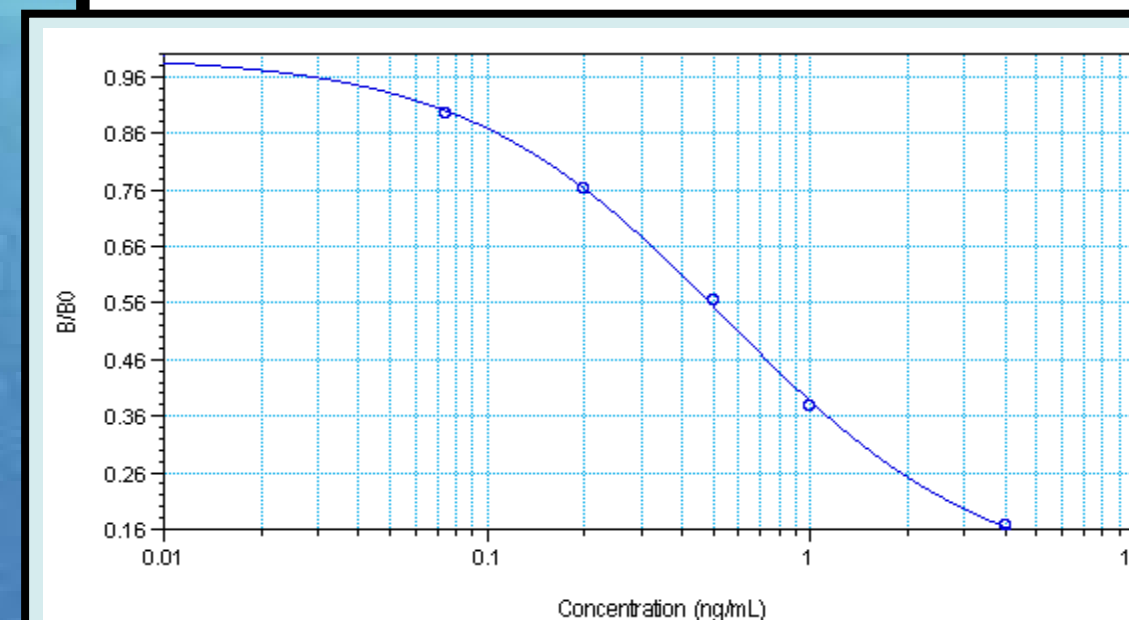


Figure 3: Typical glyphosate ELISA standard curve

### Validation of Method

To validate the matrices the following studies were conducted:

Recovery of glyphosate on each sample was conducted by spiking in glyphosate at 5 and 10 ppb. All recoveries after fortification were between 70 - 130 %.

Serial dilution was conducted on all positive samples. All sample dilutions obtained diluted in parallel.

Table 1. Glyphosate ELISA Specificity

| Compound    | 50% B/B0   | X-React. (%) |
|-------------|------------|--------------|
| Glyphosate  | 0.5        | 100          |
| Glyphosine  | 3000       | <0.02        |
| Glufosinate | 70,000     | <0.001       |
| AMPA        | >1,000,000 | <0.001       |
| Glycine     | >1,000,000 | <0.001       |

## Results & Discussion (continued)

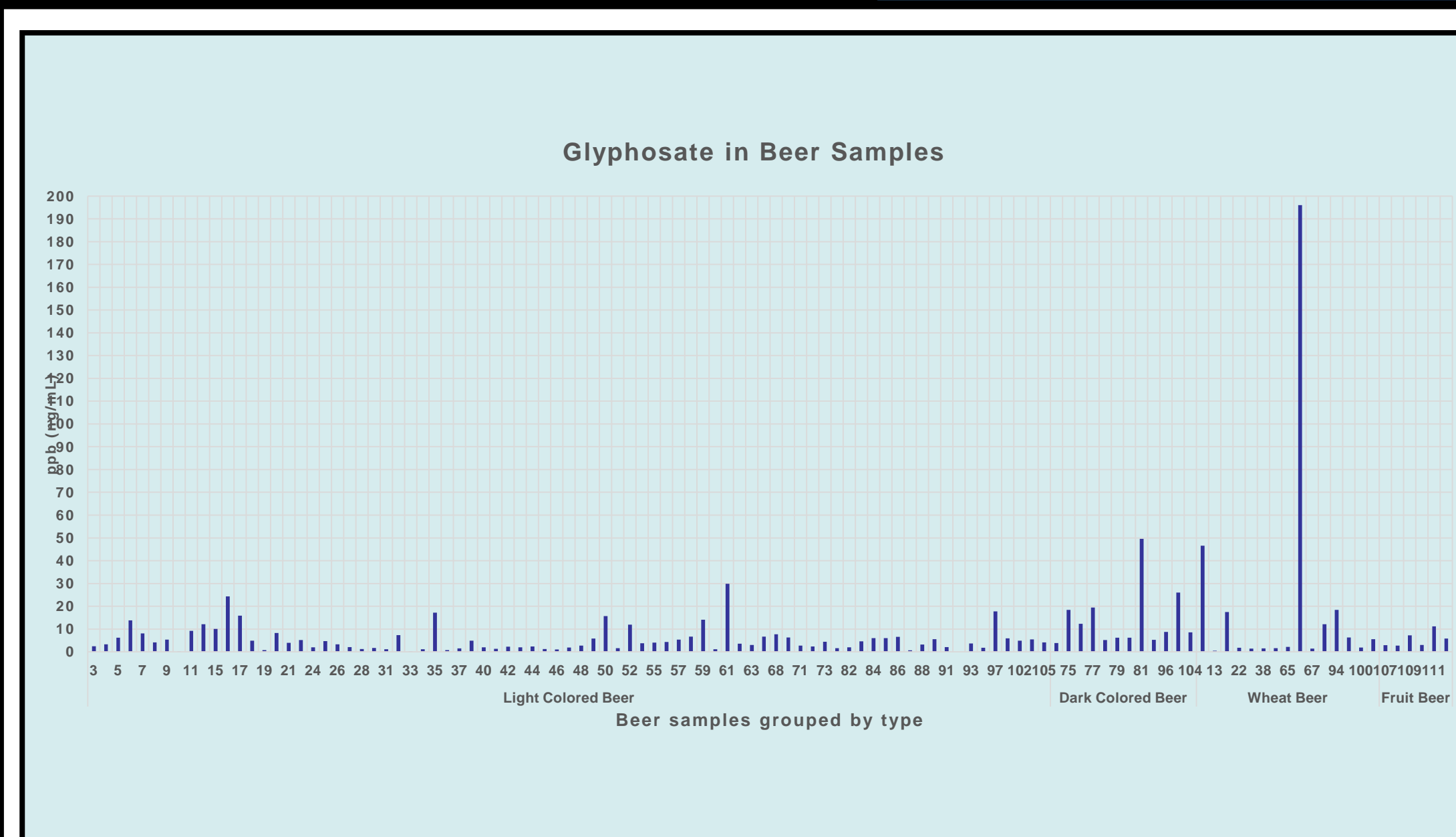


Figure 4: Concentration of glyphosate (ng/mL) obtained on beer samples analyzed.

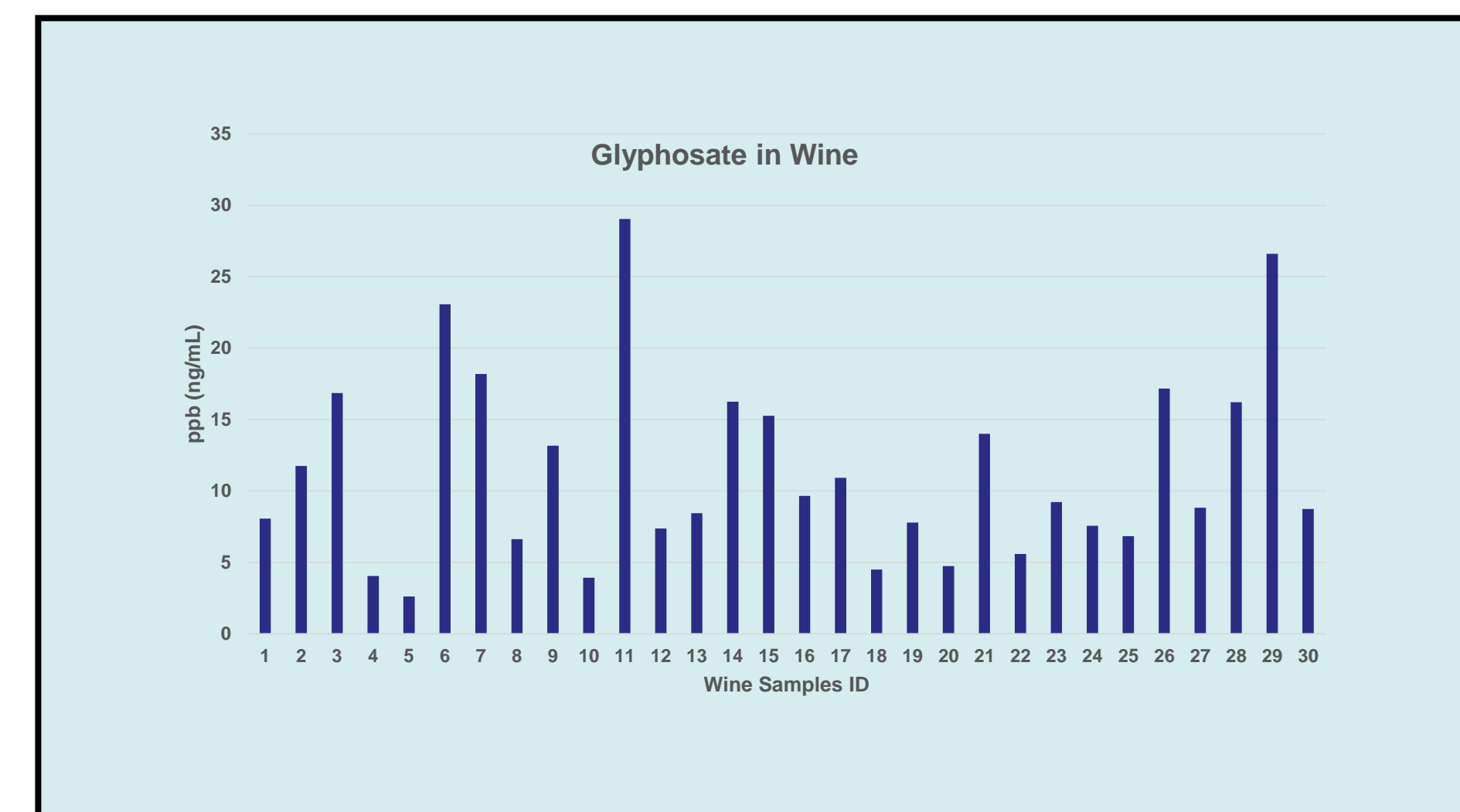


Figure 5: Concentration of glyphosate (ng/mL) obtained on wine samples analyzed.

## Conclusions

• Only 6 of the 112 beers tested exhibited glyphosate concentrations below the LOQ (0.38) ppb of the assay. The following are those beers: Miller Sharp's low alcohol; Kronenbourg 1664 Witbier Wheat; Birra Peroni Pale Ale; Amstel Brewery Light Lager; Molson Coors Pale Lager; Appalachian Brewing Company Ginger Beer. The beer with the highest glyphosate concentration (196 ppb) was Troegs Independent Brewing Co. Hefewizen.

• All 30 wine samples exhibited glyphosate concentrations from 2.6 (Cavit Pinot Grigio, Italy) to 29 ppb (Barefoot Merlot, California).

• Results obtained with additional wine samples and many types of food and beverages will be presented in the future.

## References

1. US patent 3799758, Franz JE, "N-phosphonomethylglycine phytotoxicant compositions", issued 1974-03-26, assigned to Monsanto Company.
2. Steinbüchel HG, Amrhein N (Jan 1980). "The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimate acid-3-phosphate synthase". *Biochemical and Biophysical Research Communications*. **94** (4): 1207-12.
3. Press release: IARC Monographs Volume 112: Evaluation of five organophosphate insecticides and herbicides. International Agency for Research on Cancer, World Health Organization. March 20, 2015.
4. Glyphosate: EFSA updates toxicological profile. European Food Safety Authority. www.efsa.europa.eu. Retrieved 2016-05-25.
5. A study on the contamination of German beer with the pesticide glyphosate. Published On 25 February 2016, the Umweltinstitut München e.V. (Munich Environmental Institute)