Glyphosate exposure, cardiovascular diseases and cancer risks

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Comment on: Pan L, Xu M, Yang D, *et al*. The association between coronary artery disease and glyphosate exposure found in pesticide factory workers. J Public Health Emerg 2017;1:4.

Abstract: Glyphosate (GLY) is a broad-spectrum herbicide with a unique mode of action and an apparent selective toxicity to plants. As far as human health risks are concerned, it has become one of the most controversial pesticides. In the last two years, a possible excess of risk of cancer (i.e., non-Hodgkin lymphoma) associated with exposure to GLY has taken center stage regarding public concerns on health risks posed by pesticides. A recent report by Pan *et al*. (*J Public Health Emerg* 2017;1:4) suggesting that GLY might also be a risk factor for coronary artery disease (CAD) seems to open a new chapter in the ongoing debate on GLY safety. In this article, we commented the major shortcomings of most observational studies that have investigated associations between GLY exposure and adverse health outcomes. The lack of quantitative assessments of exposure (e.g., by repeated measures of urinary levels of GLY) and the fact that studies were not designed to find out dose-response relationships are two of the major weaknesses common to all epidemiology investigations of health hazards posed by GLY exposure. Owing to these methodological limitations, current epidemiology evidence is insufficient to conclude that exposure to GLY increases the risk of cardiovascular disease and or non-Hodgkin lymphoma, or any other type of cancer.

Keywords: Cancer; glyphosate (GLY); coronary artery disease (CAD)

Glyphosate (GLY) is a broad-spectrum herbicide discovered by John E. Franz from Monsanto and first sold in 1974 (1). Owing to its unique mode of action and apparent selective toxicity to plants, GLY was a breakthrough in the research and development of pesticides. GLY selectively inhibits the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase thereby blocking the shikimate pathway, a metabolic route not found in animals that is used by plants, bacteria, fungi, and algae to synthesize aromatic amino acids (tyrosine, phenylalanine, and tryptophan) (1). As far as acute toxicity to vertebrates and environmental impact are concerned, GLY seems to compare favorably with many other herbicides (e.g., 2-4 D, paraquat, atrazine). In the last years, however, this "once-in-a-century" herbicide has become one of, if not the most controversial pesticide. A few years ago, a report by Paganelli *et al*. (2) on GLY-based herbicides induced malformations in frog and chicken embryos, and anecdotal reports saying that there was an increased incidence of birth defects in South America GM-soy crop areas where GLY is extensively used, raised concerns on a possible teratogenicity of this herbicide (2-4). Experimental studies in mammals and epidemiology investigations, however, do not support the notion that exposure to GLY during pregnancy poses teratogenic risks to the unborn child (4-6). In the last two years, a possible excess of risk of cancer associated to GLY has taken center stage regarding to health hazards posed by widespread use of pesticides (7). A recent report by Pan *et al*. (8) suggesting that GLY might be a risk factor for coronary disease seems to open a new chapter in the ongoing debate on GLY safety.

GLY and cardiovascular diseases

A prospective cohort (2 years) study by Pan *et al*. (8), in

Chinese pesticide factory workers, found associations between coronary artery disease (CAD) and GLY exposure (OR; 95% CI: 2.30; 1.075–4.92), overweight or body mass index ≥24 kg/m^2 (1.135; 1.034–1.245), hyperlipidemia (2.085; 1.005–4.328) and alcohol use (9.755; 4.127–23.057). It is of note that overweight, hyperlipidemia, and alcohol abuse (the most impressive OR) are known and independent risk factors for CAD. No association between CAD and other known risk factors for CAD such as diabetes and smoking was found in this group of workers (8). The authors also measured workers' plasma levels of GLY (11.73 µg/L) and its breakdown product aminomethylphosphonic acid—AMPA (5.29 µg/L). Nonetheless, all comparisons were made between a group of exposed workers (directly involved in GLY production lines) and a control group of theoretically unexposed workers who were not directly involved in the manufacture of GLY (8). It is unclear why the authors did not take advantage of the plasma levels of GLY to distinguish between exposed and non-exposed workers, and/or to analyze the data according to exposure level strata. Needless to comment, both groups are exposed to GLY (and AMPA) residues via food intake and this dietary exposure is a major contributor to chronic exposures and thus may be particularly relevant for the development of CAD. As commented elsewhere in this article (studies on cancer risks), lack of a quantitative and reliable assessment of exposure is a major shortcoming of most epidemiology studies that have investigated associations between GLY and adverse health outcomes. Another major weakness of Pan *et al.*'s study is the relatively short duration of the prospective cohort (2 years) to make any inference on cause-and-effect relationships, i.e., between GLY and chronic diseases. Risk factors for the development of chronic and progressive diseases such as CAD probably act much earlier in the individual life history than the preceding two years followed by this cohort study.

At any rate, as far as we are aware, Pan *et al.*'s article is the first report suggesting that exposure to GLY might be a risk factor for coronary heart diseases. Previous studies on cardiovascular toxicity of GLY-based herbicides described short term effects on the heart, such as arrhythmias and QTc prolongation, in cases of intentional (suicide attempts) or accidental poisoning with formulated products (5,9,10). Experimental studies and clinical reports, however, demonstrated that pulmonary and cardiac effects seen in acute intoxication result from ingredients of formulated products other than GLY (i.e., the surfactant polyoxyethylene amine—POEA used in most GLY-based herbicide formulations) (9,10).

An apparent increase in rabbit heart defects after exposure to GLY during pregnancy was not confirmed by most experimental studies, nor was it revealed by epidemiology investigations (4-6).

GLY and cancer risks

In March 2015, IARC-WHO put GLY into category 2A ("probably carcinogenic in humans"), a classification that considerably fueled the debate over health risks associated with exposures to this widely used herbicide (7). The allocation of a compound into 2A category means that there is "limited" evidence in humans and "sufficient" evidence of carcinogenicity in animals. In this case, "limited" human evidence refers to a report on the excess of risk of non-Hodgkin lymphoma among exposed workers (NHL, risk ratio: 1.5; 95% CI 1.1–2.0, subtype: B-cell lymphoma, 2; 1.1–3.6) found by a meta-analysis of case-control studies by Schinasi and Leon [2014] (11). A systematic review and meta-analysis by Chang and Delzell [2016] examined associations between GLY exposure and lymphohematopoietic cancers (LHC) including NHL, multiple myeloma and leukemia (12). Similarly to the previous meta-analysis, Chang and Delzell also found a weak, albeit marginally significant association of GLY with NHL (RR 1.3; 1.0–1.6), and statistically null associations with other LHCs (12).

Classification of GLY as a probable human carcinogen is at variance with previous evaluations by USEPA and the European Union and a more recent assessment by the EFSA (European Food Safety Authority). In October 2015, EFSA arrived at the conclusion that GLY is "unlikely to pose a carcinogenic hazard to humans" (13,14). In June 2016, an evaluation by JMPR (FAO and WHO Joint meeting on pesticide residues) found that GLY "is unlikely to pose a carcinogenic risk to humans from exposure through the diet" (15).

Whether or not causality is the most likely explanation for an observed association is a central issue whenever epidemiology findings have the potential to trigger public health interventions.

The remarkable reflection by Austin Bradford Hill on the aspects of an association to consider before making a causal inference from epidemiologic observations, started by emphasizing the importance of the strength of an association to exclude possible non-causal explanations (16). Along the same line, Richard Doll commented that, according to his experience in conducting epidemiology studies, when relative risks are small (e.g., ≤2:1) the problems of eliminating

bias and confounding are immense, and massive data are generally required (17). As taught by these two of the most outstanding epidemiologists of last century, one should not overestimate a possible cause-and-effect explanation for statistically significant though weak associations. As remind us Hill's lesson, as far as observational studies are concerned, systematic errors are at times more important than random errors (16).

Another major problem in concluding that the association between GLY and NHL is causal is the lack of evidence of a dose-response relationship. In both meta-analyses, RRs were marginally statistically significant for "any" versus "no use" of GLY. If the association between GLY and NHL is causal, one could expect that the heavier and the longer the exposure the higher the incidence of NHL among GLY exposed people. Nonetheless, the meta-analyses provided no evidence of a biological gradient or dose-response relationship (11,12).

Furthermore, studies included in the two meta-analyses assessed occupational and/or environmental exposures to GLY by self-administered questionnaires or telephone interviews (11,12). Non-quantitative or semi-quantitative assessments of exposure to GLY undermine a look for a dose-response relationship. Indirect assessments of exposure to GLY by responses to questionnaires or interviews, and analysis of exposure data as a dichotomous variable ("exposed" versus "not exposed" individuals) is too inaccurate, even if a number of relevant factors such as "wearing protective clothes", "diet", "type of contact" and "amount of pesticide sprayed", and others are taken into account. The absorbed or "internal dose" of GLY received by members of a population is a continuous variable and dichotomization of this variable is likely to make the study less sensitive to detect harmful effects of pesticides on human health when they do occur. GLY is used in soybean and a variety of other edible crops and the general population is exposed to its residues and breakdown products (AMPA) through the diet. Therefore, there is no strictly "unexposed" control group of people. Niemann et al.'s (18) comprehensive review of published and unpublished bio-monitoring studies revealed that urine samples of farm and non-farm individuals (in the EU and in the US) contain GLY in the ppb (μg/L) concentration range. Curwin et al. (19) also measured urinary levels of pesticides in people living in Iowa-US and found detectable levels of GLY in most (>60%) farm and non-farm participants. Along the same line, Acquavella et al. (20) studied a group of 48 US farmers and their families,

and detected GLY in 24-h urine samples of 60% of farmers, 4% of their wives and 12% of the children on the day of GLY spraying (geometric mean concentration was 3 ppb and the maximum value was 233 ppb). The authors also noted that farmers who did not wear rubber gloves had urinary GLY levels higher than those who used this protective equipment (10 versus 3 ppb). Overall, data provided by bio-monitoring studies indicated that both dietary ingestion and occupational exposure contribute to individual internal doses. Moreover, Acquavella et al. (20) data suggested that farmers' exposure is high on the day of pesticide application and that exposure via dermal route (if rubber gloves are not used) may substantially add to the background intake of GLY residues in food. According to Williams et al., estimates based on worst-case assumptions for acute and chronic GLY exposure scenarios, were 125 and 32.3 μg/kg bw/day, respectively (5). For an adult female sprayer, for instance, estimated exposure via diet would account for 23.8 μg/kg bw/day while occupational exposure would account for 56.2 (acute) and 8.5 μg/kg bw/day (chronic) exposures (5). In chronic exposure scenarios, therefore, the diet seems to be a major contributor to the absorbed or internal dose of GLY.

As far as we are aware, no study has investigated whether responses to questionnaire/interview are valid and reliable non-quantitative indicators of increased exposure to GLY.

Concluding remarks

Epidemiology evidence suggesting that exposures to GLY might increase risks of cardiovascular disease and or non-Hodgkin lymphoma, or any other type of cancer, is insufficient to conclude that the reported weak positive associations resulted from cause-and-effect relationships. Nonetheless, owing to the lack of quantitative assessments of exposure and other flaws in study design, available epidemiology studies provide no definitive evidence that GLY exposure is not associated to increased risks of non-Hodgkin lymphoma and coronary heart disease either. In conclusion, human risk assessment of GLY would greatly benefit from additional good quality epidemiology studies, particularly from prospective cohort studies with reliable quantitative estimations of GLY exposure.

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Footnote

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