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Abstract

The usage of glyphosate is increasing worldwide. Glyphosate and its major metabolite, aminomethylphosphonic acid (AMPA), are of potential toxicological concern in unknown chronic kidney disease (CKDu). As with Cd and other elements, glyphosate exposure has been reported as risk factor for CKDu in farmers. This study aimed to evaluate the influence of co-exposure to glyphosate and heavy metals in chronic kidney disease. In this study, the urine samples from 55 patients with CKD and 100 participants without CKD were analyzed for glyphosate, As, Cd, and Pb concentrations, and eGFR. Negative associations between glyphosate, AMPA, As, and Cd concentrations in the urine and eGFR were found for study subjects ($p < 0.05$). With regard to the effect of co-exposure, the odds ratios (OR) for subjects with an eGFR of < 60 mL/min/1.73 m^2 was significant because of the high Cd concentration (> 1 μg/g creatinine; OR = 7.57, 95% CI = 1.91–29.95). With regard to the effect of co-exposure, the OR for subjects with an of eGFR < 45 mL/min/1.73 m^2 was significant at high glyphosate concentration (> 1 μg/g creatinine; OR = 1.57, 95% CI = 1.13–2.16) and As concentration (> 1 μg/g creatinine; OR = 1.01, 95% CI = 1.00–1.02). These results showed that glyphosate, AMPA, As, and Cd have an effect on CKD; notably, Cd, As, and glyphosate exposure can be important risk factors after stage 3a of CKD, and that there was a co-exposure effect of As and glyphosate in CKD after stage 3b. The potential health impacts of glyphosate should be considered, especial for patients with CKD and eGFR below 45 mL/min/1.73 m^2.

Introduction

Since the early 1990s, an unknown chronic kidney disease (CKDu) with compelling tubulointerstitial presentations, called chronic interstitial nephritis, has been reported in agricultural areas of various tropical countries (Jayasumana et al. 2015b; Jayasumana et al. 2015c; Ruwanpathirana et al. 2019), particularly in developing countries without certain chronic etiologies, such as diabetes, hypertension, and glomerulonephritis (Jha et al. 2013). CKDu has spread across rural communities in South Asia, China, and Central America (Correa-Rotter et al. 2014; Jayasumana et al. 2014; Smpokou et al. 2019; Wang, D. et al. 2019). Notably, the overall prevalence of CKDu in Sri Lanka has reached 10% , and is as high as 22.9% in several communities; it is the cause of more than 20,000 deaths annually (Jayasumana et al. 2015a).

Glyphosate, an organophosphorus pesticide, is used in relatively high amounts worldwide owing to the increased planting of genetically modified seeds (Duke et al. 2008; Myers et al. 2016); further, the contamination of the crops, leaf, and seed by glyphosate can be detected in the air, water, and rain (Chang, F. C. et al. 2011; Krüger et al. 2014). Owing to its low vapor pressure (25°C, 9.8×10^{-8} mm-Hg), glyphosate is hardly vaporized into air, which can be adsorbed by the leaves of plants through the soil, and then passed down the phloem to enter the root (Helander et al. 2012). In the eco-environmental system, glyphosate and its major metabolite, aminomethylphosphonic acid (AMPA) (Bai et al. 2016), are of potential toxicological concern, mainly as a result of the accumulation of residues in topsoil (Silva et al. 2018; Yang et al. 2015) and the food chain (Bai et al. 2016). Therefore, the major exposure pathway in humans is through the ingestion of the residuals of glyphosate and AMPA in food (Bai et al. 2016; FSA 2018); the other routes of exposure are inhalation and dermal contact in occupational workers and farmers (Abdul et al. 2021; Cai et al. 2017; Jayasumana et al. 2015c).

A previous study showed that glyphosate has tumor-promoting potential in mouse skin (George et al. 2010), and the International Agency for Research on Cancer defined glyphosate as Group 2A (probably carcinogenic to humans) in 2015 (IARC 2015). However, the European Food Safety Authority has suggested that glyphosate does not appear to be genotoxic and would not be a carcinogen (EFSA 2015). Many recent studies have declaimed that glyphosate has

adverse effects on human health (Chang, Ellen T. et al. 2016; EFSA 2015; Samsel et al. 2013), including impacts on antioxidants, reproductive hormones, and gut microbiome (Ruuskanen et al. 2020), and it may cause acute kidney effects or chronic disease following short-term or long-term exposure (Schaeffer et al. 2020; Tsai et al. 2018; Wimalawansa, Shehani et al. 2014).

In sugarcane farmers with kidney dysfunction in CKDu-emerging regions of Sri Lanka, urinary beta 2-microglobulin and serum cystatin C levels were significantly correlated with urinary glyphosate levels, which is potentially relevant to the subsequent decline in kidney function, as indicated by estimated glomerular filtration rate (eGFR), and albumin creatinine ratio, and neutrophil gelatinase-associated lipocalin (Abdul et al. 2021). Herrera-Valdes (2019) suggested that glyphosate specifically affected the kidneys in which farmers/farmworkers were highly exposed. Moderate to severe coagulative necrosis of hepatocytes and glomerular and renal tubular necrosis were observed when 4.4–750 mg/kg of oral glyphosate was administered daily for 36 weeks in rats (Tizhe et al. 2020). In an *in vitro* study, glyphosate was found to reduce cell viability and induce apoptosis and oxidative stress in a dose-dependent manner in a human renal proximal tubule cell line); this was attributed to the similarity of the chemical structures of glyphosate and AMPA to glycine and glutamate, which are agonists of the N-methyl-D-aspartate receptor, and further to result to an imbalance of oxidant and antioxidative products involved in glyphosate-induced renal proximal tubule epithelium apoptosis (Gao et al. 2019). In addition, glyphosate consumption and acute kidney injury were also observed in toxic epidermal necrolysis (Indirakshi et al. 2017). Therefore, the adverse effects of glyphosate exposure on renal dysfunction should be considered.

Metal exposure has been proven to be associated with kidney dysfunction (Sabath et al. 2012; Tsai et al. 2018; Wimalawansa, S. J. 2016). In Taiwan, the importance of zinc (Zn) and chemical oxygen demand in rivers was demonstrated in a regression model of CKD; a high CKD prevalence was related to arsenic (As) contamination in groundwater in Taiwan (Chang, Kuan et al. 2018); and a high As level of ≥ 50 μg/L in drinking water was a risk factor for end-stage renal disease (Cheng et al. 2018). The higher heavy metal concentrations in farms close to patients' residences were associated with a higher risk of progression to end-stage kidney disease (Tsai et al. 2018).

Jayasumana et al. (2015b) first reported a new form of CKD among paddy farmers, termed Sri Lankan Agricultural Nephropathy in 1994. They found that multiple heavy metals and glyphosate may have a synergistic nephrotoxicity. Meanwhile, they also found that phosphate fertilizers were a major source of inorganic As, which is relevant to CKDu in the endemic areas of Sri Lanka (Jayasumana et al. 2015a). Moreover, Babich et al. (2020) showed that metals in drinking water, even at safe levels, can impede kidney development from an early age, which potentiates increased susceptibility to other agrochemicals, such as glyphosate. The effects of drinking water contaminants on mitochondria can contribute to the progression of kidney dysfunction. However, no evidence was found for the loss of kidney function in participants at risk of mesoamerican nephropathy (MeN) (Smpokou et al. 2019). However, other studies pointed out that there may have been a synergistic effect of glyphosate and hard water on renal injury through mitogen-activated protein kinases /cytosolic phospholipase A2/arachidonic acid and their downstream factors (Wang, R. et al. 2021; Zhang et al. 2021).

Above all, the concern of the risk of kidney disease caused by glyphosate is very important when glyphosate exposure is increasing annually. The aim of this study was to evaluate the influence of co-exposure to glyphosate and heavy metals on CKD. The impact of glyphosate residues on health is warranted in subgroups sensitive to kidney dysfunction, especially in those exposed to metals, which are also related to kidney illness.

Materials And Methods

Subject enrollment

For this cross-sectional study, 55 patients with chronic kidney disease were recruited from the Division of Nephrology of National Cheng Kung University Hospital (NCKUS) and 100 participants with healthy kidney function were recruited from Taiwan Biobank (TWB). The study was approved by the Ethics Committee of NCKUS (Tainan, Taiwan, encoded: A-ER-108-189) and TWB (Taipei, Taiwan, encoded: TWBR10811-05). All participants signed a consent form before sampling started.

Interviewer-administered questionnaire

Demographic information was obtained using a face-to-face questionnaire-based interview in NCKUS, and the information on the 100 healthy participants was provided from TWB. Personal characteristics (including sex, age, height, weight, occupational history, neighborhood geography, and socioeconomics), lifestyle factors (alcohol consumption, smoking habits, drinking other liquids, etc.), and dietary patterns (consumption frequency and quantities) were included in the questionnaires. Meanwhile, histories of familial disease were also recorded in the questionnaires.

Blood serum and urine sampling

In 55 patients with CKD, 1 mL samples of plasma and 7–8 mL of urine were obtained in hospital, stored at 4°C, and then kept at −80°C until analysis. For the 100 study participants from TWB, 0.8 mL plasma and 2 mL urine were stored in glass tubes in the dark and kept at −80°C before metal analysis.

Analysis of urine glyphosate concentrations

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to analyze the concentration of glyphosate and AMPA. The concentrations of glyphosate and its metabolite, AMPA, in urine samples were determined after processing through a liquid–liquid extraction in acidic conditions and analysis using a validated LC-MS/MS method. In addition, creatinine content was measured to correct for diuresis. Then, 1 mL of urine sample and 100 µL of the internal standard (IS) solution (containing 1 ng/mL of each IS) were directly extracted with 1 mL deionized water containing 2% formic acid. The mixture was then vortexed for 3 min, and 1 mL of the mixture was filtered through a 0.2-µm PTFE membrane filter before LC–MS/MS analysis. The LC-MS/MS analysis was performed using an Agilent 1200 Infinity HPLC system coupled with an Agilent 6410 triple quadrupole mass spectrometer (Agilent Technologies, Inc., Palo Alto, CA, USA). The LC separation was conducted using an IC-Pak Anion HR 6 µm, 4.6 mm × 75 mm LC column (Waters, Milford, MA, USA), at a flow rate of 200 µL/min. The mobile phases were water (A) and ACN (B), both were in 2% HCOOH. The injection volume was 20 µL. The initial gradient was 10% B; increased to 41% B at 3 min; to 70% B at 4 min; maintained at 70% B for 4 min; and then returned to 10% B at 10 min. Multiple reaction monitoring data were acquired and processed in negative and positive ESI modes. The following transitions (quantification transitions are underlined) were found to be optimal for the detection: glyphosate, 170 > 88/170 > 60; ^{13}C, ^{15}N glyphosate, 172 > 90.1/ 172 > 62.1; AMPA, 110 > 63.1/ 110> 79.1; and ^{13}C, ^{15}N AMPA, 114 > 63.0/ 114 > 79. The MS-MS nebulizer was set at 40 psi, the dry gas (N_2 99.9% pure) flow was set at 10 L/min, and the source temperature and capillary voltage were kept at 350°C and 4 kV, respectively. Glyphosate and AMPA was quantified using a developed linear calibration, which spanned over two orders of magnitude, with a working range from the

lowest reportable value (0.5 ng/mL) to the highest standard (50 ng/mL); $R^2 > 0.998$. Each analysis batch (10 samples) contained a laboratory blank, a pooled sample, a spiked pooled sample, and a repeatedly spiked sample. The concentration of glyphosate and AMPA in analytical blanks had to be lower than half of the method detection limit (MDL), at 0.003 and 0.012 µg/L, respectively. A QC check standard was analyzed for each batch to verify that the instrument remained properly calibrated and the recovery rates were 90%–111%. The recoveries of glyphosate and AMPA in the spiked samples ranged from 89%–114% and 87%–104%, respectively. The coefficients of variation of glyphosate and AMPA for the repeatedly spiked samples were both <10%.

Analysis of metal concentrations in the serum and urine samples

Blood sampling and analysis details for metal concentrations are available elsewhere (Batista et al. 2009; Palmer et al. 2006). Metal contents were analyzed using inductively coupled plasma-mass spectroscopy (ICP-MS; ICP-MS-ELAN DRC II, PerkinElmer). The recovery efficiencies for Pb, Cd, and As were measured by the addition of a standard solution to samples and the recovery rates in blood were 97% (Pb), 101% (Cd), and 102% (As) in urine, as well as MDLs were 0.047 mg/L (Pb), 0.014 mg/L (Cd), and 0.02 mg/L (As).

Statistical analysis

Metal, glyphosate, and AMPA concentrations were reported in units of µg/g creatinine in urine and µg/L in blood. SPSS 26 (IBM SPSS Statistics) were used for data management and statistical analysis. Chi-squared tests were used to examine the frequency distributions of dichotomous variables—gender, smoking status, and drinking status—in four groups of patients with different stages of CKD. The Kruskal-Wallis and Jonckheere-Terpstra test were used to compare the differences and trends in age, metal concentrations in the blood, in the urine, and glyphosate and AMPA concentrations in the four CKD groups. In addition, to assess the effects of co-exposure to glyphosate and metals, we compared using logistic regression, the odds ratios (ORs) of different CKDs for participants with different levels of exposure. Statistical significance was set at $p < 0.05$.

Results

Demographics and the description of biomarker levels of 155 subjects

The average age of the 105 men and 50 women in the study was 53.1 years of age; 43 subjects were smokers and 15 subjects were drinkers (Table 1). The median glyphosate concentration in urine samples was 0.33 µg/g creatinine (0–12.13 µg/g creatinine), and the median AMPA concentration was 0.17 µg/g creatinine (0–14.64 µg/g creatinine). For the exposure biomarkers, the median concentration of As was 34.4 µg/g creatinine (1.12–1020.73 µg/g creatinine), the Cd concentration was 0.41 µg/g creatinine (0.07–4.66 µg/g creatinine), and the Pb concentration was 4.59 µg/g creatinine (0.18–62.24 µg/L). As a biomarker of kidney function, the average eGFR was 85.12 mL/min/1.73 m^2 (standard deviation: 40.57).

The relationship between exposure biomarkers and eGFR

We further analyzed the correlation between glyphosate, AMPA, metal concentrations, and biomarkers of kidney function in Table 2. Negative correlations were shown with glyphosate (β=−0.521), AMPA (β=−0.541), As (β=−0.388), and Cd (β=−0.580) concentrations and eGFR in the urine samples from the study subjects ($p < 0.05$), separately (Table 2). In addition, significant negative associations were also found between the decrease in eGFR and glyphosate, AMPA, Cd, and As concentrations, even after adjustment for age, sex, and BMI (Table 3).

The relationship between exposure biomarkers and eGFR

Therefore, in the second stage, we categorized all participants into two groups, comprising: subjects without CKD (eGFR>90 mL/min/1.73 m^2), non-CKD; patients with CKD stage 1–3a (60£eGFR<90 mL/min/1.73 m^2); stage 3b (45£eGFR<60 mL/min/1.73 m^2); and stage 4–5 (eGFR<45 mL/min/1.73 m^2). Significant differences in age and smoking status were found among the four groups, but without a consistent increase or decrease in trends (Table 4).

For exposure biomarkers (Table 5), the average glyphosate concentrations in urine samples in the non-CKD, and stage 1–3a, 3b, and 4–5 groups were 0.38, 2.67, 2.29, and 3.62 µg/g creatinine, respectively; the AMPA concentrations were 0.25, 2.35, 2.02, and 2.09 µg/g creatinine, the average concentrations of As were 35.11, 95.96, 161.04, and 118.49 µg/g creatinine, and the average concentrations of Cd were 0.41, 1.11, 1.35, and 2.04 µg/g creatinine, respectively. There were significant differences between all four groups ($p < 0.05$). In the test for trends in biomarker exposure among these four groups, trends were found for glyphosate and Cd among these four CKD stages (Figure 1).

Further, dichotomized groups were categorized for eGFR <60, or <45 mL/min/1.73 m^2, and ORs of subjects with eGFR below or above the two groups were calculated from logistic regression analysis. Higher glyphosate values were significantly related to an increased risk of a decrease in eGFR compared with the glyphosate lower group after adjustment for age, sex, BMI, and the interaction terms of glyphosate, Cd, and As concentrations. In Model 1, the OR for eGFR<60 was significant because of the high Cd concentration (> 1 mg/g creatinine; OR = 7.57, 95% CI = 1.91–29.95) and the OR was greater than 1 for high glyphosate concentrations (> 1 mg/g creatinine; OR = 1.39, 95% CI = 0.90–2.15), but the association was not significant. In Model 2, the OR for eGFR<45 was significant because of the high glyphosate (> 1 mg/g creatinine; OR = 1.57, 95% CI = 1.13–2.16) and As (> 1 mg/g creatinine; OR = 1.01, 95% CI = 1.00–1.02) concentrations, but the high Cd concentration (> 1 mg/g creatinine; OR = 1.85, 95% CI = 0.83–4.11) was not significant. The OR for the decrease in eGFR varied because of the different CKD stages.

Discussion

Biomarker of glyphosate exposure

In the metabolism analysis, the maximum concentrations of glyphosate and AMPA were observed at 2.42–5.16 h after the intravenous injection of glyphosate (Anadon et al. 2009). A study was designed in which 12 participants consumed a test meal with a known concentration of glyphosate residue and a lower concentration of AMPA, and the results showed that the estimated elimination half-life for glyphosate was 9 h (Zoller et al. 2020). Therefore, the measurements of glyphosate and AMPA in urine samples can be a short-term marker for external exposure.

Mills et al. (2017) have reported that the average glyphosate and AMPA concentrations in the general population of USA were 0.024 µg/L and 0.314 µg/L, increasing from 0.008 µg/L to 0.401 µg/L in 1993–1996 and 2014–2016 in the aging healthy population in the United States, whereas a decreasing trend in glyphosate residues in urine in Germany youngers was found (Conrad et al. 2017). This study and the other one all found that chronically ill patients

had significantly higher urinary concentrations of glyphosate residues than healthy individuals (Krüger et al. 2014). Moreover, the glyphosate and AMPA concentrations in urine samples from adults and elders in this study were higher than those found in other countries (Krüger et al. 2014; Mills et al. 2017).

Although a review paper that collected data from seven studies revealed no health concerns because the glyphosate exposure estimation for general population was far below than "acceptable daily intake" or "acceptable operator exposure level", exposure was predominantly resulted from the occupational and dietary exposure routes in Europe and North America (Niemann et al. 2015).

Many researchers have reported that glyphosate and AMPA residues were present in soy-based infant formula, maize-derived food, beer, wine, fruit juice (González-Ortega et al. 2017; Jansons et al. 2018; Rodrigues et al. 2018), and that glyphosate was detectable in nearly all honey samples in Switzerland (Zoller et al. 2018) and in American mothers' breastmilk (Honeycutt et al. 2014). Therefore, the evaluation of glyphosate exposure via food consumption in patients with CKD is important.

With regard to As, higher exposures were found in this study compared with biological monitoring data from urine samples of other countries (Aguilera et al. 2008; Feng et al. 2015; Morton et al. 2014). For Cd exposure, the urine Cd concentrations in this study were clearly higher than those of other studies in Western countries (Aguilera et al. 2008; Baeyens et al. 2014; Heitland et al. 2006; Morton et al. 2014; Tellez-Plaza et al. 2008) and in Thailand (Nishijo et al. 2014), but were equal to those reported by Liao et al. in Taiwan (Liao et al. 2019). Overall, the concentrations of bio-exposure markers, such as glyphosate, AMPA, Cd, and As, were higher than those of other countries.

Metals, glyphosate exposure and renal function

In discussing the relationship between exposure biomarkers and eGFR, the variation in climate, temperature, air quality, water quality, and drought, and exposure to fertilizers, soil conditioners, herbicides, fungicides, and pesticides have been considered as contributing factors for the development of CKD in South Asia (Wilke et al. 2019). For example, in Thailand, the serum creatinine concentrations were associated with glyphosate use and pesticide exposure index in the occupational group (Mueangkhiao et al. 2020), which indicated that glyphosate exposure might be related to renal dysfunction. Meanwhile, an acute kidney injury developed after the ingestion of glyphosate-based herbicide, indicating epithelial injury in proximal tubules, and glyphosate mitochondrial toxicity was also found (Kimura et al. 2020). Other environmental contaminants, such as heavy metals (e.g., Cd, As, and Pb) and organic pesticides (e.g., glyphosate) in the drinking water, even at safe levels, can impair kidney development at an early age; and may play a role in the childhood onset and progression of kidney dysfunction (Babich et al. 2020). However, in three cohorts across different phases of child development, the authors confirmed detectable glyphosate in children's urine at various ages and stages of development, but found no evidence for renal injury in children exposed to low concentrations of glyphosate (Trasande et al. 2020). Meanwhile, Gunatilake (2019) suggested that glyphosate's synergistic health effects when combined with paraquat, and the continuous high temperatures of lowland tropical regions could result in renal damage. Glyphosate exposure, such as enhancing the growth of Clostridia species and ruminal metabolism *in vitro* (Riede et al. 2016), promotes As toxicity in renal dysfunction (Jayasumana et al. 2015a), and the low-dose exposure of glyphosate-based herbicides disrupted the urine metabolome and its interaction with gut microbiota has been dysregulated in related diseases through the commensal microbiome (Hu et al. 2021). Overall, several pathologies associated with MeN, a type of CKDu, may be linked to glyphosate exposure, such as altered gut microbiota (Rueda-Ruzafa et al. 2019), increased As toxicity, suppressed synthesis of adrenocorticotropic hormone, disruption of fructose metabolism, and promotion of dehydration and high serum urate (Seneff et al. 2018).

For metal exposure, the higher concentrations of Zn and nickel (Ni) in farms close to the residence of patients with CKD were associated with a higher risk of progression to end-stage kidney disease in Taiwan (Tsai et al. 2018). An epidemiology study has suggested that high plasma selenium (Se) and low red blood cell Pb levels or Cd levels can interact to increase the eGFR (20.70, 15.56–26.01 mL/min/1.73 m^2) in CKD (Wu et al. 2019). However, a report showed that the non-association between glyphosate, aluminum (Al), and As exposure and decreased kidney function in 350 young adults living in area of Central America with an epidemic of MeNs (Smpokou et al. 2019). Meanwhile, the exposure and risk assessment showed that there was no treatment risk with glyphosate (Honeycutt et al. 2014; Krüger et al. 2014; Niemann et al. 2015). All of the above studies were retrospective, and used biological biomarker data for external exposure, and APMA was not included in the above studies. However, in our study, the co-exposure of As and glyphosate was found in patients after CKD stage 3b. The alternative importance of glyphosate and Cd or As exposure in the progression of CKD have been seen in patients with CKD stage 3 or above. These results can respond to the conclusion from Seneff et al. (2018), who reported that the most likely way to prevent end-stage renal failure in sugarcane workers was to stop the use of glyphosate in Brazil, and that the progression of patients with CKD into end-stage renal failure may be prolonged by a reduction in glyphosate exposure.

Conclusions

In this study, we observed that glyphosate, AMPA, As, and Cd affected CKD; notably, Cd exposure may be an important risk factor after CKD stage 3a, and As and glyphosate have a synergistic effect following co-exposure in patents with CKD beyond stage 3b. Therefore, the potential health risks of glyphosate must be considered, especial for patients with CKD and eGFR values below 45 mL/min/1.73 m^2.

However, a comprehensive analysis of chemical contaminants in the drinking water and the effects of these compounds and their mixtures on kidney development and function is missing. Therefore, this study is a starting point for the discussion of the etiology and the progression of kidney disease; and analytical models for alternative agro-chemicals can provide other insights in future. Furthermore, the relationships should be followed up in a large population of patients with CKD to increase the power of the statistical analysis and allow consideration of more factors that co-influence CKD progression.

Declarations

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Authors' contributions

Junne-Ming Sung contributed to conceptualization, methodology, validation, subjects' recruitment; Wei-Hsiang Chang, Chung Yu Chen contributed to formal analysis, writing; Kuan-Hung Liu contributed to subjects' recruitment, methodology; Trias Mahmudiono, Ho-Chi Hsu contributed to review, sampling and formal analysis, Wan-Ru Wang, Zhen-Yi Li contributed to data curation, visualization; Hsiu-Ling Chen contributed to conceptualization, methodology, validation, writing – original draft, editing and funding acquisition.

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No.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval The study protocol was approved by the Ethics Committee of NCKUS (Tainan, Taiwan, encoded: A-ER-108-189) and TWB (Taipei, Taiwan, encoded: TWBR10811-05) in accordance with the Ethical Principles for Medical Research Involving Human Subjects.

Consent to participate

All study participants provided written informed consent.

Consent for publication

No need.

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Tables

Table 1

Demographic results of all participants

	Population (N=155)
Demographics	
Sex	
Men	105 (67.7)[a]
Women	50 (32.3)[a]
Age	53.19±16.09[b]
BMI	25.21±4.46[b]
Body Weight	69.48±16.14[b]
Smoking	43 (27.7)[a]
Drinking	15 (9.7)[a]
CKD stage	
Non CKD	99 (63.9)[a]
1	3 (5.45)[a]
2	10 (6.45)[a]
3a	11 (7.10)[a]
3b	15 (9.68)[a]
4	10 (6.45)[a]
5	7 (1.02)[a]
Exposure indicator	
Glyphosate(μg/L)	1.10 (0, 11.28)[c]
Glyphosate (μg/g creatinine)	1.16 (0, 12.13)[c]
AMPA(μg/L)	0.79 (0.01, 6.73)[c]
AMPA(μg/g creatinine)	0.86 (0, 14.64)[c]
As (μg/g creatinine)	62.34 (1.12, 508.82)[c]
Cd (μg/g creatinine)	0.78 (0.07, 4.66)[c]
Pb (μg/L)	6.11 (0.08, 62.24)[c]
Biomarker of kidney function	
eGFR (mL/min/1.73m^2)	85.06±40.70 [b]
Creatinine_urine (g/L)	1.68 (0.33, 3.81)[c]

Creatinine_serum (mg/dL)	1.38 (0.40, 9.15)[c]

[a] Number and parentheses with percentage

[b] Mean±SD

[c] Median and parentheses with Minimum and Maximum

Table 2

The correlation among glyphosate, metals and biomarkers of kidney function

N=155 r[@]	Glyphosate (µg/g creatinine)	AMPA (µg/g creatinine)	As (µg/g creatinine)	Cd (µg/g creatinine)	Pb (µg/L)	Creatinine (mg/dL)	eGFR (mL/min/1.73m^2)
Glyphosate		**0.569****	**0.477****	**0.723****	0.033	**0.538****	**-0.526****
AMPA			**0.471****	**0.653****	0.115	**0.495****	**-0.540****
As				**0.580****	0.064	**0.356****	**-0.384****
Cd					0.009	**0.550****	**-0.585****
Pb						-0.058	0.067

[@] Spearman correlation coefficient * p<0.05, ** p<0.001

Table 3

The association between different exposure biomarkers and eGFR

Independent variable	β	p-value[@]
Glyphosate (µg/g creatinine) [¶]	-5.216	**0.001***
AMPA (µg/g creatinine) [¶]	-2.315	0.070
As (µg/g creatinine) [¶]	-0.107	**0.002***
Cd (µg/g creatinine) [¶]	-18.348	**0.001***

[¶] Adjustment for age, sex, BMI

[@] Linear regression model, * p<0.05

Table 4

Demographic results of participants in different CKD stage

CKD stages	Non-CKD	1-3a	3b	4-5	P-value
N=	99	25	14	17	
Sex					0.183[c]
Men	64 (64.6)[a]	15 (60.0)[a]	12 (85.7)[a]	14 (82.4)[a]	
Women	35 (35.4)[a]	10 (40.0)[a]	2 (14.3)[a]	3 (17.6)[a]	
Age	45.98±12.51[b]	65.04±11.02[b]	71.14±15.84[b]	63.00±14.90[b]	**<0.001*[d]**
BMI	24.78±4.53[b]	25.66±4.80[b]	25.58±4.16[b]	26.72±3.59[b]	0.362[d]
Body Weight	69.83±17.52[b]	67.98±13.68[b]	67.67±11.27[b]	71.17±15.23[b]	0.893[d]
Smoking	33 (33.3)[a]	2 (8.0)[a]	1 (7.1)[a]	7 (41.2)[a]	**0.012*[c]**
Drinking	9 (9.1)[a]	2 (8.0)[a]	1 (7.1)[a]	3 (17.6)[a]	0.692[c]

[a] Number and parentheses with percentage

[b] Mean±SD

[c] Chi-square test

[d] One-way ANOVA

* p<0.05

Table 5

Difference of biomarker levels in different CKD stage

CKD stages	Non-CKD	1-3a	3b	4-5	P-value@
N=	99	25	14	17	
Glyphosate (μg/g creatinine)	0.31±0.22	2.28±2.57	2.33±2.37	3.62±3.64	**<0.001****
AMPA (μg/g creatinine)	0.22±0.57	1.95±2.96	1.99±2.34	2.09±3.62	**<0.001****
As (μg/g creatinine)	34.12±34.63	109.66±120.55	112.63±61.83	118.49±93.06	**<0.001****
Cd (μg/g creatinine)	0.36±0.21	1.46±0.96	1.01±0.70	2.04±1.25	**<0.001****
Pb (μg/L)	5.55±3.65	6.77±8.12	6.29±5.65	8.3±14.84	0.751
eGFR (mL/min/1.73m^2)	109.15±24.86	64.58±20.74	36.43±4.64	15.88±8.02	**<0.001****
Creatinine (mg/dL)	0.78±0.21	1.12±0.33	1.81±0.29	4.80±2.69	**<0.001****

Data showed as Mean ± SD, ** means statistical significant different

@ Kruskal Wallis-test

Table 6

The odds ratio (OR) of eGFR decrease by glyphosate, AMPA, Cd and As levels

N=155	OR (95% CI)	p-value@
Model 1:eGFR≧60 vs. <60 ¶		
Glyphosate (μg/g creatinine)	1.388 (0.896-2.150)	0.142
AMPA (μg/g creatinine)	0.959 (0.646-1.424)	0.836
Cd (μg/g creatinine)	7.567 (1.912-29.949)	**0.004***
As (μg/g creatinine)	1.006 (0.997-1.015)	0.208
Model 2:eGFR≧45 vs. <45 ¶		
Glyphosate (μg/g creatinine)	1.566 (1.134-2.162)	**0.006***
AMPA (μg/g creatinine)	0.998 (0.762-1.307)	0.987
Cd (μg/g creatinine)	1.851 (0.833-4.111)	0.131
As (μg/g creatinine)	1.008 (1.000-1.015)	**0.038***

¶Adjustment for age, sex, BMI and interaction terms of glyphosate, Cd and As levels

@Logistic regression model

Figures

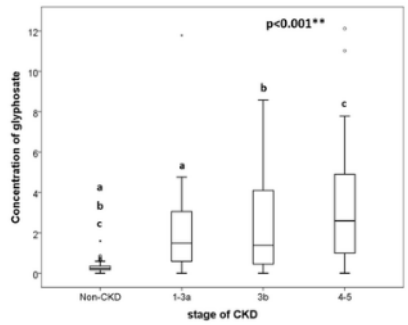


Figure 1-1

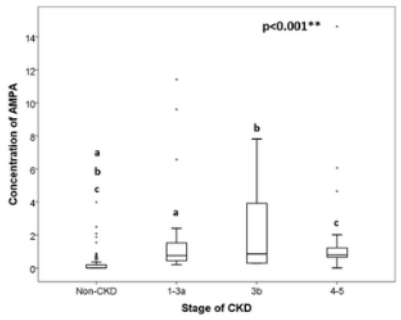
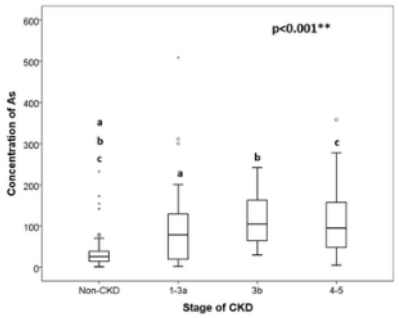


Figure 1-2



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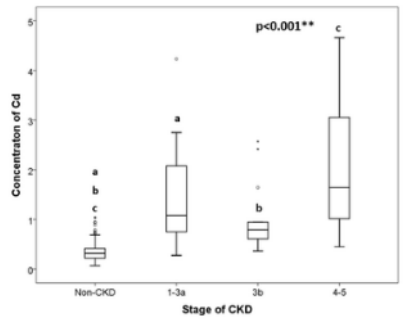

Figure 1-4

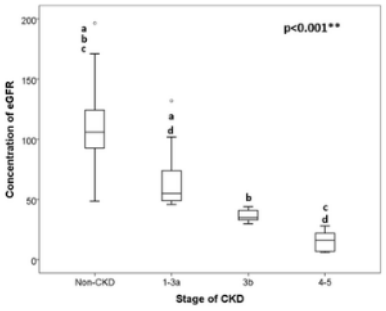

Figure 1-5

Figure 1

Trend of biomarkers of all participants in different CKD stage Note: The values with the same superscript letters are significantly different by Jonckheere Trend-test ($p<0.05$).

The Effect of Co-exposure to Glyphosate, Cadmium, and Arsenic on Chronic Kidney Disease

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