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ORIGINAL ARTICLE

Dietary Exposure of Filipinos to Ochratoxin A and Glyphosate from Commonly-Consumed Foods using Theoretical Maximum Daily Intake (TMDI) Approach

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KEYWORDS

ADI;
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ABSTRACT: Ochratoxin A (OTA) is a mycotoxin resulting from poor storage conditions while glyphosate is an herbicide used in agricultural production. Exposure to foods contaminated with these substances may result in unfavorable health problems. This study, therefore, aimed to estimate the exposure and characterize the risk of the Philippine general population, children, and women of childbearing age (WCBA) to OTA and glyphosate using the Theoretical Maximum Daily Intake (TMDI) approach based on WHO guidelines. The dietary exposure was estimated using individual food consumption data from the National Nutrition Survey 2008 combined with maximum levels set by relevant authorities. The risk was characterized by evaluating the exposure estimates against the set acceptable daily intake (ADI) for glyphosate, and permissible tolerable weekly intake (PTWI) for OTA. Results revealed that the population groups had low exposure to glyphosate at 16 to 59% of its ADI. Cereal grains and flour (98%) were found to be the major contributor to dietary intake. However, consumers among children and WCBA were highly exposed to OTA at 163 and 314% PTWI, respectively. Bread and rolls (57%) and maize (31%) were the major contributors of OTA among children and WCBA, respectively. Based on the findings, it is recommended to conduct a refined exposure assessment by analyzing actual OTA values of the identified food contributors to validate the results of this study.

INTRODUCTION

Food contamination due to chemicals from natural or anthropogenic sources is a significant source of foodborne illness worldwide, the health implication of which may range from mild to severe risks [1]. Food is susceptible to contamination during the long chain of production from the field to the plate. Environmental contaminants entering the food chain include metals, mycotoxins, antimicrobials, polycyclic aromatic hydrocarbons (PAHs), pesticide residues, and dioxins [2], in which concentration above the reference toxicological limit potentially affect human health.

Mycotoxins are naturally occurring contaminants formed through the action of fungi usually from *Aspergillus, Fusarium,* and *Penicillium* species [3, 4]. Mycotoxins, in general, have been reported to be carcinogenic, and are considered as priority food chemical contaminants based on WHO [4–6]. Ochratoxin A (OTA) is a toxic mycotoxin and the most prevalent ochratoxin, formed during storage and post-harvest [5, 7]. OTA is normally found in staple food products and highly consumed foods such as cereals, coffee, spices, dried fruits, among others [4, 5, 8]. To date, the Philippines have established

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national standards and codes of practice for the prevention and reduction of ochratoxin A in coffee and tablea [9,10], and reduction of mycotoxin in cereals [11].

Pesticides, meanwhile, are commonly applied in agricultural produce at regulated levels to mitigate crop losses due to '*pests and diseases'* and can be classified as an *insecticide, fungicide, bactericide, nematicide, herbicide, molluscicide, avicide, rodenticide, plant regulator, defoliant, desiccant, and the like* [12–14] depending on their mode of action and function. Exposure to excessive pesticides through direct contact, inhalation, and ingestion can have an acute and chronic effect such as poisoning, carcinogenic, neurological, reproductive effects, among others [13,15]. Glyphosate is a widely used non-selective herbicide used to control weeds and grasses by inhibiting the action of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) preventing protein synthesis [16–18]. Over time, there is an increase in the use of glyphosate-based herbicides (GBH) and cases of GBH residues were detected in market foods such as cereals and pulses are common [19,20]. The Internal Agency for Research on Cancer (IARC) classified glyphosate to Group 2A which meant "probably carcinogenic in humans" [21] and a recent study have suggested that increased exposure to GBH may also develop inflammatory diseases [18].

Exposure assessment, an integral part of the risk assessment process, has been widely developed as a tool in analyzing and quantifying safety concerns. The increasing count of safety issues regarding food contaminants constitutes an apparent need for this tool to assess the probability of the exposed population to potential risk. Concentration data, coupled with consumption data, is the basic requirement in the conduct of dietary exposure assessment. In the Philippines, the availability of a national exposure data is hampered by the lack of concentration data of these contaminants in foods.

The Theoretical Maximum Daily Intake (TMDI) is one of the simplest approaches in dietary exposure assessment developed by Codex Alimentarius, detailed in the Guidelines CAC/GL 3-1989. TMDI is a screening dietary exposure assessment method used for identifying risk assessment priorities for further refined exposure studies, as part of a cost-effective stepwise approach to exposure assessment. This method assumes that the concentration of contaminants in the food is at its designated maximum limit.

The study aimed to provide conservative dietary exposure of the Filipino population group to Ochratoxin A and glyphosate in commonly-consumed foods using the TMDI approach. The conduct of exposure assessment aimed to protect the public from the health concerns brought about by the consumption of high levels of harmful compounds in the diet. The results then identified the most susceptible Filipino population and determined priority areas for refined and advanced exposure activities.

MATERIALS AND METHODS

Dietary exposure of Filipinos to OTA and glyphosate was assessed in three major phases: food mapping; intake estimation; and risk characterization (Figure 1).

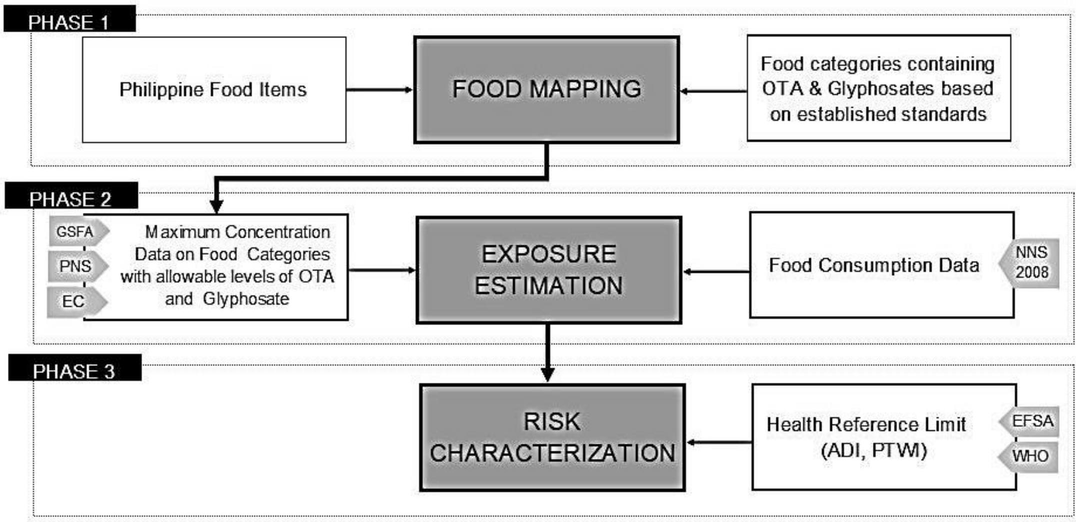

Figure 1. Conceptual framework of the study.

Food mapping

A list of food categories with possible occurrence of OTA and glyphosate was prepared and consolidated from established international and national food standards (e.g., Codex GSFA, PNS/BAFS, and European standards). Philippine food items were then 'mapped' and 'matched' into these food categories by appropriate definition and standard provisions to establish the final list of Philippine food categories. The Philippine food items used in food mapping were imported from the FNRI's food consumption database for exposure assessment, which was developed in 2012 based on WHO requirements.

Exposure estimation

Food Consumption Data

Individual two-day, non-consecutive 24 h dietary food recall from the 2008 Philippine National Nutrition Survey was used in the study. The database was harmonized with the WHO food classification system requirements for exposure assessment. Survey data contained information on the number of subjects and the number of consumers; while food consumption data (g per day) for mean and 95th percentile were statistically analyzed for the general population, children, and women of child-bearing age (WCBA).

Concentration Data of OTA and Glyphosates in Foods

The maximum residue limits (MRLs) set by different standards per food category were used as the concentration data (also referred to as occurrence data) of OTA and glyphosates in determining the exposure estimate. MRLs of OTA in different commodities were derived from the European Union Commission Regulation No. 123/2005 [23]. For glyphosates, MRLs were lifted from Codex GSFA24 and various PNS/BAFS standards 158-162:2015 depending on the food group [24–29].

Theoretical Maximum Daily Intake (TMDI)

Dietary exposure was assessed by combining food consumption data and the concentration of the permitted levels and residue limit in food. Body weights were based on the recommended weight per age and gender groups published in the 2015 Philippine Dietary Reference Intake (PDRI) [30]. The TMDI approach was applied for the calculation of dietary exposure to ochratoxin A and glyphosate. Food contaminant data is multiplied by the daily food consumption as shown in equation 1 [22].

$$\text{Dietary exposer} = \sum (\text{concentration of food contaminant in food} \times \text{food consumption}) / \text{body weight (kg)} \quad (1)$$

The contaminant intake was estimated for the general population and different physiological groups of consumers and non-consumers (all subjects), and consumers only. Assumptions made were a) all foods, in which the food contaminant is permitted, contain the additive, b) the food contaminant is always present in the maximum amount (permitted/ residual), c) the food in question containing the contaminant are consumed by people every day of their lives at mean per capita level, d) the amount of the food contaminant in the food does not change as a result of storage, cooking or processing technique, and e) all foods permitted containing the food contaminant are ingested and nothing is discarded.

The mean exposure to food additives per food category was multiplied by the mean and high (95th percentile) daily food consumption divided by the average body weight of the age group. The summation of which resulted in the estimated daily exposure for 'all subjects' and 'consumers only' of Philippine population age groups to glyphosates and OTA to commonly-consumed foods. 'All subjects' refers to all respondents in the survey which included consuming and non-consuming population, whereas 'consumers' included only the respondents which consumed the food items of interest.

Risk characterization

The level of risk was characterized by comparing the estimated intake with the relevant health reference value. The European Food Safety Authority (EFSA) (2006) has designated provisional tolerable weekly intake (PTWI) of 120 ng kg^{-1} bw week^{-1} for OTA [31]. The Joint Meeting on Pesticides Residues (JMPR), meanwhile, has designated an acceptable daily intake (ADI) of up to 1 mg kg^{-1} bw day^{-1} for glyphosate [32, 33].

RESULTS

TMDI of Ochratoxin A

The TMDI estimate of the Philippine population covered the general population, children, and WCBA to OTA (Figure 2 and Figure ***3***). Figure 2 showed the exposure expressed in nanogram per kilogram of body weight per week (ng.kg^{-1} bw.week^{-1}), while Figure 3 showed the exposure expressed as the percentage of the PTWI for OTA.

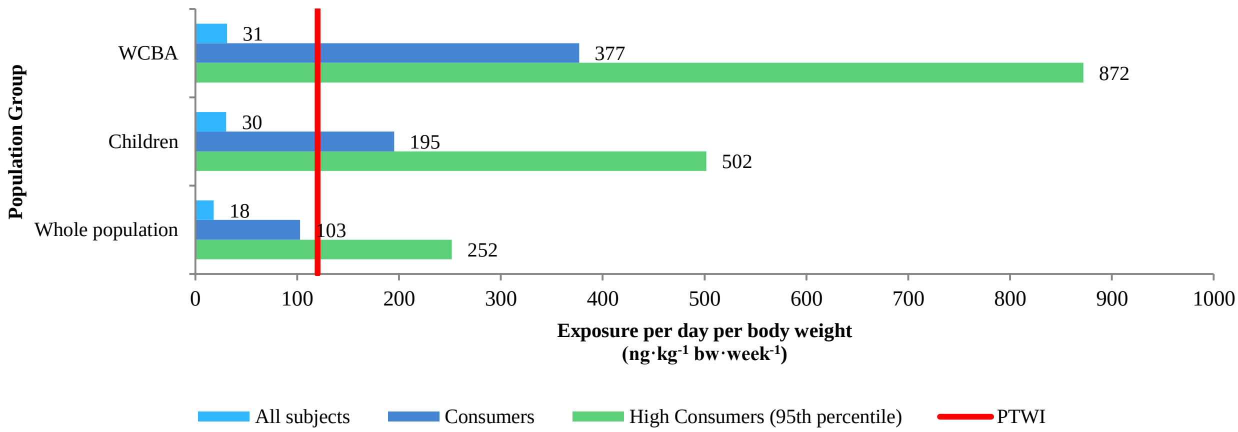

Figure 2. Dietary exposure of different Philippine population groups to ochratoxin A. expressed as ng kg^{-1} bw week^{-1} (PTWI: 120 ng kg^{-1} week^{-1})

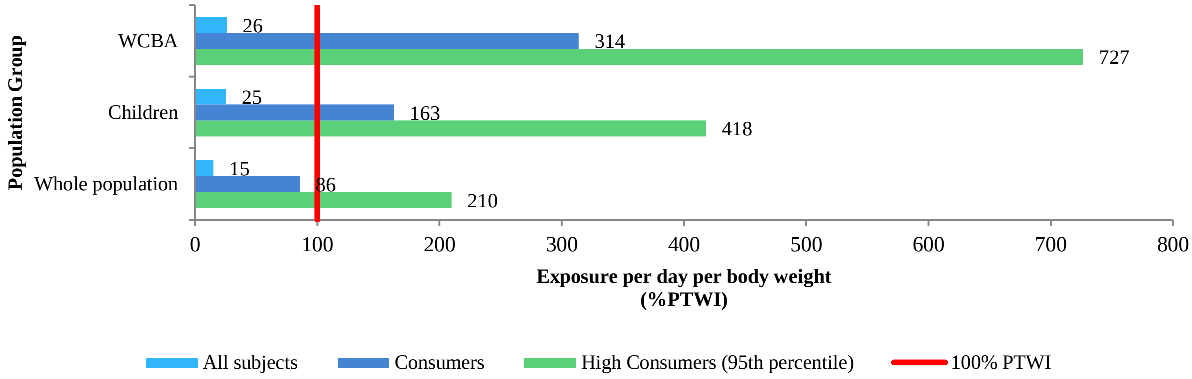

Figure 3. Dietary exposure of different Philippine population groups to ochratoxin A, expressed as %PTWI.

As shown in figures 2 and 3, results showed that the average dietary exposure of the general population comprised of both eaters and non-eaters was estimated to be at 18 ng kg^{-1} week^{-1} (expressed as 15% of the PTWI). Furthermore, no risk of exceeding the permitted weekly intake was observed for the population of both children and WCBA which were minimally exposed at levels ranging from 30 to 31 ng kg^{-1} week^{-1} at 25% and 26% of the PTWI, respectively.

For average consumers among the general population, exposure was estimated to be at 103 ng kg^{-1} week^{-1}, 86% of the PTWI. Meanwhile, average consumers among children and WCBA were exposed to mean OTA levels ranging from 103 to 377 ng^{-1} kg^{-1} week^{-1}, which exceeded the PTWI by 163 to 313%, respectively.

Consequently, high-level consumers (95th percentile) among all the population groups were highly exposed to OTA at levels ranging from 252 to 872 ng kg^{-1} week^{-1} (212 to 875% PTWI). The results indicated that average and high consumers were exposed to ochratoxin A at levels above the PTWI.

Results of the exposure assessment revealed that under a worst-case scenario of high ochratoxin A levels in food and beverages, there is a potential for both average and high-level consumers among all the population groups to exceed the permissible tolerable weekly intake.

Bread and rolls and maize were found to be the major contributor to the average OTA intake of both eaters and non-eaters among all the population groups. Other significant sources of OTA from the adult population group were from consumption of beer and malt beverages as shown in Table 1.

Table 1. Mean contribution (%) of major food categories to OTA in the TMDI approach of the Filipino population

Food category	%Mean contribution		
	General population	**WCBA**	**Children**
Bread and rolls	39	31	57
Maize	39	25	41
Beer and malt beverages	11	29	-
Others	11	15	2

TMDI of Glyphosate

The estimate of the theoretical maximum daily intake of the Philippine population composed of the general population, children, and women of childbearing age to glyphosate is presented in Figure 4 and Figure 5. Figure 4 showed the exposure expressed in milligram per kilogram of body weight per day (mg kg^{-1} bw day^{-1}), while Figure 5 showed the exposure expressed as the percentage of the Acceptable Daily Intake (ADI) for glyphosates.

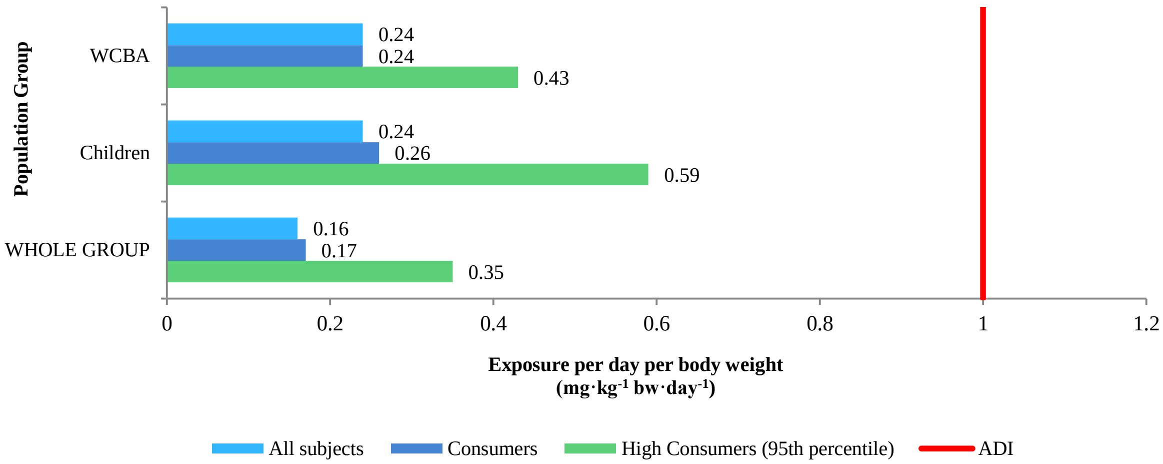

Figure 4. Dietary exposure of different Philippine age groups to Glyphosate, expressed as mg.kg^{-1} bw.day^{-1} (ADI: 1 mg kg^{-1} bw day^{-1})

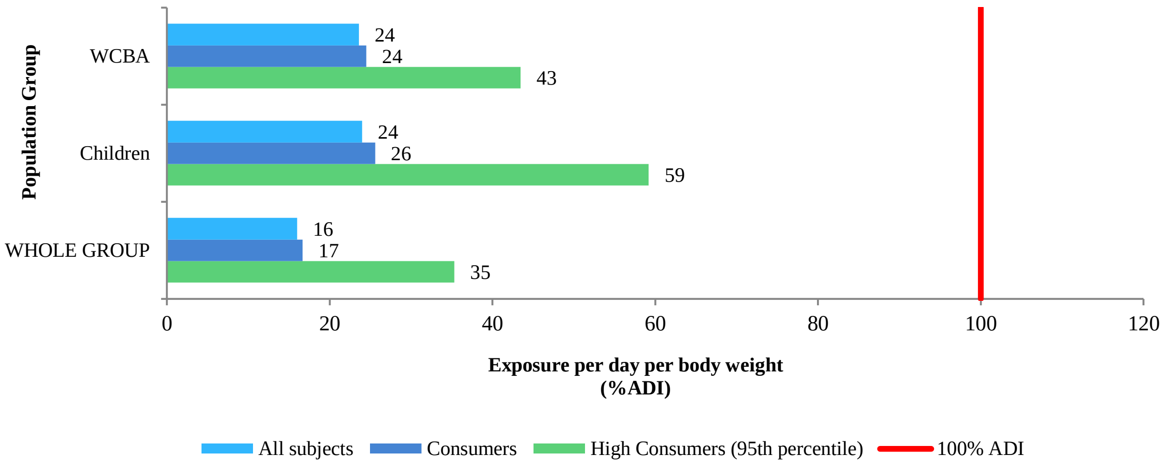

Figure 5. Dietary exposure of different Philippine age groups to Glyphosate, expressed as %ADI.

The daily average exposure of both eaters and non-eaters of the general population, children, and women of childbearing age to glyphosate revealed that among eaters and non-eaters of the general population mean exposure was estimated at 0.16 mg kg $^{-1}$ bw day^{-1} that was below the ADI, expressed as 16% of the ADI. Children and WCBA were likewise minimally exposed to glyphosate at intakes both estimated at 0.240 mg kg $^{-1}$ bw day^{-1}, representing 24% of the ADI. Results of the dietary exposure estimate implies minimal exposure of the Philippine population to glyphosate from foods that may contain the residue. It did not exceed the established acceptable daily intake for all the physiological groups.

Cereal grains and flour (99% contribution) is the main food source for glyphosate in the diet among all the population groups.

DISCUSSION

Ochratoxin A

There are different methods in quantifying exposure estimation of contaminants in food depending on the quality of data, principles, and statistical analysis applied, from conservative estimation to refine estimation [34]. Assessment of ochratoxin A occurrence and human exposure in literature made use of a variety of methods such as total diet studies, probabilistic method, analysis of OTA in food products using validated methods, among others.

Total diet studies analyzed food products as consumed, therefore the amount of OTA is affected by the processing of foods, which can either lead to an increase or decrease in the level of contaminants. There are also studies that analyzed the actual concentration of OTA in selected foods coupled with the use of food consumption databases.

In this present study, the method made use of readily available data and conservative assumptions – premises of a screening-level assessment. Table 2 showed the comparison of the method used for the evaluation of OTA exposure from various population groups, the exposure data, and the sources of OTA in their respective diet in different areas and population groups. Comparing with OTA exposures in other countries who conducted refined assessment methods, the Filipino population, specifically the general population group, were higher compared with France and the US. Similarly, consumers among the Philippine population group were higher among the majority of those shown in Table 2. However, exposure of the consumers among the whole population in the Philippines were lower compared with those reported in Vietnam total diet study (with exposure of 130 ng kg^{-1} bw day^{-1}). Since the method employed in this study was conservative in nature, there is a possibility that the levels of exposure of the Philippine population group will decrease when refined exposure studies such as probabilistic studies and total diet studies are employed.

Table 2. Comparison of Dietary Exposure of different population groups, method of exposure assessment, and main sources of OTA in the diet

			Dietary Exposure Levels												
			Adults						**Children**						
			Total			**Consumers**			**Total**			**Consumers**			
Place	**Ref**	**Method of DEA**	**ng.kg^{-1} bw day^{-1}**	**ng.kg^{-1} bw week^{-1}**	**%PTWI**	**ng.kg^{-1} bw week^{-1}**	**ng.kg^{-1} bw week^{-1}**	**%PTWI**	**ng.kg^{-1} bw day^{-1}**	**ng.kg^{-1} bw week^{-1}**	**%PTWI**	**ng.kg^{-1} bw day^{-1}**	**ng.kg^{-1} bw week^{-1}**	**%PTWI**	**Main food sources of OTA**
France	35	TDS	2.2	15.4	13%	3.6	25.2	21%	4.1	28.7	24%	7.8	54.6	46%	Cereals and cereal products (70%)
U.S.	36	Direct Analysis	0.14 -0.37	0.98 - 2.59	<2%	1.5 - 2.26	10.5 - 15.82	<2%	0.12	0.84	1%	0.42	2.94	2%	Oat-based cereal
Lebanon	5	TDS				4.28 *13.60	29.96 95.20	25% 79%							Caffeinated beverages (34.8%) Biscuits & croissants (18.1%) Alcoholic beverages (15.2%)
Czech Republic	8	Direct Analysis				1.0-2.1	7.0-14.7	<12.25%				1.0 - 4.4	7.0-30.8	<25.6%	Cereals, spices, coffee, tea, wine and beer
Vietnam	37	TDS				18.7	130.9	109%							Rice and products

*High-consumers (p95th), PTWI: 120 ng.kg^{-1} bw $week^{-1}$

Cereals and cereal-based products were the main OTA contributors to the diet for most of the studies reviewed. This includes breads, cereals, rice, biscuits, and oats. Caffeinated beverages, tea, wine and alcoholic beverages can also be a significant source of OTA when consumed in high-levels. These are mostly staple foods which are consumed largely by the population in different parts of the world.

Contamination of OTA in the commodities may happen during pre-harvest, storage, and post-harvest activities. Strategies were already studied which focused on preventing OTA contamination, decontamination or detoxification of food products, and inhibiting the absorption of OTA in the body [38]. The most fundamental preventive ways in preventing the occurrence of OTA are (1) good agricultural practices (GAP) and implementation of Hazard Analysis Critical Control Point (HACCP) system especially in the storage conditions; (2) use of fungicides; and (3) development of fungal resistant crop [39]. Reduction of OTA levels through decontamination or detoxification can be classified as physical, chemical, and biological in nature

[38]. There are also more novel ways in minimizing risks in the commodities, such as the use of microorganisms (protozoa, bacterial, yeast, and fungal species) capable of neutralizing OTA in which full mechanisms are detailed elsewhere [39].

Glyphosate

Exposure to glyphosate and other pesticides may come from sources such as the environment (air, dust, & water), direct application to crops (occupational exposure), and food [40,41]. Risks of pesticide residues exposure are then assessed using methods such as supervised trial median residues (STMRs), use of monitoring data, total diet studies, and analysis of glyphosate in the urine.

Similar with the Philippine population group, children from Argentina were also minimally exposed to glyphosate at 1.0 to 4.9% ADI, using TMDI method [42]. Whereas, the results of the Cameroonian total diet study have shown that glyphosate was not detected in the composite samples analyzed [43]. There are also studies which measured the glyphosate and its principal metabolite aminomethylphosphonic acid (AMPA) in the urine samples, which were used as markers for glyphosate exposure [19, 44, and 45]. Mean glyphosate levels in the urine were detected at 0.11 to 0.18 µ L^{-1}, 0.80 to 1.35 µ L^{-1} for German adults and Irish adults, respectively.

Most of the studies reviewed have shown low dietary exposure to glyphosate. However, there are pesticide residues that exceeded its respective ADI, and refining the standard methods widely used was the focus of some studies concerning exposure assessment in pesticides to have more realistic figures using deterministic and probabilistic models [16,17]. The results of the studies showed a large reduction in earlier conservative estimates. However, the conduct of refined methods needed extensive databases, monitoring data, processing factors, and other relevant information that may not be available in some countries.

CONCLUSIONS

The results of the exposure assessment using the TMDI approach revealed that under a worst-case scenario of high ochratoxin A levels in food and beverages - there is a potential for both average and high level consumers among all the population groups to exceed the PTWI. However, exposure of Filipinos to foods that may contain glyphosates was found to be within its established ADI thereby posing minimal risk to the exposed population. A refinement of exposure assessment of OTA in cereal-based products is therefore recommended to determine a more accurate estimate of exposure.

Author contributions

RJA for the overall implementation and design of the study, conducted dietary exposure assessment of Filipinos to ochratoxin A and glyphosates. REPG assisted in the compilation of concentration data and reference health-guidance values, conducted food matching of NNS 2008 with GSFA food categories, assisted in the conduct of dietary exposure assessment RADT assisted REPG in the compilation of concentration data and reference health guidance values, assisted in the conduct of dietary exposure assessment, prepared initial draft of manuscript EGB reviewed latest literature related to the topic, reviewed dietary exposure estimates, and assisted in the drafting of final manuscript for publication

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Conflict of interest

The authors declare that there is no conflict of interest.

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