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REVIEW ARTICLE

A critical review of glyphosate findings in human urine samples and comparison with the exposure of operators and consumers

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Abstract For active substances in plant protection products (PPP) with well defined urinary elimination, no potential for accumulation and virtually no metabolism, measuring of urine levels could be a powerful tool for human biomonitoring. Such data may provide reliable estimates of actual internal human exposure that can be compared to appropriate reference values, such as the 'acceptable daily intake (ADI)' or the 'acceptable operator exposure level (AOEL)'. Traces of the active compound glyphosate were found in human urine samples, probably resulting either from occupational use for plant protection purposes or from dietary intake of residues. A critical review and comparison of data obtained in a total of seven studies from Europe and the US was performed. The conclusion can be drawn that no health concern was revealed because the resulting exposure estimates were by magnitudes lower than the ADI or the AOEL. The expected internal exposure was clearly below the worst-case predictions made in the evaluation of glyphosate as performed for the renewal of its approval within the European Union. However, differences in the extent of exposure with regard to the predominant occupational and dietary exposure routes and between Europe and North America became apparent.

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1 Introduction

Glyphosate is one of the most widely used pesticides worldwide. In agriculture, PPPs containing glyphosate are mainly used in pre-emergence weed control for seedbed preparation and in stubble fields on cropland, for pre-harvest treatment (desiccation) and for grassland renewal. However, glyphosate itself and in particular the extensive use of glyphosate-based herbicides on genetically modified (i.e., glyphosate-resistant) crops, especially in North and South America, came in for a lot of criticism with regard to exposure of human beings and possible health effects (e.g., Antoniou et al. 2012; Samsel and Seneff 2013).

Currently, glyphosate is subject to routine reassessment within the framework of the EU evaluation of active substances according to Regulation (EC) No. 1107/2009 (Anonym 2009). Germany, acting in the European Union (EU) as Rapporteur Member State (RMS) for glyphosate, has submitted its draft of the "Renewal Assessment Report" (RAR) to the European Food Safety Authority (EFSA) in January 2014. Preparing the health chapter of this RAR, the Federal Institute for Risk Assessment (BfR) reviewed more than 150 new toxicological studies. Nearly 300 studies that had been already used for the previous evaluation (European Commission 2002) were reassessed. In addition, over 900 studies published in

scientific journals were taken into consideration. This comprehensive draft report (EFSA 2014) was reviewed by all Member States of the EU and made available for public consultation.

In the past, the detection of glyphosate in human urine samples gained considerable attention and was sometimes considered in the public to indicate or even to prove a potential health risk for humans. However, if used as an active substance in PPP in agriculture, it is inevitable that humans and animals will ingest small quantities of glyphosate via food and feed that contain residues at admissible levels. Glyphosate residues in animal products such as meat may also contribute to dietary intake by humans. In addition, humans may become directly exposed as operators, bystanders or residents when herbicides containing glyphosate are applied in the field. The amount of glyphosate that is subsequently excreted in urine might allow an estimate of previous occupational or dietary exposure. In this paper, the available findings have been critically reviewed and the exposure that may be assumed on this basis was calculated. Following the general principle of toxicological risk assessment, these exposure estimates were then compared to the newly proposed reference doses for glyphosate, i.e., an ADI of 0.5 mg per kg body weight and an AOEL of 0.1 mg per kg body weight and day (EFSA 2014).

2 Materials and methods

In total, seven studies from both the United States and Europe were identified in which glyphosate was measured in human urine samples. These studies differ very much with regard to the analytical method, the number of participants involved, the main route of exposure to be presumed and the level of detail in reporting. For instance, in some of them, no information on the background of the individuals from which samples were taken was provided or how they were recruited for the studies. Sometimes, mean and maximum glyphosate concentrations in urine are given as numeric values whereas, in other cases, their magnitude can be estimated from printed figures only.

All these studies have been reviewed and are described in Sect. 3. For a better comparability of the results, the analytical detection and/or quantification limits in urine as well as the measured and calculated concentrations are always given in μg/L although, in the original reports, sometimes the unit "ppb" has been used instead (1 μg/L = 1 ppb).

Based on urinary excretion, it is possible to roughly estimate the glyphosate "body burden" for the study participants, i.e., the internal (systematically available) dose, independent from the route of exposure. Subsequently, the approximate magnitude of exposure in mg/kg body weight (bw) may be calculated and compared to the reference doses, either the ADI or the AOEL. For this approach, certain assumptions had to be made that are explained in the following:

1. Complete excretion of the absorbed amount of glyphosate ("internal dose") via urine, no accumulation and virtually no (or very limited) metabolism

These assumptions are based on results from the multitude of kinetic studies performed by the various manufacturers of glyphosate as reported in comprehensive overall evaluations (European Commission 2002; WHO/FAO 2006; EFSA 2014) and was confirmed by the authors of the rather few articles on this issue in the public domain (Brewster et al. 1991; Chan and Mahler 1992; Anadón et al. 2009). In contrast to many other active compounds in plant protection products that are extensively metabolised and/or are subject to enterohepatic (re)circulation, these properties of glyphosate enable its reliable biomonitoring by determination of urinary concentrations.

2. Daily urine volume of 2 L for an adult person

This standard assumption is based on textbooks of human physiology (see, e.g., Tortora and Derrickson 2006; or Silbernagel and Despopoulos 1991) in which a mean daily urine excretion of 1.5–2 L is mentioned although a large variability must be acknowledged. Frequently, excretion will be less because many people will often drink a lower amount and liquid loss by other routes as sweating may be also significant but assuming a lower urine amount would simply mean that the calculated body burden of glyphosate was in fact lower. Thus, assuming 2 L will result in a conservative estimate.

3. Mean body weight of 60 kg for an adult person

Many people, in particular men and, thus, the majority of applicators, at least in Europe and North America, will weigh more but, again, a conservative estimate will result in terms of the internal dose in mg/kg bw if 60 kg is assumed.

For children, no clear-cut assumptions on urinary excretion and body weight could be made and, accordingly, respective data were assessed on a case-by-case basis if needed.

4. Oral absorption of glyphosate of 20 %

Systemic resorption of orally administered glyphosate from the gut is rather poor. The previous assumption of about 30 % (European Commission 2002) was not confirmed during the current re-evaluation. New toxicokinetic data (EFSA 2014) including information from the open literature (Anadón et al. 2009) rather suggests an oral absorption rate of about 20 %. This means that, if the body burden is mainly due to oral intake of glyphosate residues in food, the dietary exposure may have been about five times higher than the resulting internal dose.

Calculation of the (systemically available) internal dose is made as follows:

$$\text{Internal Dose}\,[\mu g/\text{kg bw}] = \frac{C_{Urine} \times V_{Urine}}{bw}$$

C_{Urine} = glyphosate urinary concentration [µg/L], V_{Urine} = urine volume per person and day [2 L per day], bw = bodyweight [60 kg per person]

For example:

- If a urinary concentration of 6 µg glyphosate/L would have been measured, a daily excretion of 12 µg can be assumed. For a 60 kg weighing person, an internal dose of 0.2 µg/kg bw would result.
- If exposure of this person can be reasonably assumed as mainly occupational or residential, i.e., occurring predominantly by the dermal and inhalative routes, this dose of 0.0002 mg/kg bw might be directly compared to the AOEL and would account for only 0.2 % of this reference dose.
- If dietary exposure is considered the more likely route of glyphosate intake, a 20 % oral absorption must be taken into account:

$$\text{External Dose}\,[\mu g/\text{kg bw}] = \frac{100 \times \text{Internal dose}}{\%\ \text{oral absorption}}$$

Therefore, an internal dose of 12 µg would be expected to result from a totally ingested amount of 60 µg, equivalent to an "external dose" of 1 µg/kg bw. This dose of 0.001 mg/kg bw would account for 0.2 % of the ADI that is, in this case, the more appropriate reference dose to compare with.

3 Results

Four of the studies have been published in scientific journals and can be assumed to have undergone a peer-review process before they were accepted. Nonetheless, the level of detail in reporting is very different. The two older ones were from the United States and the remaining, more recent, apparently report data from Europe. Table 1 summarises the available data for glyphosate concentrations in human urine samples (mean and maximum values) and resulting estimates of previous exposure compared to either the ADI or the AOEL or both.

3.1 1st study

Acquavella et al. (2004) investigated the urinary excretion of glyphosate in a so-called "Farm Family Exposure Study". Urinary concentrations were measured in 48 farmers, their spouses, and 79 children (4–18 years of age) from Minnesota (25 farms) and South Carolina (23 farms). 24-hr composite urine samples were collected for each family member the day before, the day of (day 0), and for 3 days after proven glyphosate application. Different glyphosate-based herbicides (sometimes also containing further active substances) were prepared and sprayed by the farmers themselves, perhaps assisted by family members.

The analytical method was HPLC-based, following chelation ion exchange for concentration and isolation of glyphosate, with a limit of detection (LOD) of 1 µg/L. A limit of quantification (LOQ) was not separately mentioned.

On average, sixty percent of the farmers had detectable levels of glyphosate in their urines on the day of application with a 87 % detection rate for South Carolina farmers as compared to only 36 % for samples taken in Minnesota. A similar pattern became apparent when the magnitude of urine residues was considered. The geometric mean of the concentrations for the whole group of farmers was 3.2 µg/L on the day of application but exhibited a considerable difference between the two Federal States (1.4 µg/L in Minnesota *vs.* 7.9 µg/L in South Carolina). It seems that the explanation for this gap is not in the application rates but in the different use that is made of personal protective equipment. Farmers who did not wear rubber gloves had higher urinary concentrations than those found in the other men (nearly 10 µg/L as compared to 2 µg/L) and, in fact, use of rubber gloves was much more common in Minnesota.

In all participants, a decline over the next days was observed.

The maximum value, measured in a sample taken from a farmer from South Carolina, was 233 µg/L.

Table 1 Glyphosate concentrations in human urine samples (mean and maximum values) and resulting estimates of previous exposure, compared to ADI or AOEL

References	Analytical method, LOD/LOQ	Participants	Urine concentrations [μg/l]		Estimated exposure or systemic dose [μg/kg bw]		Percentage of ADI or AOEL [%]
			Mean	Maximum	Mean	Maximum	
Acquavella et al. (2004)	HPLC following ion exchange LOD 1 μg/L	**48** male farmers from Minnesota and South Carolina (USA), **their spouses** and **79** children	**3.2**	**233** (farmer) **29** (child)*	**0.11** (systemic dose)	**8.3** (systemic dose)	**8.3** % of AOEL (maximum value), ca. **0.1** % of AOEL (mean value)
Curwin et al. (2007)	Immunoassay (fluorescent microbeads) LOD 0.9 μg/L	**48** women, **47** men, **117** children from "farm" and "non-farm" households in Iowa	**1.1–2.7** (in different groups)	**18** ("farm child")*	**0.5** (dietary exposure highest mean) **0.1** (systemic dose highest mean)		**0.1** % of ADI
Mesnage et al. (2012)	HPLC–MS LOD 1 μg/L LOQ 2 μg/L	**1** farmer, his wife and **3** children, presumably Europe	**n.a.** (only single values available)	**9.5** (farmer) **2** (child)*	**0.33** (systemic dose)		**<0.4** % of AOEL
Hoppe (2013)	GC–MS/MS following derivatisation LOQ 0.15 μg/L	**182** citizens from 18 European countries	**0.21**	**1.82**		**0.3–0.4** (dietary exposure)	**<0.1** % of ADI
Markard (2014)	GC–MS/MS (presumably) LOQ 0.15 μg/L	**40** male and female German students	**n.a.** (22 samples above LOQ)	**0.65**		**0.13** (dietary exposure)	≪**0.1** % of ADI
Krüger et al. (2014)	ELISA partly validated against GC–MS LOD/LOQ not given	**>300** (mostly from Germany)	**≤2**	**5**		**0.83** (dietary exposure)	**<0.2** % of ADI
Honeycutt and Rowlands (2014)	ELISA LOQ 7.5 μg/L	**35** women, men and children from USA	**n.a.** (13 samples above LOQ)	**18.8**		**3.3** (dietary exposure) **0.66** (systemic dose)	**<0.7** % of ADI, **<0.7** % of AOEL

n.a. not applicable

* For children, comparisons to reference values were not performed since age, body weight and urine volume were not known

(Based on all the available studies, this is the highest urinary concentration that was ever measured in a man so far.) On post-application day 3, the urinary concentration was still 68 μg/L. It is remarkable that the same man's teenage son had the highest urine concentration among all children, i.e., 29 μg/L.

Of all children who had been enrolled in the study, 12 % (all from South Carolina) had detectable glyphosate concentrations in their urines on the day of application. All but one of these children either had helped with the application or they were at least present during herbicide mixing, loading, or spraying. Among spouses, only very few (4 %) had detectable levels in their urine on the day of application, but not later, with the maximum value being 3 μg/L.

Taking all this information together, it seems that exposure to glyphosate in this study was predominantly occupational by the dermal and inhalative routes and that dietary intake was of very minor, if any, relevance. Therefore, as a worst-case scenario, the maximum value of 233 μg/L may be used to estimate the internal (systemic) dose and compare it to the AOEL.

The study authors themselves calculated for this highly exposed farmer an average systemic dose of 0.004 mg/kg bw/day for the first three days post application, taking into account the decline over this time, assuming excretion of 2 L per day and making some corrections for incomplete excretion and pharmacokinetic recovery. This dose would cover 4 % of the newly proposed AOEL. If, however, only the extraordinarily high concentration on the day of application itself is taken into account, the systemically available amount of glyphosate would be at least 466 μg (rounded for 500 μg in case that not all of it had been excreted in urine the same day) giving, for a 60 kg weighing person, a systemic dose of 0.0083 mg/kg bw. Even this dose would account for only 8.3 % of the proposed AOEL of 0.1 mg/kg bw/day on the first day and this percentage would become lower from day to day after application. Exposure of the man's son must have been much smaller since his urinary concentration of glyphosate was by 10 times lower. With the geometric mean of 3.2 μg/L for the whole group of farmers, the systemic dose will be hardly above 0.1 % of the AOEL and even in South Carolina average exposure would not exceed 0.3 %.

3.2 2nd study

A second study from the US partly confirmed these findings but revealed also certain differences and, in addition, included a group of people for whom occupational exposure was unlikely. Curwin et al. (2007) analysed urine samples that were obtained in 2001 from farm and non-farm households in Iowa for residues of four pesticides including glyphosate. 24 men ("fathers"), 24 women ("mothers") and 66 children (37 boys and 29 girls) living on a total of 25 farms were enrolled in the study. The control group comprised 23 men, 24 women and 51 children (32 boys and 19 girls) from "non-farm" households. Glyphosate analysis was performed by means of a fluorescent microbead covalent immunoassay that was claimed (but not shown) to have been validated before. The LOD was 0.9 μg glyphosate/L whereas a separate LOQ was not mentioned.

In more than 60 % of the samples taken from adults and in more than 80 % of the samples obtained from children, urinary concentrations of glyphosate were above the LOD. The mean values were in the range of 1.1 to 2.7 μg/L for the different groups (categorised according to sex, adult/child, farm/non-farm). Thus, in principle, mean glyphosate concentrations in urine were in a similar magnitude as determined by Acquavella et al. (2004) in their study. Even the highest measures in farm children in both studies (29 and 18 μg/L, respectively) were well in line with each other even though extraordinarily high levels in individual farmers as seen in South Carolina were absent among the samples taken in Iowa. However, the more frequent detection in children was surprising. More striking, there was no statistically significant difference in glyphosate concentrations between study participants from farm and non-farm households, neither for adults nor for children. This finding was in clear contrast to what was found for atrazine, chlorpyrifos, and metolachlor in the same collection of samples since concentrations of these three substances were higher in urine samples taken from farm people.

However, in this study from Iowa, there is less precise information on actual use of glyphosate than in the study of Acquavella et al. (2004). It seems at least that, on some farms, there was custom application of the pesticides instead of spraying by the farmers themselves. Residential use of glyphosate in the neighbourhood might be an additional explanation for the lacking difference between farm and non-farm households in this study. Last but not least, it cannot be excluded that dietary exposure to glyphosate residues will have also contributed to the measured urinary concentrations.

Because of the lacking difference in glyphosate excretion between "farm" and "non-farm families", it

is more appropriate to compare the presumed dietary exposure to the ADI than to calculate the internal dose and confront it with the AOEL. Based on a (rounded) maximum concentration of 3 μg/L, intake of glyphosate residues in the food might be as high as 30 μg/day/person resulting in a mean dose of 0.0005 mg/kg bw, i.e., 0.1 % of the ADI. Nonetheless, for the maximum value of 18 μg/L as measured in a "farm child", occupational or residential exposure may be assumed but the percentage of AOEL that is covered by this dose would be lower than in the worst-case scenario in the study by Acquavella et al. (2004) mentioned above and, thus, was not of concern.

3.3 3rd study

In contrast, the study by Mesnage et al. (2012) is more a case report. Urinary concentrations were measured in a farmer, presumably in Europe (country not mentioned), on the day before and two days after spraying an herbicide containing glyphosate. The analytical method was HPLC with ion trap mass spectrometry with an LOD of 1 μg/L and an LOQ of 2 μg/L.

The urine concentrations of glyphosate found were in the same order of magnitude as those reported by Acquavella et al. (2004). Seven hours after commencement and three hours after termination of spraying, the farmer had 9.5 μg glyphosate/L in his urine and, two days later, the concentration had fallen to 1.9 μg/L. According to a figure in the article, no glyphosate was detected in the urine samples which were taken on the day before glyphosate use on the crops. For the first day, an internal dose of 0.3–0.4 μg/kg bw may be estimated covering 0.3–0.4 % of the AOEL. From the description in the study, it seems that the farmer had taken adequate protective measures.

Biphasic excretion was seen that might, according to the authors, reflect the sequence of rapid inhalative (or oral) intake and (delayed) dermal absorption.

Surprisingly, a similar concentration of 2 μg/L was measured on day 2 after spraying in one of the farmer's children living 1.5 km away from the treated fields while no glyphosate was found in urine samples obtained from his wife and their two other children. These findings cannot be explained with certainty but might be either due to dietary intake (although it would be surprising then that the mother and siblings had none in their urines) or to track-in of traces of the herbicide by the father resulting in residues, e.g., in house dust or yard dirt.

In this study, urine was also analysed for aminomethylphosphonic acid (AMPA), i.e., a plant and soil metabolite of glyphosate. AMPA is normally found at very low levels in conventional plants, however, several genetically engineered varieties of glyphosate-tolerant plants degrade glyphosate very quickly giving higher amounts of this metabolite. In mammals, AMPA is formed only in traces, most likely due to the activity of intestinal bacteria (Brewster et al. 1991; EFSA 2014). Thus, it is not surprising that Mesnage et al. (2012) could not detect AMPA in any sample since the amount of glyphosate received was relatively small and the main exposure route, at least for the father, was certainly dermal and/or inhalative but not by ingestion.

3.4 4th study

A much more comprehensive study with regard to the number of participants was recently published by Krüger et al. (2014). Several hundred human urine samples were analysed by means of an ELISA (Abraxis, USA). Unfortunately, an LOD or LOQ was not mentioned and no information regarding linearity or cross-reactivity of the assay given. However, in the analytical part of this article, a comparison between values obtained by this ELISA and a GC–MS method was provided revealing a sufficient correlation (R^2 of 0.87 for human urine). Thus, the measured values may be considered reliable.

The mean glyphosate concentration for all samples was slightly lower than 2 μg/L with a maximum of ca. 5 μg/L. A weakness of this brief publication is that only figures were printed instead of giving precise numbers in the text.

Furthermore, it does not become entirely clear from this paper how many subjects had been actually involved and how they were recruited for the study. On one hand, glyphosate concentrations in 99 urine samples from humans on conventional diet were compared to 41 samples obtained from people who had claimed to eat mainly "organic". As to be expected because of extensive use of glyphosate in conventional agriculture, the mean (nearly 2 μg/L) and maximum values (around 4 μg/L) in the "conventional diet" group doubled those in the "organic eaters" group. Here, the mean was in the magnitude of 1 μg/L and the peak values were apparently below 2 μg/L. The resulting difference between both groups was statistically significant.

On the other hand, urine concentrations of glyphosate in 102 "healthy" and 199 "chronically diseased" people were compared. In the "healthy"

population, the mean was, again, slightly below 2 μg/L with a maximum of less than 4 μg/L. In the "sick" group, the mean value appeared to be slightly above 2 μg/L and a maximum of about 5 μg/L was measured. Also this difference was statistically significant ($p < 0.03$). However, it was not reported if the included groups (separated by nutritional preferences and health status) were overlapping or not. In addition, there was no information on the participants of the study given, neither with regard to age, gender and the chronic diseases they suffered from nor with regard to occupational or social background or residential status (town or countryside). It is even not mentioned in which year and country the samples had been taken. Based on the mean urine concentrations, it may be assumed at least that the predominant route of exposure was dietary.

Based on the maximum concentration of 5 μg/L, a totally ingested amount of 50 μg may be assumed resulting in an "external" exposure of about 0.8 μg/kg bw for an adult of 60 kg. This dose would account for less than 0.2 % of the proposed ADI of 500 μg/kg bw.

The studies that are reported in the following have not been published in scientific journals yet and one of them (Markard 2014) is not available to the public so far but was exclusively submitted to the German Federal Institute for Risk Assessment.

3.5 5th study

A Europe-wide, exploratory biomonitoring study (Hoppe 2013) was performed on behalf of the non-governmental organisation (NGO) "Friends of the Earth" and its German partner organisation "Bund für Umwelt- und Naturschutz Deutschland" (BUND). The results were published on the Internet and the original study provided on request to the Federal Institute for Risk Assessment.

182 frozen urine samples from 18 European (EU and non-EU) countries (6–12 per country) were examined for glyphosate and AMPA by means of a modern and selective analytical method, i.e., transformation of both compounds to two different derivatives followed by GC–MS/MS. The LOQ for both, glyphosate and AMPA, was 0.15 μg/L. As in the previous studies by Acquavella et al. (2004) and Curwin et al. (2007), creatinine was also measured as an internal proof for the validity of the urine measurements.

For glyphosate, nearly 44 % (80 samples) and, for AMPA, more than one-third (65) of the participants had urine concentrations above the LOQ. Maximum values of 1.82, 1.64 or 1.55 μg/L for glyphosate were found in samples obtained from Latvia, the UK, and Malta, respectively, but the mean value of 0.21 μg/L was much lower. (For calculation of the mean, the study author had apparently included the samples with values below the LOQ. He assumed a concentration of 0.075 μg/L, i.e., half the LOQ.) For AMPA, the maximum values of 2.63, 1.26, and 0.89 μg/L were measured in samples from Croatia, Belgium, and Malta with a mean urinary concentration of 0.18 μg/L for all involved people. It was surprising that in more than 30 cases the AMPA concentrations were higher than those of glyphosate, sometimes by 10 times or more. In a few samples, AMPA values were rather high with glyphosate concentrations below the LOQ.

Apart from this data, a "reference value" for glyphosate in urine of 0.8 μg/L was mentioned. This figure was explained to be based on analytical investigations in a total of 90 people from a not further described "urban collective" from the region of the German city of Bremen. This figure was the 95th percentile of the individual values and was established in 2012 in preparation of the main study. For AMPA, a "reference value" of 0.5 μg/L was given.

The glyphosate in the urine samples, most likely, will have resulted from dietary intake. The maximum value of 1.82 μg/L might indicate the ingestion of a total amount of up to 20 μg glyphosate via food commodities. This would equal a dose of nearly 0.0003 mg/kg bw for a 60 kg weighing adult person, representing less than 0.1 % of the ADI.

Obviously, there must have been also some exposure to AMPA although its origin is less clear. Based on animal data (EFSA 2014), significant transformation of glyphosate to AMPA in the human body is very unlikely. The amount of AMPA residues in the average European diet, presumably from genetically modified crops, is not known. There might be additional sources than the agricultural use of glyphosate. However, the same ADI as for glyphosate is also applicable to AMPA (European Commission 2002; EFSA 2014). The measured AMPA concentrations in urine will not account for more than 0.2 % of this ADI.

Due to the limited number of samples and the absence of information on study participants (such as age, gender, body weight, social background, origin from urban or rural environments, nutrition habits) and the way how they were recruited, the study is far from being representative. Nonetheless, the results of this study are interesting because they provide a first idea of the actual glyphosate intake throughout Europe although the mean dietary exposure levels cannot be estimated on this basis, neither for a single country nor for Europe in its whole. Moreover, no

conclusion can be drawn to which extent the apparent differences in urinary levels of glyphosate in the samples might reflect the actual use of glyphosate in the different countries or the residues in imported food. (It was reported, e.g., that 8 out of 10 samples from Austria and 10 out of 12 from Switzerland were below the LOQ in contrast to only 3 of 10 from the UK or even 1 of 10 from Malta. However, this distribution might well be a random one.)

3.6 6th study

To support the ongoing evaluation of glyphosate, so far unpublished data was submitted by the German Federal Environmental Agency (Markard 2014) providing some support for the results of Hoppe (2013). Frozen urine samples had been collected for other purposes in 1996 and 2012 in the city of Greifswald in the north-eastern part of Germany and its surrounding region and were now analysed in retrospect for glyphosate residues. In each of the two sampling years, urine analysis for glyphosate and AMPA was performed in samples from ten male and ten female students (age 20–29 years at the time of sampling). The LOQ of the test method (presumably gas chromatography) of 0.15 μg/L was exceeded for glyphosate in 22 of the totally 40 samples. The maximum value was 0.65 μg/L. The resulting dietary dose would be well below 0.1 % of the ADI. There was a tendency towards an increase in glyphosate concentrations in urine in the 2012 samples compared to those from 1996, possibly reflecting a more frequent use of glyphosate in agriculture resulting in a higher dietary intake. The LOQ was exceeded more frequently and individual values tended to be higher.

Again, there were indications that AMPA concentrations in the urine may be higher than those of glyphosate. 10 out of 40 results were above the LOQ of 0.15 μg/L with a maximum value of 1.31 μg/L. However, in contrast to glyphosate, the AMPA concentrations appeared to decrease between 1996 and 2012 suggesting that there is poor correlation between glyphosate and AMPA residues and that other routes or sources for exposure to AMPA than by (plant) metabolism of glyphosate should be considered. In addition, the stability of glyphosate in deep-frozen urine over more than 16 years was not investigated, maybe resulting in a shift of the glyphosate/AMPA ratio.

3.7 7th study

The most recent data on glyphosate findings in urine came from the United States again. On behalf of two NGOs ("Moms Across America" and "Sustainable Pulse"), 35 urine samples obtained from women, men and children (4 to 71 years of age) from 14 Federal States were analysed and the results published by Honeycutt and Rowlands (2014) on the Internet. The authors themselves quoted that this "initial testing" was "not meant to be a full scientific study. Instead it was set up to inspire and initiate full peer-reviewed scientific studies on glyphosate, by regulatory bodies and independent scientists worldwide".

Analysis was performed in a commercial laboratory in St. Louis (Missouri) by means of a not further specified ELISA with a rather high LOQ of 7.5 μg/L. This value was exceeded in 13 samples with individual concentrations ranging from 8.1 μg/L for a 6-year old boy to 18.8 μg/L for a 26-year old woman, i.e., in a range that seems high but not implausible against the background of the previous American studies (Acquavella et al. 2004; Curwin et al. 2007) reported above. Unfortunately, no further information on study participants is available that would allow to specify the most likely route of exposure. Under the assumption of dietary exposure being the relevant route, the maximum excretion of nearly 19 μg/L might result from ingestion of up to 200 μg glyphosate. A daily dose of 3.3 μg/kg bw (0.0033 mg/kg bw) may be calculated that would account for nearly 0.7 % of the ADI of 0.5 mg/kg bw, which is still a very low percentage. If the exposure would have been occupational or residential, the internal dose of approximately 0.66 μg/kg bw would cover <0.7 % of the AOEL of 0.1 mg/kg bw/day. However, the measured top concentration was by about ten times higher than the maximum one detected by Hoppe (2013) in Europe by a presumably more sensitive method and also clearly above the ELISA results of Krüger et al. (2014).

4 Discussion and conclusions

Application of PPP will usually result in residues in crops and in commodities intended as human food or feedstuffs for animals. The dietary intake of such residues by humans is predictable and maximum residue limits (MRLs) for each active substance have been established. Since urine is a major elimination route for glyphosate, it is not surprising that certain amounts can be detected in human urine samples. However, if the estimated exposure is clearly below science-based trigger values (i.e., the ADI or AOEL), there is no health concern for consumers.

Since oral absorption of glyphosate is known (about 20 %) and the substance is virtually excreted

chemically unchanged (Brewster et al. 1991; EFSA 2014), the measured glyphosate concentrations in urine could be used to estimate the previous exposure of both operators and consumers and to compare it to the reference values. This exercise was made for all seven studies that were available to us. If the presumed exposure was expected to be mainly via food, it was compared to the ADI. If it was expected to be predominantly occupational, the appropriate reference value to compare with was the AOEL. If not known or different exposure scenarios have to be considered, comparison to both reference values was made.

From the data reported in this critical review, the following conclusions were drawn:

- Current analytical techniques allow the detection and determination of much lower amounts of glyphosate in human urine than in the past. The results obtained with different methods are not that much different and, to some extent, confirm each other.
- Positive glyphosate findings in human urine are quite common and may result from occupational or residential exposure, from dietary intake or from both. The origin may often not be clearly distinguished and will probably overlap sometimes.
- Urinary concentrations in operators after application of plant protection products tend to be higher than those resulting from dietary intake of glyphosate by consumers.
- The by far highest concentrations were measured in the urine of one operator and his son and may indicate that the recommended protective measures were not properly taken.
- Although the available data is not representative, mean urine concentrations measured in the US appear higher than those found in Europe. The assumption of this difference is based mainly on data reported by Curwin et al. (2007) and, more recently, by Honeycutt and Rowlands (2014) when compared to Hoppe (2013); Markard (2014), or Krüger et al. (2014). This finding is likely to reflect differences in the agricultural use of glyphosate-based herbicides and the plantation of glyphosate-resistant, genetically modified crops in North America.
- As suggested by the data of Markard (2014) and Krüger et al. (2014), there might be a trend towards increasing glyphosate concentrations in measured urine samples also in Europe, probably reflecting more sensitive analytical techniques, more frequent use in agricultural practice in Europe or higher residues in imported foodstuffs.
- All measured values, even the highest, were of no health concern. The calculated human exposures were at least one order but mainly two or more orders of magnitude lower than the ADI and AOEL.
- The same holds true if urine concentrations of AMPA are taken into account. However, correlation between glyphosate and AMPA in urine is poor suggesting that other sources of AMPA than metabolism of glyphosate in plants should be considered.

The expected exposure of consumers to glyphosate was predicted in the worst-case scenarios for risk assessment as outlined in the RAR (EFSA 2014). Based on average residues in all crops that may be treated with glyphosate and taking into account the nutrition habits (Banasiak and Hohgardt 2007), the 'International estimated daily exposure (IEDI)' and the 'National estimated daily exposure (NEDI)' have been determined. Based on the EFSA PRIMo (EFSA 2007), the highest potential dietary exposure in Europe was calculated for Danish children in the magnitude of 2.5 % of the ADI. In Germany, based on the NVS II Model (BfR 2011), an exposure of up to 1.5 % of the ADI might be expected. However, urinary excretion data suggest that actual exposure is much lower (see Table 1). Accordingly, the dietary risk assessment as currently performed in the EU appears sufficiently conservative. This is well in line with results obtained in Australia where McQueen et al. (2012) calculated an exposure of pregnant women due to glyphosate residues in composite food samples that accounted for not more than 0.4 % of the ADI in the average (2 % at maximum) whereas 4–5.5 % had been predicted.

Regarding operator safety, an exposure to glyphosate of about 1 % of the AOEL has been assumed in the RAR (EFSA 2014). The urinary excretion data for situations in which exposure was mainly occupational suggest this calculation to be realistic on condition that glyphosate-containing herbicides are properly used.

Thus, the results of this review of urine analysis data confirm the conclusion drawn during re-assessment of glyphosate (EFSA 2014) that the dietary intake as well as occupational exposure is unlikely to present a public health concern.

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