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Testbiotech
Institute for Independent
Impact Assessment in
Biotechnology

**Testbiotech comment on the
German Renewal Assessment Report (RAR)
on the active ingredient glyphosate**

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October 2014

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Summary

In December 2013, the German authorities completed a Renewal Assessment Report (RAR) for the re-approval in the European Union of the active ingredient glyphosate. The main outcomes were outlined by the German Federal Institute for Risk Assessment (BfR) as follows:

- Available data do not show that glyphosate is carcinogenic or mutagenic or that glyphosate is toxic to fertility, reproduction or embryonal/foetal development in laboratory animals.
- Evaluation of literature however showed that the toxicity of some herbicides containing glyphosate can be higher than just the active ingredient because of co-formulants (added ingredients) such as e.g. tallowamines used as surfactants.
- A research project initiated by BfR shows that the active ingredient glyphosate and the co-formulants (added ingredients) do not have a negative effect on the microflora in the rumen of ruminant animals.

The completed RAR was passed on to the European Food Safety Authority (EFSA) for further assessment. During the course of the public participation process, Testbiotech has since commented on the completed report, focussing thereby on newer more recent studies published in 2013 and 2014. Some older studies, not in the RAR, were also referred to as and when appropriate, but not systematically researched. It appears highly probable that a lot more relevant data is available for evaluation, that neither appears in the RAR nor been looked at by Testbiotech.

The following conclusions can be drawn from the Testbiotech assessment:

- The German authorities failed to evaluate several relevant studies that were available before the RAR was completed.
- A number of new relevant publications have been published since the RAR was completed, and should be taken into account for the overall evaluation of glyphosate.
- Based on these data, it is evident that there is new information on e.g. subchronic toxicity, long-term toxicity, genotoxicity, endocrine effects and ecotoxicity which must be taken into account in a new re-assessment.
- Data on glyphosate residues and effects on plant metabolism (especially in genetically manipulated plants) must be looked at in much more detail than appears in the current RAR.
- The data specified in the RAR on the possible effects of glyphosate on the microflora in the gut have not yet been published, and appear to touch only partially on the relevant questions. Special attention must therefore be paid to an assessment made by the Aarhus University in Denmark that concludes further investigations are needed.
- Glyphosate is the most frequently used herbicide and residues from spraying can be found regularly in food and feed, surface water and soil etc., making it necessary to systematically test for additive effects and synergies with other herbicides.

Overall, the results of the German RAR on glyphosate have to be regarded as preliminary and inconclusive.

Zusammenfassung

Im Dezember 2013 stellten die zuständigen deutschen Behörden den Entwurf des Bewertungsberichts (RAR) für die Neuregistrierung des Wirkstoffs Glyphosat in der Europäischen Union fertig. Die wichtigsten Ergebnisse wurden vom Bundesinstitut für Risikobewertung (BfR) zusammengefasst:

- Die verfügbaren Daten zeigen keine Hinweise auf krebserzeugende, mutagene, reproduktionsschädigende oder fruchtschädigende Wirkung bei Versuchstieren.
- Aus der ausgewerteten Literatur ergibt sich jedoch, dass die Toxizität bestimmter glyphosathaltiger Pflanzenschutzmittel aufgrund der darin enthaltenen Beistoffe (z.B. Tallowamine als Netzmittel) höher sein kann als die des Wirkstoffes.
- Ein vom BfR initiiertes Forschungsprojekt zeigt, dass der Wirkstoff Glyphosat und die Beistoffe keinen negativen Einfluss auf die Mikroflora des Vormagens haben.

Dieser Bericht wird nun von der Europäischen Behörde für Lebensmittelsicherheit (EFSA) bewertet. Testbiotech hat den RAR im Rahmen des öffentlichen Beteiligungsverfahrens kommentiert und sich dabei auf neuere Studien konzentriert, die in den Jahren 2013 und 2014 veröffentlicht wurden. Ältere Studien, die im RAR keine Erwähnung finden, wurden bei Bedarf verwendet, aber nicht systematisch recherchiert. Es ist sehr wahrscheinlich, dass deutlich mehr relevante Daten zur Verfügung stehen, die weder von Testbiotech, noch im RAR erwähnt werden.

Auf Basis der von Testbiotech bewerteten Studien ergeben sich folgende Schlussfolgerungen:

- Die deutschen Behörden haben mehrere relevante Studien nicht bewertet, die bis zur Fertigstellung des RAR bereits veröffentlicht waren.
- Seit Fertigstellung des RAR sind weitere Publikationen erschienen, die berücksichtigt werden und in die Gesamtbewertung von Glyphosat einfließen müssen.
- Auf Grundlage dieser Daten wird deutlich, dass es neue Erkenntnisse unter anderem zu Themen wie subchronischer Toxizität, langfristiger Toxizität, Genotoxizität, endokrinen Effekten und Ökotoxikologie gibt, die in die Neubewertung einfließen müssen.
- Daten zu Glyphosat-Rückständen und Auswirkungen auf den Pflanzenstoffwechsel (vor allem in gentechnisch veränderten Pflanzen) müssen sehr viel detaillierter betrachtet werden als im aktuellen RAR.
- Die im RAR genannten Daten zu den möglichen Auswirkungen von Glyphosat auf die Mikroflora des Darms sind nicht veröffentlicht und scheinen überdies nur einen kleinen Teil der relevanten Fragen zu betreffen. Daher ist besondere Aufmerksamkeit auf eine Bewertung der Universität Aarhus (Dänemark) zu legen, die zu dem Schluss kommt, dass weitere Untersuchungen erforderlich sind.
- Da Glyphosat das am häufigsten verwendete Herbizid ist und Rückstände regelmäßig unter anderem in Lebens- und Futtermitteln, Oberflächenwasser und Böden auftreten, sollten Synergien mit anderen Pestiziden und additive Effekte systematisch getestet werden.

Insgesamt müssen die Ergebnisse des deutschen RAR zu Glyphosat als vorläufig und nicht schlüssig betrachtet werden.

1. Introduction

In December 2013, the German authorities completed a Renewal Assessment Report (RAR) for the re-approval in the European Union of the active ingredient glyphosate. The main outcomes have been outlined by the Federal Institute for Risk Assessment as follows:

“In conclusion of this re-evaluation process of the active substance glyphosate by BfR the available data do not show carcinogenic or mutagenic properties of glyphosate nor that glyphosate is toxic to fertility, reproduction or embryonal/fetal development in laboratory animals. As a result of the re-assessment for the active substance BfR proposes slight amendments of the reference values. BfR believes that there is convincing evidence that the measured toxicity of some glyphosate containing herbicides is the result of the co-formulants in the plant protection products (e.g., tallowamines used as surfactants). Therefore BfR calls special attention to the co-formulants and incorporated a toxicological assessment of tallowamines in its draft report. A research project initiated by BfR and performed by the University of Veterinary Medicine in Hanover investigated the influence of a glyphosate containing herbicide on microbial metabolism and communities in ruminants. The results of this study are summarised in the draft suggesting that there is no negative impact on the microflora in the rumen.”¹

Testbiotech commented on the RAR in the course of the public participation process foreseen by EU regulations. Because of the size of the RAR and the massive amount of literature available for glyphosate, Testbiotech decided to focus on recent studies that were published in 2013 and 2014. Older studies were considered when appropriate.

2. Short-term effects

The results of short-term toxicity tests with glyphosate are illustrated in the RAR as follows:

“The short-term toxicity of glyphosate was rather low in all species under study and by all routes of administration”.

It is further stated that most studies were conducted by industry.

However, several peer-reviewed studies were not assessed in the RAR. Some were published during or after the preparation of the RAR, whereas others were omitted for unknown reasons. Some studies were conducted with the ai (active ingredient = glyphosate) and show significant results (such as DNA damage or biochemical modifications) e.g. Larsen et al. (2012) or Mañas (2014).

In the Larsen et al. study for example, rats were exposed for 30 or 90 days to the highest level (0.7 mg/L) of glyphosate allowed in water for human consumption (in the US) as well as a 10-fold higher concentration (7 mg/L). Biochemical modifications were shown, even at 3–20-fold lower doses than the US oral reference dose of 2 mg/kg/day. The authors asked for clarification regarding the toxicological significance of their findings.

Several other studies not mentioned in the RAR were conducted with glyphosate formulations and also show significant results (for example Tizhe et al., 2013 a,b,c).

In conclusion, several studies were not taken into account in the RAR.

¹ http://www.bfr.bund.de/en/the_bfr_has_finalised_its_draft_report_for_the_re_evaluation_of_glyphosate-188632.html

3. Genotoxicity / Cytotoxicity

According to the RAR, the

“potential genotoxicity of glyphosate was tested in an adequate range of in vitro and in vivo studies providing no evidence of a genotoxic potential.”

Later in the text, this summary is clarified further:

“Taking a weight of evidence approach, it may be concluded that there is no in vivo genotoxicity and mutagenicity potential of glyphosate or its formulations to be expected under normal exposure scenarios, i.e., below toxic levels.”

Studies on mammals:

However, several peer-reviewed studies were not assessed in the RAR. Some were published during or after the preparation of the RAR, whereas others were omitted for unknown reasons. Several of the studies not mentioned in the RAR indicate that glyphosate may have genotoxic properties *in vitro* and *in vivo*.

Alvarez-Moya et al. (2014) examined the genotoxicity of glyphosate isopropylamine in different concentrations in human lymphocytes, erythrocytes of fish species *Oreochromis niloticus* *in vitro* and staminal nuclei of a plant species (*Tradescantia*) *in vivo*. Significant genetic damage was observed *in vivo* and *in vitro* in all cell types and organisms tested. According to the authors, *“results indicate that glyphosate is genotoxic in the cells and organisms studied at concentrations of 0.7-7 µM.”*

Further, in a study conducted by Mañas et al. (2013), mice were administered technical grade glyphosate (40 or 400 mg/kg/day) or AMPA (100 mg/kg/day) in drinking water for 14 days. In the comet assay there were statistically significant differences in all the treatments and tissues studied in comparison to control animals. The major results of this study were that glyphosate and AMPA induced a significant increase in DNA damage in liver and blood. DNA effects on liver and blood indicate that these compounds could be of concern in terms of potential damage to genetic material. These results are of concern regarding the genotoxicity of glyphosate, especially as this study is an *in vivo* study.

Studies on organisms other than mammals:

Several studies aimed to investigate potential genotoxic effects of glyphosate on aquatic organisms. For example, a study on tropical fish *Prochilodus lineatus* (Moreno et al., 2014) revealed that both glyphosate itself and the formulation Roundup Transorb were genotoxic to gill cells and erythrocytes of *P. lineatus*. Guilherme et al. (2012) not only confirmed the genotoxicity of Roundup® for the fish species *Anguilla anguilla*, but also demonstrated the genotoxic potential of glyphosate and POEA.

Other studies not assessed in the RAR also show genotoxicity (De Souza Filho et al., 2013) or cytotoxicity of glyphosate formulations (Chaufan et al., 2014).

In conclusion, several relevant studies that were not taken into account in the RAR show genotoxicity or cytotoxicity of glyphosate or glyphosate formulations.

4. Long-term toxicity and carcinogenicity

According to the RAR,

“glyphosate was considered unlikely to pose a carcinogenic risk in humans [...]”

It is further stated that in

“epidemiological studies in humans, there was no evidence of carcinogenicity [...]”

However, several peer-reviewed studies were not assessed in the RAR, which were published during or after the preparation of the RAR. These studies show associations of glyphosate with various types of cancer.

For example, Kachuri et al. (2013) found a “nearly significant” association between glyphosate and multiple myeloma in Canadian male pesticides users, when these used glyphosate over a period of over 2 years.

Overall, uncertainties remain regarding the long-term toxicity and carcinogenicity of the active ingredient glyphosate. Contrary to the conclusion of the RAR, there are studies that indicate long-term health risks of glyphosate or glyphosate-based herbicides.

5. Reproductive toxicity and endocrine disruption

According to the German RAR, no evidence of reproductive toxicity was observed in multi-generation studies. Further, studies in developmental toxicity and teratogenicity found no teratogenic potential in rats. According to the German authorities, published data also contained no clear evidence for the association of glyphosate with negative reproductive outcomes in *in vivo*, *in vitro* studies or in epidemiological studies.

However, several recent studies were not assessed in the RAR, which were published during or after the preparation of the RAR.

a) *In vitro* studies

One *in vitro* study found an association between glyphosate and breast cancer cells (Thongprakaisang et al., 2013). The results indicated that low concentrations of glyphosate possess estrogenic activity at low and environmentally relevant concentrations which lead to the stimulation of breast cancer cells.

b) *In vivo* studies

In another study not mentioned in the RAR, a pesticide mixture containing glyphosate led to severe oxidative stress (OS) in testicles of rats (Astiz et al., 2013).

Other studies with glyphosate formulations have also suggested adverse effects on male reproductive performance (Caglar and Kolankaya, 2008; de Liz Oliveira Cavalli; Omran et al., 2013). A review by Basrur (2006) showed that glyphosate can act as an endocrine disruptor and an estrogen-like compound in domestic animals and humans.

Reproductive and endocrine effects were also found in aquatic animals (see chapter 7).

In conclusion, reproductive toxicity and endocrine disrupting potential of the active ingredient glyphosate should be assessed in more depth.

6. Neurotoxicity

According to the German RAR, glyphosate was not neurotoxic in rodents. However, a recent study by Cattani et al. (2014) not presented in the RAR shows that a glyphosate-based pesticide is associated with neurotoxicity.

There is an ongoing scientific debate regarding possible effects of glyphosate on microbial communities in animal or human intestines. In the RAR, this area of risk assessment is also filed under “neurotoxicity”.

In a recently published *in vitro* study, Chłopecka et al. (2014) found that even very low doses of glyphosate (0.003 g/L) caused negative effects on the motoric activity of intestine. The authors conclude:

“The gathered data suggests that glyphosate impairs gastrointestinal strips’ motility at concentration that are noticed in human exposed to non-toxic doses of glyphosate.”

In another *in vitro* study, Schrödl et al. (2014) examined the intestines of dairy cows on farms in Germany and found that glyphosate appears to modulate the fungal community.

Apart from new literature not assessed by the German authorities, the RAR also presents conclusions from studies that were obviously still ongoing when the document was being finalised.

In Annex 6.1 “Toxicology and metabolism”, it is stated that the results of a BfR study on the effects of glyphosate on microorganisms in the rumen of ruminants are not available. In the Summary report however, the following is stated:

“Because the enzyme (EPSPS) as in plants might be inhibited in many bacteria, there was concern about possible alterations in the composition of microbial communities and microbial metabolism in the rumen eventually resulting in disease. However, a new study in an artificial rumen has shown no adverse effects on animal health are to be anticipated” [Summary report, page 36].

The findings of the study conducted by the University of Veterinary Medicine in Hanover were also presented prominently in the press release by the Federal Institute for Risk Assessment (BfR) which accompanied the finalisation of the RAR. Testbiotech is of the opinion that it is unacceptable to present unpublished results from a study and declare them conclusive for a crucial area of risk assessment. Further data on other livestock (such as poultry and pigs) were not assessed nor was the impact on human gut microflora.

In assessing the current data, special attention should be given to the hypothesis developed by the Aarhus University (Sørensen et al., 2014). The experts from Denmark suggest – based on available data - that health effects on livestock from microbial populations and / or changes in the mineral status of the animals can be a cause of effects on health. They conclude further studies are necessary, using animal feed sprayed and unsprayed with glyphosate. They propose to run targeted studies with, for example, weaning pigs.

7. Aquatic organisms

Fate and behaviour in water

According to the German RAR, it is assumed that glyphosate as well as its main metabolite AMPA are quickly dissipated from the water phase by adsorption to sediment or degradation by micro-flora. However, a recent study by Mercurio et al. (2014) that was not assessed by German authorities shows that glyphosate and AMPA may persist much longer in water than previously thought.

From the perspective of risk assessment, it is noteworthy that the RAR acknowledges very high concentrations of glyphosate and AMPA in European surface water. According to the RAR:

“Maximum glyphosate and AMPA concentrations in European surface waters as measured in comprehensive monitoring campaigns (Horth, 2012, BVL no 2310291) range between 1.3 microgramm/L and 0.22 > 200 microgramm/L for glyphosate and AMPA, respectively. It has to be noted that glyphosate acid and AMPA in surface water (PEC_{sw}) calculated using the FOCUS (2000) surface water models, even though worst case applications was assumed.”

Concentrations as mentioned in the RAR are likely to have negative impacts on aquatic organisms (see below). Therefore the conclusion of RAR that based on these findings, *“the risk for aquatic organisms is acceptable”* has to be rejected. In addition, several recent studies showing risks of glyphosate for aquatic organisms were not assessed as necessary. Amongst others, the following studies were not taken into account:

Fish

Fan et al. (2013) found toxic effects of glyphosate, POEA and Roundup® on the goldfish, *Carassius auratus*, after 7 days exposure. Fish were exposed separately to concentrations of 0.16, 0.032 and 0.0064 mg/L of Roundup® [containing 41% isopropylamine salt of glyphosate and 18% polyoxyethylene amine (POEA)], Glyphosate isopropylamine salt (G.I.S) and POEA. Amongst others, effects on acetylcholinesterase (AChE) activity were measured as a common indicator of toxicity. According to the authors:

“All tests revealed a significant inhibition of AChE activity during 7 d exposure. The inhibition percentages range from 28%–40% in Roundup®, 5%–23% in G.I.S, and 20%–50% in POEA.”

In a study with the fish species *Danio rerio*, Lopes et al. (2014) found that sperm membrane and DNA were impaired following short-term exposure to pure glyphosate. Both concentrations (5 mg/L and 10 mg/L) also caused a reduction of mitochondrial functionality and sperm motility. According to the authors:

“The results showed that glyphosate can induce harmful effects on reproductive parameters in D. rerio and that this change would reduce the fertility rate of these animals.”

Moreno et al. (2014) found genotoxic effects when the fish species *Prochilodus lineatus* was exposed to glyphosate and the formulation Roundup Transorb. According to the authors, results revealed that both glyphosate itself and Roundup Transorb

“were genotoxic to gill cells and erythrocytes of P. lineatus, suggesting that their use should be carefully monitored considering their potential impact on tropical aquatic biota.”

Also Uren Webster et al. (2014) demonstrated that glyphosate caused reproductive toxicity in zebrafish at high concentrations.

Further, toxic effects were found in different glyphosate formulations. Nwani et al. (2013) found genotoxicity damage in the fish species *Channa punctatus* following exposure to commercial formulations of carbosulfan, glyphosate and atrazine. Richard et al. (2014) found an impaired immune system following exposure of European Sea Bass (*Dicentrarchus labrax*) to a glyphosate-based herbicide.

Negative effects (behavioural changes) were also found in African catfish (*Clarias gariepinus*) by Akinsorotan et al. (2014). Genotoxic effects of Roundup were shown in the fish *Corydoras paleatus* by de Castilhos Ghisi & Cestari (2012). DNA and chromosomal damage induced by AMPA were found in fish *Anguilla anguilla* by Guilherme et al. (2014). Finally, changes in the ovaries of zebrafish exposed to a glyphosate formulation were found by Armiliato et al. (2014).

Erhunmwunse et al. (2013) found pathological alterations in the liver of post-juvenile African Catfish exposed to sublethal concentrations of the glyphosate, whereas Gholami-Seyedkolaei et al. (2013) found changes in hematological and biochemical as well as an inhibition of the AChE activity in tissues of gill, liver, muscle and brain of *Cyprinus carpio*.

Other negative effects on fish species were for example detected by De Souza Filho et al., (2013), Vera-Candioti et al., (2013), Guilherme et al., (2012), Hued et al., (2012), and Gluszcak et al., (2011).

Other aquatic organisms

Other aquatic organisms such as mussels (Sandrini et al., 2013) were shown to be sensitive to glyphosate. Sandrini et al. exposed brown mussel *Perna perna* and fish species *Danio rerio* and *Jenynsia multidentata* to different glyphosate concentrations. Results demonstrated that cholinesterase from different fractions of all species tested was inhibited by glyphosate.

In the study by Avigliano et al. (2014a), crayfish (*Cherax quadricarinatus*) were exposed for 60 days to 10 and 40 mg/L of pure glyphosate (acid form) in freshwater. Mortality was 33 % at the highest concentration. Amongst others, results suggest chronic stress associated with glyphosate exposure. Besides, a possible metabolic depression in muscle and possible damage to several other tissues are suggested.

Avigliano et al. (2014b) found that glyphosate as well as Roundup Ultramax caused negative reproductive effects in the crab species *Neohelice granulata*.

The authors used concentrations of glyphosate and Roundup which may be expected in runoff water from crop fields. Reductions in the number of hatched larvae were noted from exposure to Roundup, whereas larvae abnormalities were seen in pure glyphosate (2.5 mg/L) and the formulation. As the authors noticed a stimulation of ovarian maturation by pure glyphosate, they hypothesise that “exposure to glyphosate disrupts the hormonal system controlling reproduction“.

Several publications that were not assessed in the RAR also show risks for other aquatic organisms from glyphosate formulations.

For example, according to recent research not mentioned in the RAR, amphibians might be even more at risk than usually estimated if the terrestrial life stages are taken into account. Berger et al. (2013) investigated whether amphibians migrating through arable fields might be exposed to glyphosate and found that “amphibians regularly co-occur with pre-sowing/pre-emerging glyphosate applications to maize in spring and with stubble management prior to crop sowing in late summer and autumn.“

The authors suggest *“in-depth investigations of both acute and long-term effects of glyphosate applications on amphibian populations not only focussed on exposure during aquatic periods but also terrestrial life stages.”*

In 2013/14, many studies were published on the effects of glyphosate formulations on different aquatic organisms such as amphibians, shrimps, mussels or water insects. Examples of such studies are: Brodeur et al., 2014; Syedkolaei et al., 2013; dos Santos & Martinez, 2014; Dornelles & Oliveira, 2013; Yadav et al., 2013; Janssens & Stoks, 2013; Güngördü, 2013; Lancôt et al., 2013; Wagner et al., 2013; Kumar et al., 2013; Meza-Joya et al., 2013; Cuhra et al., 2013; Lajmanovich et al., 2013; Mona et al., 2013; Yadav et al., 2013; Relyea, 2012; Moore et al, 2012; Plötner & Matschke, 2012.

Several of these studies show that pesticide mixtures or combined exposure of aquatic organisms to multiple stresses increase negative effects. In consequence, the conclusions of the RAR on aquatic organisms are not conclusive and need extensive reassessment.

8. Arthropods / Insects

According to the RAR, glyphosate has very low toxicity for bees. Therefore, it is claimed that risks for bees are acceptable.

However, recent research indicates that risks for insects such as bees might be underestimated. Herbert et al. (2014) found that pure glyphosate caused negative effects on the learning abilities of bees.

“Altogether, these results imply that GLY [glyphosate] at concentrations found in agro-ecosystems due to standard spraying can reduce sensitivity to nectar reward and impair associative learning in honeybees.”

The authors are concerned about possible long-term negative consequences on bee colony performance. As bees provide important ecosystem services, risks should be assessed very carefully. The example of systemic insecticides shows that fixation on toxicological effects is likely to underestimate long-term risks for pollinators. Long-term studies with glyphosate and glyphosate-based herbicides should therefore be conducted to investigate behavioural effects.

9. Other relevant issues

Metabolism in plants

According to the German RAR, glyphosate and its metabolites are found at low levels in different crop species. In the case of desiccation however, residue values up to 21 mg/kg were found in oats, rye, wheat, and barley. Recent studies that were not assessed in the RAR have also shown very high levels of glyphosate and metabolites in glyphosate-tolerant soybeans (Bohn et al., 2014; Testbiotech, 2013; Sørensen, 2014).

Further, whereas several studies are mentioned in the RAR, no comprehensive assessment of studies was presented on the metabolic changes in glyphosate tolerant soybeans, although a large body of evidence suggests that changes in plant composition (see table 1) may be attributed to glyphosate.

Study	Compound	Result
Barbosa et al., 2012	Protein: malondialdehyde, ascorbate peroxidase, glutathione reductase, and catalase	Elevated (seed)
	Actin fragment, cytosolic glutamine synthetase, glycinin subunit GI, and glycine-rich RNA-binding protein	Expressed differently (seed)
Bellalui et al., 2008	Protein	Protein elevated (seed)
	Oil	Reduced (seed)
	Oleic acid	Elevated (seed)
	Linolenic acid	Reduced (seed)
	Nitrogen assimilation, as measured by in vivo nitrate reductase activity (NRA) in leaves, roots, and nodules	Reduced
Bellalui et al., 2009a	Protein	Elevated (seed)
	Amino acids	Elevated (not significant) (seed)
	Oil	Reduced (seed)
	Oleic acid	Elevated (seed)
	Linolenic acid	Reduced (seed)
	Prolin	Elevated (seed)
	Nitrate reductase activity (NRA)	Reduced
Bellalui et al., 2009b	Ferrum	Reduced (seed)
Bott et al., 2008	Root biomass and root elongation	Depressions of plant growth in the GR soybean cultivar Valiosa strongly dependent on the selected culture conditions
	Shoot biomass	In soil culture, shoot biomass production declined by approximately 15–30% in glyphosate treated plants grown on an acidic Arenosol but not on a calcareous Loess sub-soil, while root biomass was not significantly affected
	Nitrogen fixation	No effect of glyphosate application on nitrogen fixation as measured by acetylene reduction assay, soybean yield, or seed nitrogen content

Study	Compound	Result
Cavalieri et al. 2012	effect of glyphosate formulations on nutrient accumulation and dry matter production on the shoot of two glyphosate-resistant (GR) soybean cultivars	Roundup Original®, Roundup Transorb® and Roundup WG® caused the greatest damage to nutrient accumulation and dry matter production. It was concluded that nutrient accumulation and dry matter production in the shoots of the soybean plants are affected by glyphosate application, even for GR cultivars.
Duke et al., 2003	Daidzein	Elevated (seed)
Duke et al., 2012 (review)	Plant mineral nutrition	Rigorous field studies on different soil types (including those highly susceptible to inducing Mn or Fe deficiency in soybeans) are needed to resolve the issue of whether glyphosate might have adverse effects on mineral nutrition of GR crops.
Reddy et al., 2000	Shoot and root dry weights	Reduced (greenhouse)
Reddy et al., 2003	Chlorophyll content, root and shoot dry weight, or nodule number	No effect
	Nodule biomass	Reduced by 21 to 28% 14 d LPOST Soybean recovered by 14 d.
	Seed protein	Reduced when two applications were made (seed)
Reddy et al., 2004	AMPA, Shikimate, glyphosate levels in plants	Chlorosis, effects measured till 22 DAT
Serra et al., 2011 Greenhouse	Mn	No effect V8 (20 days after application)
	N, Mn, Cu, Zn and Fe	Reduced
	Nodes	Reduced
	Dry mass	Reduced
Zablotowicz et al., 2007	Seed nitrogen	Reduced, when high doses of glyphosate were applied (seed)
Zobiole et al., 2010a	Number and weight of seeds per plant	Reduced by 25% and 13% (seed)
	17:1n-7 (not essentiell)	Increased (by 30.3%) (seed)
	18:1n-9 (not essentiell)	Increased (by 25%) (seed)
	Linoleic acid (18:2n-6)	Decrease (2.3%) (seed)
	Linolenic acid (18:3n-3)	Decrease (9.6%) (seed)
Zobiole et al., 2010b	Photosynthetic parameters	Decrease
	Biomass production	Decrease

Study	Compound	Result
	Water absorption	Decrease
	Photosynthesis	Decrease
	Water use efficiency	Decrease
Zobiolo et al., 2010c	Shoot and root dry biomass	Decrease
	Photosynthetic parameters	Decrease
	Micronutrients (Zn, Mn, Fe, Cu, B) in leaves	Decrease
	Macronutrients (N, P, K, Mg, Ca, S)	Decrease (but no effect on N)
Zobiolo et al., 2010d	Photosynthetic parameters (A, SPAD, Fo, Fm)	Linear decrease R1 growth stage → long-term physiological impacts
	Macronutrients (N, P, K, Mg, Ca, S)	Decrease: effects in the following order: Ca > Mg > nitrogen (N) > S > K > P
	Micronutrients (Fe, Co, Zn, Mn, Cu, Mo, B)	Effects of single application in the following order: Fe > Mn > cobalt (Co) > Zn > Cu > boron (B) > molybdenum (Mo) Two applications: Fe > Co > Zn > Mn > Cu > Mo > B
	Shoot, root and total biomass dry weight	Reduced proportional to glyphosate dose.
Zobiolo et al., 2010e	Photosynthetic rate	Severely decreased RR2 more sensitive than RR1
	Leaf area and shoot biomass production	Severely decreased RR2 more sensitive than RR1
Zobiolo et al., 2010f	Photosynthetic rate	1800 dose: reduction 33 and 31%.
	Lignin and amino acid content	Reduced (linear)
	Height and total (shoot and root) dry weight	Reduced (linear)
Zobiolo et al., 2010g	Nodule number	Reduced
	Ni concentration	Reduced
	Chlorophyll (SPAD units)	Lower in RR soybean Even lower when treated with glyphosate
		Chlorotic symptoms, non persistent

Study	Compound	Result
Zobiolo et al., 2011a RR1 and RR2	Fusarium spp.	Increased in RR1 and RR2 soybean
	Mn reducers/Mn oxidizers ratio	Decreased in RR1; The greatest reduction in the ratio of potential Mn reducers/Mn oxidizers occurred when glyphosate was applied at early (V2) compared with later growth stages (V4 and V6)
	Root and shoot dry weight	<p>Decreased in RR1 and RR2 soybean</p> <p>Earlier glyphosate applications caused greater decreases in root dry weight</p> <p>Shoot dry weight of both varieties was most reduced when glyphosate was applied at the V6 growth stage and least at V4 and V2 stages, with the GR2 cultivar affected more than the GR1 cultivar (Fig. 6). In general, GR2 produced less biomass (shoot and root) than GR1 when glyphosate was not applied</p>
Zobiolo et al., 2011b RR1 and RR2	Chlorophyll	Decrease
	Macro and micronutrient accumulation	<p>Decrease</p> <p>All macro- and micronutrients, with exception of N and K, accumulated more in RR1 than RR2 (Figs. 2 and 3 and Table 1). This result may be an individual cultivar characteristic, but it suggests that the RR2 cultivar was inefficient in nutrient uptake and translocation or was unable to rapidly recover from potential chelating effects of glyphosate</p> <p>In the present experiment, glyphosate apparently remained active in soybean through R1 growth stage or later as indicated by decreased nutrient accumulation. It is known that glyphosate and its metabolites can remain within the plant until complete physiological maturity.</p>
	Nodule dry weight and number	Reduced tendency for late applications to have less effect than early applications

Study	Compound	Result
	Shoot biomass	Decrease higher percent reduction associated with late than with early glyphosate applications
	Chlorosis	The new generation RR soybeans also showed undesirable glyphosate effects as “yellow flashing”.
Zobiolo et al., 2012 RR2	Photosynthetic rate	Severely depressed
	Macro- and micronutrient accumulations	Proportionally reduced as glyphosate rates increased and applications were delayed Macronutrient and all micronutrient concentrations except Cu were within the nutrient-sufficiency ranges for soybean Concentrations of Ca, Mg, S, and Cu were significantly ($p < 5\%$) lower in glyphosate-treated soybean yet all values were within the sufficiency ranges for those nutrient concentrations to provide acceptable soybean growth. Concentrations of P and Fe appeared to be increased by glyphosate.
	nodule number and dry weight	Significantly decreased In contrast with other results, a tendency was noted for reduced effects at late applications compared with early applications
	Root dry weight	More severely depressed with glyphosate applied at V2 growth stage compared with V6 growth stage
	Leaf area and shoot dry weight	More strongly decreased at the late growth stage than at the early stage

Table 1: Impact of spraying genetically modified plants with a glyphosate-based herbicide (quoted from Testbiotech, 2012)

In this regard, the RAR is not acceptable because it does not deal with the residues from spraying and the impacts thereof to the extent necessary. Relevant findings were omitted.

Furthermore, since glyphosate is the most frequently used herbicide and can be found regularly in food and feed, surface water, soil etc., synergies and additive effects have to be tested systematically with other compounds that very often occur together with glyphosate (such as residues from spraying with other herbicides).

10. Conclusions

- › The German authorities overlooked several relevant studies that were available at the time the RAR was being prepared.
- › A number of new relevant publications have been published since the RAR was completed and need to be taken into account.
- › On the basis of these data it is evident that, amongst others, subchronic toxicity, long-term toxicity, genotoxicity, endocrine effects, as well as ecotoxicology have to be reassessed and cannot be concluded from the RAR.
- › Other data on glyphosate residues and effects on plant metabolism have to be assessed in much more detail.
- › The data in the RAR on the effects of glyphosate on the microflora in the gut have not yet been published and appear to only briefly touch upon the relevant questions. Therefore special attention has to be given to the assessment made by Aarhus University in Denmark that concludes further investigations are needed.
- › Since glyphosate is the most frequently used herbicide and residues from spraying can be found regularly in food and feed, surface water, soil etc., synergies and additive effects need to be tested systematically with other compounds that often occur with glyphosate (such as residues from spraying with other herbicides).

Overall, the results of the German RAR on glyphosate have to be regarded as preliminary and inconclusive. It has to be emphasised that the studies assessed in the RAR will need further careful re-evaluation, and the data derived from the publications presented here can be useful in re-assessing the conclusions and uncertainties in the RAR.

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