RAGES
RISK ASSESSMENT OF GENETICALLY ENGINEERED ORGANISMS IN THE EU AND SWITZERLAND

Serious shortcomings in the European risk assessment of herbicide tolerant GE plants for human health

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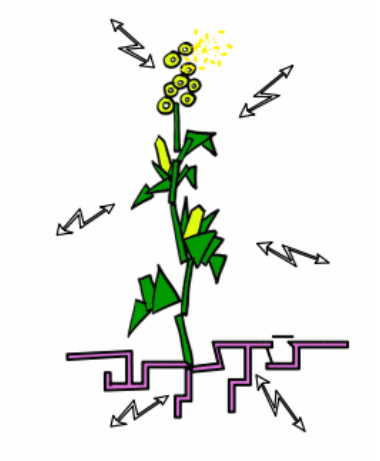

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Content

Summary

Herbicide tolerant genetically engineered (HT GE) plants have been engineered to tolerate herbicides and are in real-life never grown without being sprayed. As the claimed benefits arise from the application of the herbicide and not of the GE crop plants in isolation, so the risks and safety issues must be considered in combination. The global use of glyphosate has increased dramatically with HT GE plants as a main driver. This is in itself an important environmental problem. Moreover, the increased use of glyphosate represents a selective pressure that accelerates the evolution of glyphosate resistant weeds. As a response, farmers in the US, Argentina and Brazil have increased, over the last 20 years, their spraying of HT GE soy. The result is that farmers now spray rates more than twice as high as those originally recommended. Similarly, the number of glyphosate applications has increased from one or two, to four applications per year, which imply more spraying late in the growing season. In addition, the GE plants are made resistant to other complementary herbicides.

This promotes higher residues from glyphosate and/or other complementary herbicides in HT GE soybeans, which dominate the global export market, including to Europe, for use in food and feed products. This raises questions about health effects for consumers.

The basis for risk related research on, and risk assessment of, HT GE plants is plant samples produced in field trials. A key problem is that these plants are sprayed with much lower, i.e. not representative, doses of the complementary herbicides compared to doses that farmers use in commercial production. Using these plant samples for risk assessment can therefore lead to wrong conclusions and underestimate the actual risks. First, the load of residues is much higher in commercially produced plants. Secondly, the plant composition may be altered by a more intense spraying regime. Thirdly, combinatorial effects can arise from interactions between plant constituents and herbicide residues. Such changes may potentially cause health effects such as toxicological, hormonal or immunological reactions at the stage of consumption.

The above-mentioned risks to ecosystems and human health, arising from glyphosate tolerant GE plants, are in the process of being replicated and exceeded with new ‘stacked’ HT GE plants that are tolerant to multiple herbicides such as glufosinate ammonium, 2,4-D, dicamba and isoxaflutole, in addition to glyphosate. These herbicides will be sprayed together and result in new and untested ‘cocktail’ mixes and co-exposure, both in the environment as well as in food and feed.
The toxicity of mixes, interactions and combinatorial effects of these substances are difficult to study and unknown to a large extent. Hence, these new HT GE plants cannot be considered to be safe. We illustrate with two current case study examples (triple resistant HT GE soy plants intended for import to the European market) how the European risk assessment system, as implemented by EFSA, fails to perform relevant risk assessment of HT GE plants.

We argue that the underlying causes of these flaws in the risk assessment come from lack of independent research, lack of relevant data (in particular on herbicide residues) and the separation of the HT GE plant and its co-technology (complementary) herbicide in risk assessment, i.e. the division of the assessment of the plant, performed by the EFSA Panel on Genetically Modified Organisms (the GMO-Panel), and assessment of the pesticide, performed by the EFSA Panel on Plant Protection Products and their Residues (the PPR-Panel).

To demonstrate safety of HT GE plants, the two areas of risk assessment need to be combined to assess the overall risks of the consumption of food and feed products derived from the HT GE plants. This need is reflected in COMMISSION IMPLEMENTING REGULATION (EU) No 503/2013, which states that the field trials with HT GE plants should compare and test plant

products with and without the complementary herbicide being applied. Furthermore, potential synergistic or antagonistic effects resulting from the combination of the transformation events should be included, and the data provided for risk assessment should be sufficient to conclude whether the expected agricultural practices influence the studied endpoints.

The most important gaps in the overall risk assessment of HT GE plants are:

EFSA does not take into account that test samples from field trials are sprayed with much lower rates of herbicides as compared to current agricultural practices.

- If feeding studies are performed, there is no requirement that the tested plant material is produced under realistic conditions (i.e. in accordance with current agricultural practices).
- Not all residues and the patterns that result from spraying with the complementary herbicides, as well as metabolites that specifically emerge in the HT GE plants, are assessed.
- There is no assessment of the residues from herbicide formulations and relevant additives and adjuvants used in other countries. Thus, HT GE plants with unknown concentrations of herbicide residues are imported into Europe from regions with deviating and weaker herbicide regulations than those in the EU.
- HT GE plants that tolerate a mixture of complementary herbicides (stacked events) have an increasing trend in the global market. Potential combinatorial effects of the expected mixtures of herbicide residues are not investigated.
- The compositional analysis and assessment of phenotypical characteristics of HT GE plants do not consider the dose, the number of sprayings or the timing of herbicide application, although these factors can influence the plant and product quality and safety.
- Long-term effects of the consumption of products and their impact on the immune system, the endocrine system and the gut microbiome escape the risk assessment completely or to a large extent.

In conclusion, the current practice of risk assessment for HT GE plants in Europe fails to assess identified and real risks. Thus, the approval process for HT GE plants is, in its current form, both inadequate and misleading. It is a further step in the wrong direction that new stacked HT GE plants are likely to introduce untested herbicide-cocktails into European food chains.
With this background, no further HT GE plants should be approved for import based on the current practice. And events which are already allowed for import need to be re-assessed.

1. Introduction

Based on the EU GMOs register[1], which lists the majority of authorized GE plants following Regulation (EC) 1829/2003, 53 of the 62 allowed GE plants (85 %) are herbicide tolerant with 35 (56 %) being single, 16 (26 %) double and 2 (3 %) triple herbicide tolerant. The numbers for the 24 pending applications reinforce this trend: 21 GE plants (88 %) are herbicide tolerant with 7 (33 %) being single, 10 (48 %) double, 2 (9 %) triple and 2 (9%) quadruple herbicide tolerant. This shows that a large majority (two thirds) of the herbicide tolerant GE plants in the pipeline are tolerant to several herbicides.

Herbicide tolerant (HT) genetically engineered (GE) crops survive spraying with one or several herbicides in the growing season. At least in the short-term, this has made farmers able to combat weeds in a cost-efficient way in large-scale industrial agriculture (Brooks and Barfoot 2017). The flip side of the coin is increased use of these herbicides in the environment, rapid resistance evolution in weeds and new exposure pathways of toxic chemicals in the food chain. The latter represents new risks to consumers.

[1] https://webgate.ec.europa.eu/dyna/gm_register/index_en.cfm.

HT GE plants are predominantly grown in Argentina, Brazil, and the USA. Glyphosate tolerant (GT) soybeans have been on the market for more than 20 years and have led to an accelerated use of glyphosate-based herbicides (GBH, Benbrook 2016), leading both to a “pesticide treadmill”, and an “arms race” to combat resistance with the use of more GBH or alternative herbicides (Binimelis et al., 2009).

A number of weed species, i.e. 43 species registered at present, globally (Heap 2019), have adapted to the massive use of GBH and have become resistant. These glyphosate- and, more generally, herbicide-resistant weeds are an increasing problem in countries where the HT GE crops are grown. Hence, Monsanto’s assessment of 20 years ago (Bradshaw et al. 1997) that GBH are different from other widely used herbicides in that they will not readily select for resistance due to glyphosate’s mechanism of action and the lack of plant metabolism has clearly been proven false, and those who raised this issue early on were proven right (e.g. Shaner 2000).

The response from biotechnology companies to the severe problems with resistant weeds of today has been to insert or cross-breed multiple transgenic constructs coding for herbicide tolerance into the same plant (stacked events). This allows the farmer to spray their crops with multiple herbicides during the growing season. The agricultural practice of using such stacked events will extend herbicide use to multiple toxic chemicals used at the same time. As further outlined in this report, this will have consequences for the content of the harvested products as well as for weeds in the fields.

As shown for glyphosate tolerant GE soy, residues of herbicides accumulate in the soybeans (Bøhn et al., 2014; Duke et al., 2003, 2017), and at a concentration that is orders of magnitude higher than for other pesticides, i.e. in the mg/kg level rather than µg/kg. Instead of such residues coming from a single herbicide family, stacked GE plants will, in the future, be expected to contain cocktail mixes of different herbicide families. Especially since these toxic chemicals are brought to the consumers through the food chain, there is an urgent need to develop analytical methods to monitor residues of new herbicide and metabolite mixes in food and feed, and to test for combinatorial health effects of such co-exposures and potential additive or synergetic effects (see for example de Arcaute et al., 2018). At present this is not done, although several new stacked events are on their way to the European market (see case studies in this report). These illustrate major weaknesses in the current risk assessment system.

The business model for HT GE crops is simple: Selling patented seeds and complementary herbicides in an inseparable double pack. The paradox is that what is an inseparable pack for the farmer, the environment and the consumer, is highly separated in the risk assessment system. EFSA is the responsible unit in Europe that should safeguard our food chains. In addition, the EU Commission, in their decision-making, should only allow GE products that are proven to be safe to reach the market (Regulation 1829/2003).

Risk assessment of HT GE as currently performed by EFSA is divided into the assessment of the organism, performed by the GMO-panel and assessment of the pesticide, performed by the plant protection products and their residues (PPR) panel. However, this separation should not result in increased risk for consumers or justify ‘gaps’ in the assessment of the safety of the GMO sprayed with the pesticide. The risk assessment needs to make sure that the safety of the organism as a whole (as well as food and feed derived from it) is shown and is not reduced to the assessment of some single parts or pieces.

Therefore, the two areas of risk assessment need to be combined: the risk assessment of the complementary pesticides and their specific effects on the HT GE plants. However, as exemplified in this report, there are major gaps in the overall risk assessment of HT GE plants.

We argue that the current system is suffering a policy-imposed weakness in its science: The deliberate disconnection of the risk assessment of the pesticide (typically glyphosate/GBH for single GE events) and the HT GE plant of which this pesticide is an essential component. For non-scientific reasons, EFSA and the EU Commission are separating the assessment groups and thereby jeopardizing good scientific principles (Bøhn 2018). This effectively limits relevant questions about interactions between the herbicide(s) and the plant, and questions about combinatorial effects on health. With stacked HT GE plants on the market, tolerant to several herbicides, combinatorial effects become crucial.

Even on a more basic level, data to assess the problems of residues are largely missing. For example, in 2015, no samples of imported soybeans were analysed for residues from spraying with glyphosate (EFSA, 2017a).

In this report, we discuss serious shortcomings and problems in the risk assessment of HT GE plants in the European context. We use two current case examples for illustration: both are HT GE soybeans, triple resistant to herbicides. These soybeans may soon be approved by EFSA for import into the EU and be eaten by European consumers.

2. Toxicity and health effects of complementary herbicides being applied on HT GE plants

Although it is beyond the scope of this report to review the toxicity of the complementary herbicides used together with HT GE plants, we briefly highlight some of the documented effects of the herbicides relevant to HT GE plants (approved or for approval) intended for the European market in the near future.

We are aware of losses in biodiversity and high levels of environmental exposure to terrestrial and aquatic systems, to the soil and the rural population, in countries where HT GE plants are cultivated.

For example, glyphosate can eliminate necessary host plants for butterflies. The monarch butterfly uses the milkweed plant as a key host plant, and this plant resides largely inside agricultural fields in the US. The extensive use of glyphosate in soy and maize field in the US has resulted in a dramatic decline in the monarch (Pleasants & Oberhauser, 2012, Schütte et al., 2017). This case exemplifies how the landscape-level, ecological context of an agricultural practice, which include multiple stressors, sub-lethal effects, interactions etc., is often omitted from analyses of risk (Bøhn and Lövei, 2017).

However, since these plants are not growing in the EU, we set aside most details of environmental risk assessment, since the purpose of this project is the current practice of GMO risk assessment in the EU. So far, more than 50 events of HT GE plants are allowed in the EU for import, but none for cultivation.

2.1 Glyphosate and Roundup toxicity and health effects

Glyphosate is the declared active ingredient in about 750 broad-spectrum herbicides such as Roundup, the major pesticide in the world. Like any pesticide, GBH contain undeclared and hence unknown mixes of co-formulants often called adjuvants, as well as additional chemicals including heavy metals (Defarge et al., 2018), making it difficult to characterize the actual herbicides used by farmers. It is documented in several models that formulations are more toxic than pure glyphosate (reviewed in Mesnage et al., 2015). The differences in toxicity between a formulation and the active ingredient appears to be a general pattern in pesticides, including in the ones described in the following paragraphs (Mesnage et al., 2014). The key difference comes from the co-formulants. For example, polyethoxylated tallowamine (POEA), a classical adjuvant family in Roundup, was shown to be more than 1000 times more toxic than glyphosate in human cells (Mesnage et al., 2013, Defarge et al., 2015). Formulations containing tallowamine were recently banned in France, but not in countries where HT GE plants are cultivated (see also Mesnage et al 2019).

Indeed, the toxicity of glyphosate and GBH is still highly debated (Portier at al., 2016), particularly since glyphosate was classified as probably carcinogenic by IARC (2015). Glyphosate and GBH products (such as Roundup) were previously claimed to be “practically non-toxic” to non-target organisms. This claim was in part based on toxicity experiments performed by the industry on the water flea *Daphnia magna* in the 1970-80s (KcKee et al., 1982; McAllister and Forbis, 1978) and presented in widely cited reviews which found little or no concern regarding human health (Williams et al., 2000), and little or no ecotoxicological effect in the receiving environment (Giesy et al., 2000).

Cuhra et al. (2013) re-tested the toxicity of glyphosate with the same type of assay for acute toxicity (EC_{50}), in the same *D. magna* model, as these old studies. The results showed that pure glyphosate IPA (i.e. the active ingredient, glyphosate isopropylamine salt) was 100-300 times more toxic (Cuhra et al., 2013). In the new studies, the EC_{50} (defined as the concentration that provokes a response halfway between the baseline and maximum response) was below 10 mg/L, compared to the early reference value of 930 mg/L.

Cuhra et al. (2013) also found serious negative effects (reduced reproduction) at a concentration of 0.45 mg/L, and reduced body size of offspring at 0.05 mg/L, i.e. at lower concentrations than the accepted limit for surface waters in the US (0.7 mg/L), and lower than the newly introduced limit in Switzerland (0.36 mg/L). Cuhra et al.’s experiments indicate that the accepted environmental concentrations of glyphosate in the US and Switzerland are too high to safeguard key freshwater species like water fleas. Water fleas have important roles in the aquatic ecosystem as they graze and thereby clean lakes for algae/bacteria and transfer energy from the primary production level to higher trophic levels, such as fish.

From Argentina, even higher concentrations of glyphosate have been measured in aquatic systems near to agricultural fields with glyphosate tolerant soybean production. For example, in the tributary of Pescado, concentrations of glyphosate between 1.8 and 10.9 mg/kg were measured (Ronco et al. 2008).

Glyphosate and GBH use from the adoption of HT GE ‘Roundup Ready’ soybean production in Argentina is causing an appreciable level of stress to the overall ecosystem with specific adverse effects on non-target aquatic biodiversity, especially on invertebrates (Ronco et al., 2008). GBH in fresh-water and marine ecosystems can have significant negative effects on non-target organisms, e.g. on aquatic microbial communities (Perez et al., 2007), macrophytes (Lockhart et al., 1989; Simenstad et al., 1996), cnidaria (Demetrio et al., 2012), sea-urchin embryogenesis (Marc et al.,

2004), fish (Servizi et al., 1987), amphibians (Mann et al., 2009; Relyea, 2005) and planktonic algae (Perez et al., 2007; Peterson et al., 1994). Other publications report low toxicity, which correspond to conclusions in published reviews of GBH ecotoxicity potential (Dill et al., 2010; Giesy et al., 2000). A review of GBH effects in aquatic ecosystems gives a comprehensive overview of individual studies for most investigated taxonomic groups (Perez et al., 2011). Furthermore, a recent study shows genotoxicity in brain, liver, kidney and gills of fish (de Oliviera et al., 2019)

As for health effects, glyphosate is documented to be toxic to the male rodent's reproductive system (Cai et al., 2017). This conclusion was based on results showing reduced sperm counts in rats from several studies (meta-analysis). Even ultra-low doses (i.e. half of the 100 ng/L allowed in tap water in the EU, and 10,000 times below the acceptable daily intake for European citizen set at 0.5 mg/kg body weight per day) of Roundup in the drinking water resulted in liver and kidney damage, as indicated by gene expression changes in female rats being exposed chronically over long time (Mesnage et al., 2015). An integrated analysis of their liver molecular profiles (transcriptome, proteome, metabolome) revealed non-alcoholic fatty liver disease and its progression to non-alcoholic steatohepatosis (Mesnage et al., 2016).

Recently, cell culture studies have for the first time shown that glyphosate induces DNA damage and methylation (epigenetic changes) in human cells (Kwiatkowska et al., 2017). The authors argue that further studies, in other cell types as well as in vivo, should be performed to confirm their findings.

Glyphosate can cause changes in the gut microbiota of animals (Lozano et al., 2018; Mao 2018), possibly promoting disease. It has been shown that the use of glyphosate can lead to changes in the composition of microbial soil flora (EFSA, 2012). Additionally, glyphosate has an antibiotic effect on specific bacteria such as *E. coli* (Forlani et al., 1997; Carlisle & Trevors, 1988). Therefore, it seems obvious that permanent exposure to glyphosate can cause changes in the gut flora of animals including humans.

In recent years, glyphosate has faced heavy criticism – and was classified as probably carcinogenic to humans by the International Agency for Research on Cancer (IARC, 2015), which is part of the World Health Organisation (WHO). EFSA, following the German agency BfR in charge of the evaluation of the solely declared active ingredient, but not taking into account the other ingredients in the full formulations, does not agree with the IARC classification (EFSA 2015). Nonetheless EFSA acknowledges major uncertainties regarding the import of food and feed derived from GE HT plants (ESFA 2018a, see also below).

2.2 Toxicity of 2,4-D

Tolerance to 2,4-D in GE plants comes from the insertion of the *aad-12* gene from *Delftia acidovorans.* 2,4-D is a systemic herbicide that leads to uncontrolled growth and death in broad leaf plants. HT GE plants such as DAS-44406-6 that inherit the enzyme AAD-12 are also tolerant to further herbicides named pyridyloxyacetate. Substances such as triclopyr, fluroxypyr and MCPA are members of this group.

2,4-D can be found in different chemical forms: as acid (basic form), inorganic salts, amines or esters (Munro et al., 1992). Plants absorb 2,4-D through roots and leaves within 4-6 hours, the chemical follows the phloem of the plant and mimics the role of auxins (plant hormones) leading to disturbances, abnormal growth and eventually death (Mullison, 1987). The herbicide 2,4-D is 75 times more toxic to broadleaf plants than glyphosate (Schütte et al., 2017), while grasses and cereals

like corn, oat, rice and wheat have relatively high tolerance to 2,4-D, giving the option of using 2,4-D as a post emergence herbicide on selected crops.

Technical grade 2,4-D acid, esters and salts show similar toxicity in rats, with some effects on liver and kidney at doses 15 mg/kg/day and higher (Gorzinski et al., 1987). A study requested by the Industry Task Force II on 2,4-D, reports a no observed effect level (NOEC, 13 weeks) in dogs on 1.0 mg/kg/day (Garabrant and Philbert, 2002).

In humans, use or mixing of 2,4-D is linked to the cancers Non-Hodgkins's Lymphoma (NHL) and Soft Tissue Sarcoma (STS), although co-exposure to 2,4,5-T and TCDD in some cases make establishing the cause of disease difficult (Garabrant and Philbert, 2002). 2,4-D was classified by WHO/IARC in 2015 as a *possible carcinogen to humans* (Guha et al., 2016). Recent publications suggest that carcinogenic metabolites from 2,4-D are produced in genetically engineered plants (Lurquin, 2016).

The interdisciplinary field of toxicogenomics may be helpful to understand gene-environment interactions and pathways that are affected by specific chemicals. For example, yeast cells exposed to 2,4-D re-model cell walls that are important in the protection of membranes with multiple functional roles (Viegas et al., 2005). Further, 2,4-D is shown to give several stress responses in yeast, including signaling pathways, cell growth, nutritional regulation, amino acid depletion and oxidative stress (Teixeira et al., 2007).

2,4-D has relatively low toxicity in aquatic systems. For example, the EC50 for the cyanobacteria *Anabaena* CPB4337 was 25.23 mg/L. When this cyanobacteria was pre-exposed to the surfactant perfluorooctanic acid (PFOA), the toxicity of 2,4-D increased, illustrating the important topic of interacting multiple stressors (Rodea-Palomares et al., 2015). In *Daphnia magna*, the LC50/EC50 acute toxicity is relatively low, i.e. in the range 144 – 248 mg/L for 24 h, and 25 mg/L for 48 h, respectively (Lilius et al., 1995; Toussaint et al., 1995).

2.3 Toxicity of dicamba

An increasing number of GE crops are tolerant to dicamba: HT GE cotton (MON88701), HT maize MON87419 (dicamba and glufosinate ammonium resistance) and GE soybean as single event (MON87708), or in 2-, 3- or 4-fold stacks combined with glyphosate resistance, changed fatty acid content and insect resistance. In all events dicamba tolerance is transferred to a *dmo gene*, the coding sequence for it is from *Stenotrophomonas maltophilia*. The herbicide dicamba is 400 times more toxic to broadleaf plants than glyphosate (see Schütte et al., 2007). Both dicamba and 2,4-D are highly volatile, thus increasing the potential for damage to non-target organisms due to spray drift. Sensitive crops, vegetables, ornamentals, and plants in home gardens could be damaged and both plant and arthropod communities in field edges and semi-natural habitats affected (see Schütte et al., 2007). As for the health effects, Dicamba is a suspected endocrine disruptor (Zhu et al., 2015).

2.4 Toxicity of glufosinate ammonium

GE plants that contain the *pat* gene from *Streptomyces viridochromogenes* become tolerant to herbicides containing glufosinate ammonium. Glufosinate ammonium belongs to a class of herbicides that is classified as showing reproductive toxicity.[2]

[2] http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN

Glufosinate ammonium is harmful through inhalation, swallowing and by skin contact. Serious health risks may result from exposure over time. Observations of patients poisoned by glufosinate ammonium have found that acute exposure causes convulsions, circulatory and respiratory problems, amnesia and damages to the central nervous system (CNS) (Hung, 2007; Watanabe and Sano, 1998). Chronic exposure in mice has been shown to cause spatial memory loss, changes to certain brain regions, and autism-like traits in offspring (Calas et al., 2008; Laugeray et al., 2014).

Effects on humans and mammals include potential damage to the brain, reproduction (including effects on embryos), and negative effects on biodiversity in environments where glufosinate ammonium is used (Hung, 2007; Matsumura et al., 2001; Schulte-Hermann et al., 2006; Watanabe and Sano, 1998). A literature review by EFSA on the risk of glufosinate ammonium has concluded it is especially harmful to mammals (EFSA, 2005). According to EFSA, the use of glufosinate ammonium will lead to exposure to farm workers that exceeds acceptable exposure levels during application. Its agricultural usage is no longer allowed in the EU, EU approval of glufosinate expired on 31 July 2018.

2.5. Toxicity of isoxaflutole and its metabolites

Isoxaflutole belongs to a class of herbicides that causes the plants to “bleach” and die (so-called HPPD inhibitors). Bayer used genetic engineering technology to develop the FG72 soybean (Balance Bean) made tolerant to applications of glyphosate as well as to isoxaflutole-based herbicides. Other HPPD inhibitors that could be used in the cultivation of soybeans have so far been ignored. According to available publications, at least one further active substance, mesotrione, could also be used in the future (Schultz et al., 2015). Isoxaflutole is classified as a “suspected human carcinogen” (EFSA, 2016). Gonçalves et al. (2016) concluded that isoxaflutole causes morphological changes in the digestive system of the generalist predatory insect *Podisus nigrispinus*. Metabolites from isoxaflutole have so far not been found in conventional soybeans, but are known to occur in HT GE plants (EFSA 2016). According to EFSA (2016) the available data are not sufficient to assess the toxicity and potential health impacts of these metabolites.

2.6 Mixed herbicide toxicity and health effects

This brief summary on potential harm from herbicide exposure, both in the environment and in the food chain, indicates what we can expect to happen if we approve and accept new stacked HT GE plants for the European market. These plants will promote increased use of the relevant complementary herbicides and these will ultimately end up in our food chain.

Please note, our summary above **only discussed direct effects of a single substance at a time**. HT GE plants with stacked herbicide traits must be expected to have more serious effects as co-exposure to several herbicides, in consumers, can cause **combinatorial effects.**

The field of combinatorial effects of stacked GE plants is currently not addressed by EFSA (Bøhn, 2018). EFSA has even ignored new empirical data and findings on combinatorial effects. i.e. that the toxicity of Bt-toxins was modified by exposure to Roundup (Bøhn et al., 2016, Bøhn, 2018), and failed to assess combinatorial effects in triple resistant HT GE soybeans intended for the European market (see case studies later in this report).

One lesson to be learned from glyphosate tolerant GE plants is that new stacked HT GE plants will increase the use of the herbicides relevant for them. This will promote the evolution of resistance to

these herbicides, which again will increase their use.

With the advent of HT GE crops that are tolerant to glyphosate and 2,4-D, scientists expect that relying on weed management with herbicide mixtures will favour and select for weeds with multiple resistances (Mortensen et al., 2012). That could be by the accumulation of specific mechanisms against each target site or by a more general mechanism, e.g. by confining herbicides in cell vacuoles which would render them resistant to further herbicides, leaving hardly any chemical management options for farmers (for different resistance mechanisms see Schütte et al., 2017).

Herbicides used with stacked events are expected to be found in increasing concentrations in our food chain. At present, from a European food chain perspective, the next generation of herbicides being used in HT GE crops, in addition to glyphosate and glufosinate, are 2,4-D, dicamba, isoxaflutole and others. Thus, approval and use of new HT GE plants, will over time lead to an extended exposure to these chemicals including their breakdown products. This will affect European consumers if these HT GE plants are authorized to enter the EU as food and feed, even if they are not authorized in the EU for commercial cultivation. Ultimately, we may expect to find these chemicals in our food chain, in various concentrations, being consumed by animals and humans. This is a substantial risk. Rather than EFSA accepting the exposure of European citizens to such risks, EFSA should conduct a proper and comprehensive risk assessment to ensure the safety of these new products and their derivatives, regardless any other (economic or political) issue.

3. Specific problem 1: Application rates of glyphosate on HT GE soybeans: Farmer use versus field trial use

To make sure a plant product is safe, the material used for testing needs to be representative for the large bulk of material that is produced, sold and consumed. This need is reflected in COMMISSION IMPLEMENTING REGULATION (EU) No 503/2013, which requests that the field trials with HT GE plants should compare and test plant products with and without the complementary herbicide being applied. The data provided for risk assessment should allow the regulator to conclude whether the expected agricultural practices influence the expression of the studied endpoints.

This is unfortunately not the case for HT GE plants under the existing EFSA risk assessment process.

The farmers using glyphosate tolerant GE soy have consistently exceeded the recommended rates of glyphosate use (1.72 kg/ha) (Duke et al., 2017). Official statistics (Benbrook 2016) confirm that the rates of glyphosate use in Argentina and Brazil, two of the dominating countries for soy production, have increased significantly from 1996 to 2014 with an almost perfect linear fit (R^2 = 0.98 and 0.91, respectively, $p<0.0001$, linear regression), reaching 3-4 kg/ha in later years (Fig. 1). Thus, these farmer rates are more than twice as high as the recommended doses used in most field trials.

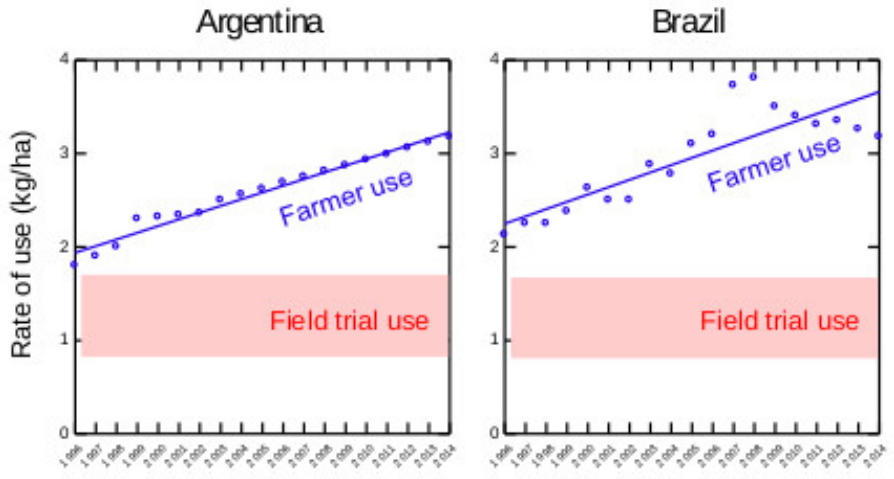

Figure 1. Rate of glyphosate use in HT GE soybean production, Brazil and Argentina 1996 – 2014. Data from Benbrook 2016. Field trial use as recommended by Duke et al., 2017 is shown as reference.

It is unacceptable that field trials, which are the basis for the safety assessment and quality testing of the plants, are sprayed according to an agricultural practice that deviates significantly from the reality of commercial farmers. **Consequently, these field trials produce unrealistic and irrelevant test samples for risk assessment, which lead to systematically underestimated risks.**

This exemplifies how an essential principle of good science – as realistic test-conditions as possible – fails in the European risk assessment system (Bøhn, 2018). Moreover, when test plants are grown specifically for use in toxicological feeding studies, we document that spraying with herbicides is done with even lower rates or is totally missing for some herbicides (see case studies in this report). In other cases, no feeding study is performed at all (see case studies in this report).

The average number of applications of glyphosate, sprayed by Argentinian and Brazilian farmers, also show a steady increase, from about 2 per year in 1996 to more than 4 applications from 2007-2010 and later (Fig. 2). Again, the relationship was highly linear (R^2 = 0.97 and 0.74, $p<0.0001$, linear regression) for Argentina and Brazil, respectively.

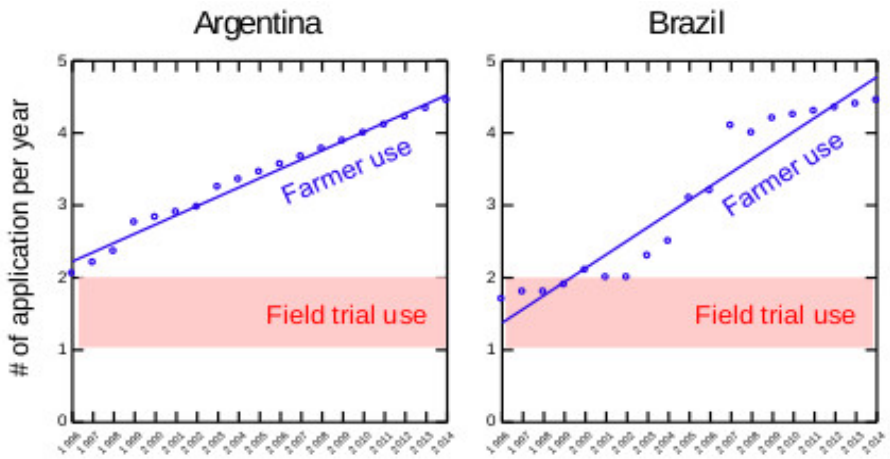

Figure 2. Number of glyphosate applications on HT GE soybeans in Brazil and Argentina 1996 – 2014, data from Benbrook (2016). Field trial use as recommended by Duke et al., 2017 is shown as reference.

Figures 1 and 2 illustrate how the farmers respond dynamically to increasing problems with resistant weeds. Moreover, these trends demonstrate the lack of follow-up in the field trials to match the practice of the farmers. The added applications performed by the farmers, i.e. sprayings no. 3 and 4, are most likely happening late in the season. Research has shown that it is the late-season-spraying that magnifies the residues up by a factor of 10 or more in soybean (Duke et al., 2003). This is further discussed in Specific Problem 2 (below).

These data still might not represent the agricultural practices under real conditions in many regions. For example, a study from Argentina reports the glyphosate application rate of no less than 10 kg /ha (Avila-Vazquez et al., 2018). Almeida et al. (2017) analysed the herbicide use in Brazil between 2000 and 2012 and reported an increase to more than 9 kg/ha per hectare for soybeans. A similar trend can also be observed in USDA (2018) data which shows applications of 7 kg /ha. Based on the data available, we conclude that it can be assumed for post-emergence applications that 3-4 kg (of active ingredient, a.i.)/ha of glyphosate from two to three sprayings is a reasonable rate. For the overall dosage (pre- and post-emergence), 6-7 kg (a.i.)/ha seems to be relatively common and even 8-10 kg (a.i.)/ha can be expected under current farming conditions in some regions (such as Argentina, Brazil and Paraguay).

The European risk assessment system, including EFSA, overlooks this key risk factor. These agricultural practices will not only cause high residue level of herbicides present in HT GE soybeans on the commercial market; they are also likely to cause changes in the plants composition and cause higher mixed toxicity (Miyazaki et al., 2019). The gaps in risk assessment are analysed in more detail by two relevant case studies with soybeans from Bayer and Dow, both triple tolerant to herbicides and meant for the European market (see below).

4. Specific problem 2: Assessment of herbicide exposure pathways in the food chain and relevant legal requirements

The excessive use of glyphosate-based herbicides in the countries of production of HT GE crops, has resulted in significantly higher residues of these herbicides in feed and food. The near ubiquitous presence of glyphosate shows that increasing herbicide use is not only an agronomic and an environmental problem (as described above), it also represents an ignored health risk to consumers. Residues from spraying with glyphosate in plant products are particularly relevant for soybeans since about 80 % of the soybean imports into the EU come from shipments which include glyphosate tolerant GE soy (EU Commission, 2016).

Studies that have sprayed HT GE soy with a single dose of glyphosate, i.e. with about 0.8-1.2 kg/ha active ingredient, have measured a residue level (glyphosate + AMPA, its main breakdown product) of about 1 mg/kg (Duke et al. 2003, 2018). When soy is sprayed late in the growing season, the residues can be much higher. This was illustrated by Duke and co-workers when they sprayed HT GE soybean a single time at full bloom, i.e. 8 weeks after planting: the residues of glyphosate and AMPA (summed) increased from about 1 mg/kg, to 9 and 28 mg/kg for the two sites tested (Fig. 3) (Duke et al., 2003).

Bøhn et al., (2014) showed that HT GE soy samples for 10 US farmers contained on average 9.0 mg/kg of glyphosate + AMPA. This contrasted with conventional and organic soybean samples from the same area; these contained no glyphosate.

Thus, when soy samples have been taken directly from farmers, the residue level of glyphosate and AMPA is rarely below 1 mg/kg, rather in the range 8-15 mg/kg (Bøhn et al. 2014). Samples from Argentina show even higher residue levels, i.e. up to 72.8 mg/kg (maximum) and 31.7 mg/kg (average) concentration of glyphosate residues (glyphosate and AMPA) (Testbiotech, 2013). These data are summarized in Figure 4 and indicate that farmers in all dominating soy producing countries, in practice, spray HT GE soy multiple times and also late in the growing season, resulting in much higher residue levels in soybeans as compared to soybeans grown in field trials for research purposes.

It is important to underline that in the case of imported soybeans, the residues from spraying always come from commercial formulations such as Roundup, and not only the single active ingredients – such as glyphosate.

Apart from the active ingredients, adjuvants (additives and wetting agents) are added to complete the commercial formulations that the farmers use. The adjuvants may enhance the toxicity compared to the single active substance, by a factor of up to 1000 times (Mesnage et al. 2014). For this reason, the use of particularly problematic adjuvants, such as polyethoxylated tallowamine, is either restricted or prohibited in several EU countries.[3]

Nevertheless, the application of POE-tallowamine, is not prohibited in countries where HT GE plants are grown.[4] From existing data, it has to be assumed that in commercial mixtures applied in the fields in Argentina, around 50 percent consist of glyphosate and about 15 percent are tallowamine (Perez et al., 2011, see also Mesnage et al., 2019). The exact formulations sprayed on the plants are kept secret and treated as confidential business information.

In consequence, it can be expected that HT GE soybeans for import to Europe have been sprayed with formulations that are not allowed in the EU, but are being used in countries such as Argentina, Brazil and the US.

As a publication by Kleter et al. (2011) shows, new patterns of herbicide residues in HT GE plants, resulting in new and increased exposures, are presently not taken into account in regular pesticide registration:

> *"1. GE herbicide-resistant crops can change the way that herbicides can be used on these crops, for example:*
> *(a) post-emergent over-the-top applications (i.e. on the crop itself) instead of directed sprays, avoiding herbicide contact with the crop; or*
> *(b) pre-emergent and pre-harvest applications made to the conventional crop and not, or in different quantities, to the GE crop.*
> *2. The residue profile of the applied pesticide may have been altered based on the nature of the modification.*
> *3. The overall pattern of pesticides applied to the particular crop may have been altered, leading to different exposure to pesticide residues overall."*

Thus, due to the new and specific agricultural practices that go along with the cultivation of HT GE plants, there are specific patterns of applications, exposure and occurrence of metabolites that require special attention.

These issues are particularly relevant for the risk assessment of imported HT GE plants. For market

[3] https://ec.europa.eu/germany/news/glyphosat-eu-staaten-schr%C3%A4nken-beistoffe-und-nutzung-ein_de
[4] http://dip21.bundestag.de/dip21/btd/18/073/1807373.pdf

authorization of HT GE plants, we highlight the following relevant provisions of the Pesticide Regulation:

- Article 29 of Regulation 1107/2009: active substances and synergists have to be approved, the maximum residue levels for the specific agricultural products have to be determined;
- Article 4 of Regulation 1107/2009: pesticides must not have any harmful effects on human or animal health, taking into account known cumulative and synergistic effects;
- Recital 5 of Regulation 396/2005: residues should not be present at levels presenting an unacceptable risk to humans and, where relevant, to animals;
- Recital 10 of Regulation 396/2005: specific maximum residue levels (MRLs) for each pesticide in food and feed products have to be established;
- Recital 26 of Regulation 396/2005: MRLs have to be set for food and feed produced outside the Community if produced by different agricultural practices as regards the use of plant protection products;
- Article 14 of Regulation 396/2005: the presence of pesticide residues arising from sources other than current plant protection uses and their known cumulative and synergistic effects have to be determined; any potential risks to consumers with a high intake and high vulnerability have to be taken into account.

The need for risk assessment of HT GE plants, taking into account the application of the complementary herbicides is recognized in the COMMISSION IMPLEMENTING REGULATION (EU) No 503/2013 which requires that field trials with HT GE plants have to be performed with and without the complementary herbicide being applied and to provide data which make it possible to assess whether the expected agricultural practices influence the expression of the studied endpoints.

Consequently, even if an active ingredient such as glyphosate is authorized for use in the EU, *detailed investigation of the residues is required for imported food and feed products if the plants were grown under a different regulatory regime/agricultural practice.* However, also according to EFSA's Pesticide Panel, the existing data are insufficient to come to assess health risk assessment of HT GE plants at the stage of consumption (see problem 3, this report).

5. Specific problem 3: Glyphosate and human health safety after consumption of products derived from HT GE plants

The multiple sources and exposure pathways of glyphosate bring this herbicide into consumers in measurable concentrations. For example, 99.6 % of spot urine samples from Germany were found to be positive for glyphosate in 2011 (Krueger et al., 2015) ("Urinale 2015"), mean concentration 1.08 ug/L. In this study, children (0-9 years) had higher concentrations than adults.

Another study showed higher levels of glyphosate in urine from children as compared to mothers (1.96 vs 1.28 ug/L) (Knudsen et al., 2017). The same study also showed positive correlations between different pesticides, those exposed also had higher concentrations of other substances.

People in Europe show a high presence of glyphosate in their urine. In a study in Portugal, 100 % of people tested showed a positive detection, with 0,31 ug/L on average.[5] In other countries 10-90 % of people tested have shown positive detection for glyphosate (Hoppe 2013).

[5] Portuguese No GMO Coalition, 2019. https://www.stopogm.net/contaminacao-cronica-por-glifosato-em-portugal

Mills et al. (2017) tested the amount of glyphosate in urine in older adults in California. The mean values in urine in 1993-96 versus 2014-16 were 0.024 and 0.314 ug/L, a factor of 13 times higher in recent years. For AMPA, the levels increased from 0.008 to 0.285, a factor of 36 times higher. Chronically ill humans have been shown to have significantly higher levels of glyphosate in their urine compared to healthy individuals. However, humans eating predominantly organic food had much lower glyphosate levels in their urine (Kruger et al., 2014).

Glyphosate is also found in farm animals. Danish cows have shown significantly higher glyphosate content in their urine compared to German cows (about 40 and 20 ug/L). In GE free zones in Germany the level was significantly reduced compared to conventional husbandry cows (Kruger et al., 2013). Moreover, glyphosate was extracted from different animal organs: intestine, liver, muscles, spleen and kidney (Kruger et al., 2013), and has also been detected in the bones of rats (Brewster 1991). The maximum residue levels in Europe (EU MRLs)[6] for glyphosate in crops are several orders of magnitude higher than for any other pesticide (approximately 200 times). These reflect and effectively authorize massive spraying of GBH, with high level residues thus arising in feed for cattle. These also lead to high MRLs in food (as an example 20 mg/kg is allowed in bovine kidney). Glyphosate cannot be washed away and is not broken down by boiling (EFSA 2009).

Given the spread and concentrations of glyphosate globally, detailed studies of health impact from glyphosate residues must be initiated and glyphosate regulations need to be critically re-evaluated. It is not clear to what extent in the EU the import of products derived from HT GE plants are contributing to the overall glyphosate load of livestock and health effects in humans and animals. While EFSA considers this problem as being of minor relevance (EFSA 2018a), we argue that significant uncertainties are evident and the amount of residues being imported in HT GE plants is likely to be substantial.

However, there are only limited data upon true exposures in the food chain: EFSA, in its 2015 European Union report on pesticide residues in food (EFSA 2017a), showed that in the whole of the EU no samples at all were taken to investigate residues from spraying soybeans with glyphosate. As EFSA states:

> *„Since soybeans are an important globally traded commodity on which glyphosate is frequently used, more detailed information on the occurrence of glyphosate residues would be desirable.“*

Further EFSA, in its 2018 report admits (EFSA 2018a):

> *“For genetically modified crops, data were sufficient to derive MRL for sweet corn (EPSPS modification) and cotton seed (EPSPS modification), noting that MRLs should be tentative pending on the submission of confirmatory methods for enforcement of AMPA and N-acetyl-glyphosate. For sugar beet roots, maize and soybeans (EPSPS modification), soybeans (GAT modification) and rapeseeds (GOX modification), the available data were insufficient to derive MRLs and risk assessment values.”*

Also, in its risk assessment of glyphosate, EFSA (2015) stated that not enough data were available on the application of glyphosate to glyphosate tolerant GE (GM) plants:

> *«In the framework of the renewal, representative uses were proposed for conventional crops only and residue trials on glyphosate tolerant GM crops were not provided.”*

[6] http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.selection&language=EN

This is the reason why EFSA's risk assessment on effects on health from glyphosate is limited to conventional crops:

> *«Based on the representative uses, that were limited to conventional crops only, chronic or acute risks for the consumers have not been identified."* (EFSA 2015)

Further, EFSA (2015) states that more investigations are needed, for example, concerning the carcinogenicity of the formulations that are applied commercially:

> *«In particular, it was considered that the genotoxic potential of formulations should be addressed; furthermore, EFSA noted that other endpoints should be clarified, such as long-term toxicity and carcinogenicity, reproductive/developmental toxicity and endocrine disrupting potential of formulations."*

As stated above, it must be expected that the HT GE soybeans for import have been sprayed with formulations that are not allowed in the EU, but are being used in countries such as Argentina, Brazil and the US.

In summary, our findings show that since 1996, the EU never performed a risk assessment on the imported food and feed derived from HT GE soybeans as requested by EU regulations.

Stacking of herbicide tolerance traits in the same plant (see the two case studies in this report) adds substantial complexity to the problem. Not only by adding one or more herbicides to what used to be a single substance (stressor) present, but also by creating complex 'cocktails' of stressors with increased risk for combinatorial effects.

6. Specific problem 4: Comparative studies of plant composition and mixed toxicity

The European system for risk assessment, as implemented by EFSA, fails to recognize that spraying a HT GE plant with its (single or multiple) complementary herbicide(s), and with increasing but varying doses and number of applications, as done by farmers in commercial use, may significantly alter the composition of the plant.

Bøhn et al. (2014) demonstrated that the nutritional and elemental composition of soybeans produced by different agricultural practices in the US (GE, conventional and organic), resulted in substantially different nutritional composition. Even with the glyphosate residues omitted from the analysis, the authors were able to differentiate (with discriminant analyses) the compositional elements of the soybeans, without any overlap between the soybean types.

In another study, deviating composition (disturbed metabolism) between NK603 GE maize and its near isogenic counterpart was shown with an integrated multi-omics analysis: significantly higher levels of 2 isoamines, namely N-acetyl-cadaverine and N-acetylputrescin were found in the GE maize (Mesnage et al., 2016).

Since glyphosate may reduce photosynthesis, even in HT GE plants, Zobiole tested whether spraying of 0.8, 1.2 and 2.4 kg/ha of glyphosate affected the nutritional status of the plants. This study showed that increasing glyphosate applications and spraying plants in later growth stages decreased nutrient accumulation, nodulation, leaf area, and shoot biomass production in the plants (Zobiole et al, 2012). Moreover, HT GE soy has been shown to have significantly reduced micro-

and macronutrients in leaves and shoots (Zobiole et al., 2011). Glyphosate application on HT GE soybean has also been shown to significantly decrease the polyunsaturated Omega-3 and Omega-6 fatty acids, but increase monounsaturated fatty acids (Zobiole et al., 2010).

One relevant hypothesis to explain patterns of reduced nutritional elements in HT GE plants is the chelating properties of glyphosate. Chelating agents bind micro- and macronutrients, many of which are essential in many plant processes, including processes that protect the plant from pathogens and disease (Mertens et al., 2018). It is also shown that glyphosate interacts strongly with phosphate (P) in the soil, resulting in altered toxicity of the glyphosate, depending further on the soil type (Bott et al., 2011).

Miyazaki et al (2019) give an overview on changes in the plant composition as found in available publications, listing more than 100 significant findings. Such changes may be particularly relevant for soybeans because they contain a naturally high concentration of phytoestrogens and allergens. These highly bioactive substances can be modified and potentially increased by interactions with herbicides, degradation products of herbicides or additives. For example, studies have found disturbances in the endocrine system of young rats when fed with soymilk in combination with glyphosate (Nardi et al., 2016).

There are many other biologically active substances known in soybeans, having toxicological, hormonal, allergenic or pharmaceutical potential (Kurosu, 2011; Cabrera-Orozco et al., 2013). In this context, there are strong indications that the EPSPS enzyme, which confers glyphosate tolerance, is also interfering with the auxin metabolism in the plants (Fang et al., 2018). Auxin is involved in multiple metabolic pathways in plants. Thus, changes in the auxin content can also result in changes in plant composition that can raise safety concerns. For example, there are indications that the content of highly toxic gossypol is enhanced in stacked HT GE cotton made tolerant to glyphosate (Then & Bauer Panskus, 2018).

These examples illustrate direct or indirect ways that herbicide used on HT GE plants may cause further unintended risks to health. The use of herbicides may also alter the composition of a HT GE plant in more complex ways, i.e. through interactions between the herbicides and the plant itself. Duke et al. (2012) rejected the hypothesis that the mineral content of HT GE soybeans changed by the use of (relatively low rates of) glyphosate, i.e. 0.86 kg/ha, applied once or twice early in the season. However, we have shown that such dose levels are outdated in comparison to what farmers use in practice, i.e. about 4 kg/ha (c.f. Fig. 1).

However, EFSA never assessed the interrelation between the application of glyphosate (dosage, number of applications and timing) and the gene expression and changes of metabolism in GE HT plants.

Internationally agreed lists of nutritional/compositional parameters analysed in soybean matrices for food, cover only a limited number of the known biological substances. In the suggested list by the OECD (2012), testing the concentrations of allergens is not required. Moreover, pharmaceutically active ingredients such as saponins are not mentioned, and not all known plant estrogens and known toxic substances are included. Other biologically active molecules discovered more recently are also missing from the analyses (see for example Cabrera-Orozco et al. 2013).

For stacked GE plants, the potential interactions between herbicides and the plant will be much more complex, but also more likely. For example, the use of glyphosate, glufosinate and isoxaflutole, sprayed in various concentrations and with multiple applications, constitute complex

mixes with a range of modes of action that may interact with the plant and its environment.

Although the COMMISSION IMPLEMENTING REGULATION (EU) No 503/2013 requires that field trials with the GE plants have to be performed with and without the complementary herbicide being applied, EFSA does not require that the sprayings applied in the field trials have to be in accordance with *current* agricultural practices. In consequence, the compositional analysis and assessment of phenotypical characteristics as performed by EFSA do not consider the doses, the number of sprayings and the timing, although these factors may alter the plant's characteristics and quality.

In summary, risks to health coming directly from herbicide residues, or through interactions with the HT GE plant, are not considered in the EU approval process to the extent necessary. We argue that the above-mentioned gaps in the current risk assessment are due to the EU Commission's attempts to separate the question of herbicide residues from the question of the HT GE plant approval. This is the approach followed by the EU Commission in, for example, the amendment of Annex 2001/18 of the EU Release Directive 2001/18. The EU Commission is prepared to defend this separation even in cases brought to the European court (C-82/17 P).

7. Case studies in a European context: soybeans with triple tolerance to herbicides

These two case studies illustrate how EFSA fails to perform relevant risk assessments of new HT GE plants that were approved for import into the European market in 2017. Both cases deal with GE soybean plants that have "stacked traits" – they are each tolerant to three different types of herbicides (see Table 1 for details).

Table 1: HT GE soybeans from Bayer and Dow that are tolerant to three herbicides

Company	Name of HT GE soybean	Tolerant to complementary herbicides (group)	Herbicides tested in field trials
Bayer	FG72 x A5547-127 trade name: 'Balance Bean'	glyphosate glufosinate HPPD inhibitors	glyphosate glufosinate isoxaflutole
Dow AgroSciences	DAS-44406-6 trade name: 'Enlist™ traits'	glyphosate glufosinate phenoxy herbicides	glyphosate glufosinate 2,4-D

Bayer and Dow AgroSciences have received approval to import into the EU the HT GE soybeans "Balance Bean" (also named FG72 x A5547-127) and „Enlist" (DAS-44406-6), respectively. "Balance Bean" is made resistant to glyphosate, glufosinate and HPPD inhibitors (such as isoxaflutole), „Enlist" to glyphosate, glufosinate and phenoxy herbicides (such as 2,4-D). Food and feed products derived from these plants were accepted by the EU Commission at end of the year 2017.

7.1. Triple tolerant HT GE soybean "Balance Bean" from Bayer

Bayer first developed the FG72 soybean (Balance Bean). This new event is not only tolerant to applications of glyphosate – it is also tolerant to HPPD inhibitors, such as isoxaflutole, that cause the plants to "bleach" and die. The FG72 soybean was allowed for import into the EU in 2016. In an extension of its business model, Bayer crossed the FG72 soybean with another HT GE event,

in order to make the plants additionally tolerant to glufosinate ammonium. This latter herbicide is in formulations known under brand names such as Liberty or Basta. The harvest of the soybean (FG72 x A5547-127) has been authorised for use in the food and feed industry and for import into the EU. It is the very first time that approval is given to a GE plant tolerant to three different herbicides. Analysis of the relevant data provided to EFSA by the companies has revealed major gaps in EFSA's risk assessment (EFSA 2017b & c).

Based on documents filed for the approval of soybeans FG72 and FG72 x A5547-127, it is evident that the risks associated with these HT GE soybeans were not assessed under realistic practical conditions. The following specific conditions were chosen for the field trials:

- In field trials with FG72, the soybeans were sprayed with around 1 kg glyphosate/ha.
- In field trials with FG72 x A5547-127, 0.863 kg of glyphosate/ha was applied to the soybeans.
- A maximum amount of 70 mg/ha of isoxaflutole was applied to FG72 and FG72 x A5547-127.
- An additional 448g/ha of glufosinate was applied to FG72 x A5547-127.
- The plants were only sprayed once at a relatively early growth stage.

The amount of glyphosate used in the field trials (0.86 - 1.0 kg/ha) was distinctly below that of the amounts used by commercial farmers (3 - 4 kg/ha) (cf. Fig 1). Similarly, in contrast to Bayer's field trials with a single spray application, commercially grown HT GE plants are sprayed 3 - 4 times or more (cf. Fig. 2).

In the US, Monsanto officially recommends that dosages of around 3 kg glyphosate per ha are applied to the soybeans (as a maximum applied directly sprayed onto the soybeans during cultivation), with additional spraying around 5 kg/ha before and after cultivation.[7] Interestingly, according to a patent application filed by Monsanto for how to use glyphosate, the amount of glyphosate sprayed onto fields with HT GE soybeans can sum to a total of 8 kg/ha.

Thus, the rates of glyphosate used for Bayer's field trials are unrealistically low and not at all representative for the soy products that will come to the commercial market.

A similar problem exists in the case of glufosinate. For the use of glufosinate in the cultivation of soybeans, Monsanto (WO2008051633)[8] recommends up to 905 g/ha. However, in the field trial, Bayer used a single dose only, with 448 g/ha. No specific data were made available concerning the metabolites emerging in the soybeans from the application of glufosinate.

Another problem emerges from the application of isoxaflutole. This herbicide is classified as a "suspected human carcinogen" (EFSA 2016). By application of isoxaflutole on the GE HT soybeans, new degradation products are known to emerge in relatively high concentrations. These metabolites are not known from conventionally grown soybeans but are only found in the genetically engineered plants. EFSA (EFSA, 2016) was unable to evaluate risks to health from these new substances due to a lack of necessary data. Therefore, the EU authority was also not able to set maximum limits for the amounts of these new residues in the harvest even though this is a legal requirement (Regulation 396/2005).

Furthermore, there are also other herbicides from the group of HPPD inhibitors. According to available publications, at least one further active substance, mesotrione, can be used directly on

[7] https://assets.greenbook.net/16-50-19-15-03-2018-63045R2-9_PMAXII_SpecimenLabel_2017.pdf
[8] https://patentscope.wipo.int/search/en/search.jsf?docId=WO2008051633&tab=PCTBIBLIO&maxRec=1000

these plants (Schultz et al., 2015). Over a dozen other HPPD inhibitors and their application on Bayer herbicide tolerant plants are claimed in patents held by the company.[9] Each one of these substances can leave specific residues in plants and/or lead to changes in their metabolism. However, the only substance applied during the field trials was isoxaflutole.

In summary, i) no data are available on residues of isoxaflutole or any of the other HPPD inhibitors, ii) no potential combinatorial effects resulting from the residues in the plants were assessed, and iii) Bayer did not perform any rodent feeding study with its triple tolerant HT GE soybean to investigate potential health impacts from consumption.

7.2. Triple tolerant GE soybean “Enlist” from Dow AgroSciences

The “Enlist” soybean (DAS-44406-6) from Dow AgroSciences is engineered to be tolerant to three different herbicides/classes of herbicides (EFSA 2017c). These are glyphosate, glufosinate and the group of auxin herbicides, from which 2,4-D is one specific substance.

As patent application WO2007053482[10] filed by Dow AgroSciences shows, the “Enlist” soybean, by inheriting the gene that produces the enzyme AAD-12, is also resistant to further herbicides named pyridyloxyacetate. Substances such as triclopyr, fluroxypyr and MCPA are members of this group and may also leave residues, either of their active ingredients or further breakdown products. None of these substances were tested in the trials for risk assessment and they were not even mentioned in EFSA’s evaluation of the “Enlist” plant.

Metabolites stemming from 2,4-D such as 2,4-dichlorphenol are regarded as being more toxic than the herbicide itself (EFSA 2017d). Recent publications suggest that carcinogenic metabolites can be produced in HT GE plants (Lurquin, 2016). However, these were not assessed by EFSA. In their field trials Dow applied herbicides more in line with farmer practices than Bayer did, i.e. they were spraying several times and using more realistic dosages.

Dow also performed a 90-day rat feeding study to investigate health risks. However, the soybean material used in the feeding study was not taken from the field trials as mentioned above. Instead Dow used other test samples from soybean that were sprayed with much less herbicide than the field trial: Glyphosate and 2,4-D were sprayed with less than half and less than a third of the dose from the field trials, respectively. Moreover, glufosinate was not sprayed at all on the samples for the feeding study (see table 2 below).

Thus, the “Enlist” soybean samples for the toxicological feeding studies were not representative of real-life samples expected on the market. Further, the feeding study suffers also from other major deficiencies: for example, no dose-dependent effects were investigated. Instead, only one low level dose of the soybeans was mixed into the diet.

Thus, arguably, this feeding study is meant to create the impression that health risks at the stage of consumption were investigated. In fact, the study is irrelevant as the test material is not representative for plant material that will come to the European market. Nevertheless, the study was accepted by EFSA.

[9] See patent application WO2017042259

[10] https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2007053482&tab=PCTBIBLIO&maxRec=1000

Table 2: Comparison of data on herbicide dosages (active ingredients, a.i. or acid equivalents, a.e.) applied in field trials for Dow´s genetically engineered "Enlist" soybean (DAS-44406-6) used in the field trials and as applied on the soybeans used in the toxicological feeding study

	Field trial DAS-44406-6	Feeding study with DAS-44406-6
Glyphosate	Three sprayings, with 3780 g a.e./ha in total	Two sprayings, with 1680 g a.i./ha in total
2,4-D	Three sprayings, with 3360 g a.e./ha in total	One spraying with around 1000 g a.i. /ha
Glufosinate	Two sprayings, with 800 g a.i./ha in total	None

7.3 Conclusions for the two case studies

One crucial aspect in risk assessment is the dosage of the herbicides and the number of sprayings applied in the field trials. This aspect is relevant for the burden of residues found in the harvest, potential combinatorial impacts on health, as well as for effects on overall plant composition. The dosages applied by Bayer are much lower than those normally applied under practical conditions, and they did not perform any feeding study to test for toxicological health effects.

Interestingly, Dow AgroSciences used much higher concentrations of the complementary herbicides in their field trials than Bayer. These soybean samples should have been used in their rat feeding study. However, Dow used other test samples for their toxicological feeding trials, samples that were sprayed with a limited set of herbicides: specifically, they omitted the use of glufosinate, which has documented serious health effects on mammals (see for example Calas et al., 2008; Laugeray et al., 2014). Moreover, glyphosate and 2,4-D were sprayed with much lower doses.

Metabolites with potentially serious health effects are known to occur in the GE HT soybeans, in particular related to the use of isoxaflutole and 2,4-D. However, these metabolites were not assessed by EFSA in detail. Moreover, the data were not sufficient to set MRLs in the case of isoxaflutole.

A further gap in risk assessment concerns the specific herbicides used in the field trials. In this regard, the genetically engineered soybeans from Bayer and Dow were made tolerant to more herbicide substances than tested in the field trials: the herbicides 2,4-D and isoxaflutole belong to larger groups of chemicals that encompass many other herbicides that can also be sprayed onto the plants during cultivation. Any relevant data for the risk assessment of spraying crops with these other herbicides are completely missing.

We conclude that the risks of "Balance bean" and "Enlist" soybeans were not sufficiently investigated and that there is huge uncertainty regarding actual health risks from consumption of these new GE soybean plants with triple herbicide tolerance. These soybean events therefore cannot be considered safe. On the contrary, there is a high risk that consumption of these soybeans may lead to harmful effects on health.

8. General conclusions

In the EU, risk assessment of HT GE crops as currently performed by EFSA is divided into the assessment of the organism (GMO-Panel) and the assessment of the pesticide (PPR-Panel). This separation unfortunately leads to serious gaps in the assessment of the safety of HT GE plants.

The two areas of risk assessment need to be integrated as already reflected in COMMISSION IMPLEMENTING REGULATION (EU) No 503/2013, which requires that the field trials with the GE plants test both sprayed and unsprayed plants, to take into account potential synergistic or antagonistic effects and to provide data which make it possible to assess whether the expected agricultural practices influence the studied endpoints.

However, in the current practice, there are major gaps in the overall risk assessment of HT GE plants:

- EFSA does not take into account that test samples from field trials are sprayed with much lower rates of herbicides as compared to current agricultural practices.
- If feeding studies are performed, there is no requirement that the tested plant material is produced under realistic conditions (i.e. in accordance with current agricultural practices).
- Not all residues and the patterns that result from spraying with the complementary herbicides, as well as metabolites that specifically emerge in the HT GE plants, are assessed.
- There is no assessment of the residues from herbicide formulations and relevant additives and adjuvants used in other countries. Thus, HT GE plants with unknown concentrations of herbicide residues are imported into Europe from regions with deviating and weaker herbicide regulations than those in the EU.
- HT GE plants that tolerate a mixture of complementary herbicides (stacked events) have an increasing trend in the global market. Combinatorial effects of mixtures of herbicide residues are documented (see for example de Arcaute et al., 2018), but this is not investigated in the current European risk assessment.
- The compositional analysis and assessment of phenotypical characteristics of HT GE plants do not consider the dose, the number of sprayings or the timing of herbicide application, although these factors can influence the plant and product quality and safety.
- Long-term effects of the consumption of products and their impact on the immune system, the endocrine system and the gut microbiome escape the risk assessment completely or to a large extent.

Regulation 1829/2003 requires that genetically engineered plants can only be approved for import if they are deemed to be safe. After having analysed the European regulatory system for HT GE plants, and how EFSA has performed its risk assessment analyses of the “Balance bean” and “Enlist” soybeans in particular, we argue that current and past approval practice of such GE plants contradicts EU laws on GMOs.

In the current practice of risk assessment for HT GE plants in Europe, many of the actual risks are framed out and systematically excluded from the evaluation. Crucial data on herbicide residues and potential changes in plant constituents are missing. Possible interactions and combinatorial effects are ignored. Against this background, no further HT GE plants should be approved for import until the health risks associated with the residues have been comprehensively investigated. The approval process for HT GE plants, is at present both inadequate and misleading. Finally, we argue, the problems described add up to a ‘mission failed’ for the core of EFSA’s responsibility: to safeguard European food systems and consumers.

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