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

Insecticidal Bt crops

EFSA's risk assessment approach for GM Bt plants fails by design

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(Dedicated to Frieder Hofmann who passed away far too early and is dearly missed)

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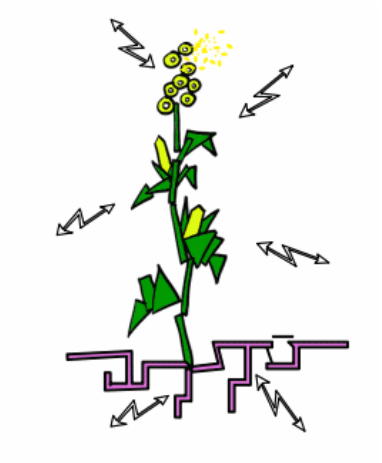

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Summary

A risk conclusion is typically based on combining information and data obtained from understanding the various routes of exposure to a particular stressor and the potential adverse effects this stressor may cause. The stressors in our case are genetically modified (GM) crop plants expressing toxins from the bacteria *Bacillus thuringiensis* (Bt). Exposure is evaluated in terms of quantity and quality of the stressor (concentration of Bt toxins) and duration/type of exposure to this stressor. However, the framing (problem formulation) and implementation of the risk assessment, including the assumptions made, the selection/limitations of the research results available and the interpretation of these data, is fundamental in determining what risks can be identified and what risks will be excluded right from the start.

We unravel and illustrate how EFSA's interpretation and implementation of the EU regulations for risk assessment is narrow and, thus, fails at its core. Instead of assessing the real, living GM plant within its complex network of ecological interactions in the real world, EFSA limits the focus primarily to the 'added chemical substances', i.e. Bt-toxins, and arrives at its conclusions regarding risks based mostly on data produced with Bt toxins isolated from an artificial bacterial surrogate system – hardly the GM Bt plant, except for occasional tests with pollen or leaves. Thus, effectively, EFSA is assessing the GM *organism*, here a plant, as an isolated, single chemical. The framing (serving as justification) of this narrow interpretation is based on the conceptual model called 'substantial equivalence' (renamed as 'comparative (safety) assessment') which means that a GM plant is nothing more than the original unmodified parent plant with the chemical, here the Bt toxin, added like a spray-on pesticide. The second assumption is that Bt-toxins have a single target specific mode-of-action, at least for non-target organisms. Both assumptions are rooted in outdated science which leads to sweeping/generalized safety claims that lack robust scientific evidence in support, or even face scientific evidence to the contrary. Nevertheless, both assumptions form the pillars for assessing GM Bt crop plants primarily like pesticidal chemicals and applying standard first-tier protocols developed by the OECD for synthetic pesticides[1] for their regulatory safety assessment.

In contrast to EFSA's narrow approach, leading to 'no risk' conclusions, we show that there are a multitude of pathways exposing many organisms, the vast majority of them being non-target organisms to both the GM plants and their Bt toxins. These pathways extend from terrestrial to aquatic systems along a diversity of exposure routes, including the recent discovery of intergenerational exposure from parent non-target organisms to their offspring. A myriad of non-target organisms are exposed to, i.e. ingest, Bt toxins, either from live or dead Bt plant material or free Bt toxins leached from live and dead Bt plants in significant quantities. Furthermore, these Bt toxins are persistently present above- and below-ground, throughout the growing season and beyond, including in aquatic ecosystems such as headwater streams running through the agricultural landscapes where Bt crops are grown. Hence, Bt toxins are highly ubiquitous in large amounts in those agroecosystems where Bt crops are grown, i.e. on more than a 100 million hectares across all continents. From there, these Bt toxins spread to aquatic systems via water transport processes within the soil or from water runoff from fields under Bt crop production.

We further describe how the single target specific mode-of-action paradigm is outdated; instead more models of modes-of-action are proposed and accepted today than there were decades ago when Bt crops were introduced. Consequently, an increasing number of non-target organisms are reported to be affected in many ways, outside of what used to be considered a limited range of target organisms. We call this the 'out-of-range paradigm'. We list 39 peer-reviewed publications that report significant (adverse) effects of Bt toxins on many 'out-of-range' species, including representatives from non-

1 short term, acute testing of isolated active ingredients on a selection of universal testing organisms (typically less than 10 organisms chosen by the applicant) that hardly if at all occur in the agroecosystem - https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems_20745761

arthropod taxa, like snails, crayfish, and bacteria. While this list of studies is not comprehensive (not a review), it does illustrate the growing diversity of affected species and effects arising from Bt toxins that researchers have observed and reported, most of which cannot be detected in short-term acute direct toxicity tests that are applied in the first tier OECD testing protocols. In other words, these adverse effects of Bt-toxins will be, and probably are already being, missed when using EFSA's approach of reducing biology to chemistry.

We conclude that EFSA systematically excludes important exposure pathways and interactions of Bt-toxins with whole communities of organisms in whole environmental compartments (e.g. aquatic ecosystems). Moreover, EFSA applies extra scrutiny to scientific evidence of potential harm to non-target organisms, using non-scientific arguments to justify their dismissal while claiming to have considered them. The outcome is that no further studies are recommended by EFSA, and relevant scientific uncertainty is not resolved but simply declared irrelevant. Therefore, EFSA's risk assessment of Bt plants fails – by design.

1. Introduction

Bacillus thuringiensis (Berliner) is a bacterium found foremost, but not exclusively, in soils (de Maagd et al., 2001). The bacterium produces a range of so-called crystal (Cry) proteins, each with somewhat different characteristics and specificities (Schnepf et al., 1998; de Maagd et al., 2001, Økstad and Kolstø 2012). Since its discovery over a hundred years ago, research into *B. thuringiensis* has focused almost exclusively on its potential for pest control (Hilbeck and Otto, 2015; Økstad and Kolstø, 2012; Sanchis, 2010). Historically, crystal protein and spore preparations of distinct strains of *B. thuringiensis* have been used as biocontrol agents in forestry, in agriculture, and in public health applications against vectors of human diseases, such as mosquitoes (van Frankenhuyzen, 2013). When genetic engineering tools became available, biotechnologists engineered the Bt toxin coding genes from *B. thuringiensis* into a number of commodity crops. The expression of one or more Bt toxins aims to make the GM plants able to defend themselves against pest insects of certain taxa, foremost lepidopteran and coleopteran species. The aim of Bt crops is to overcome the limitations of Bt pesticides – leading to persistence and increased efficacy by expressing the mostly activated forms (Latham et al. 2017). While both persistence and increased efficacy are promoted as beneficial for target pest control, they continue to be ignored for non-target safety assessment purposes. Also, the continuous exposure to Bt toxins, due to these being expressed within the entire plant throughout the season, represents a new dimension of prophylactic persistence and exposure compared to the pulsed spraying of Bt crystals, and often also spores only, when a pest problem arises and exceeds a threshold. This leads to chronic exposure of all organisms feeding on these plants, in addition to the target pests. Continuous exposure raises additional concerns regarding resistance development in target pest species, and thus the sustainability of the technology. However, in this report, we do not address pest resistance because this has been the only 'risk' factor that has been included in all pre-release 'applications'.

We also omit or only briefly allude to combinatorial effects stemming from customary application of all sorts of agro-toxins applied against other biotic problems beyond the target pests of Bt toxins and against weeds. This report deals with the regulations of GM crops and their deficiencies and the consequences of these deficient regulations. Of course, this is not where it ends and we are cognizant of this fact but the limits of this project did not allow us to include additional aspects here. They remain to be addressed in further critical analyses.

In Europe and most countries of the world, GM plants are subject to regulations, which require risk assessments for human health and the environment. Here, we focus on environmental risk assessment (ERA). In the EU, the relevant regulations encompass the Directives 2001/18 (environmental release) and Regulation (EC) 1829/2003. The environmental risk assessments as conducted by the EU

authority, i.e. the GMO panel of the European Food Safety Agency (EFSA), have remained highly controversial since the beginning for the reasons detailed below. After two decades into commercial production and use of Bt crops, we feel it is time for a stock taking exercise regarding the persisting controversies over EFSA's risk assessments. We describe what effects have been documented and reveal why EFSA's approach could not and will not capture potential adverse effects and the resulting risks – by default - through its interpretation and implementation of EU regulations.

Overview of insecticidal Bt crops imported to and grown in Europe

According to industry figures, genetically modified (GM) crop plants expressing insecticidal proteins from *Bacillus thuringiensis* (Bt) are grown predominantly in industrial production systems of 26 countries since 1996 (ISAAA 2016). In the European Union, only one Bt crop event is commercially cultivated to date, event MON810 maize expressing the Bt toxin Cry1Ab. On the other hand, over 40[2] applications for food and feed uses, import and processing of insecticidal Bt crops have been approved in the EU (Annex, Table 3). Thereof, only 6 are single trait Bt crops (MON810, Bt11, MON88701, 1507, MIR604, MIR162). The rest are GM crops with stacked traits, expressing insecticidal toxins for Lepidopteran and/or Coleopteran control, as well as herbicide tolerance traits conferring increased tolerance to glyphosate and/or glufosinate herbicides. Other added traits are meant to help select the transformed plants. These include antibiotic resistance, visual markers and mannose metabolism. Thereby, some of these stacked trait crops have up to eight transgenes introduced. The insecticidal Bt genes introduced include crystal (cry) and vegetative (vip) proteins derived from various subspecies and strains of *Bacillus thuringiensis*, as well as synthetic genes such as mcry3A, ecry3.1Ab and cry1Fa2. Furthermore, one approved GM crop includes a non-Bt insecticidal gene: the dvsnf7 gene from the Western Corn Rootworm (*Diabrotica virgifera virgifera*). This gene induces RNA interference in Coleopteran insects by down-regulation of the function of the targeted Snf7 gene.

Additionally, over 30 insecticidal Bt crops are currently awaiting EU approval (Annex, Table 4). Of these, only 5 include applications for cultivation. The application for renewal of authorisation of continued cultivation of MON810 is the only single trait crop in the pipeline. The stacked trait crops express the same traits mentioned above, but also include some additional traits such as increased tolerance to the herbicides Dicamba and 2,4-D or drought stress tolerance.

Apart from the crops approved or still in the pipeline for approval, 27 insecticidal Bt crops have been withdrawn from the EU pipeline before the finalisation of the risk assessment process, or later on from the market, for example, due to a commercial decision by the applicant (Annex, Table 5). In contrast to the crops approved or in the pipeline for approval, almost half of these withdrawn applications included application for cultivation.

2 The real number of insecticidal Bt crop plants allowed to be marketed in the EU is much higher, since the scope of some of the approval applications also include several sub-combinations of the evaluated multi-stack event. For these, EFSA followed a weight-of-evidence approach, and concluded that they are expected to be as safe as the multi-stack event. Hence, no additional data was required for the sub-combinations.

2. Hazard Identification – an ecologically unrealistic approach

The hazard identification step, by design, defines the scope and boundaries of an ERA. What is excluded here will not make it into the assessment later. Consequently, this step is the most critical step for determining the rigor and breadth of the entire ERA. Hence, unsurprisingly, the dichotomy between EFSA's exclusive, narrow approach and a more inclusive, broader ERA approach favoured by other scientists and the public begins right here.

How EFSA is reducing biology to chemistry

The EFSA GMO panel embedded this first step of the ERA within a step called 'Problem formulation', which includes identifying the actual stressor, i.e. the subject of the assessment (EFSA, 2010). The EFSA GMO panel has reduced the hazard identification process to only identify certain differences between the GM plant and its comparator[3], presumably the parental plant (if available), called the 'comparative safety assessment' (EFSA 2010) or the 'comparative approach' (EFSA 2011). This is based on a simple analysis of the basic compounds constituting any plant (or organism) (e.g. amino acids, protein, water, ash content), notably, including any variability of these basic compounds ever reported for this plant species in history. We have prepared a detailed critical appraisal of the comparative approach/safety assessment in a separate document (submitted for publication). Here, we focus on the aim of identifying the differences deemed relevant for assessment of Bt-plants.

The aim of the reduction, or rather exclusion exercise that EFSA performs in the first step of their problem formulation, is "*to focus the ERA on the potential environmental consequences of these differences.*" They continue that: "*while some differences may be deemed irrelevant to the assessment, others will need to be assessed for their potential to cause harm.*" (EFSA, 2010).

Consequently, based on this approach to the hazard identification step, the sole difference 'deemed relevant' and, therefore, the subject of the assessment, is the novel compound, typically tested in a decontextualized fashion from the GM plant. The novel substance, here artificial Bt-toxins purified from microbes, is treated as an 'added' chemical compound and tested in isolation from the actual subject of regulations, the whole GM plant. Moreover, since the Bt toxin is a pesticidal compound, it is tested like a synthetic pesticide where an applicant – at its discretion - can chose toxicity tests from a prescribed OECD list of testing protocols for synthetic pesticides (EFSA 2010, page 65-66; EFSA 2011, Table 2, page 11). The pesticide-testing regime is based on the use of universal surrogate testing species many of which have little if any ecological relevance to any receiving agro-ecological environment. These pesticide testing regimes start with so called 'first tier' tests based on protocols that are narrowly designed to test direct, immediate and acutely toxic effects of the purified, isolated single product. Only if these first tier tests deliver data of concern – which is also a flexible definition with a lots of space for interpretation – are higher tier tests, including more ecological realism triggered, which rarely happens.

Some illustrative examples of such ecotoxicity studies accepted by EFSA (2013) include a 48 hour lower-tier study with a mosquito species (in EFSA 2013, page 40), where even in highly susceptible target pests the mode of action takes longer to lead to definitive responses. Another example is a tier 1a study with 5 day-old honeybee larvae (EFSA 2013, page 43), clearly being in their late and, therefore, less susceptible larval stage if not already non-feeding prepupae (no study details provided in EFSA, 2013). According to Hendriksma et al. (2011), who also tested the impact of Bt-toxin containing maize pollen, their honeybee larvae reached the non-feeding pre-pupal stage on day 5 after hatching. Hence, unless reared at suboptimal low temperatures, the 5-day old honeybee larvae possibly were not even feeding anymore when exposed to the diet containing Bt toxins. Likewise, in

3 *'A comparison of the characteristics of the GM plant with those of its appropriate comparator (plant) enables the **identification of differences** in the GM plant that may lead to harm'EFSA Guidance 2010, page 14*

a tier 1a test with the predatory bug species *Orius laevigatus*, 4-5-day old nymphs were also used, which EFSA speculates are 'probably late second instars' – possibly even already in their 3rd larval stage depending on rearing temperature (Sanchez and Lacasa 2002). Significant effects on development time were detected nevertheless. Interestingly, EFSA then did recognize the rather old age of the nymphs but only to dismiss the differences as 'not biologically relevant' owing to possibly accumulated variability during their long development time prior to the test (pure speculation), instead of questioning the robustness and relevance of the test protocol in the first place or simply assigning it to what the test was designed to test, the impact of Bt toxins (EFSA 2013, page 41).

> All three tests were accepted as part of the evidence that "*the available data show that the expression of the Cry34Ab1/Cry35Ab1 proteins in maize 59122 has no toxic effect on non-target terrestrial arthropods …*" (EFSA 2013). In reality, these studies do no such thing.

Detailed critiques of the separation of the Bt-toxin, or any other added compound coded by the transgenes, from the GM plant have been published multiple times in the scientific literature (e.g. Hilbeck et al. 2011, Dolezel et al. 2011, Andow and Hilbeck 2004) - to little avail. Under a published, inclusive ERA approach, the whole Bt plant, i.e. the actual GM Organism (as laid down in the respective Directive) in its receiving environment is the subject of the ERA (Hilbeck et al. 2011). This published inclusive approach leads to fundamental differences in the number and types of identified hazards that should be subjected to a risk assessment compared to EFSA's exclusive approach.

However, as a result of this persistent criticism, the revised EFSA Guidance for ERA in 2010 contains some improvements. For example, since 2010, additional first tier tests (1b) may be required that actually use some GM plant material "*that guarantees exposure to both transgene products and the plant*". This GM material can, in theory, include anything from pollen, leaves to roots or the whole GM plant but in practical terms pollen is the primary GM plant test material of choice in regulatory studies, with nontarget organisms or dried and powdered leaf material mixed into artificial diets, if necessary (e.g. EFSA 2013 59122 incl. supplementing statement of the same year, EFSA 2011 MON88017). No mandatory minimum set of experiments with living whole plants in the closed environment, nor really at the open field trial stage, are required for biosafety testing purposes with nontarget organisms. At best, and at the applicant's discretion, data are submitted on surveys of nontarget organisms from field trials carried out for multiple purposes – typically, collecting material for compositional analyses, agronomic and phenotypic data. As collateral, some basic inventory lists of observed insects are compiled. Because many insect species are often present only in low numbers at a given moment in time (snapshot), or not all, or are present as transient visitors only, typically large variability exists in these kinds of generic studies that allow very little meaningful data to be obtained, which, on top of this, are often associated with low power of detecting existing difference. Consequently, a living GM organism is mostly assessed like a pesticidal chemical.

EFSA overlooks modifications of Bt toxins in GM plants

As a prerequisite for first tier testing, i.e. testing of isolated purified toxins from microbes, it is required that purified metabolites are '*in the same form as expressed the plant'* (EFSA 2010). Data provided to establish whether or not the purified toxins fulfil this requirement, comparative molecular characterization, has to be carried out for the purified proteins from the microbes and the GM plant. In basically all cases, the applicant and regulator (EFSA) determined that the measured differences, that always exist, were biologically irrelevant and based their conclusions on the '*weight of evidence'* confirming the presence of the expected bands/signals and justifying ignoring the unexpected ones – see the following example.

Example DAS 59122 maize assessed by EFSA (2013 referring to its assessment in 2007, page 11): *"... In addition, a band was observed at ca. 40 kDa for the P. fluorescens-derived Cry35Ab1 protein*

preparation, most likely produced by cleavage of the Cry35Ab1 peptide by proteases removing the C-terminal 4 amino acid peptide. ... Unidentified peptides were observed and some expected peptides were not detected, due to random cleavage or formation of fragments during hydrolysis. However this analysis provides good indicators on a high degree of homology between proteins from both sources." Furthermore, the first amino acid (methionine) was missing for both the *P. fluorescens* and GM maize derived Cry34Ab1 protein as compared to the wild-type protein. It is speculated that aminopeptidases can cleave peptides from the N-terminal end in both types of transgenic organisms. No further meaning is assigned to it, the overall conclusion rests on the '*weight of evidence'* which fits the expectation declaring that *'the P. fluorescens-produced Cry34Ab1 and Cry35Ab1 proteins used in the experimental studies are equivalent to those extracted from leaf material of maize event 59122*.'

Latham et al. (2017) analysed in detail a total of 23 applications submitted to regulatory agencies in the US, EU and Australia/New Zealand. Regulators and applicants alike build on the old narrative (dating well back into the last century) that Bt toxins from its native bacteria, *Bacillus thuringiensis*, have 'a history of safe use' as formulated microbial pesticide. Notwithstanding all kinds of alterations – intended and unintended – during the various steps of the genetic engineering process (incl. cloning, construction of transgene constructs, transformation of plasmids of vectors and recipient plants, etc.) the common industry and regulator narrative maintains that none of these alterations matter much when it comes to environmental and human and animal health safety issues, while they do induce significant novelties and changes with regard to patents and efficacy. GM Bt crops contain alterations in the gene sequences coding for the expression of the Bt toxin that differ substantially from those in the native parental *B. thuringiensis* or, to put it in other words, have never occurred in nature and are, therefore, substantially novel toxins (without this novelty, patenting would not be possible). These alterations include truncations, mutations and additions, in comparison to the native versions of the genes, which may alter, or are designed to alter, their activity and/or specificity compared to their natural originals (Latham et al. 2017). The purpose of some of these modifications is to impact new 'target' insects or increase toxicity towards target insects, these include events containing Bt toxin molecules with swapped domains between different Bt toxin types to change the spectrum of activity: this is the case for Cry1A.105 (*Genuity VT Double Pro* maize from Monsanto) and eCry3.1Ab (*Agrisure Duracade* maize from Syngenta).

In addition, parts of Bt genes are often combined to produce hybrid expression products, i.e. transgenic molecules that also do not exist in nature. And all GM Bt proteins studied by Latham and colleagues (2017) were additionally altered inside plant cells. It seems that the GM crop plant itself invariably also creates changes in Bt toxins. Latham et al. (2017) concluded that not a single one of the 23 commercial Bt crop lines that they analysed was identical to natural or historically used versions of Bt toxins. Consequently, Latham et al. (2017)'s findings on DAS 59122 maize differ from those of EFSA (2013) above: *" ...DAS-59122 maize Cry34Ab1 exhibits forms at 60, 50 and 42 kDa in addition to the expected 13.6 kDa protein… . … applicants may refer to unexplained bands as 'impurities', even when detected by their specific antibodies and not present in the control lanes* (see Latham et al. 2017 for details and also how patent descriptions provide additional reason for doubting the toxicological equivalence between wild-type and GM plant Cry proteins – page 22).

In almost every case where novel or otherwise unexpected Bt toxin forms were found, they were omitted from further analysis. Latham et al. (2017) concluded that "*both applicants and regulators appear to have largely lost sight of the fact that establishing the absence of unexpected fragments in plants is the goal. Verification of expected molecules is not*."

Although these variations in specificity/efficacy in comparison to the Bt bacteria-produced Cry toxins may also include insects that are not targeted by these toxins (i.e. non-target organisms including beneficial insects), they are hardly ever tested for effects on non-target organisms (except for the

exception that some GM plant material is used for testing, mostly pollen), nor even discussed within the context of possible effects on the environment/biodiversity. Furthermore, the structure of the DNA sequences that would be necessary to inform about differences between the native Bt toxin and the Bt toxin as produced in the plants, is kept as confidential business information by EFSA. This is unacceptable since all information that is related to risk assessment of GMOs has to be made public in the EU.

Complex modes of action versus EFSA's specificity narrative

Much of the argument for omitting safety testing of non-target species, or doing so with bacteria-produced toxins in isolation from the GM plant, rests on an outdated narrative of specificity and postulated mode of action for Bt toxins. The crucial point here is that one specific mode-of-action, as described for target organisms, is misused to assume no other modes-of-action exist. For a detailed, in-depth critique, we refer the readers to publications by e.g. Hilbeck and Otto (2015), Vachon et al. (2012), and Latham et al. (2017). Hence, although the science on the modes-of-action of Bt toxins and its range of affected species clearly has moved on and is also controversially debated (Vachon et al. 2012 – see below), EFSA still bases its risk assessments on scientifically outdated paradigms and claims that can hardly be reconciled with the new emerging scientific evidence that a broad spectrum of organisms are affected, based on a diversity of modes-of-actions. In the following, we will only summarize the most recent findings in these fields.

Based on research with the original bacteria *B. thuringiensis*, it was believed that, for example, Bt toxins as used in the first generation Bt crop, MON810 (Cry1A), were only active against specific orders of insects, such as Lepidoptera. Today, for the most extensively studied Bt toxins, adverse effects beyond its presumed target species range have been reported for 69 different insect taxa (van Frankenhuyzen, 2013), hardly supporting a narrow specificity narrative.

Nevertheless, EFSA continues to argue that effects documented on groups of non-target organisms outside of the old postulated target orders, for example *Daphnia magna* (Bøhn et al. 2016) are not expected, simply because of the phylogenetic distance between the target pest and the non-target *D. magna*:

> *"**Based on the known spectrum of activity of the Cry1Ab protein and its selectivity to lepidopteran species**, and the phylogenetic distance between D. magna and target species (pests of the family Lepidoptera), **susceptibility of daphnids to the Cry1Ab protein is not expected** at field concentrations."* (EFSA, 2016) (emphasis added).

EFSA is writing this as if the observed negative effects of Bt-toxins on *D. magna* may not exist. However, it has previously been documented that *D. magna* suffered higher mortality and other stress effects from feeding on real Bt-plant material (Bøhn et al., 2008, 2010, Holderbaum et al. 2015), i.e. at realistic field concentrations of Bt-toxins. And EFSA is aware of these published studies as they have written a full report on the first study and referenced several of them in their own work (EFSA 2016). What EFSA is doing here is rejecting (after 'assessing' the evidence) documented and published scientific evidence of effects on non-target organisms, not only from feeding studies with real plant material but also dose-response effects of purified Bt-toxins (increased mortality and reduced growth/fecundity) (Bøhn et al. 2016). This is also an example of how EFSA holds on to their own *a priori* expectations, which imply that Cry-toxins will not harm non-target organisms with a certain phylogenetic distance to Lepidoptera, regardless of the scientific evidence suggesting otherwise. By such actions, EFSA is avoiding the key question: the need to investigate further the potential harm from Bt toxins to non-target organisms (Bøhn 2018).

EFSA has performed similar exercises in several other cases where even the applicant submits data that contradict the narrow specificity narrative. For example, in first tier screening tests with microbially produced, presumably only coleopteran-active, Cry34Ab1/35Ab1 proteins and 59122

maize pollen, significant adverse effects were detected in three Lepidopteran species: *Pieris rapae, Sitotroga cerealella* and *Ostrinia nubilalis* (EFSA 2013, pages 29-30 and 35). Again, EFSA simply argued/speculated that these statistically significant (and surprising) effects were either '*not considered as biologically relevant'* because they were '*small'* (EFSA 2013, page 30) or that they were '*most likely due to impurities (salt and buffer constituents) in the lyophilised purified Bt-protein powders used as test material*' (EFSA 2013, page 35), although no evidence was provided to support these (wild) speculations. Firstly, this could be avoided when actually using the GM Bt plant and, secondly, apparently the wrong control treatments were chosen, which did not control for the effects of buffer and salt, which points to a truly 'poor study design' that apparently escaped EFSA's attention. At the same time, they invent 'poor study design' arguments where they do not exist – consistently only when the results do not fit their expectations (see below).

In contrast to EFSA's 'expectations' and conclusions, the scientific literature is increasingly questioning the toxicological and ecological safety of Bt toxins, i.e. by showing significant effects on a much wider group of insect taxa (cross-order), in particular for the Cry1Ab toxin that is active across 6 insect orders (van Frankenhuyzen, 2013). Since most Bt toxins have been tested with species from one or two insect orders only, cross-activities may be even higher (Glare and O'Callaghan, 2000; Hilbeck and Otto, 2015; van Frankenhuyzen, 2013). However, this is less surprising when the full range of proposed modes of action reported in the scientific literature is appreciated. Ignoring all other (non-target) species that may also have some or all preconditions necessary for being affected, at least to some degree, is neither in line with the current scientific state-of-knowledge nor with the precautionary principle.

In target insects susceptible to Cry1 toxins (the most studied class of Bt toxins), the classical mode of action model (Pietrantonio and Gill 1996) starts with the step-wise activation of crystalline protoxins by several enzymes active in the midgut lumen under favourable chemical conditions (e.g. alkaline pH in the Lepidopteran insect mid gastrointestinal tract). None of this is relevant in the case of GM plants that produce already (largely or fully) activated Bt toxins. Most if not all experiments conducted with a small group of non-target organisms, however, were performed using the bacteria-produced Bt crystal protein.

The second step implies a specific binding step(s) of the activated Cry toxins to specific receptors bound to the membrane on the apical side of midgut epithelial cells. These receptors include cadherins, aminopeptidases, alkaline phosphatases, ABC transporters and glycolipids, their nature being species/genus/class/order-specific, which jointly determines specificity, at this level.

The extremely diverse group of non-target insects (many of which are important pollinators) remain un(der)studied despite increasing reports about Bt effects on non-target organisms (van Frankenhuyzen, 2013). Nonetheless, research shows that susceptibility to Bt does not rely solely on this binding step, but also is dependent on other steps of the mode(s) of action. Following the binding step described above, two mode of action models (aiming at complementing or challenging the classical MOA) are proposed:

1. The formation of transmembrane pores resulting in osmotic shock and lysis of the cells, which in turn will allow gut bacteria (including Bt bacteria and spores) to reach the hemolymph where they will multiply. The insect will die from septicaemia, although to a lower extent and in a slower manner if the gut bacteria were partially or totally destroyed by the addition of antibiotics in the food (see Broderick et al. 2006, 2009, Mason et al., 2011 and other refs therein).

2. The classical model, also called "Pore Formation Model" (PFM), and the Sequential Binding Model" (SBM) agree on this MOA to a large extent, with only the first activation and binding steps in the classical model being complemented/detailed by additional steps in the SBM.

Under the Signalling Pathway Model (SPM), it is hypothesized that the stimulation of a G-protein signalling pathway results in increase of cAMP and functionalization of the protein kinase A effector-mediated apoptotic metabolism. These are of particular interest in non-target insects where binding with Bt and/or formation of pore may not occur (Jurat-Fuentes and Crickmore, 2017), although this was never properly studied in insects outside of the 'expected' range of taxa.

Given the level of detail required for studying such molecular mechanisms, only the real Bt plant material or Bt toxins as produced in these GM plants should be used for testing. To date, however, this is rarely, if ever, the case as purified Bt toxins are not extracted from plant material but produced in the surrogate transgenic Bt *Escherichia coli* (or other) bacteria. The transformation processes of *E. coli* and of crop plants, even with the same transgene construct, was shown to yield significantly different end-products, i.e. Bt toxins (Latham et al. 2017).

Moreover, there is an outright dispute over the question of whether or not *B. thuringiensis* is even a full pathogen in its own right or an 'impotent pathogen', as Raymond et al. (2009) have put it (Broderick et al. 2006, 2009). Under the latter model, Bt toxins are ascribed only a support role by forming pores in the gut epithelium and, ultimately, allowing for the translocation of other microbes from the gut into the hemocoel. In the hemocoel it is then the other microbes that induce septicaemia (i.e. the commensal-to-pathogen switch; Mason et al. 2011, Graf 2011) which ultimately kills the insect, rather than Bt cells or toxins. However, while it seems from the published controversy that no party contests that gut microbiota have a role in the impact or rather the unfolding of the impact of *B. thuringiensis* bacteria, spores and toxins on lepidopteran larvae, the core of the controversy seems to centre around the question of whether or not *B. thuringiensis* and its by-products can actually kill the target insect on its own even in the absence of other gut bacteria? There have been conflicting studies published wherein this was investigated when adding broad spectrum antibiotics to the Bt-containing diet of the test insects. While the researchers discussed above have on-going arguments regarding the specific mechanisms, all studies report that antibiotics mask or even eliminate the effect of Bt toxins. In a study using actual GM Bt maize plants, Hilbeck et al. (2018) confirmed that the addition of antibiotics to the test diets masks Cry toxin effects significantly by reducing and delaying the onset of the impact. But regardless of the final outcome, the bacterial community in the individual insect gut seems to be a key factor contributing to the function of Bt-toxins, consequently, a new layer of complexity must be recognized for a reliable risk assessment This has, in particular, implications for the ecotoxicity testing of nontarget effects when using pollen or purified proteins in artificial diets laced with antibiotics as has been done by Li et al. 2014 and Ali et al. 2016a,b – the claimed lack of observed effects in these studies were at the very least confounded by the (very) high concentrations of antibiotics in the diets and probably masked by them. To our knowledge to date, EFSA has neither taken these methodological flaws into account in their (re-)assessments nor critically evaluated these altered protocols as they routinely do when the outcomes do not fit their 'expectations'. In fact, in a recent statement, EFSA relied on these (flawed) studies when deflecting the relevance of other studies that did not meet their 'expectations' (see below).

3. Exposure Assessment – how EFSA ignores exposure pathways of Bt toxins

Bt-toxins are gut poisons. Consequently, ingestion is the relevant route of exposure to Bt-toxins for both target and non-target organisms. With the production of GM Bt crop commodities on vast industrial scales came an unprecedented spatio-temporal expansion of exposure and inundation of agroecosystems with these highly bioactive (activated) bacterial toxins. Using the MON810 Bt maize as an example, a single gene Bt plant with relatively low expression of Bt compared to other events (especially stacked with multiple Bt toxins), the following simple calculation illustrates how many more Bt toxins will take part in environmental interactions as well as in the human food chain.

Humans have historically been exposed unintentionally to Bt toxins by eating insignificant amounts of soil (raw food) or by inhalation of dust. Heinemann (2009) conservatively estimated the concentration of Bt spores at 1000 spores per gram of soil and a maximum estimate of Bt toxin at 0.7 ng/g soil (without Bt plants present). With the highest daily average intake of soil for an adult (625 mg) the exposure to Bt toxins would be to consume Bt toxins at 0.0004 ug/day.

In comparison, by eating MON810 based corn products (containing 0.29 ug/g of Bt-toxin), even Americans with a small proportion of their diet coming from maize, would consume 10 ug of Bt toxin per day. In exposure terms for Bt toxins before Bt plants were introduced this is comparable to eating 14 kg of soil per day (Heinemann 2009).

For single gene Bt plants, the concentration of Bt toxins is highly variable (range 0.19 – 115 ug/g in seeds), depending on both the event and the type of Bt toxin produced (Heinemann 2009). Using the rough average of 40.5 ug/g, Bt maize plants produce 179 g of Bt toxins per ha. With 60 million ha of Bt maize plants (ISAAA 2016), more than 10,000 tonnes of pure Bt toxins are produced in the agroecosystems and brought into global food chains. When the current trend to replace GM plants expressing only one Bt toxin with GM plants expressing multiple (2-6) Bt toxins (stacked events) simultaneously, the amount of Bt toxins will have to be multiplied by a factor of 2-6 (Bøhn 2018). The key point here is to bring to attention the massive and unprecedented increase in the amount and concentration of Bt toxins that animals, humans and ecosystems are exposed to where Bt crops are grown.

EFSA, however, working under its narrow ERA model, largely ignores the resulting multitude of pathways through which non-target organisms (or humans for that matter) can be exposed to Bt-toxins in terrestrial, soil or aquatic environments. EFSA also does not carry out separate ERAs for so-called stacked events that combine several Bt toxins in one plant, even when they contain novel Bt toxins that, for one, do not exist in nature nor, secondly, have been approved as a separate event before (Latham et al. 2017). Under a broader, ecologically more relevant ERA model, such exposure pathways must be captured and it must be understood how Bt toxins move through food webs. In the following, we will do what EFSA does not: a) explore the possible diversity of exposure pathways in the field, b) determine the concentrations of the exposure to Bt toxins, and c) assess the spatio-temporal extent of the exposure of non-target organisms to Bt-toxins.

Pathways of exposure in terrestrial food-webs

There are many pathways through which non-target organisms can be exposed to Bt-toxins simultaneously. Firstly, by directly feeding on Bt-plant material including leaves, fruits, nectar, pollen etc. This is called the direct or bitrophic exposure pathway. Secondly, non-target organisms can also be exposed to Bt-toxins by uptake when predaceous or parasitic non-target organisms feed on prey/hosts that have ingested Bt-toxins. This is called the tritrophic exposure pathway. Another potential pathway of exposure is the intergenerational transfer of Bt-toxins, with parents transferring the toxins to their offspring. All of these possible exposure pathways have been studied at least to some extent in field and laboratory experiments and will be discussed in the following sections.

Bitrophic exposure in laboratory experiments

In the scientific literature, different laboratory studies have confirmed that direct consumption by non-target herbivores of Bt-plant material, via artificial diets containing activated Bt-toxins, as well as pure Bt-toxins, results in exposure to Bt-toxins (Howald et al., 2003; Ludy and Lang, 2006a,b; Obrist et al., 2006b; Paula et al., 2014; Raps et al., 2001; Torres et al., 2006; Zhang et al., 2014, Svobodova et al. 2017). Paula et al., (2014) showed that third instar larvae as well as the adults of the non-target butterfly *Chlosyne lacinia* have Cry1Ac receptors in the midgut and confirmed uptake of Cry1Ac by adults as well as larvae. Raps et al. (2001) and Howald et al., (2003) detected Cry1Ab and Cry1Ac, respectively in larvae and faeces of the herbivore *Spodoptera littoralis* and *Athalia rosae*, showing that the toxins are detectable after ingestion and excretion by herbivores. They suggest that Bt toxins in faeces may affect the ability of parasitoids to locate hosts. Other studies showed that omnivorous predators such as *Orius majusculus* or different spider species can be directly exposed to Bt-toxins when feeding on pollen containing the toxin or being mixed with the toxin (Ludy and Lang, 2006; Obrist et al., 2006a; Peterson et al., 2016; Zhang et al., 2014). Direct toxin ingestion from purified Cry1Ac diluted in water was confirmed in a laboratory study with *G. punctipes* (Torres et al., 2006).

Tritrophic exposure in laboratory experiments

It took at least a decade of commercial production before the first data were published confirming what some researchers have always correctly expected, but others denied, that Bt toxins will spread in significant amounts throughout the food web in agroecosystems where Bt crops are grown – within and beyond the arable field. Howald et al. (2003) assumed that since the Bt toxins are not completely degraded during digestion, natural enemies could be exposed to the Cry1Ac protein by feeding prey. Tri-trophic laboratory and greenhouse studies have indeed shown that tritrophic consumption of Bt-toxins by non-target organisms results in exposure (Chen et al., 2009; Gao et al., 2010; Obrist et al., 2006a, 2006b; Peterson et al., 2016; Svobodova et al. 2017; Tian et al., 2012; Torres et al., 2006; Zhang et al., 2006a; 2006b; Zhou et al. 2014). Peterson et al. (2016) showed that spider predators can be exposed to Bt-toxins via prey that fed on Bt maize leaves and pollen. Likewise, Chen et al., (2009) demonstrated that the spider predator *Pirata subpiraticus* can take-up Bt-toxins when feeding on Bt-rice fed *Cnaphalocrocis medinalis* (Rice leafroller).

Tritrophic exposure to Bt-toxins was also demonstrated in the green lacewing, *Chrysoperla carnea* (Obrist et al., 2006b), *Orius majusculus* (Obrist et al., 2006a) and the coccinellid predator *Propylaea japonica* (Zhang et al., 2006a; 2006b). In contrast to other studies finding no Bt toxins in aphids (Raps et al. 2001, Dutton et al. 2002), Zhang et al. (2006a,b) did demonstrate tritrophic uptake of Bt-toxins by *P. japonica*, when reared on Bt-cotton fed cotton aphids, *Aphis gossypii* during their entire adult life from hatching (Zhang et al., 2006a). Tritrophic uptake of Bt-toxins by *P. japonica* was also demonstrated in larvae, when reared on 24h old larvae of the Oriental Leafworm moth, *Spodoptera litura* (Zhang et al., 2006b). Gao et al. (2010) further showed that egg parasitoids such as *Anagrus*

nilaparvatae are exposed to Cry1Ab when reared on the eggs of Bt-rice fed planthoppers, *Nilaparvata lugens*. Today it is well established that Bt toxins can be detected at all trophic levels of the foodweb, and at high concentrations also when feeding on stacked Bt maize plants combining up to 6 Bt toxins, known as Smartstax (Svobodova et al. 2017). The coccinellid species, *Harmonia axyridis*, ingested the highest total concentrations of total Bt toxins up to >35 microgram/g dry weight from feeding on pollen of Smartstax Bt maize alone increasing to > 72 micrograms/g dry weight when feeding on the herbivorous prey *Tetranychus* urticae feeding on Smartstax Bt maize (Svobodova et al. 2017). Also, the lacewing larvae of *Chrysoperla carnea* was reported to contain >10 microgram/g dry weight when reared on *T. urticae* prey feeding on Smartstax Bt-maize, or even when fed Smartstax pollen – which is surprising for a non-pollen feeder.

Evidence of exposure to Bt toxins in field food-webs

Field studies are necessary to confirm exposure of NTOs to Bt-toxins in the field. Field exposure has been demonstrated in Bt maize, Bt cotton, and Bt soybean fields. Harwood et al. (2005) for the first time confirmed the long suspected but denied presence of Cry1Ab in non-target arthropod food-webs in the field. Different herbivore species, including the maize flea beetle, *Chaetocnema pulicaria*, as well as predator species, including several spiders and coccinellids, contained Cry1Ab above the threshold of 0.5 ng Cry1Ab per g fresh weight. It is not clear whether the presence of Cry1Ab in predators resulted from direct feeding on plant material or consumption of prey that contained the Bt-toxins or, probably, from both. The authors concluded that long-term exposure to Bt-toxins indeed occurs in the field and should be considered in the risk assessment of GM insecticidal crops to non-target organisms. In 2007, Harwood et al. reported temporal variability of Bt-toxin presence in adult coccinellids collected in Bt maize fields. The presence of Bt toxins could not directly be correlated with anthesis, pointing towards tri-trophic interactions. In 2005, Zwahlen and Andow presented the first evidence that ground beetles are exposed to Bt toxins in the field. They suggested that Bt maize residues are a significant source of exposure, although other exposure pathways, including feeding on living plant tissues, or on prey containing Cry1Ab, were also considered possible. In another field experiment with Bt maize, Obrist et al., (2006a) detected Cry1Ab in several different non-target arthropods, including herbivores such as spider mites, chrysomelids and mirids and predators such as *Stethorus punctillum*, *Orius spp*., and *Chrysoperla spp*.. Torres et al. (2006) confirmed the presence of Cry1Ab in Lepidopterans collected in Bt cotton fields, including *Spodoptera eridania, P. includens* and *S. exigua*. Cry1Ab was further detected in two out of seven assayed predators collected in the field. Yu et al. (2014) demonstrated presence of Cry1Ac in several non-target arthropod species from nine orders collected in Chinese Bt soybean fields. These include herbivores as well as predators. Peterson et al. (2016) further showed that spider predators, representing the fourth or higher trophic level, are exposed to Bt-toxins in the field, probably by feeding on maize pollen and on prey (which includes other predators). They demonstrated the presence of different Bt-toxins in various spider species and different ecological guilds.

Fate of Bt toxins in the food chain and intergenerational exposure

Although most research efforts have focussed on bitrophic and tritrophic exposure of nontarget organisms to Bt-toxins, recent studies suggest that intergenerational transfer could be another exposure pathway. Preconditions for intergenerational transfer of Bt toxins are: 1) toxin uptake by the parents 2) toxin bioaccumulation in the parents, and 3) toxin sequestration.

According to Paula and Andow (2016), uptake of a toxin must not be confused with presence. Uptake means that the substance is not only absorbed but also incorporated into the organism. Thus, the substance is still present after exposure has stopped (Paula and Andow, 2016). While lab and field studies have focused on demonstrating presence or absence of Cry toxins in non-target organisms, uptake has rarely been considered. Recent studies do however suggest that Cry toxins are in fact taken

up by non-target organisms (Gao et al., 2010; Paula et al., 2014; Paula and Andow, 2016; Zhang et al., 2006a).

Bioaccumulation means that less substance is being excreted than being taken up and, thus, the substance accumulates in the organism. As with uptake, bioaccumulation of Cry toxins in non-target organisms has been mostly overlooked in risk assessment. In 2016, Paula et al. demonstrated bioaccumulation of Cry1Ac and Cry1F in the coccinellid predator *Harmonia axyridis*. Since the amount of toxin was similar in pupae and adults the authors suggest that the toxin content does not decline during pupation. Zhang et al., (2006a) showed that the amount of Bt-toxin in the coccinellid predator *Propylaea japonica* increased over time when fed Bt-cotton reared aphids.

Toxin sequestration, the deposition or storage of Bt-toxins in the insect predators after uptake, has been observed by Gao et al., (2010), Paula et al. (2015) and Zhang et al., (2006a).

Different studies have shown that intergenerational transfer of Bt-toxins is indeed possible. Paula et al. (2014) showed that the non-target Lepidopteran *Chlosyne lacinia* is able to take up and transfer activated Cry1Ac to its eggs. Transfer of Cry1Ab from a herbivore to its eggs has also been suggested by Gao et al., (2010). They found Cry1Ab in the egg parasitoid *Anagrus nilaparvatae* when reared on the eggs of Bt-rice fed *Nilaparvata lugen*s.

In 2015, Paula et al. confirmed for the first time that intergenerational transfer of Bt-toxins to higher trophic levels is possible. They showed that the coccinellid predator *Harmonia axyridis* is able to sequester Cry1F from its prey and transfer it to its offspring. Thereby, the Cry1F concentration in the parents was positively related to the Cry1F concentration in their offspring. Intergenerational transfer of Cry toxins has further been demonstrated in a second coccinellid predator, *Propylaea japonica* (Zhang et al. 2006a). These results suggest that intergenerational transfer of Bt-toxins may be a common route of exposure which has not been considered in EFSA's environmental risk assessment of Bt-toxins to date, partially also due to their 'out-of-range' non-target species postulation.

When EFSA was mandated to analyse the publications by Paula et al. 2015 and Paula and Andow 2016 regarding '*whether they contain elements that could lead the GMO Panel to reconsider its previous scientific opinions on the cultivation of genetically modified (GM) maize MON810*' (EFSA 2019), the GMO panel's conclusion was that both studies '*do not reveal new scientific information that would invalidate the previous risk assessment conclusions on maize MON810 made by EFSA and its GMO panel*', citing two questionable reasons. Firstly, the GMO panel argued that the findings reported by Paula et al. 2015 and Paula and Andow 2016 '*have no direct relevance for the environmental risk assessment of maize MON810 because none of the Cry proteins evaluated ... correspond to the protein expressed in maize MON810, i.e. Cry1Ab.*' This means, no conclusions can be drawn from studies using Cry1Ac or any other Cry toxin for Cry1Ab regarding risks. However, vice versa, EFSA does draw conclusions for safety from studies that do not use Cry1Ab toxins if it meets their expectations of the 'known': for example, '*Based on the known spectrum of activity of Cry1Ac and Cry1F proteins and its selectivity to lepidopteran species and the phylogenetic distance between ladybird beetles and target species (pests of the order Lepidoptera), susceptibility of H. axyridis to Cry1Ac and CryF proteins is not expected at field concentration*s' and '*Similar findings* (no adverse effects) *have been reported in the scientific literature for Cry1F and other Bt-proteins on this ladybeetle. In direct feeding assays, ingestion of biologically-active purified Cry1Ac, Cry2Ab, Cry1Ca, Cry1F or Vip3A proteins by H. axyridis larvae did not negatively affect their development, survival or weight (Ali et al. 2016)*.' Notably, Cry1Ab was not included in these experiments. Furthermore, EFSA entirely ignored that the cited research by Ali et al. 2016a, b (and also by Li et al. 2014) has been revealed to have used questionable testing protocols which include unexplained excessive amounts of antibiotics known to significantly alter the impact of Bt toxins on tested insect larvae (Hilbeck et al. 2018, Latham 2019).

> '*The documented impact of antibiotics on the efficacy of Cry toxins has important potential ramifications for ecotoxicity testing of nontarget organisms using artificial diets. In two recently published reports testing the ecotoxicity of Bt toxins on beneficial insects, high amounts of antibiotics were added to the test diets. While both Li et al. [28] and Ali et al. [29,30] based their recipes on those developed originally by Cohen and Smith [27], they deviated most significantly from the original recipe with regard to the addition of antibiotics. While Cohen and Smith [27] added antibiotics (streptomycin and chlortetracycline) at a concentration of 50 mg/100 g diet, Li et al. [28] more than tripled the amount to 180 mg/100 g diet (streptomycin and cephalosporin) and Ali et al. [29,30] even raised the concentration to an astounding 800 mg/100 g diet (streptomycin and penicillin). This significant deviation from the original recipe by Cohen and Smith [27] remains unexplained and unaccounted for. ... Also, van Frankenhuyzen [34] declared in a paper reporting about a study that addressed and expanded the experiments by Broderick et al. [19,20], that the 2 mg/mL total antibiotic used by Broderick et al. [19,20] as "high". He further stated in that paper: "It is clear that the choice of antibiotics can profoundly affect experimental results" [34]. We would like to add that not only the choice but also the quantity of antibiotics will likely affect the outcome such as larval weight gain and survival.*' (excerpt from Hilbeck et al. 2018)

Hence, double standards are being applied in what constitutes legitimate 'bridging data' and what does not and, also, grossly different levels of rigour are applied regarding the quality of considered published research in support of EFSA GMO panel claims.

Quantitative estimation of exposure to Bt toxins in the food chain

The actual level of exposure depends on a number of factors: the toxin expression levels in the crop, which again depends on the crop variety, the growth stage of the plant, the different plant parts and environmental conditions for the non-target organism, its feeding behaviour and life stages; the pathway of exposure and the availability of alternative food sources. In the case of predators, the feeding ecology of its prey also has an important role.

Obrist et al. (2006b), for example, reported an approximately 10 times higher Bt toxin concentration in the prey species *Tetranychus urticae* than in *Spodoptera littoralis*. Accordingly, the predator *Chrysoperla carnea*, in which approximately half of the concentration in the respective prey was measured, demonstrated a 10 times higher exposure to Bt toxins when feeding on Bt-fed *T. urticae* than on *S. littoralis*.

Obrist et al. (2006a) also found Bt concentration levels to depend on the predator's feeding habits as well as on the feeding ecology of its prey. They reported significant Bt toxin levels in *Chrysoperla spp., Stethorus spp*. and *Orius spp.* larvae. The authors suggested that Bt maize pollen was responsible for the Bt-toxin found in *Orius spp.*, while feeding on available arthropod prey would only lead to a minor exposure. Interestingly, Bt toxin concentration in predators was generally higher in pre-imaginal stages than in adults, when being fed the same prey items. This is relevant since pre-imaginal stages are known to be much more susceptible to Bt toxin than adults and this should be taken into account in the risk assessment of Bt-crops. In herbivores, the concentration of Cry1Ab was especially high in spider mites. Toxin levels three times higher than measured in the plant leaves were detected in spider mites when sampled later in the season. The authors conclude there is a high likelihood that spider mites can transfer the toxin to higher trophic levels. Also, Zhang et al. (2006b) found the Bt toxin concentration to be 10 times higher in the predator larvae than in the Cry1Ab Bt-cotton leaves they fed on and 3 times higher than in the Bt leaves containing Cry1Ac.

Yu et al. (2014) found Bt concentrations to vary depending on arthropod species, life stage and the growth stage of the plants. The highest concentration was found in the grasshopper *Atractomorpha sinesis*, which contained about 50% of the concentration in soybean leaves. Other herbivores contained 1 and 10% of the Bt toxin found in the plant. Torres et al. (2006) reported mean seasonal concentrations in herbivores in the field as high as 17% (*P. includens*), 42% (*S. eridania*) and 50%

(*S. exigua*) of the amount found in the plants. Furthermore, 8.3% (*P. maculiventris* adults) and 29% (*C. rufilabris* larvae) were still detectable in predators. In the greenhouse study with *S. exigua*, concentrations reached 78% in 4-day old and 10-day old larvae, respectively. They found Cry1Ac levels to vary between species, depending on prey species and amount of prey consumed as well as during the season. Similarly, Harwood et al. (2007) reported seasonal variability in the proportion of adult coccinellids containing significant levels of Bt toxins. In spiders, exposure to Bt toxins reportedly varies based on sampling period, transgenic line, as well as the functional guild and species of the spiders (Peterson et al. 2016, Zhou et al. 2014).

Field studies are highly relevant to estimate realistic exposure levels of non-target species. This is highlighted in the following example: concentrations of Cry1Ab were shown to be four times higher in spider mites collected in the field (almost 17 ug/g DW) than in those used in the laboratory that were kept on Bt maize (Obrist et al., 2006a). The authors reported relatively high (ca. 0.5 ug/g DW) toxin levels in *C. carnea* larvae that possibly fed on spider mites. Moreover, *Stethorus punctillum*, a coccinellid specialist that exclusively feeds on spider mites, had the highest toxin concentration of all predators analysed (ca. 2.5 ug/g DW). Thus, real-life exposure to Bt toxins of spider mites and their predators might be underestimated in the lab.

In-plant Bt toxins represent expanded spatio-temporal environmental exposure

In terrestrial systems, conventional Bt insecticides have a low persistence on the surface of plants after spray application, due to UV degradation or removal by rain (Behle et al. 1997). They also do not reach sap- and cell sucking herbivores such as aphids, thrips, spider mites or pests that feed inside crops like stem borers. These shortcomings were overcome with the introduction of GM crops expressing Bt-toxins. Bt crops synthesize the toxins in all plant parts and throughout the entire lifetime of the crop. This not only led to chronic exposure to Bt-toxins but also broadened the spatio-temporal exposure to include all insects feeding on Bt maize plants, but also most species that are members of the Bt maize field food-web. Such exposure is also recognized by EFSA (2009):

> *"Harwood et al. (2005) and Zwahlen and Andow (2005) studied exposure to the Cry1Ab protein (event Bt11) for several groups of non-target organisms and reported levels of Bt-protein observed in non-target herbivores and their natural enemies under field conditions. The authors showed that significant quantities of the Cry1Ab protein can move into higher trophic levels."*

Exposure of pollen-feeding insects to Bt toxins

Through genetic engineering it became possible to equip pollen of crop plants with insecticidal toxins and to do so at a vast scale. This is a significant change for pollen-feeding insects, many of which are also important pollinator species. Hence, within a few years, a broad range of pollen-feeding insects were exposed to insecticidal substances when foraging in Bt crop stands. These Bt toxins act as further stressors in addition to, for example, exposure to other regular synthetic pesticides used in industrial crop production (including Bt crops), such as those from neonicotinoid seed treatments.

Therefore, exposure of pollen-feeding insects has been the subject of intense debates, with competing models for exposure and concentration calculations. Hofmann et al. (2014) modelled maize pollen deposition in relation to distance from the nearest field and concluded that a power function actually describes pollen deposition better than the model currently applied by EFSA. They concluded that maize pollen can travel up to the kilometre range. Pollen dispersal up to several kilometres, and with a very large variation, has previously been confirmed in the field (Brunet et al., 2003, Bøhn et al. 2016). Hofmann et al. (2014) questioned the buffer zone distances of 20-30 meters between GM maize and protected habitats as applied by EFSA. In their reply (EFSA, 2015), the EFSA GMO panel,

however, concluded that their previous recommended isolation distance still remains valid as several factors of uncertainty need to be considered. To be able to do so, EFSA coined an "effective exposure" term of non-target organisms to maize pollen (EFSA, 2015), based on a weak mechanism of expert judgement. In essence, this is yet another example of the reversal of the burden of proof and the precautionary principle: it means that until proof-of-damage is established and accepted by EFSA (not the same), the small isolation distance of 20-30 meters is upheld despite the evidence of possible risks.

This narrow assessment seems in stark contrast to the widely held and growing consensus that in face of insect biodiversity collapse and, in particular, of the indispensable service pollinators provide to humans, they deserve special attention and utmost precaution. EFSA, however, assumes that pollinators are mostly represented by *Apis mellifera* – other taxonomic groups are hardly tested and no specific test protocols are available. Furthermore, methods to measure toxin concentrations in pollen are poor and lack a scientific standard. Additionally, the large total toxin load in plant material of some of the stacked events remains unaddressed to date – certainly when evaluating applications for import as food and feed. EFSA should at least address the issue of combinatorial effects for simultaneous cultivation of different single-gene events (e.g. Bt11 or MON810 and 1507 Bt maize). For example, based on industry data, we calculated a total possible Bt toxin load ranging from 100 to 200 ng/mg pollen dry weight (about 70 to 150 µg/g fresh weight) for the stack called 'Smartstax' (Table 1), based on (applicant) data from Phillips (2008) and Stillwell & Silvanovich (2007).

Non-target organisms may be affected when feeding not only on pollen in Bt maize fields but also in areas next to the crop. For Bt maize, EFSA has neglected field evidence from European field studies of the exposure of non-agricultural land with maize pollen (Hofmann 2007) up to 2015. By doing so, pollen dispersal to larger distances has been systematically underestimated by using an exponential function of pollen decline with distance since 2015. In practice use of the exponential function means effectively cutting off exposure of in distances larger than 15-20m, whereas evidence from field measurements clearly demonstrates that pollen is deposited in the field over the km range (Hofmann et al. 2007, 2009, 2014).

After EFSA acknowledged the power function in 2015 (EFSA 2015), it downsized a rather arbitrarily defined "realistic exposure" by the use of exposure reducing factors. Some of these factors are likely correlated, whilst other factors are in conflict with existing literature. In summary, EFSA used its so-called uncertainty factors in a one-sided way to decrease the exposure estimate.
Kruse-Plass et al. (2017) replied to the use of pollen deposition data and challenged the assumptions made previously by EFSA. A critical issue in this respect is that empirical field data of pollen deposition on leaves exceed the assumptions of EFSA. Many of the "uncertainty" factors of EFSA, used to downsize the quantity of pollen on leaves, are implicit in the field data (e.g. movement and loss of pollen; rain events) whereas other factors seem to be auto-correlated or in disagreement with scientific literature (e.g. avoidance of leaf veins; see Lang & Otto 2015).
The critique of the Hofmann working group of this procedure (Kruse-Plass et al. 2017) was addressed by EFSA in an inappropriate way i.e. without direct consultation of the Hofmann working group and without addressing the core points of the different approaches to estimate pollen deposition between Hofmann et al. (2016) and EFSA.

Table 1: Maximum Cry toxin concentrations [µg/g] measured in field-grown 'SmartStax' maize; several US locations in 2006*;

FW: fresh weight; DW: dry weight;

Bt toxin	Leaves		Pollen		Kernels	
	DW	FW	DW	FW	DW	FW

Cry1A.105	210	34	21	16	4.9	4.3
Cry2Ab2	350	60	2.3	1.8	7.5	6.7
Cry1F	31	4.7	32	25	7.4	6.7
Total Lepidopteran-active Cry	591	99	55	43	20	18
Cry3Bb1	490	92	24	19	26	23
Cry34Ab1	279	42	117	90	94	85
Cry35Ab1	158	24	0.5	0.4	2.3	2.0
Total Coleopteran-active Cry	927	158	142	109	122	110
TOTAL	1518	257	197	152	142	138

*Source: compilation based on Phillips (2008) and Stillwell & Silvanovich (2007)

EFSA recognizes input of Bt toxin into aquatic systems but downplays exposure to non-target organisms

Bt-plants represent a new exposure route for Bt toxins into aquatic systems, in streams and ponds near agricultural fields, and their highly diverse ecological communities. In contrast, sprayed Bt insecticides hardly if ever reach aquatic systems before they are degraded, unless directly applied to aquatic systems for mosquito control. Thus, this massively increased exposure of Bt toxins through GM plant material needs to be captured in ERAs.

In the scientific literature, there is growing recognition that water bodies near agricultural fields can receive significant amounts of run-off and crop residues that contain Bt toxins (Böttger et al. 2015, Li et al. 2013), suggesting that the aquatic ecosystem is a relevant context for risk assessment and testing of transgenic crops with insecticidal traits (Venter and Bøhn, 2016). The relevance of exposure of aquatic ecosystems under common maize cultivation conditions for central Europe has been demonstrated in a pilot study in Germany (Kratz et al. 2010; Hofmann et al. 2013). Aside of crop detritus from the surrounding farmland, other natural links between terrestrial and aquatic systems are, for example, insects spending different life-stages in aquatic and terrestrial environments or fish that feed on terrestrial insects.

Carstens et al. (2012) differentiated between entry routes and exposure pathways, where the ways and means by which Bt crop materials (plant material, Bt proteins and transgenes) end up in aquatic ecosystems represent the entry routes, and the routes by which organisms may come into contact with this material and be affected by it, represent the exposure pathways (Carstens et al., 2012). However, in their proposed ERA model, Carstens et al. (2012) focused on entry routes, established worst case scenarios of Bt maize inputs - based on pond or ditch models developed, again, for chemicals and not for plant residues - which then was followed by elaborate argumentation and mathematical deduction using all kinds of diluting factors. The entry routes were considered in isolation and converted into 'degradation routes' based on selected studies supporting the (preconceived and unproven) assumption of fast degradation and inactivation of the Cry toxins in soil or water environmental media. Consequently, this inevitably led to negligible exposure conclusions. However, during storms or floods, the amount of crop plant material brought to a local stream or pond can be massive (Venter and Bøhn 2016). Therefore, dilution in relation to the volume of water must be taken into account when considering Bt toxins in aquatic systems, as well as temperature and oxygen content for further degradation processes. The range and maximum concentrations of Cry toxin that can enter aquatic ecosystems under such diverse real-world conditions are not known but are certainly higher than speculated in these low-exposure models. It has been shown that within the first hour of aquatic exposure, 61 % of the Cry1Ab toxin leached from Bt maize leaves, however, the resulting Cry1Ab concentration in the water was not determined (Griffiths et al. 2009). Strain and Lydy (2015) similarly reported that Cry1Ab rapidly leached or degraded from leaves in their experiments, with a half-life

of approximately 2 hours, but that the concentration of the protein in the water peaked about one day after initial exposure. Consequently, organisms inhabiting aquatic environments adjacent to Bt crops will be exposed to Bt-containing plant material and Bt toxins at varying concentrations, depending on their feeding habits, the type of crop and cultivar, the age and breakdown rates of the plant material, and the properties of the water and sediment of the aquatic environment (Venter and Bøhn 2016). Douville et al. (2007, 2005) were the first to detect Cry-toxins in the aquatic environment. Later, Tanks et al. (2010) and Jensen et al. (2010) confirmed the presence of GM maize detritus containing Bt toxins in streams near US agricultural fields (also reviewed in Viktorov 2010). EFSA finally recognized the fact that Bt toxins are found in aquatic systems and lead to exposure of aquatic organisms but immediately concluded based on the above construction of arguments that "*exposure of NTOs to the Cry1Ab protein in aquatic ecosystems is likely to be very low due to its rapid degradation*" (EFSA Opinion 2016). EFSA states this without validation of its assumptions and, thus, again goes in line with the industry, but not with the precautionary principle.

4. Adverse effect assessment - How EFSA tries to rescue the outdated 'out-of-range paradigm'

Another argument for rejecting possible risks to non-target organisms - even if extended spatio-temporal exposure is finally admitted - is by arguing that the exposed non-target organisms, regardless of which environmental compartment they occupy, cannot be affected as they are not declared target organisms (see quote above) and, thus, not *'expected'* to exhibit susceptibility. This argumentation we call the 'out-of-range paradigm'. Statements like these make explicit the rejection of data and results that do not meet preconceived assumptions or beliefs, which brings EFSA's assessments beyond its 'scientific' mandate. What is normal in science and would be expected from EFSA is that 'unexpected' novel findings are used to initiate further studies or to inform and update their knowledge, rather than to fall back on untested or outdated assumptions (Bøhn 2018). Further investigations are always necessary as they may clarify causal factors and mechanisms and, thus, improve our understanding step by step. The history of science is the story of new findings that detail, disprove and replace (convenient) old knowledge.

Over the past decade and longer, increasingly broader spectra of non-target species have been reported to be affected by Bt toxins of microbial or GM plant origin in one form or another, e.g. Lövei & Arpaia (2005), Hilbeck & Schmidt (2006), Marvier et al. (2007), Bøhn et al. (2008), Lövei et al. (2009), van Frankenhuyzen (2013), Hilbeck & Otto (2015).

In Table 2, a selection of 39 peer-reviewed publications is listed that report significant (adverse) effects of Bt toxins on many 'out-of-range' species, including representatives from non-arthropod taxa, like snails or crayfish or bacteria. While this list of studies is not comprehensive (not a review), it does illustrate the growing diversity of affected species and effects arising from Bt toxins that researchers have observed and reported, most of which cannot be detected in short-term acute direct toxicity tests that follow first tier OECD toxicity protocols. In other words, these Bt effects will be and probably are being missed – by default (or design) - when using EFSA's approach of reductionism and denial. This list also complements the more comprehensive catalogue of non-target (or 'cross-order') effects of Bt toxins maintained by van Frankenhuyzen (2013) that focuses on microbe-produced Bt toxins, by adding documented non-target effects caused by GM plant-produced Bt toxins.

Table 2. Documented diversity of reported sublethal and lethal effects attributed to Cry proteins from plants or pesticides on non-target organisms

#	Species	Order	Parameter affected	Bt protein type & source	Reference
	Bt from GM plants				
	Predators				
1	*Chrysoperla carna*	Insecta, Neuroptera	Mortality (juvenile)	Cry1Ab, GM maize (via two prey species)	Hilbeck et al. 1998a
2	*Chrysopa sinica*	Insecta, Neuroptera	Mortality, development time (juvenile)	Cry1Ab, GM cotton (via prey)	Guo et al. 2004
3	*Chrysopa formosa*	Insecta, Neuroptera	Mortality, development time (juvenile)	Cry1Ab/Ac fused, GM cotton (via prey)	Guo et al. 2004
4	*Propylea japonica*	Insecta, Coleoptera	Development time (juvenile), number ovipositing adults, body mass	Cry1Ab/Ac fused, Cry1Ab GM cotton (via prey, aphids)	Zhang et al. 2006a

#	Species	Order	Parameter affected	Bt protein type & source	Reference
5	*Propylea japonica*	Insecta, Coleoptera	Body mass and body length, development time, mortality (juvenile)	Cry1Ab/Ac fused, Cry1Ab GM cotton (via prey, S. litura)	Zhang et al. 2006b
6	*Harmonia axyridis, carabid beetles*	Insecta, Coleoptera	Lifespan adults, population densities (in field)	Cry3Bb, GM maize (via prey)	Stephens et al. 2012
7	*Coleomegilla maculata*	Insecta, Coleoptera	Development time (juvenile)	Cry1Ab, GM maize	Moser et al. 2008
	Aquatic herbivores				
8	*Helicopsyche borealis*	Insecta, Trichoptera	Mortality (juvenile)	multiple Cry toxins unknown, GM maize	Rosi-Marshall et al. 2007
9	*Lepidostoma liba*	Insecta, Trichoptera	Development time (juvenile)	Cry1Ab, GM maize	Chambers et al. 2010
10	*Chironomus dilutus*	Insecta, Diptera	Mortality	Cry3Bb1 GM maize	Prihoda & Coats 2008
11	*Chironomus dilutus*	Insecta, Diptera	Mortality	Cry1Ac GM cotton	Li et al. 2013
12	*Daphnia magna*	Crustacea, Cladocera	Mortality, age at maturation, fecundity (all)	Cry1Ab, GM maize kernels	Bøhn et al. 2008
13	*Daphnia magna*	Crustacea, Cladocera	Mortality, fecundity and population growth rate (all)	Cry1Ab, GM maize kernels	Bøhn et al. 2010
14	*Daphnia magna*	Crustacea, Cladocera	Growth, fecundity, chronic stress response (resting eggs)	Cry1Ab, GM maize	Holderbaum et al. 2015
15	*Orconectes rusticus*	Crustacea, Decapoda	Mortality	Cry1Ab, GM maize	Linn & Moore 2014
	Mollusca				
16	*Cantareus aspersus*	Mollusca, Pulmonata	Growth	Cry1Ab, GM maize	Kramarz et al. 2009
	Annelida				
17	*Eisenia fetida*	Annelida Haplotaxida	Growth, reproduction, enzyme activity	Cry1Ab GM maize (Bt11 and MON810)	Shu et al. 2015
18	*Lumbricus terrestris (earthworm)*	Annelida Haplotaxida	Weight growth	Cry1Ab GM maize (Bt 11)	Zwahlen et al. 2003
	Bacterial communities				
19	Bacterial communities of *Eisenia fetida* casts (compost-worm)	Bacteria	Changes in the bacterial community of E. fetida casts	Cry1Ab GM maize (Bt11 and MON810)	Shu et al. 2017
20	Soil microbes	Bacteria, enzymatic activities	Soil microbial biomass suppressed, enzyme activity affected;	Cry1Ac GM cotton	Chen et al. 2017

#	Species	Order	Parameter affected	Bt protein type & source	Reference
			Bt toxin accumulation in soil over years		
21 22	Soil microbes	Bacteria, enzymatic activities	Soil microbial biomass carbon, microbial activities, soil enzyme activities significantly decreased	Cry1Ac and CpTI stacked GM cotton	Chen et al. 2011 Chen et al. 2012
23	Soil microbes	Bacteria enzymatic activities	Species diversity differed	Cry1Ab GM maize (MON 810)	Van Wyk et al. 2017
24	Soil microbes	Actinomyces Species diversity	Negative effect on organic carbon	Cry1Ac Bt-Brinjal (eggplant)	Singh et al. 2013
	Insect communities				
25	Aquatic insect communities	Insecta, Coleopteran, Trichoptera, Plecoptera, Ephemeroptera	Various, community composition differences	Cry3Aa, GM populus trees	Axelsson et al. 2011
26	*Scarabaeidae communities*	Insecta, Coleoptera	Various, community composition differences	Cry1Ab and Cry1F maize	Campos & Hernandez 2015
27	*Pycnopsyche sp., Caecidotea communis*	Insecta, Isopoda,	Abundance differences	Cry1Ab, Cry3Bb1 GM maize	Swan et al. 2009
	Behavioral differences				
28	*Phytoseiulus persimilis*	Arachnida, Mesostigmata	Avoidance of Bt-contaminated prey	Cry3Bb1, Cry1Ab stacked GM maize	Prager et al. 2014
29	*Chrysoperla carnea*	Neuroptera, Insecta	Avoidance of Bt-contaminated prey	Cry1Ab GM maize	Meyer & Hilbeck 2001
30	*Phytoseiulus persimilis*	Arachnida, Mesostigmata	Avoidance of Bt-contaminated prey	Cry3Bb GM eggplant	Zemkova Rovenska et al. 2005
	Bt proteins (purified or insecticides)				
31	*Chrysoperla carna*	Insecta, Neuroptera	Mortality (juvenile)	Cry1Ab	Hilbeck et al. 1998b
32	*Chrysoperla carna*	Insecta, Neuroptera	Mortality (juvenile)	Cry1Ab, Cry2A, (via prey)	Hilbeck et al. 1999
33	*Adalia bipunctata*	Insecta, Coleoptera	Mortality (juvenile)	Cry1Ab, Cry3Bb	Schmid et al. 2009
34	*Adalia bipunctata*	Insecta, Coleoptera	Mortality (juvenile)	Cry1Ab,	Hilbeck et al. 2012
35	*Cheilomenes sexmaculatus*	Insecta, Coleoptera	Mortality (juvenile)	Cry1Ab, Cry1Ac, (via prey)	Dhillon & Sharma 2009
36	*Propylea japonica*	Insecta, Coleoptera	Body mass and body length, development time (juvenile)	Cry1Ac (via prey)	Zhang et al. 2006c
37	*Mytilaster minimus,*	Mollusca, Bivalvia	Various eco-physiological parameters, mortality	Commercial Bt kurstaki (Cry1) based pesticide	Manachini et al. 2013

#	Species	Order	Parameter affected	Bt protein type & source	Reference
	Brachydontes pharaonis				
38	*Daphnia magna*	Crustacea, Cladocera	Mortality	Cry1Ab, Cry2Aa	Bøhn et al. 2016
39	*Dragonflies and damselflies*	Odonota	Indirect food web effect decrease in prey	Bt israelensis	Jakob & Poulin 2016.

In the following, we highlight a few illustrative cases in more detail for which by now a body of evidence, stemming from several studies, has accumulated. They allow us to deconstruct and reveal a repeating pattern of argumentation by EFSA when confronted with research data that are not in line with the outdated narratives and scientifically unfounded dogmas on which EFSA's risk assessments are still based (i.e. 'expectations').

Case example 1: EFSA in denial of documented negative effects on lacewings

Larvae of the beneficial non-target predator called the green lacewing (*Chrysoperla carnea*) were shown, in a series of three publications, to be adversely affected by Bt toxin regardless of whether the Bt toxin was produced by bacteria, or by GM maize, or was fed via prey (target and nontarget) or directly (Hilbeck et al. 1998a,b, 1999). These studies by the Hilbeck group triggered a series of counter studies by Dutton et al. (2002), Romeis et al. (2004), and Rodrigo-Simon et al. (2006), trying to disprove the findings by the Hilbeck group. In their review, Hilbeck and Schmidt (2006) comparatively evaluated all relevant scientific aspects of these studies in detail: statistical design, methods applied, materials used, results reported, interpreted and conclusions drawn. They found that methodological differences were significant and that the studies did not support conclusions of providing counter evidence or proving opposite findings. They are, at best, complementary studies. However, EFSA consistently followed the arguments by those disputing the adverse effects (see EFSA Opinion 2009), and ignored or dismissed (denied) all evidence pointing to the complementary nature of these studies, while, simultaneously, accepting data submitted by the applicants that were clearly based on flawed protocols (Hilbeck et al. 1998b, Hilbeck and Schmidt 2006).

For example, for years regulators in the US and the EU accepted studies using known flawed protocols which had been pointed out to them early on (1998):

> "*A major obstacle in conducting feeding bioassays with C. carne has been that the larvae typically suck their food from within a substrate. In previous studies investigating direct effects of B. thuringiensis proteins on C. carnea, the surface of Sitotroga cerealella (Olivier) eggs was coated with B. thuringiensis proteins and subsequenty fed to C. carnea larvae (Croft 1990, Sims 1995). However, because C. carnea suck out the egg contents without ingesting the shells, they probably ingested little or no B. thuringiensis protein*." … "*In another study, B. thuringiensis-containing pollen of transgenic plants was provided to test for side effects of Cry1Ab on C. carnea larvae (Pilcher et al. 1997). However, ... predaceous C. carnea larvae feed only to a very limited extent if at all on pollen*."(Hilbeck et al. 1998b)

Finally, from 2002 onwards, at least the US EPA concurred with this criticism raised years earlier by Hilbeck et al. (1998b) (US EPA 2005, 2007):

> *"In some of the dietary toxicity tests conducted with green lacewing larvae, the Bt protein was presented to lacewings in a moth egg (Sitotroga sp.) diet.* ***However, the Bt protein may bind to the surface of the moth eggs, resulting in limited exposure to lacewings that feed with piercing-sucking mouthparts*** *(EPA-SAP 2002). Therefore,* ***it is inappropriate to test the activity of insecticidal proteins, such as a Bt protein, by incorporating the Bt protein into a moth egg diet****. Furthermore,* ***because green lacewings do not consume***

much pollen in the field *(...) and are not exposed to Bt proteins via consumption of aphids (...), other generalist predators should be considered for Tier I tests.*" US EPA 2007 (emphasis added).

And:

"The August 27, 2002 SAP concluded that green lacewing (Chrysoperla carnea) is not an appropriate test species for several reasons. Green lacewing are difficult to test in the laboratory because of a high rate of mortality. For example, in the study outlined above (MRID 457904-07), the test was terminated after 10 days because there was >28% mortality in the negative control. ***In addition, it is questionable whether green lacewings ingest the protein on coated moth eggs, since green lacewing have piercing-sucking mouthparts and do not consume the external surface of eggs.***" (US EPA 2005) (emphasis added).

Interestingly, Hilbeck et al. (1998b, 1999) never reported such high control mortality values in their first-tier studies using artificial diet. But instead of reviewing and reconsidering the science brought forward up to then in light of these now recognized and finally accepted shortcomings, EPA decided to drop this organism from the list of test species and suggested instead another test organism from a taxonomic group that had already been demonstrated to be unaffected by Bt toxins - coincidentally or not, also by the Hilbeck group (Zwahlen et al. 2000).

"*For these reasons, [the applicant should conduct a laboratory insect toxicity test on an alternate organism, such as the minute pirate bug (Orius insidiosus).] This egg predator, which feeds on pollen when prey is scarce, is typically found in corn fields. An appropriate evaluation would involve feeding O. insidiosus pollen, a natural food source, and purified protein in diet in two separate diet bioassays.*" (US EPA 2005) (emphasis added).

In contrast, although rejected by the EPA already in 2002 (see above quote), EFSA continued to accept the lacewing studies within the applicant's package, without even addressing the flaws that the US EPA has meanwhile admitted for many more years, and rejected documented adverse effects on spurious grounds (as revealed in Hilbeck and Schmidt 2006)

In 2009, EFSA stated: *"Another frequently used predator species in laboratory tritrophic bioassays to investigate potential effects of Bt-crops on predators via dosed prey is lacewing Chrysoperla carnea (Lövei and Arpaia, 2005). Hilbeck et al. (1998a,b, 1999) indicated significantly prolonged larval development and increased mortality when immature Chrysoperla carnea was fed lepidopteran larvae reared on Cry1Ab expressing maize under laboratory conditions. The authors suggested a possible chronic effect of Cry1Ab protein, while Romeis et al. (2004) indicated possible indirect effects due to poor prey quality. Rodrigo-Simón et al. (2006) reported that the Cry1Ab protein does not show specific binding in vitro to brush border membrane vesicles from the midgut of Chrysoperla carnea larvae, which is considered as a prerequisite for toxicity."*

"Moreover, no acute adverse effects were reported when Chrysoperla carnea larvae were fed non-susceptible Tetranychus urticae containing large amounts of biologically active Cry1Ab protein (Dutton et al., 2002)." (EFSA 2009).

Again, EFSA cites these studies without acknowledging that Hilbeck et al. (1998b) had also demonstrated an acute toxic effect when using activated Cry1Ab toxins fed directly to *C. carnea* larvae without any mediating prey.

"In the field, Chrysoperla carnea larvae are known to feed mainly on aphids and lepidopteran larvae are not considered an important prey, especially after their first moult (Romeis et al., 2004). Therefore, the continuous exposure of Chrysoperla carnea to diets exclusively based on lepidopteran larvae is unlikely under field conditions where a variety of prey is available (Dutton et al., 2003), ***though chronic effects cannot be excluded completely****."* (EFSA 2009).

When confronted with the arguments contained in the review by Hilbeck and Schmidt (2006) earlier, EFSA (2009) was unimpressed by the significant methodological differences shown, ignoring all of them, and aligning solely with the arguments by the contesting group:

*".. the key experiments on **what caused the significantly higher mortality in Bt-exposed lacewings larvae in these studies** are still missing to date. ... **Even though chronic effects cannot be completely ruled out**, the GMO Panel emphasizes that the continuous exposure of C. carnea to diets exclusively based on lepidopteran larvae is unlikely under field conditions (Canard, 2001; Dutton et al., 2003)."*(emphasis added).

Reluctant admittance (after years of denial) of a reported effect (see emphasis added in the quote above) is followed by dismissal based on optimistic speculation without documentation. Where aphids are not present, the polyphagous *C. carnea* larvae feed on everything else including any lepidopteran or coleopteran larvae available or even their own species, many of which have been documented to contain Bt toxins (see above). In contrast, studies are accepted without scrutiny and criticism that are obviously flawed as long as they fit the 'out-of-range paradigm':

For example: *"In addition, Li et al. (2008) demonstrated **that adults of C. carnea are not affected by Bt-maize pollen** and are not sensitive to the Cry1Ab and Cry3Bb1 proteins at concentrations exceeding those observed in pollen of Bt-maize."(emphasis added)* (EFSA 2009)

This reveals that EFSA (2009) seems ignorant, for one, of the fact that *C. carnea* larvae cannot feed on pollen given their piercing-sucking mouthparts, which EPA had acknowledged already in 2002 (see quote above) and, secondly, that adult stages of all species including target pests are known to be much less susceptible to Bt toxin, if at all. Hence, this finding by Li et al. (2008) is both unsurprising and irrelevant to the disputed case.

"No negative effects on C. carnea have been documented in the field; sampling from Cry1Ab-expressing maize fields has not shown a decline in their abundance (Bourguet et al., 2002; Eckert et al., 2006)."(EFSA 2008)

The cited field studies did not address and, therefore, neither confirm nor refute the findings by Hilbeck. This is explained at length in Hilbeck and Schmidt (2006) but ignored by EFSA.

Eventually, almost a decade later, EFSA also had to recognize the indisputable facts and '*did not consider that "the test species [C. carnea] was sufficiently exposed to the Cry3Bb1 toxin"* (EFSA 2011 – MON88017, page 35) and '*it is questionable whether green lacewings ingested the Cry34Ab1/Cry35Ab1 proteins coated moth eggs. On account of the feeding mode of C. carnea larvae, which have piercing-sucking mouthparts and therefore do not consume the external surface of eggs, exposure to Cry34Ab1/Cry35Ab1 when feeding on the treated lepidopteran eggs is likely to be low*' (EFSA 2013 - Maize 59122, page 42).

Following the EPA, EFSA eventually also dropped *C. carnea* from the list of first tier test studies by assigning it low relevance ('*lacewing larvae will not be exposed much (if at all) to Cry3Bb1 in the field, this study was considered of less relevance*'). They further followed the precedent set by the EPA and also accepted, even 'recommended', the *O. insidiosus* feeding study with old immature life stages (up to 5 days old, late second instars – see below) (EFSA 2011, EFSA 2013, page 42).

All of the above arguments document serious bias and selective citing behaviour by EFSA that is neither in line with their mandate of an independent scientific assessment body, nor with good and fair scientific practice – let alone precaution.

Case example 2: EFSA in denial of documented negative effects on ladybeetles

In 2009, a report was published where another non-target predator species, the two-spotted ladybeetles (*Adalia bipunctata*) exhibited significantly higher mortality rates when raised on a Bt-

toxin laced diet (Schmidt et al. 2009) (Table 2). As in the lacewing case above, a counter-study was published shortly thereafter disputing the effects (Alvarez-Alfageme et al. 2010). Two years later, however, the Hilbeck group published new evidence (Hilbeck et al. 2012a) and a commentary explaining and revealing the underlying reasons for these seemingly contradictory studies – supported by new experimental data (Hilbeck et al. 2012b). In these publications, Hilbeck et al. (2012a,b) deconstructed the counter study and showed that they did, in fact, not repeat and, thus, not refute the Schmidt et al. study. More importantly, the significant changes made to the testing protocols of the counter-study were shown, with new experimental data, to be the underlying reasons for failing to find the same results. When following these altered protocols, not even the target pest species could be killed anymore (Hilbeck et al. 2012a). The Schmidt et al. (2009) study was credited with being a key study that triggered the national bans for Bt maize in Germany and, subsequently, France (Ricroch et al. 2010). In contrast to these national regulatory decisions (that are in effect until today), EFSA maintained that these studies did not in any way affect their safety assessment and claimed the following (highlighted in text below), notably without any reference to scientific data or scientific articles in support of their claims:

> *"... neither a dose-response relationship, nor sublethal effects (on developmental time and adult body weight) on surviving specimen were observed;* ***both these features represent a typical response of sensitivity to Cry proteins.*** *The higher toxicity of a Lepidoptera-specific Cry1Ab reported on Coleoptera in comparison to the more Coleoptera-specific Cry3Bb is an outcome that needs to be confirmed based on more quantitative data (both on food intake and actual protein concentration). [The EFSA GMO Panel is of the opinion that these data are not sufficient to identify a hazard] or indicate a new mode of action of Cry proteins on the coccinellid species tested."* EFSA 2009 (emphasis added).

However, on a more encouraging note, in an exceptional move, EFSA acknowledged in a 2013 published Supplementing Statement (EFSA 2013b) complementing their earlier, typical optimistic opinion on the Bt maize 59122 application (EFSA 2013a) that regulatory lab studies conducted for risk assessment with *Coccinella septempuncata* remained lacking to the degree that the EFSA GMO panel had '*to reconsider its previous recommendation*' of no case-specific monitoring being necessary. The submitted data was not able to resolve the '*remaining scientific uncertainty on the potential toxicity of the binary Cry34Ab1/Cry35Ab1 protein on Coccinella septempunctata or other ladybirds*.' They suggested instead a laboratory study with pure Cry34Ab1/Cry35Ab1 proteins and a tri-trophic study with a spider mite-consuming ladybird.

Case example 3: EFSA in denial of documented negative effects on *Daphnia magna*

The water flea *Daphnia magna* is one of the most commonly used model organisms in ecotoxicology. *D. magna* has been used for testing both whole plant material that expresses Cry1Ab-toxin (Bøhn et al. 2008, 2010, Holderbaum et al. 2015), and purified Cry1Ab and Cry2Aa toxins (Bøhn et al. 2016). When *D. magna* were fed with flour from MON810 maize kernels, expressing Cry1Ab-toxin, in 42 day life-cycle experiments, the animals showed early maturation with the trade-off cost of reduced survival and fecundity in later life-stages, compared to animals fed with near-isogenic maize flour (Bøhn et al., 2008). This was interpreted as a weak toxic effect of the Bt-maize. When, in addition, test animals were exposed to smell from a fish predator (three-spined stickleback), the differences in fitness (population growth rate) increased between animals fed Bt-maize and non-Bt-maize (Bøhn et al., 2010). Also, Bt-transgenic leaves have been tested with *D. magna.* Powder from maize leaves also produced negative effects (on growth and late-life fecundity) as well as stress responses (production of resting eggs) after chronic dietary exposure (Holderbaum et al., 2015).

D. magna was also tested for effects after exposure to several Cry-toxins. One study tested exposure to 0.75 mg L-1 of microbially produced Vip3A toxin for 10 days and showed that the body size of the test animals was significantly reduced (Raybould and Vlachos, 2011). Another study showed that life-time exposure to purified Cry1Ab and Cry2Aa toxins (0.75 – 4.5 mg L-1), resulted in dose-response

effects on mortality, growth and reproduction. When animals were exposed to both toxins at the same time, effects were additive (Bøhn et al., 2016).

EFSA dismissed the results of these studies, again based on the 'out-of-range paradigm' and claimed that methodological weaknesses did not allow proper interpretation of the effects observed, and that several uncertainties remained. In sum, EFSA considered that their own risk assessment conclusions on maize MON810 and Bt11 for cultivation, made by the GMO Panel, remained valid and applicable (EFSA 2016).

> *'Based on the limited exposure to significant levels of the Cry1Ab protein via intact plant material and particulate organic matter in the water column, the* ***known spectrum of activity of the Cry1Ab protein and its selectivity to lepidopteran species, and the phylogenetic distance between D. magna and target species, EFSA does not consider D. magna the most representative NT aquatic organism for testing.*** *However, EFSA acknowledges that D. magna represents a member of a taxonomic group not typically tested for terrestrial NTOs, and that early-tier tests with daphnids can inform ERAs by determining the activity spectrum of the Cry1Ab protein, and by corroborating or rejecting the risk hypothesis of no harm to D. magna. Overall, EFSA is of the opinion that the study reported in the publication by Bøhn et al. (2016) addresses an objective relevant to the NT risk assessment of aquatic organisms in the frame of maize MON810 and Bt11 for cultivation'*(EFSA 2016) (emphasis added).

In a recent publication responding to this assessment by EFSA, Bøhn (2018) shows in detail how, firstly, EFSA speculates without scientific documentation to create a false positive explanation, i.e. that a buffer in the experiment might have caused the demonstrated negative effects rather than the Cry toxins tested. This misunderstanding by EFSA could have easily been clarified as irrelevant if EFSA had contacted the author – as they often do when questions arise with applicants. Secondly, EFSA omits key publications in the literature, e.g. Raybould and Vlachos (2011) that support the finding of toxicity in *D. magna* of purified Cry toxins at the same concentration as Bøhn et al. (2016) used, i.e. 0.75 mg/L. Thirdly, EFSA dismisses completely the key results of the paper by Bøhn et al. (2016), i.e. the combinatorial effects shown for Cry1Ab and Cry2Aa, as well the combinatorial effects of a single Cry toxin together with Roundup herbicide. EFSA holds on to their 'expectation' that *Daphnia* is an 'out-of-range' taxa and, therefore, cannot be susceptible to Bt toxins. Finally, EFSA fails to recommend any further studies, which would be normal scientific practice in case of uncertainty, a practice that contradicts the precautionary principle as laid down in European legislation.

With their response to all three case examples described above, EFSA documents their unwillingness to recognize contingency or complexity, e.g. in how chemicals and organisms have interactive effects. All cases document how reductionism and denial lead to serious flaws in the ERA and put EFSA on the defensive. This again highlights the prevailing working basis of the EFSA, where anything (including scientific evidence) that is not in line with old, preconceived narratives (i.e. 'expectations') and preconceived safety assumptions is met with rejection no matter how outdated or how overwhelming the counter evidence is. Effectively, thus, EFSA is relieving the applicants from their obligation to prove the safety of their products based on new data and the most recent science and is placing the burden of proof on independent scientists with extremely limited funding. Requiring independent scientists to prove that commercialized GMO products are unsafe is the exact opposite of what the Precautionary Principle calls for and what the mandate of EFSA supposedly is. To what degree the 2013 move to revise and reconsider previous typical safety conclusions (EFSA 2013b), based on a more critical evaluation of protocols of studies that did deliver the 'expected' outcome, will become a new standard remains to be seen.

5. Emerging hazards – The case of teosinte in Spain

Up until 2016, hazards arising from gene flow of the insecticidal Bt trait in GM maize to wild and weedy relatives were dismissed on the basis that there are no wild or weedy relatives of currently cultivated GMOs, i.e. Bt maize MON810, in Europe. This situation changed dramatically when, in 2009, Spanish farmers discovered a new, fast spreading and highly destructive weed in their maize fields (Pardo et al. 2014, Trtikova et al. 2017). As it turned out, the new weed is the ancestor of maize, teosinte, except the exact species remains unclear, as does the route of introduction. Civil society organisations quickly called on the EU Commission to take note of this worrisome development threatening to spread to other European countries and requested that EFSA be tasked to re-do its risk assessment in light of this new situation[4]. In September 2016, EFSA published a Technical Report (EFSA 2016b) wherein the GMO panel concluded that: *"…it is unlikely that environmental harm will be realised"*.

In 2017, Trtikova et al. published the first study of its kind reporting the findings of a genome-wide analysis of single-nucleotide polymorphism data for the teosinte found in Spain as well as sympatric populations of maize and samples of reference teosinte taxa. In 2018, a group of authors, led by an EFSA GMO panel member and an industry representative, re-formulated the earlier findings and arguments of no concern of the Technical Report by EFSA in light of the new publication by Trtikova et al. (2017), and published this in a peer-reviewed journal (Devos et al. 2018). Given that much of the content of the Devos et al. (2018) paper is identical or builds on and further develops the EFSA Technical Report of 2016, we focus our analysis on this latest publication vis-à-vis the Trtikova et al. (2017) publication.

The Devos et al. (2018) narrative builds on two critical elements, one is the highly normative framing of the problem including a likewise narrow and normative definition of harm, and the second is the assumption that the teosinte found in Spain is either the *mexicana* teosinte or the *parviglumis* teosinte native in Mexico, and is therefore identical in its biology and ecology to either of these species.

The industry value frame – Pre-empting precaution and the reversal of burden of proof and responsibility on farmers' shoulders

Key to the Devos et al. (2018) assessment is the application of what the authors call the 'conservative hypothesis' of no risk. The chemical-intensive, industrial form of agriculture as practised in the affected Spanish regions which the authors declare as 'conventional' agriculture, acts as the comparator. Hence, this form of agriculture serves as the 'gold standard' of comparison with respect to environmental harm for all of Europe. No scientific hypothesis or any scientific evidence is offered in support of this normative choice of the declared 'gold standard'.

> *"A crucial step of problem formulation for an ERA is to identify what qualifies as harm.." ... "When defining harm, an important consideration is whether the proposed activity may lead to new harms, or only to different ways of causing harm that already result from current practices" … "Hence, definitions of harm for ERA for GM crops really are statements about what would be considered unacceptable increases in the frequency or severity, or both, of harmful effects if a particular GM crop was to be used instead of a similar conventional crop."* (Devos et al. 2018, p 20)

In other words, according to Devos et al. (2018), as long as a proposed activity is not more destructive than the current destructive practice, the new destructive practice is acceptable and a priori a 'no risk' conclusion is warranted. This is not only non-inspiring and non-aspiring from a visionary perspective regarding the future of agriculture but also strongly ideological) and vested interest-guided (Syngenta affiliated author). Consequently, similar statements like these lack references that would at least

4 http://www.ifoam-eu.org/sites/default/files/ngos_policy_teosinte_in_spain_open_letter_20160224.pdf

suggest ideological support. Hence, it reflects solely the authors' personal values and world vision. However, it does follow EFSA's likewise unaspiring guidance on problem formulation based exclusively and expressly on the same vested-interest industry thinking as Devos et al. (2018), i.e. the corporate concept developed by Syngenta (Raybould 2006, 2007 - see also quote below from problem formulation section of EFSA Guidance on ERA of GM plants, EFSA GMO Panel 2010[5]). Other (non-corporate) conceptual approaches to problem formulation, available to both the EFSA GMO panel and Devos et al. (2018) at all times, were and continue to be systematically ignored. We list them here again for transparency and evidence purposes (Capalbo et al. 2006, Hilbeck et al. 2004, Nguyen et al. 2008, Nelson et al. 2004).

The narrative of Devos et al. (2018) builds on these pillars of assuming that teosinte will pose *a priori* no risk, guided by a definition of 'harm' that considers only effects that are more destructive than the existing destructive form of agriculture practised in the affected regions in Spain, riddled by exotic weed problems already. Adding to or replacing existing problems is deemed an 'acceptable' risk that farmers have to deal with on a daily basis. Thus, dealing with this proven serious novel weed is put squarely into the responsibility of farmers only. Needless to say, farmers were not consulted in reaching this conclusion.

'*Iberica teosinte*' – a teosinte of its own

The fundamental scientific flaw of the Devos et al. (2018) analysis is that the authors presume and treat the teosinte found in Spain as if it were either a '*mexicana*' or a '*parviglumis*' teosinte subspecies, despite clear evidence to the contrary published by Trtikova et al. (2017). Consequently, they built their speculative and (industry) normative ERA exclusively on the knowledge available for these two distinct, known teosinte subspecies from their native home ranges in Mexico.

Trtikova et al. (2017) published the first genome data of the teosinte found in Spain and compared it to six other known and sequenced teosinte sub-species (Fig. 1). The single most important key message of this publication was that the parents of the teosinte found in Spain are unknown (Trtikova et al. (2017). The authors showed that no gene sequences from '*parviglumis*' were identified, nor of other sequenced and known teosinte sub-species they compared them to, except some minor traces of the '*mexicana*' teosinte sub-species (Figure 1). But these traces neither explain nor define the teosinte found in Spain as is clearly stated in Trtikova et al. (2017): *'...results are not compatible with the published suggestion that teosinte plants observed in Spain represent Z.m. ssp. mexicana'.*

Hence, until proven differently, it is a teosinte type of its own and in order to make this clear, we call the teosinte found in Spain hereafter '*iberica* teosinte'.

5 "*Problem formulation is generally performed on the basis of a conceptual model and an analysis plan (EPA, 1998, Hill and Sendashonga, 2003, Raybould and Cooper, 2005, Raybould, 2006, 2007, Romeis et al., 2008, Storkey et al., 2008, Raybould, 2009, Raybould et al., 2009, Wolt, 2009, Wolt et al., 2010*)." Page 15, EFSA GMO Panel 2010

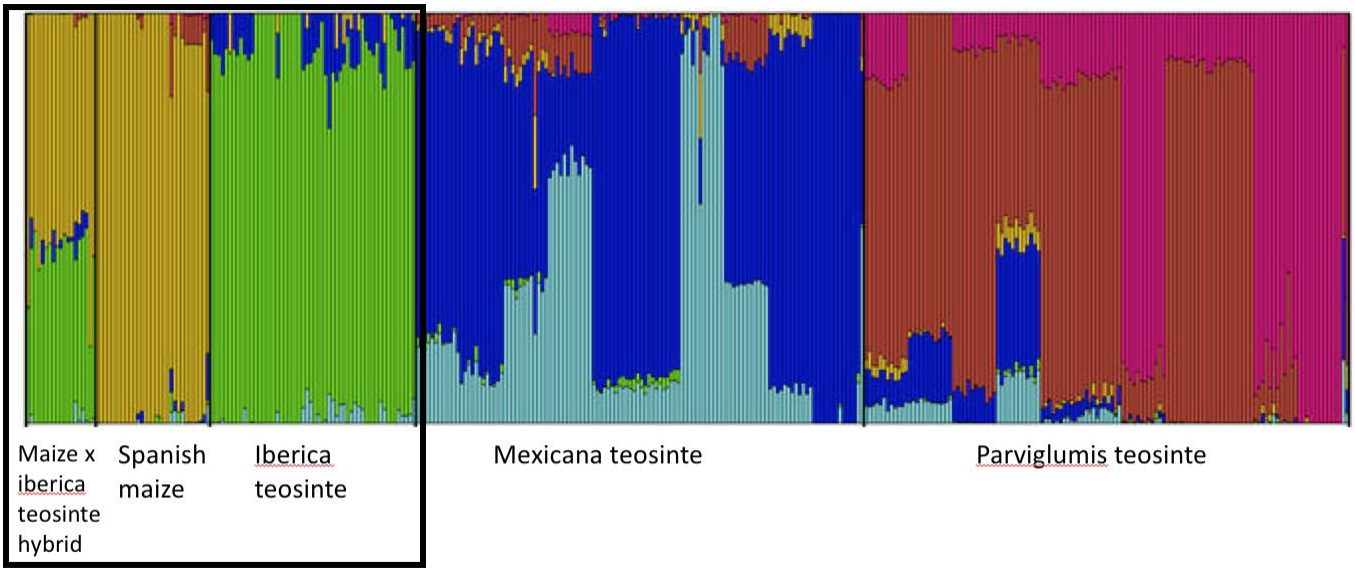

Figure 1. STRUCTURE analysis of Spanish maize varieties, hybrids with *iberica teosinte*, *iberica teosinte* (*Z.m. iberica*), *Z.m. ssp. mexicana* and *ssp. parviglumis*. Bar plot of assignment proportions at K = 6 combining own SNP data and other publicly available data (27'476 SNPs and 360 individuals). (After Trtikova et al. 2017)

The consequences of this finding are significant and explain why the '*iberica* teosinte' deviates in critical characteristics from either of the other teosinte species. This demonstrates that many assumptions made by Devos et al. (2018) are speculative and require detailed follow-up research to understand the development and potential risks with the '*iberica* teosinte' on a new continent.

In fact, Trtikova et al. (2017) hypothesized that the '*iberica* teosinte' is possibly the product of hybridization and backcrossings with maize which has one of its parents in the past as 'mexicana'. The hybridization process leading to '*iberica* teosinte' probably happened some time ago, somewhere in the world (not necessarily Mexico nor Spain). '*Iberica* teosinte' combines traits which make it far more weedy and proliferous than has been reported for other teosintes like '*mexicana*' or '*parviglumis*', producing long-lasting seed banks and proving difficult to control by both chemical and cultivation control measures (personal observation and first-hand information from affected farmers in Spain).

While it is true that hand pollination of '*iberica* teosinte' with maize pollen resulted in viable progeny in exploratory crossing experiments to a much lower extent, it proved possible nevertheless, although this is not the prime issue of concern at this point. Yet, Devos et al. (2018) almost exclusively focus on this pathway of gene spread while ignoring that '*iberica* teosinte' is fully cross-fertile with maize when '*iberica* teosinte' is the pollen donor (Trtikova et al. 2017). It is this feature that makes '*iberica* teosinte' such an extraordinarily destructive weed. '*Iberica* teosinte' has been forming its own teosinte-to-maize hybrid populations already, as documented by Trtikova et al. (2017) (see Figure 2), and together with the '*iberica* teosinte', they are indistinguishable from maize until tasselling and cob development. The cobs of both '*iberica* teosinte' x maize hybrids and '*iberica* teosinte' are much smaller than those of maize, and show highly variable kernel traits ranging from fully encased dark-brown teosinte-like kernels to yellow, non-encased maize-like kernels on one cob. They shatter much more easily and the kernels germinate quickly and are highly vigorous. Nothing is known to date regarding the cross-fertility between these '*iberica* teosinte'-to-maize hybrids and '*iberica* teosinte'. It is feasible that these serve as hybrid-bridges, the likelihood and consequences of which cannot be speculated about from knowledge about any other teosinte sub-species or other hybridization experiments carried out with known teosinte sub-species and maize in the Americas.

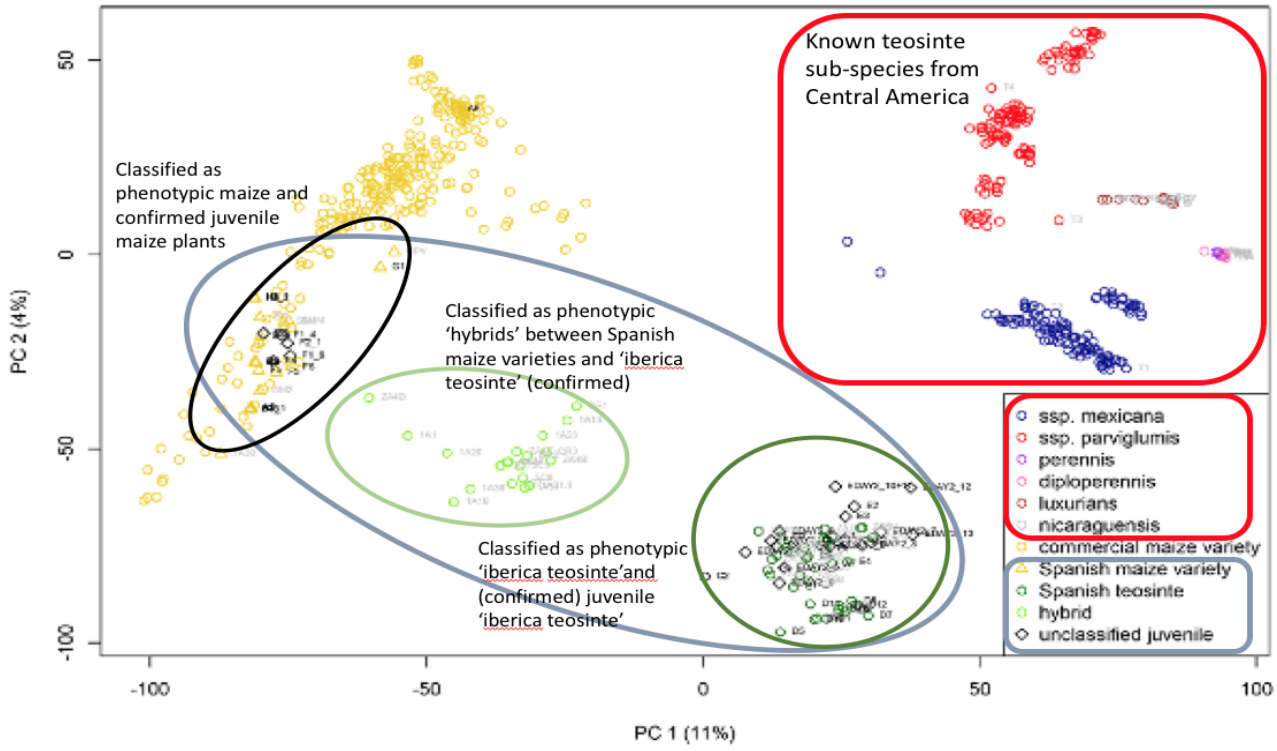

Figure 2. PCA of maize, teosinte and hybrid samples collected in Spain. Samples were collected from plants growing in the field (black labels), from seeds collected in the field and subsequently grown in a climate chamber (grey labels) or from reference material (grey labels). Labelled symbols represent own data that were compared to other publicly available data (without labels) (27′476 SNPs and 662 individuals). (From Trtikova et al. 2017)

Whether or not '*iberica* teosinte' has higher levels of resistance against herbivores feeding on it is also pure speculation without basis, scientific or anecdotal. As this plant is entirely novel in the European context, there is no basis for any of the many 'expectations' formulated by Devos et al. (2018). If the insecticidal Bt transgene from the widely produced Bt maize in Spain outcrosses to '*iberica* teosinte' – which we consider not a question of 'if' but only 'when' and 'how frequently' it will happen – it must undoubtedly be expected that, if the Bt transgene is expressed in '*iberica* teosinte' as in Bt maize, it confers a further competitive advantage to these already highly competitive weeds. It is these competitive traits (listed above) of the '*iberica* teosinte' and its hybrids with maize that have brought maize production to a grinding halt in the most severely affected areas (Pardo et al. 2016a,b). To completely ignore the destruction reported by Spanish farmers and speculate on a hypothetical basis that has already been rejected with experimental data by Trtikova et al. (2017) – '*iberica* teosinte' is not identical to any other teosintes – is grossly negligent towards farmers and the environment alike. It also sheds a dubious light on the peer-review process this paper supposedly went through at the journal that published this paper, as these basic flaws were overlooked – it could have qualified as an opinion or a 'wishful thinking' piece at best.

6. Conclusions - The core problems that arise from the regulatory system in Europe

When applying its narrow ERA model that effectively denies relevant biological complexity and contingency, such as interactions between Bt-toxins, GM plants and ecological communities in the environment, EFSA will continue to fail to meet its objective of protecting human health and the environment from damage by i) insisting on applying an outdated understanding of the modes of action of Bt toxins, ii) refusing to accept the substantial differences between native Bt proteins and those expressed by synthetic transgenes in GM crop plants and iii) applying a decision-making concept where a 'risk conclusion' is hardly possible.

Consequently, EFSA is not able to uphold the scientific standards they claim are at the core of their work. For example, EFSA clearly and deliberately fails to follow its own published requests for combinatorial effects research. Ultimately, EFSA is at risk of jeopardizing its own scientific and public reputation and failing its main mandate – to protect human and ecosystem health.

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