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Testbiotech comment on risk assessment and confined field trials with genetically engineered Bt cowpea conducted in Ghana

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Data provided and the design of field trials in Ghana were insufficient to conclude on risks to the environment and human health

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Table of Contents

Summary

The application for the field trials of genetically engineered (GE) Bt cowpea in Ghana did not inform the authorities fully and correctly about the following:

- the risks of non-stable gene expression,
- the potential of invasiveness of the GE plants,
- the risks to non-target organisms,
- the specific health risks triggered by potential combinatorial effects caused by mixing the Bt proteins with cowpea proteins, which are classified as potential allergens.

Furthermore, the design of the field trials was not adequate to generate the necessary data for an application and approval for agricultural cultivation and food production in Ghana. As explained by the applicant, the trials were meant to provide data only concerning agronomic performance, such as efficacy concerning protection against *Maruca vitrata* infestation and to generate seeds for further trials. In this regard, an environmental risk assessment was not taken into account by the field trials as designed. Consequently, the data available do not take into account the different biogeographic regions, their climate, biodiversity, wild relative species and regional cowpea varieties in Ghana. Therefore, it is not possible to conduct and conclude an environmental risk assessment for any release of the GE cowpea in Ghana.

Overall, the application and the design of the field trials were not sufficiently based on sound science and did not take into account intended and unintended effects. Some of the information presented in the application (on invasiveness and abundance of wild relatives) could even be regarded as misleading.

In conclusion, the application and the design of the field trials as conducted cannot be considered as lawful, adequate and sufficient to assess the risks of release, cultivation and consumption of GE Bt cowpea. Therefore, based on the data available, the field trials should not have been allowed, and cultivation and consumption of Bt cowpea in Ghana should not be approved.

Introduction:

Testbiotech acts as an independent institute for impact assessment of biotechnology and GE organisms. Testbiotech is entirely independent from any interest in the development and application of GE organisms and has extensive experience in risk assessment of GE plants as conducted in the European Union. More information about our activities in this field can be found in our database (www.testbiotech.org/en/database) and our publications (www.testbiotech.org/en/publikationen).

At the request of Food Sovereignty Ghana (FSG), Testbiotech analysed the application for field trials of the CSIR-Savanna Agricultural Research Institute to the Ghanian National Biosafety Committee (NBC) as filed with the NBC and the risk assessment as conducted by the NBC.

Subject and reason for the analysis:

Testbiotech was informed that the applicant of the field trials with GE cowpea in Ghana, based on the outcome of the field trials, would use this as a basis to obtain approval for cultivation and the use for food and feed and processing purposes of the GE crop plants. In this context, critical questions have been raised regarding whether the field trials can be considered as adequate to deal with the specific environmental and health risks associated with the introduction of the GE cowpea into the food and farming systems of Ghana.

Objective of the analysis

The question raised by FSG was whether the application and the design of the field trials could be regarded as lawful, adequate and sufficient to assess the risks to justify approval for field trials themselves as well as the release, cultivation and consumption of GE Bt cowpea.

Accessed documents:

To conduct the assessment, Testbiotech had access to the following documents:

- Application form for a confined field trial in Ghana for Maruca-Resistant Cowpea project filed by CSIR-Savanna Agricultural Research Institute to the NBC (received on 2011-05-04) but without the annexes;
- Summary on Risk assessment as conducted by the NBC;
- Schedule to the Approval of the field trials by the NBC and
- Trial Managers Handbook.

Testbiotech was not able to access information on the data and results of the field trials. Testbiotech was also not able to access the full text of the dossiers for risk assessment. Testbiotech considers restricted access to data and lack of transparency as unfortunate and regrettable since the field trials were proposed as a public interest intervention, driven by concerns regarding food security and not by commercial interests. Testbiotech regrets that access to information was limited and is aware of the fact that this has meant that relevant information could not be included in this analysis.

Methodology:

In performing the analysis of the available documents, Testbiotech followed standard and best practice procedures with regard to risk assessment. We started from the biology of the plants (taking into account gene flow), the description of the intended genetic modifications, the description of the plant components and known environmental interactions of the plants with the environment, including the food web and non-target organisms. In addition, some considerations concerning health aspects at the stage of consumption were also included in the assessment, given the importance of cowpea in the Ghanaian diet.

Testbiotech identified and described potential hazards and resulting risks, which in our expert opinion, should have been taken into account in the application and design of the field trials and the overall risk assessment. Risks identified by us were compared with the application and the design of the field trials, as filed to the NBC in 2011 and discussed in the light of the decision of the NBC.

A) Identification and description of hazards and risks

Testbiotech focused on the following topics:

- Molecular data, genetic stability and unintended effects;
- Gene flow to other cultivated subspecies or wild relatives;
- Impact on non-target organisms; and
- Health risks at the stage of consumption.

Molecular data, genetic stability and unintended effects caused by the process of genetic engineering

Since *Agrobacterium tumefaciens* was used for the transformation of the plants, the site of the insertion, the number of inserted copies and the final structure of the DNA as inserted cannot be predicted (Gelwin 2017), as *Agrobacterium* transformation is known to lack precision. Besides, gene expression of the additional inserted genes or changes in the original plant genes can be impacted by several causes, also including epigenetics (Jupe et al., 2019).

This is especially relevant for the Bt cowpea since two gene products are intentionally expressed in the plants. In addition to the Bt toxin, an enzyme is produced by the plants conferring resistance to antibiotics (NptII).

Therefore, to assess genetic stability, gene expression, unintended gene products, etc., specific data are needed, including the following:

- the number of copies inserted (several copies might have been inserted);
- the structure of the copies inserted (there might occur deletions or inversions);
- the place of insertion (there might be plant genes that may be disrupted); and
- the expression of the genes in the various plant tissues, during the season and in reaction to changing environmental conditions.

As available peer reviewed publications (Adamczyk & Meredith, 2004; Adamczyk et al., 2009; Chen et al., 2005; Dong & Li, 2006; Huang et al., 2014; Luo et al., 2008; Nguyen & Jehle, 2008; Then & Lorch, 2008; Trtikova et al., 2005) demonstrate with the examples of Bt genes, gene expression can be impacted by:

- the genetic or epigenetic background (the specifics of the plant's genome and its gene regulation) of the varieties into which the additional genes are inserted;
- interaction with environmental factors and stressors; and
- the different stages during vegetation due to growing, flowering, ripening.

Furthermore, the number of gene constructs, their place of insertion and their interaction with the plant’s genome can cause unintended changes in the plant’s composition and its phenotypical characteristics, such as seed dormancy, number of pollen and seeds, pollen viability and reaction to biotic or abiotic stressors.

Unintended effects caused by the insertion of the additional genes may for example, trigger unexpected high or low levels of the Bt toxin in the various tissues of the plants with significant consequences for the environment and food production. A too low level can cause the plants to be insufficiently protected against infestations of the larvae of *Maruca vitrata*. Further, under these conditions, the insect pests can more rapidly evolve to become resistant to the expressed Bt toxin. A high concentration of Bt toxins, on the other hand, is relevant for assessing risks to non-target organisms and food safety (see below).

Furthermore, unintended changes in the plant composition, it's metabolism and signalling pathways may affect organisms that carry out essential ecosystem services, such as pollinators or associated soil organisms. Other examples of potential adverse effects might include the higher vulnerability of the plants to stressors or enhanced gene flow and spread into the environment.

If fitness is lowered due to fitness costs of the Bt protein, the Bt cowpea might become more susceptible to other pest insects, mites, bacterial and fungal diseases, viruses and parasitic weeds (for a list of relevant plant diseases and pest insects see OECD, 2015, table 6). In this regard, pest replacement has also to be considered: While *Maruca vitrata* abundance might be reduced, other pest insects can show higher rates of infestation. Such effects are known to occur in Bt maize and Bt cotton already (Catangui & Berg, 2006; Dorhaut & Rice, 2010; Lu et al., 2010; Wang et al., 2008).

Finally, changes in the plant composition can impact food safety. The composition of cowpeas concerning proteins, anti-nutritional compounds and biologically active compounds is complex, also including potential allergens (see below).

Therefore, essential data have to be made available by the applicant, before approving any field trials, concerning the insertion of the genes, the gene expression, the concentration of the additional proteins in the various plant tissues and the impact of the gene insertions on the activity of the plant genes, plant composition and its biological characteristics.

Furthermore, the design of the field trials should aim to provide data that record the reaction of the plants to specific abiotic or biotic stressors that are known to occur in the region or, for example, are likely to emerge due to climate change.

Gene flow to other cultivated subspecies or wild relatives

Ghana and India are the countries with the earliest evidence of cowpea (*Vigna unguiculata*) cultivation in the world, more than 1500 years ago (D'Andrea et al., 2007). Therefore, Ghana can consider the history of cowpea as being a significant national heritage, being closely related to its history.

Cowpea is morphologically variable and adapted to different environments, resulting in a wide range of local varieties (OECD, 2015). The nutritional composition of cowpea is impacted by genetic characteristics, agro-climatic conditions, biotic stresses and postharvest management (OECD, 2018). Interestingly, despite the considerable morphological diversity, limited genetic diversity occurs among cultivated cowpea varieties owing to a single domestication event that has given rise to all cultivated varieties (OECD, 2018). Therefore, epigenetic effects (gene regulation) seem to have major impacts on biological characteristics of the various subspecies.

The *Vigna unguiculata* species complex is currently divided into eleven subspecies. Ten of the subspecies are perennial, and one subspecies is annual (OECD, 2015). There are no apparent barriers to hybridisation or recombination between members of the different cultivar groups or with the wild cowpeas (*var. spontanea*) in the subspecies *unguiculata* (OECD, 2015). As stated in OECD (2015): "The overall message is that crosses appear possible among all members of the *Vigna unguiculata* complex, but they vary from being easy to being difficult."

Wild relatives subspecies belonging to the group *Vigna unguiculata var. spontanea* are known to occur in Ghana as well as in many other West African countries. In Ghana, *Vigna unguiculata var. spontanea* can be found mostly in disturbed areas (such as fields, field margins, roadsides and fallows) but could also be found in natural ecosystems, such as observed in Cameroon, Uganda and Ethiopia (OECD, 2016).

Gene flow between wild subspecies can be observed; its frequency is largely dependent on the subspecies, the size and location of the area of cultivation, and the occurrence of insects. While often considered as a rare event, according to a research project in Western Africa published in 2012, there were findings of gene flow between cultivated and wild relatives in each of the populations investigated (Kouam et al., 2012). As the OECD (2016) summarizes, cultivated cowpeas readily cross with wild cowpeas in the same subspecies (i.e. *var. spontanea*) and can be crossed with members of the other subspecies of *Vigna unguiculata* but with varying degrees of difficulty.

There are several pollinating insects involved in the distances and success rates of gene flow while their specific role still needs to be investigated in many cases: As it is stated in OECD (2015): "Cross-pollination is usually less than 1%, but will vary somewhat with the cultivar and, more particularly, with the population of some insects. In several cases, the pollinators are not known, but honeybees (*Apis mellifera*) have been observed around cowpea flowers and thus have been implicated in pollination (…) In coastal Kenya and Burkina Faso, several large carpenter bee species (*Xylocopa spp.*) and leafcutter bee species (*Megachilidae spp.*) were considered potential cross-pollinators of cowpea (...), and it was shown that these same leafcutters and carpenter bees were the likely pollinators of the wild progenitor of cowpea (…) Casual observations made in California, Texas and Nigeria indicate that large bumblebees (*Bombus spp.*) may be responsible for the cross-pollination that occurs in cowpeas in these regions."

Under the conditions of the confined field trials it might be possible to prevent such gene flow due to the mechanisms of self-fertilising plants, isolating zones and controls to prevent seed dispersal by human activities. However, since field trials are never fully contained, gene flow cannot be excluded per se. Gene flow will occur under field conditions when the GE cowpea is grown in close vicinity to regional varieties and without sufficient distance from wild relatives in other bio-geographical zones. For example, as NEPAD's African Biosafety Network of Expertise (NEPAD-ABNE), explains on their website, only 90 percent of the pollination in cowpea stems from self-fertilisation[1], meaning that there will be a 10 percent possibility for gene flow to occur. Importantly, insects, in particular, can enable outcrossing to wild relative species.

Therefore, agricultural cultivation of the Bt cowpea will cause the introduction of the artificial genes into regional varieties, whether intentionally or unintentionally. Also, gene flow to wild relatives is very likely to occur in the longer term. Hence, the additional inserted gene constructs will be introduced into plants with quite heterogeneous genetic and epigenetic backgrounds.

In some regions, hybrid crossings of cultivated varieties and wild subspecies are even grown deliberately as fodder plants (OECD, 2016). Often wild cowpea plants are not uprooted from the field, and appear to be tolerated in the agroecosystem. The hybrid progenies may even end up being used by farmers for sowing and may be considered as fodder landraces (OECD, 2016).

Therefore, a risk assessment should consider effects on hybrid offspring and the effects on the next generation, considering regional varieties as well as wild relatives and their interactions with the environment. Crucial risks include the effects on the following: genetic stability, gene expression, gene function, pleiotrophic effects, persistence, and invasiveness. These biological mechanisms are known to be impacted by the genetic or epigenetic background of the genome into which the additional genes are introduced by gene flow as well as by interactions with the environment. There is evidence that the biological characteristics of the offspring generation in many cases cannot be predicted from the original genetically engineered event (Bollinedi et al., 2017; Cao et al., 2009; Kawata et al., 2009; Lu & Yang, 2009; Vacher et al., 2004; Yang et al., 2017). If the offspring can persist and propagate in the environment, interactions with the environment or changes in the environmental conditions can, in addition, play a major role in triggering unintended biological effects (Zeller et al., 2010; Matthews et al., 2005; Meyer et al., 1992; Trtikova et al., 2015; Then & Lorch, 2008; Zhu et al., 2018; Fang et al., 2018).

1 http://nepad-abne.net/biotechnology/gm-crops-under-research-in-africa/cowpea/

Relevant risks posed to the environment are, for example, changes in the plant composition of the hybrid offspring. This can involve their metabolism and signalling pathways in such a way that essential ecosystem services performed by pollinators, or associated soil organisms can be impacted. Other examples of potential adverse effects include the higher vulnerability of the plants' offspring to stressors, or enhanced gene flow and spread into the environment. Furthermore, effects observed in hybrid offspring are unexpectedly high or low levels of the Bt toxin as produced in the various tissues of the plants (see above).

It should be taken into account that if Bt toxins are produced in wild relatives of the cultivated cowpea, this is likely to confer higher fitness compared to wild-types, which can finally result in replacement and displacement of the native species and far-reaching disruptive consequences for the associated ecosystems. According to NEPAD-ABNE, wild cowpea has to be considered as an invasive and allelopathic species with much more extended seed dormancy compared to cultivated varieties. This underlines the risk of Bt cowpeas becoming invasive and replacing and displacing natural populations and thereby becoming disruptive for ecosystems beyond the fields. This is especially relevant since West Africa is considered as a centre of biological diversity of cowpea.

Therefore, the risk assessment should take into account the presence and occurrence of regional varieties and wild relatives, their biological characteristics and their genetic differences compared to the variety used for the field trials. To obtain reliable data, experimental crossings should be conducted under contained conditions such as a closed greenhouse, before any field trials take place.

Impact on non-target organisms

There are many publications showing that Bt toxins have several modes of action (for overview see: Hilbeck & Otto, 2015; Vachon et al., 2012), impacting on a broader range of non-target organisms than previously thought, and that also do not follow the boundaries of taxonomy (Hilbeck & Otto, 2015; van Frankenhuyzen, 2013). This is also the case with the Cry1Ab toxin produced by the Bt cowpea plants which, as far as we know, has not been tested on a broader range of endemic African species.

Further, the structure of the toxin is relevant for its toxicity and selectivity, if for example its DNA has been modified or truncated due to the process of genetic engineering (Hilbeck & Schmid, 2006; Latham et al., 2017). Small changes in the structure of the protein can cause major changes to its biological characteristics, such as toxicity (see, for example, Gomez et al., 2014).

Furthermore, selectivity and efficacy can be influenced by many co-factors (Hilbeck & Otto, 2015; Then, 2010). In the case of cowpea, plant compounds such as the trypsin inhibitor can cause the Bt toxin to degrade much slower than for example, in maize. This can result in much higher toxicity of the Bt toxin if it is taken up together with the plant tissue, compared to the toxin in isolation (Zhang et al., 2000; Zhu et al., 2007; Pardo Lopez et al., 2009).

Thus, if plant material is taken up by insects, pollinators, and wildlife species such as birds or other non-target organisms, this may have severe negative effects on ecosystem services, the food web and biodiversity. OECD (2015) provides a list of non-pest arthropods associated with cowpeas, including many pollinating and beneficial species that are natural enemies of cowpea pests and general predators (Table 8).

In regard to the food web, it has to be taken into account that the Bt toxins might accumulate in higher concentrations within the tiers of the food web, especially if taken up by predatory insects, such as beneficial predator wasps, feeding on the larvae of *Maruca vitrata* (Obrist et al., 2006a and 2006b; Paula & Andow, 2016; Zhang et al., 2006; Zhou et al., 2014). An impressive list of parasitoids and entomoviruses attacking the podborer *Maruca vitrata* in West Africa can be found in OECD (2015, table 7). As stated in OECD (2015), in addition to parasitoids, generalist predators also feed on cowpea insect pests. These include mites, beetles, ants, bugs and spiders.

Therefore, a list of organisms that could potentially be exposed to the plant material directly or indirectly in Ghana should have been provided before the field trials were authorised. These organisms should be subjected to specific tests in the laboratory or a greenhouse, to provide the relevant data concerning toxicity and impact of the plant material, before any field trials might commence.

Taking into account the rich biodiversity in Ghana as well as the current threats to its conservation (Republic of Ghana, 2016), these questions are of high priority to the environmental risk assessment of the GE cowpea. It has to be taken into account that several bio-geographical zones in Ghana exist (Republic of Ghana, 2016), which show substantial differences regarding fauna and flora. This is especially relevant since small-scale farmers might cultivate the beans in highly diverse regions, and gene flow is likely to cause exposure to the Bt toxins in ecosystems beyond the fields (see above).

Health risks at the stage of consumption

As mentioned above, there are many publications showing that Bt toxins have several modes of action, impacting on a broader range of non-target organisms, not respecting the boundaries of taxonomy. This is also the case with Cry1Ab. Furthermore, selectivity and efficacy can be influenced by co-factors and changes in the structure of the Bt protein (see above). In the case of cowpea, plant compounds such as the trypsin inhibitor can cause the Bt toxin to degrade much slower than for example, in maize. This can result in much higher toxicity of the Bt toxin if it is taken up together with the plant tissue, compared to the toxin in isolation (see above and also: Then & Bauer-Panskus, 2017). Therefore it is evident that, for example, feeding studies using the isolated protein or feeding studies using other Bt staple food such as maize or soybean, are not reliable when it comes to assessing the potential health effects of Bt cowpea at the stage of consumption. Whole plant feeding studies should instead be conducted with the Bt cowpea in question.

There are several publications showing that toxicity of Bt toxins has to be investigated in more detail in regard to human health (for an overview see: Then & Bauer-Panskus, 2017). In this context, it is a matter of specific concern that at least some Bt toxins are likely to cause non-allergic immune reactions, so-called adjuvant effects (Finnamore te al., 2008; Moreno-Fierros et al., 2000; Vázquez et al., 1999; Legorreta-Herrera et al., 2010; Jarillo-Luna et al., 2008; E. González-González et al., 2015; Ibarra-Moreno et al., 2014; Guerrero et al., 2007; Guerrero et al., 2004; Moreno-Fierros et al., 2013; Rubio-Infante et al., 2018; Rubio-Infante et al., 2016) which might contribute to chronic diseases or enhance immune reactions. While most of the findings so far concern the Bt protein Cry1Ac, it is generally acknowledged that more data are needed on adjuvantic effects and Bt proteins (see for example, Rubio-Infante, 2016; Santos-Vigil et al., 2018). In any case, the Bt toxin Cry1Ab as expressed in the cowpea should be tested in detail in regard to its potential immune reactions: According to NEPAD-ABNE, some proteins as naturally produced in the beans of cowpea are regarded as possible allergens. Furthermore, some allergies were already identified to occur, although so far being rare for cowpea. According to OECD (2018), a research published in 2000 reported that serum from six individual patients that were allergic to cowpea identified 41 kDa and 55 kDa proteins to be the major allergens of cowpea. Thus, combining these proteins with immune reactive Bt proteins might cause the emergence of new allergens.

In regard to the cowpea, it has to be taken into account that its uptake as a main staple food in many regions might cause high exposure of humans to the Bt toxin. Therefore, the risk assessment should take into account the concentration of the Bt toxins and its degradation throughout the relevant stages of processing of all parts of the plants (pods, beans and leaves) meant for human consumption. For human consumption, cowpea is mainly grown for grain (dry and fresh) and sometimes for fresh pods but also leaves (OECD, 2018). In general, the green and fresh edible parts of the plants (pods, leaves and beans) will undergo less processing of the food compared to dried beans and therefore might show higher concentrations of the Bt toxin. Also in this context, it has to be taken into account that several bio-geographical zones in Ghana exist (Republic of Ghana, 2016), which show substantial differences not only regarding fauna and flora but also regarding agricultural and cooking practices. For example, in Ghana, the cooking time of cowpeas is traditionally reduced by cooking them with a naturally-occurring alkaline rock-salt known as ‘kanwa' (OECD, 2018). Furthermore, soaking before cooking is widely used to reduce cooking time. These traditional practices may also impact the degradation or non-degradation of the Bt protein in the diet.

Furthermore, the composition of cowpeas concerning proteins, anti-nutritional compounds and biologically active compounds is complex. NEPAD-ABNE lists phytic acid, trypsin inhibitor, raffinose, stachyose, verbascuose, hemagglutin as the most relevant anti-nutrients. Similarly, OECD (2016) lists hemagglutinin, tannin, trypsin inhibitors, oxalate, phytate, polyphenols and oligosaccharides. Therefore, any changes in plant composition that might be caused by the insertion of the additional genes and their impact on food safety have to be assessed thoroughly.

Consequently, any application for field trials or agricultural cultivation needs to be accompanied by data regarding food and feed safety, encompassing long term (chronic) feeding studies with whole plant food and feed.

B) Can the data as provided by the applicant and the design of the field trials be regarded as lawful, adequate and sufficient to assess the risks of release, cultivation and consumption of GE Bt cowpea?

Testbiotech did not have the possibility to analyse the national biosafety law of Ghana. Therefore we refer to general aspects regarding the lawfulness, adequacy and sufficiency of the field trials. In this context, it is a generally accepted principle that GE organisms (or Living Modified Organisms (LMOs) as referred to by the Cartagena Protocol on Biosafety, to which Ghana is a Party) can only be released into the environment if an adequate risk assessment was performed beforehand.

To be considered as adequate, risk assessment has to be based on sound science, consider intended and unintended effects and should be sufficiently robust and conclusive. This is the only way to address the precautionary principle underlying the Convention on Biological Diversity and the Cartagena Protocol. Furthermore, the objective of risk assessment under the Protocol is to identify and evaluate the possible adverse effects of LMOs on the conservation and sustainable use of biological diversity, also taking into account risks to human health.

To assess if the field trials were lawful, adequate and sufficient, the risks as identified above can be used to compare with the data provided by the applicant and the design of the field trials.

Molecular data, genetic stability and unintended effects caused by the process of genetic engineering

The data, as submitted by the applicant, provide some information about the gene construct prepared for insertion. These data show that the Bt toxin, as expressed in the plants, is not identical to the Bt toxin as found in natural sources, the soil bacteria. Instead, it was "modified to improve its expression and stability in the plant cell." This information is very relevant for the assessment of risks to non-target organisms and food safety, since, as explained above, small changes in the structure of the protein can have a significant impact on its toxicity. In any case, data on the natural variants of Cry1Ab toxin regarding the mode of action, selectivity and toxicity are not sufficient to demonstrate the safety of the Bt toxin, as expressed in the GE cowpea.

No data and information are made available on the actual number of gene sequences inserted. Nor in relation to whether the sequences were altered by the process of insertion, whether additional sequences were inserted without being intended, whether the place of the insertion of the gene into the genome is likely to affect the expression of other genes or might trigger unintended gene products in the plants. At least, none of this relevant information can be deduced from the accessible documents.

Regarding gene expression data, the concentrations of the Bt proteins in the different plant tissues were not reported. In the application, it is explicitly stated that no data on gene expression were provided and it was not deemed necessary. However, as shown above, without such data, no conclusion can be made on gene stability, as well as concerning risks to wildlife species, pollinators, and food safety.

Furthermore, by analysis of the design and the goal of the field trials, we could not identify any effort to generate data concerning gene expression in the various tissues of the plant during the periods of vegetation and growth of the plants and their reaction to specific environmental conditions. Similarly, data on the expression of the Bt toxin in the transgenic crop plant, after crossing with regional varieties or gene flow to wild relatives are not available and are not taken into account. Only some very few agronomic characteristics (such as yield) are mentioned as a goal of the field trials, but no data on plant composition and other biological characteristics were made available.

Gene flow

As mentioned above, the applicant did not provide any data on gene expression after gene flow to regional varieties or wild relatives. Instead, it only refers to the biological mechanisms of self-fertilisation of the cowpeas and it was assumed that no gene flow beyond the field trials will occur.

Furthermore, as mentioned, according to the information provided by NEPAD-ABNE, cowpea is classified as a species with invasive and allelopathic characteristics. Moreover, as shown in the publications quoted above, several wild subspecies can also be found in Ghana. However, this information is not even mentioned and considered in the application and the design of the field trials. Instead, the applicant inaccurately and misleadingly stated that no wild relatives of cowpea would occur in Ghana and that cowpea would not be regarded as an invasive species.

This illustrates that the applicant did not fully and correctly inform the authorities in Ghana. Since this information was not provided in the application for field trials, the purpose, design and safety standards could not be defined properly.

It has to be taken into account that the offspring of crossing with wild relatives are likely to show a higher degree of fitness (due to the production of the Bt toxin) and therefore, might become invasive, spreading more rapidly than the wild type plants. As explained above, the ecosystems can be damaged severely by the uncontrolled spread of the transgenes.

It is instructive to note that the European Food Safety Authority (EFSA) is requesting more data on GE plants in cases where gene flow to wild relatives is possible and is likely to result in plants with enhanced fitness, weediness and invasiveness (EFSA, 2010).

In any case, much more data should have been generated and provided by the applicant to assess the potential effects of hybridization, before field trials commenced. To be more explicit, to assess the risks properly and in detail, experimental crossings of the GE plants with wild relatives should have been conducted before any of the GE plants were allowed to be grown outside the greenhouse. Similarly, gene flow to regional varieties in Ghana should have been investigated.

In regard to effects of gene flow to regional varieties, it has to be considered that the lines of Bt cowpea used for the field trials were imported from other countries. Depending on the origin of the parental plants used for the genetic transformation, there might be significant differences in the genome and epigenome of the varieties used for introgression, compared to regional varieties grown in Ghana.

Also within Ghana, there is quite a range of varieties being marketed such as varieties called Togo, Bawku red, Bawku white, Lagos, Niger, Ejura white, Ejura red, Burkina and Red beans (Quaye et al., 2011). Crossings between the GE cowpea and these varieties can promote hybrid effects in ensuing generations such as higher fitness and unexpected and non-predictable biological characteristics, which may disturb or disrupt agro-ecological systems.

Regarding the design of the field trials, we could not find any evidence that efforts were made to generate any data concerning gene flow and the characteristics of hybrid offspring.

Non-target organisms

The applicant did not provide any data concerning relevant non-target organisms being abundant in the specific region. Furthermore, no data were made available on the susceptibility of the abundant species such as listed by OECD (2015). This is surprising since Ghana is the centre of origin of more than 20 endemic butterfly species and home to several hundred butterfly species (Republic of Ghana, 2016) which belong to the same group of insects (*Lepidoptera*) as the target species.

Since experimental field trials are meant to prepare an application for agricultural cultivation, more information on the potential impact on non-target species is not only essential but indispensable. Further, during the field trials, birds, insects and rodents cannot be prevented from entering the field trials completely. However, no information on the range of non-target species that might be exposed in the region, during the trials, was made available.

As explained above, selectivity and efficacy of the Bt toxin as expressed in the plants can be largely impacted and modified compared to natural sources of Bt toxins. Relevant factors include especially the structure of the toxin, and combination with other stressors or plant components. Furthermore, the expression of the Bt toxin in the plant tissue results in new paths of exposure, including changes in quality and quantity, compared to the natural variants of the Bt toxin. However, in the application for the field trials, the applicant only refers to knowledge about the natural variants of the protein Cry1Ab. Consequently, the authorities in Ghana were not fully and correctly informed about these crucial aspects that should have formed part of the risk assessment.

Regarding the design of the field trials, there is no mention of any monitoring or investigation of any non-target organisms entering the field trials. Therefore, no specific data on non-target species, which are abundant in Ghana, will be generated. The application only states that netting will be used from the onset of flowering, in order to measure the specific damage as caused by *Maruca*. This illustrates that the effects on non-target organisms were never intended to be investigated.

Human health

In the application, it is stated that no allergies against cowpea have been observed. There is no mention of the potential interactions of the newly produced proteins (especially Bt proteins) with plant constituents such as potential allergens. Without any mention of the proteins with potential allergenicity as identified by NEPAD-ABNE and OECD (2018), cowpeas are simply declared by the applicant to be non-allergenic. Further, there are no data regarding potential changes in food composition, which, for example, could cause increased levels of anti-nutrients.

There is no mention of generating data on potential health effects during or after the field trials. All plant material, except the harvested seeds, was supposed to be destroyed after the field trials. Therefore, no analyses, involving edible parts of the plants before harvest (such as green or fresh beans, pods or leaves) were planned to be undertaken during or after the field trials.

Since the seeds are stored after harvest, the beans might have been used for further health risk assessment. But this seems to be unlikely, because the only purpose of the storage as mentioned, is to store the seeds for further field trials.

The storage of seeds, not being destroyed after trials, raises further concerns. Losses or misappropriation of seeds during the field trials might be minimized by taking strict measures as requested by the authorities of Ghana. However, its further usage after the termination of the field trials, might no longer be subjected to sufficiently strict controls. This underlies the need to demonstrate full food safety before the commencement of field trials.

Discussion

As explained by the applicant, the trials were intended to provide data on agronomic performance and efficacy in regard to *Maruca* infestation and to generate seeds. It is astonishing and a matter of concern that neither before the field trials nor during the field trials, were the relevant data for risk assessment considered, provided or generated.

Furthermore, it is a matter of deep concern that the application for the field trials did not inform the authorities fully and correctly about the risks with regard to non-stable gene expression, the potential emergence of invasive GE plants, the risks posed to non-target organisms and the specific health risks triggered by potential combinatorial effects of adding the Bt protein with the cowpea proteins, known as potential allergens.

There are further issues that show that the field trials were not adequately designed and performed. The NBC of Ghana, in the summary of their risk analysis of the risk assessment, identify reasons for concern, and which were not considered in any detail by the applicant:

For example, in their decision regarding the field trial, they do not exclude potential health damage by allergies or toxicity or changes in the bioavailability of the nutrients. They consider these risks to be acceptable only in the case where a food safety assessment is conducted. However, concerning the material as accessed by Testbiotech, no such food safety assessment, including all relevant parts of the plants, could be identified. The entire assessment regarding food safety as presented by the applicant is solely based on assumption, and not on any experimental data.

Furthermore, the authorities consider the loss of nitrogen fixation as a relevant risk factor that has to be examined during the field trials. However, we cannot find any indication that the design of the field trials would have foreseen the generation of such data.

Most of the concerns as posed by the NBC are directed at gene flow and unintended dispersal of plant material. The NBC considers these risks to be acceptable if the measures for containing the material were complied with. However, the applicant did not inform the authorities fully and correctly about the risks of gene flow. Therefore, the question arises: would the NBC still have accepted the data as provided, if they been aware of the full range of relevant hazards and risks? From our findings above, it can be concluded that, for example, experimental data on gene expression, hybrid effects and invasiveness should have been requested by the authorities before taking any decision on the field trials.

In any case and especially in regard to any application for allowing the general cultivation and consumption of the GE cowpeas, the data as provided by the applicant for the field trials and the data generated during the field trials do not sufficiently address the factual risks posed to the environment, food safety and food security in Ghana.

Testbiotech is aware that further information was provided to NBC by the applicant, which could not be accessed by us. Due to this lack of transparency, we could not fully assess all the details. Nevertheless, there is no indication that any such further information would have fully addressed our findings and conclusion.

Conclusion on the application and the design for the field trials

The data provided for the field trials, and the design of the field trials do not allow for the undertaking of a full and complete risk assessment in regard to the potential likely adverse impacts on the environment, biodiversity, food safety and food security in Ghana. As explained by the applicant, the trials were intended only to provide data on agronomic performance and efficacy in regard to *Maruca* infestation and to generate seeds for further trials.

Consequently, no data are available concerning environmental risks, taking into account the different biogeographic regions, their climate, biodiversity, wild relative species and regional varieties in Ghana. Therefore, it is not possible to conduct and conclude on an environmental risk assessment based on sufficiently sound science.

Furthermore, the application for the field trials did not inform the authorities fully and correctly about the

- risks of non-stable gene expression,
- the potential of invasiveness of the GE plants,
- the risks to non-target organisms, and
- the specific health risks triggered by the potential combinatorial effects of mixing the Bt toxin with the proteins of the cowpea, which are potential allergens.

Some of the information provided by the applicant on these issues should even be considered as misleading.

In conclusion, the application and the design of the field trials as conducted in Ghana cannot be considered as being lawful, adequate and sufficient to assess the risks of release, cultivation and consumption of GE Bt cowpea in terms of national and international biosafety law.

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