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Abstract

This report seeks to contribute to the operationalization of the concepts of "sustainable development", "societal benefits" and "ethical justifiability" of the Norwegian Gene Technology Act. In order to achieve this, the report addresses the questions surrounding the sustainability of herbicide-tolerant crops identified by the Norwegian Biotechnology Advisory Board. The report uses the case of Intacta™ Roundup Ready™ 2Pro—a genetically modified stacked crop–used in Brazilian agriculture. This report summarizes, consolidates and contrasts the information provided by the dossier on the environmental and food biosafety of Intacta™ Roundup Ready™ 2Pro submitted by the applicant to the Brazilian authorities, as well as different findings reported by relevant empirical studies. The compiled information is organized in two sections. The first part addresses ecological aspects of the genetically modified herbicide tolerant plant and its related herbicide (glyphosate and related commercial formulations). The second section describes social and economic issues. Of particular importance, the report contrasts the applicant´s conclusions regarding the absence of adverse effects against the literature's findings on the existence of an array of possible harmful impacts. Given the current emphasis on types of production systems, the set of indicators used, and the focus on specific characteristics of herbicide tolerant crops, it can be recognized that the current state of knowledge on the sustainability of herbicide tolerant crops is incomplete.

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Abbreviations

AMPA	Aminomethyl phosphoric acid (the main glyphosate metabolite)
CTNBio	National Biosafety Technical Commission
DNA	Deoxyribonucleic acid
DT_{50}	Time it takes for a chemical to degrade by 50 percent
EIQ	Environmental Impact Quotient
GAP	Good agricultural practices
GHG	Greenhouse gases
GM	Genetically modified
GMO	Genetically modified organism
GR	Glyphosate resistant
GT	Glyphosate tolerant
HGT	Horizontal gene transfer
HR	Herbicide resistant
HT	Herbicide tolerant
IPM	Integrated pest management
Intacta RR2 Pro	Intacta™ Roundup Ready™ 2Pro genetically modified soybean
mRNA	Messenger ribo deoxyribonucleic acid
NBAB	Norwegian Biotechnology Advisory Board
POE-15	Polyethoxylated tallowamine
POEA	Polyethoxylated tallowamine
RR	Roundup Ready
RR1	First generation (single trait) Roundup Ready® tolerant crop
RR2	Second generation (stacked trait) Roundup Ready® tolerant crop
USDA	United States Department of Agriculture
WHO	World Health Organization

Preface

Background

The Norwegian Biotechnology Advisory Board (NBAB) was commissioned by the Norwegian Environment Agency to develop a guidance document, in line with the Norwegian Gene Technology Act, to assess the sustainability of genetically modified (GM) herbicide-tolerant (HT) plants, having as main intended audience the administrative staff supporting the processes of decision-making on the biosafety of genetically modified organisms. For this purpose, the project "Herbicide-resistant genetically modified plants and sustainability" was carried out from 2012 to 2013, which was a continuation of previous work done also by the NBAB on the operationalization of relevant concepts embraced by the Norwegian biosafety framework, such as sustainable development, societal benefits, and ethical considerations. The main achievement of the project was the identification of a set of parameters that are instrumental in establishing whether GM HT plants contribute to sustainable development. For further details on the project and its outcomes, see NBAB (2014). That set of parameters was translated into guiding questions to put into practical terms the information needed to evaluate the ecological and socio-economic sustainability of GM HT plants. The report presented here is based on those guiding questions as framed by the NBAB.

Objective and scope

The main goal of this report is to contribute to the operationalization of the concepts of sustainable development, societal benefits and ethical justifiability upon which the Norwegian Gene Technology Act and its central provisions are based. With this aim, this report compiles information relevant for evaluating the sustainability of GM HT plants based on the guiding questions identified by the NBAB, as mentioned earlier. This compilation is limited to the sets of questions that should be answered by applicants requesting approval of GM plants in Norway. However, in this report, the answers are not restricted to the information provided by the applicant; it also includes findings reported by other researchers and sources available in the literature. As such, this document aspires to provide decision-makers with comprehensive insights for analyzing the sustainability of GM HT crops.

In order to make the compiled information relevant to current biosafety assessments, this report focuses on Intacta™ Roundup Ready™2Pro (Intacta RR2 Pro) soybean cultivation in Brazil, upon suggestion from the Norwegian Environment Agency. Intacta RR2 Pro is a GM stacked crop tolerant to the herbicide glyphosate and resistant to Lepidopteran insects. Since the guiding questions framing this report are on sustainability of HT plants, the answers provided are limited to the GM HT trait only.

As indicated above, the document´s main goal is the consolidation of relevant existing information for answering the specific set of pre-determined guiding questions by the NBAB.

Accordingly, it is mainly a descriptive and not a normative analysis document on the information gathered.

Organization of the report

The main body of this report consists of the answers to the guiding questions provided by the NBAB in the document "Herbicide-resistant genetically modified plants and sustainability" (NBAB 2014) (see the whole set of guiding questions in the Annex).

The report presented here is organized into two main sections:

- Environmental sustainability and ecology, which covers the GM HT plant and the herbicide for which it presents tolerance; and
- Social and economic sustainability.

A set of related guiding questions divides each section in different topic-specific units. Each section includes a summary of the information reported from the applicant and the findings from other sources. As indicated earlier, the complementary information from different researches has the purpose of providing insights for a comprehensive and comparative biosafety analysis.

Sources of information

The main sources of information for answering the guiding questions of the NBAB are:

- *The dossier on Intacta™ Roundup Ready™2Pro presented by the applicant*. This document, entitled "Environmental and Food Biosafety of MON 87701 and MON 89788", was presented by *Monsanto do Brasil Ltda*. to the Brazilian National Biosafety Technical Commission (CTNBio, according to its name in Portuguese) in 2009. This dossier was accessed under the Federal Brazilian Law No. 12.527 of November 11, 2011, which mandates transparent management of the information relevant to the public (except information declared as strictly confidential), ensuring its availability to the citizens (National Congress of Brazil 2011). This access to information is via an online citizens' service available at www.acessoainformacao.gov.br.

  The relevant literature consulted, mostly scientific peer-reviewed articles and book chapters, was retrieved via the multidisciplinary academic searcher Scopus (www.scopus.com), and to a lesser extent from Google Scholar (www.scholar.google.com). The literature search was carried out using key words corresponding to the topics of each set of guiding questions. When no information on Intacta™ Roundup Ready™2Pro or equivalent stacked GM soybean varieties was found, studies on single-stacked GM HT soybean (i.e. RR1, genetic trait 40-3-2) were used. When no specific research on Brazil was found, the geographically closest cases, or other relevant studies on GM HT soybean, were consulted.

- *Field observations and empirical experiences gathered by the author*. The full extent of on-field and real-time performance of GM HT plants is still not documented by the scholarly research. The author's experiences are included in some sections of the report, indicating whether they were obtained via field observations or direct communication with local actors.

In the majority of the cases, the comparative description of impacts and considerations reported here relate to GM HT crops and the corresponding conventional production system. This is due to the lack of information on other possible comparators, such as integrated pest management, organic or agroecological farming systems.

I Introduction

1.1 *The regulatory context*

The Norwegian Gene Technology Act of 1993 regulates the production and use of genetically modified organisms (GMOs). It is rooted in the notion that potential approvals can be granted in Norway if the GMO in question is demonstrated not to have detrimental effects to the environment or human health, and that the production or use of the GMO is "in accordance with the principle of sustainable development", and is socially and ethically justifiable (*Act of 2 April 1993 No. 38*).

The Norwegian biosafety framework's requirements make it a unique regulatory instrument with a broad approach towards sustainability, welfare and ethical issues, as opposed to the common limited risk-assessment evaluations of most biosafety regulations, particularly with regard to socio-economic issues (Spöek 2010).

The operationalization of sustainable development, societal benefits and ethical justifiability under the Norwegian biosafety regulation has posed a number of challenges due to: (i) the multiple and intertwined dimensions its components refer to, reflecting the complex realities in which GMOs are to be introduced; (ii) the differing perspectives that assessments, in this case of GMOs, are commonly based on parameter-specific risk assessment and not on comprehensive technology appraisals (Wynne 1975), and (iii) the fact that the Norwegian authorities had essentially never received the necessary information from the applicants in order to test comprehensively its biosafety regulation.

As indicated previously, the guiding questions identified by the NBAB for GM HT plants and the answers provided in this report aim at contributing to the operationalization of the keystone concepts of the Norwegian Gene Technology Act.

1.2 *Key conceptual terms*

1.2.1 Sustainable development

For the Norwegian regulatory framework, sustainable development is interpreted according to the World Commission on Environment and Development's (WCED) 1987 definition, which refers to the possibility of people to satisfy their needs without compromising the needs fulfillment of future generations (WCED 1987). Therefore, it involves a generational responsibility for preserving the ecological and social basis for welfare in the long-term. The sustainability approach is applied both to the Norwegian biosafety regulation, and also to the guiding questions for appraising the sustainability of GM HT crops. The latter is based on three analytical pillars: environmental/ecological, social and economic.

Some have criticized this definition of sustainable development and the focus on three inter-related dimensions (see, for instance: Dovers and Handmer 1993; and Lele 1991). However, the notion of sustainable development has contributed to the recognition of the intertwined relations between environmental and socio-economic dynamics; the need for long-term approaches; and the relevance of appraising ethical aspects of the Nature-Society interactions (Euston and Gibson 1991; Dovers and Handmer 1993; Lele 1991).

1.2.2 Societal benefits

In the framework provided by the Norwegian Gene Technology Act, societal benefits refer to public and community utility and welfare considerations regarding the adoption of a GMO. In the case of GM plants, these aspects refer to concrete production-related issues (such as access to seeds, or changes in income), as well as large-scale social considerations (differences in global agricultural structures and farming systems, the equity between the Global North and South, etc.). Other aspects considered are the social acceptability, need and demand for the GMO; the kind of agricultural, ecological and social problems it intends to solve; and the problems that may arise from its adoption, use and consumption (*Act of 2 April 1993 No. 38*; NBAB 2009; Rosendal 2008).

1.2.3 Ethically justifiable

The Norwegian Gene Technology Act is explicit in its aim of ensuring that the production and use of GMOs should take place in an ethically justifiable manner (*Act of 2 April 1993 No. 38*). The question of ethics is approached from three different angles: (i) the sustainable development perspective, which is considered an intrinsic moral value of respect and care for current and future generations of living organisms, including humans (Euston and Gibson 1991), (ii) the social benefits approach that comprises justice and the pursuit of the common good, and (iii) the situational reasoning and implementation analysis in terms of the alternatives, parties involved (also considering their norms, conflicts, advantages and disadvantages), and conflicts among intrinsic values (e.g. the intrinsic value of Nature) and other fundamental principles (e.g. solidarity and equity) (NBAB 2009).

1.2.4 Precautionary principle

Under the Norwegian biosafety legislation, the precautionary principle applies when there is scientific uncertainty and reasonable doubt concerning the absence of harm or adverse effects with the adoption and use of a GMO. According to Norwegian regulation, the precautionary principle is entrenched in the sustainable development approach (NBAB 2009).

The use of the precautionary principle was a central issue during the public hearing related to the Norwegian Gene Technology Act before its final completion and entering into force in 1993. Most institutions, organizations and the Ministry of Environment were clearly in favor of a rather strict use of the principle regarding the environmental introduction and use of GMOs (Ministry of Environment 1993).

1.3 GM HT soybean production in Brazil

Soybean cultivation in Brazil started in 1882 with seeds introduced from the United States to Bahia. In 1914, the first commercial statistics of this crop were recorded; and in the 1940s, Brazilian soybean became part of international trade. In the 1970s, production increased due to technological changes—mainly the use of new varieties better adapted to southern latitudes—concentrating the production in the South of Brazil. In the following decades, soybean expansion occurred primarily in the Central-West of the country, and by the 1990s, soybean production in this region surpassed production in the South. In the past few decades, the new regions of soybean expansion have been the North and Northeast, entering into the Cerrado and Amazon regions (Bonato and Bonato 1987; Schlesinger 2006; Salin 2013).

Globally, approximately 50 percent of soybean production is used for edible oil production. The second most important use is animal feed and the food industry (refined oil and soy lecithin being the main products). Approximately 34.5 percent of global soybean production is exported to China, the European Union, Japan and Mexico, the major importers. In Brazil, the majority of soybean production is destined for international markets, mainly China (74 percent of the total production in the first half 2014) and the European Union (Salin 2013; Salin 2014).

GM soybean production in Brazil was given preliminary authorization in 2003. The final approval was issued in March 2005 (National Congress of Brazil 2005). Since its authorization, GM soybean production has expanded quickly at the expense of other crops and forests (Catacora-Vargas *et al*. 2012). By 2012, 80 percent of the total production of soybean in Brazil was GM (ABRANGE 2012). Intacta™ Roundup Ready™2Pro (Intacta RR2 Pro) was approved in the country in 2010 via a Technical Opinion issued by the CTNBio. The approval was given for open-field production, human consumption and animal feed (CIB 2014).

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1.4 *Soybean imports to Norway*

According to the statistics of the Food and Agriculture Organization (FAO) of the United Nations, during the last decades the imports of soybean to Norway increased significantly, from approximately 220 thousand tons in 1994 to about 420 thousand tons in 2011 (FAOSTAT 2014a), with an average range in the last ten years of 350 to 400 thousand tons. The majority of the soybean imports to Norway are destined for animal feed (livestock production and fish farming) (Garberg *et al*. 2014).

Based on Norwegian regulations and market preferences, soybean imported to Norway needs to comply with sustainability and corporate responsibility standards, and be of non-GM production (Garberg et al. 2014; Norwegian Ministry of Foreign Affairs 2009). Brazil is considered a strategic commercial partner to Norway (Norwegian Ministry of Foreign Affairs 2011), and is the only country in the South American region producing a significant amount of non-GM soybean. However, the share of this type of soybean within Brazil is decreasing, from 29 percent of the national soybean production in 2010 to 20 percent in 2012 (ABRANGE 2012; Catacora-Vargas *et al*. 2012).

The Brazilian State of Mato Grosso is an important non-GM soybean provider in the country, and the largest source of non-GM soybean introduced to Norway. In 2013, 71 percent of the soybean imports by the major Norwegian soybean trader, Denofa, originated from this state. An important soybean production area in Mato Grosso concentrates in the Cerrado region, a savannah ecosystem between the Pantanal (wetlands) and the Amazon rainforest (Irauí Cremaq 2010).

Hogan (2014) indicates that an increase of non-GM soybean of up to 50% of the total harvest in the State of Mato Grosso is enough to fulfill the European demand. However, such a percentage may be unattainable since the share of GM soybean is rising due its expansion in cultivation area (Catacora-Vargas *et al.* 2012).

1.5 *Intacta™ Roundup Ready™2 Pro*

Intacta RR2 Pro soybean is a GM HT variety developed by the company Monsanto. It results from the cross breeding of two GM parental lines, one expressing tolerance to the herbicide glyphosate and the other a resistance to Lepidopteran insects (Berger and Braga 2009). See section 2.1.1 ("Characterization of the genetically modified (GM) herbicide tolerant (HT) plant") for further details.

Brazil was the first country to approve Intacta RR2 Pro soybean for cultivation in 2010. Only three other countries authorized it for this purpose: Argentina and Uruguay in 2012, and Paraguay in 2013. Hence, nowadays the production of Intacta RR2 Pro concentrates in the South American region. As of September 2014, this GM HT soybean was not approved in the United States, the home country of the biotech-company that developed it. Another seven countries authorized Intacta RR2 Pro for direct use as food and/or feed only, among them the major Brazilian soybean markets, the European Union (in 2012) and China (in 2013) (ISAAA 2014).

The introduction of Intacta RR2 Pro soybean into the Brazilian market took place despite disputes among farmers and trading firms with Monsanto due to the considerable increase in royalty fees (Rural BR 2013) (see section 3.4.4 "Ownership rights" for details). This explains its low adoption (4 percent) in the inaugural season in 2013/2014. However, it is expected that in 2014/2015 the adoption of Intacta RR2 Pro will reach 25 percent of the total Brazilian soybean area (Bonato and Stauffer 2014).

II Environmental Sustainability and Ecology

2.1 *The genetically modified plant*

Guiding questions:

a. Has the GM HT crop been thoroughly genotyped and/or phenotyped?
b. Are the genome, gene expression and properties of the GM HT crop stable over time and through several generations?
c. Is the GM HT crop substantially equivalent to the unmodified parent plant with the exception of the inserted gene and the protein it expresses, and does the answer apply irrespective of cultivation site and conditions?
d. Is the GM HT crop tolerant to more than one herbicide?
e. Does the GM HT crop have a gene for resistance to antibiotics?

Summary:

- The ***genotypic and phenotypic characterization*** of the modified plant *Glycine max* (L) Merrill is well documented. The GM HT soybean Intacta^{TM} Roundup Ready^{TM}2Pro results from the crossbreeding of two GM parental lines: MON 89788 expressing the gene *cp4 epsps* that defines tolerance to the herbicide glyphosate, and MON 87701 expressing the gene *cry1Ac* determining resistance to insects.

- The ***stability of the genome, genetic expression and properties*** was evaluated by the applicant. The evaluation reports stable integration of the gene expression cassettes through five breeding generations. Protein expression was evaluated only in two seasons, showing different levels of concentration in different plant tissues. The studies presented are based on limited criteria and a short-term appraisal. Moreover, data from field trials in different ecosystems are presented as aggregated values. Finally, the stability issues related to the promoter used in one of the GM parental lines (MON 89788) are not addressed.

- ***Substantial equivalence*** is assessed through compositional comparative analysis of 64 components. Among other research limitations, the control comparators are unspecified. The applicant concludes that substantial equivalence exists among the varieties compared. However, current consulted literature concludes that there is a statistically different composition between different soybean varieties and cultivation systems (implying no substantial equivalence).

- Intacta^{TM} Roundup Ready^{TM}2Pro is made ***tolerant to the herbicide glyphosate*** (besides resistant to Lepidopteran insects).

- The applicant reports no use of ***antibiotic resistance marker genes***. However, the applicant and another study (see reference below) indicate the presence of *aadA* genes (conferring resistance to spectinomycin and streptomycin) during the cloning process to generate the plasmid vectors used in the genetic transformation of MON 89788 and MON 87701. Southern blot analyses were performed to confirm the absence of such antibiotic-resistance encoding genes in the final event; however, such tests have important limitations in detecting the actual transfer of genetic material into the resulting GM plant genome.

2.1.1 Characterization of the genetically modified herbicide tolerant plant

Genotypic and phenotypic characterization

The GM soybean Intacta RR2 Pro was developed to control a broad spectrum of weeds and Lepidopteran insects in soybean production. Genotypically, the modified plant is *Glycine max* (L) Merrill, a tetraploid diplodized (2n=40) from the leguminous family. Further information on the chromosomic and genomic characteristics of the modified plant is provided by Gurley *et al.* (1979) and Windels *et al.* (2001).

The GM variety results from the crossbreeding of the following two GM soybean parental lines:

- ***MON 89788*** that contains the gene *cp4 epsps* (originally from *Agrobacterium* sp. cepa CP4) to synthesize the protein CP4 EPSPS that defines tolerance to the herbicide glyphosate. In the development of MON 89788, the new gene was inserted in the genome of the Asgrow Seed Company cultivar A3244 through *Agrobacterium tumefaciens* mediation (directly into the plant meristem) using the plasmid PV-GMGOX200 as the transformation vector and FMV (Figwort Mosaic Virus) as the promoter.

- ***MON 87701*** that includes the gene *cry1Ac* (originally from *Bacillus thuringiensis* var. *kurstaki*) that determines Lepidopteran insect resistance. This gene was initially inserted in the genome of the Asgrow Seed Company soybean cultivar A5547 maturation group V directly to meristem cells through *Agrobacterium tumefaciens* mediated transformation using the plasmid PV-GMIR9 with two T-DNAs.

In the development of Intacta RR2 Pro, the two T-DNAs from the plasmid PV-GMIR9 (used in the production of MON 87701) serve to introduce two expression cassettes in independent loci: the expression cassette for the *cry1Ac* gene in T-DNA I and the expression cassette of the *cp4 epsps* gene in T-DNA II. After crossbreeding the parental lines, the plants identified as containing both genes were segregated for classical genetic improvement (Berger and Braga 2009).

Among the phenotypic characteristics, Intacta RR2 Pro inherited the main features of the A5547 cultivar, which are: (i) resistance to stem canker (*Diaporthe phaseolorum* var. *caulivora*) and cyst nematode (*Heterodera glycines),* (ii) non-specific specific resistance to phytophthora rot (*Phytophthora megasperma* var. *sojae*), (iii) susceptibility to sudden death syndrome, (iv) high yield potential in environments similar to the Mid-South and East Coast of the United States, and (v) good competition against weeds (Rhodes 1997). As for cultivar A3244, no specific information on its particular characteristics was found in the consulted literature.

The applicant included the following phenotypic field test results for Intacta RR2 Pro: (i) growth stages, (ii) initial stand, (iii) emergence, (iv) vigor, (v) flowering of 50 percent of the plants, (vi) color of the flowers, (vii) height, (viii) color of pubescence, (ix) lodging, (x) dehiscence of the pods, (xi) final stand, (xii) grain yield and weight, and (xiii) number of nodules and nodules dry weight. These phenotypic and agronomic characteristics were evaluated during two growing seasons (2007/2008 and 2008/2009) in four different locations in Brazil: Sorriso in Mato Grosso; Cachoeira Dourada in Minas Gerais; Rolândia in Paraná; and Não-Me-Toque in Rio Grande do Sul. The phenotypic evaluation involved a comparative assessment using the GM soybean in question; the parental lines (MON89788 and MON87701); and a control (a variety unspecified by the applicant).

The use of GM parental lines only, instead of, or in combination with, the conventional ones (A3244 and A5547) contradicts the standard guidelines for selecting the right comparator, as established by the European Food Safety Authority (EFSA) (EFSA Panel on Genetically Modified Organisms (GMO) 2011). According to EFSA, "field trials for comparative assessment should include the GM plant containing stacked events under assessment and its conventional counterpart. In case a conventional counterpart is not available […] the conventional counterpart can be substituted, on a case-by-case basis, by another non-GM line derived from the same breeding scheme used to develop the GM plant" (EFSA Panel on Genetically Modified Organisms (GMO) 2011, p. 13).

The agronomic characteristics evaluated overlap to a certain extent with the phenotypic studies, and include: (i) initial stand count of plants, (ii) rate of seedling vigor, (iii) days to 50 percent flowering, (iv) plant height, (v) lodging rate, (vi) rate of dehiscence of pods, (vii) final stand count of plants, (viii) seed moisture, (ix) seed weight, (x) test weight, and (xi) yield. From these tests, the applicant concludes that no phenotypic or agronomic changes are expressed in Intacta RR2 Pro, and accordingly, this HT crop is unable to become a weed (further analysis on this is presented in section 2.1.3 "Gene flow", see "Seed-mediated gene flow").

The applicant determined that, based on the findings, the agronomic and phenotypic characteristics of the Intacta RR2 Pro soybean do not differ from its GM parental lines; and hence, it is not adversely impacted by the introduction of stacked traits (Berger and Braga 2009). It is important to note that this conclusion is based on restricted observations: only two consecutive growing seasons from field trial plots comprising eight lines of ten meters, of which only two were intended for phenotypic observations. Moreover, the results presented are derived from combined analysis of the experimental fields at Rolândia in the State of Paraná with those of Não-Me-Toque in the State of Rio Grande do Sul; and combined analysis of Sorriso in the State of Mato Grosso with Cachoeira Dourada in the State of Minas Gerais, according to the maturation group of soybean cultivars used at these locations. Since all of these sites belong to different agroecosystems, such combined data analysis is inappropriate. Finally, as indicated before, the comparison of the agronomic and phenotypic characteristics of Intacta RR2 Pro with its GM parental lines, instead of the available non-GM cultivars (A3244 and A5547), makes the evaluation unsuitable under the

EFSA guidelines. This is because using only GM plants will not be possible to identify intended and unintended differences between the GM and non-GM varieties (EFSA Panel on Genetically Modified Organisms (GMO) 2011).

Additional field experiments were conducted in 2008/2009 at three locations: Sorriso (Mato Grosso), Santa Elena de Goiás (Goiás) and Morrinhos (Goiás). These field experiments evaluated yield; ripening; plant height; and oil and protein content. In these field trials, the control used was not specified.

Soybeans
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Stability of the genome, genetic expression and properties

The applicant reports stable integration of the gene expression cassettes (*cry1Ac* and *cp4 epsps*) in the soybean genome based on the DNA characterization of soybean Intacta RR2 Pro (MON 87701 x MON 89788). The stability of the dominant *cry1Ac* gene was evaluated along five different breeding generations, as was stability of the inheritance of resistance to insects, both assessed using Mendelian inheritance as model. The expression of the proteins Cry1Ac and CP4 EPSPS was tested through ELISA (enzyme-linked immunosorbent assay) in tissues of Intacta RR2 Pro soybean, and the corresponding GM parental lines in Brazil and the United States (Berger and Braga 2009). Overall, the genetic stability was evaluated based on limited criteria and in the short term, using mostly the GM single trait parental lines instead of the new stacked event compared to conventional counterparts (e.g. A3244 and A5547), or near isogenic lines.

The expression of Cry1Ac varies in different plant tissues, according to what is reported by the applicant from studies for two harvest seasons (2007/2008 and 2008/2009) in four test locations (Sorriso in Mato Grosso, Cachoeira Dourada in Minas Gerais, Rolândia in Paraná, and Não-Me-Toque in Rio Grande do Sul). Although the tests were conducted at four sites, there is no specification of the results at each site. The applicant reported only aggregated protein concentration values in the grains, which ranges from 3 - 9 μg/g on a dry matter basis, and from 120 - 390 μg/g in the leaves. As for the expression of CP4 EPSPS, it ranges from 57 - 140 μg/g in grains and from 170 - 330 μg/g in leaves on dry matter basis.

An important issue not addressed by the applicant in relation to the genetic expression and properties of Intacta RR2 Pro soybean is the safety and stability in GM plants developed with promoters from the genus of the Cauliflower Mosaic Virus 35S (P35S), such as the Figwort Mosaic Virus (FMV) promoter. This promoter regulates the *cp4 epsps* gene from MON 89778. Podevin and Jardin (2012) state that P35S variants contain open reading frames that could lead to “unintended” phenotypic changes when expressed.

Substantial equivalence

Comparative compositional analysis was performed between Intacta RR2 Pro (MON 87701 x MON 89788), *Bacillus thuringiensis* (Bt) soybean (MON 87701), Roundup Ready® (RR) soybean (MON 89788), an unspecified conventional comparator, and locally produced commercial varieties. Sixty-four different components were analyzed, of which 53 presented values higher than the minimum quantification limit and were included in the statistical analysis. The applicant concludes that the evaluated soybean varieties are substantially equivalent in composition (Berger and Braga 2009). However, based on what is reported, this study presents the following limitations: (i) it does not include the conventional cultivars used for developing the GM parental lines of Intacta RR2 Pro, (ii) it is based on field trials and not on real agricultural production conditions (including pesticides applications), which may impact the compositional properties due to the management practices (Bøhn *et al*., 2014), (iii) it does not include a chemical analysis of the herbicides related to the genetic modification, and (iv) it does not take into account the characteristics and expression of the genes inserted; of the genetic modification components; and of the secondary effects, as indicated by the OECD (OECD 1993). The common exclusion of the latter is an important limitation of studies rooted in the substantial equivalence concept (Millstone *et al*. 1999).

Contrary to the applicant´s report, a recent comparative study on the compositional characterization of commercially produced GM soybean tolerant to glyphosate, conventional non-GM and organic soybean production, shows significant differences. In contrast to the conventional and GM soybean, the organic soybean had higher concentrations of sugars (glucose, fructose, sucrose and maltose), total protein and zinc; and less total saturated fat, omega-6 fatty acids and fiber (Bøhn *et al*., 2014). These findings contradict an earlier study by Harrigan *et al.* (2007), whose field trials conclude no compositional difference between GM soybean tolerant to glyphosate and its conventional counterpart. Bøhn *et al*. (2014) also found high residues of glyphosate and AMPA (aminomethyl phosphoric acid, the main glyphosate metabolite) in GM soybean ready for sale. These results point out that the compositional profile of GM soybeans may not be substantially equivalent to non-GM counterparts, and will be affected by the agricultural production system and cultivation conditions. Other changes in the metabolism and chemical composition of HT plants due to agroecological conditions are described in section 2.1.2 ("Interaction between the HT plant and the environment").

Herbicide resistance

Intacta RR2 Pro is tolerant to the herbicide glyphosate, as explained in section 2.2 ("The herbicide").

Presence of antibiotic resistance marker genes

Genes conferring resistance to antibiotics used as markers in the development of GM plants have raised concerns over their possible adverse effects on human and animal health, as well as risks to the environment (e.g. antibiotic resistance transferred to bacteria). The applicant indicates no use of antibiotic resistance marker genes, but only the use of the trait of herbicide tolerance and, accordingly, the presence of the gene cp4 epsps as a molecular marker. However, the applicant and another risk assessment analysis (Bøhn et al. 2010) mention that in the cloning process for the plasmid vectors used in the transformation of the Intacta RR2 Pro parental lines MON 89788 (encoding the protein CP4 EPSPS for glyphosate tolerance) and MON 87701 (encoding the protein Cry1Ac for Lepidopteran resistance) the bacterial gene marker aadA was used. This gene confers resistance to the antibiotics spectinomycin and streptomycin. The applicant indicates that since this and other genetic elements are outside the border regions of the plasmid used for the genetic modification, it is unlikely that antibiotic resistance genes would have been transferred to the Intacta RR2 Pro soybean genome. However, the literature indicates that although the frequency of transfer of genetic material outside the border regions of the plasmid used is lower than that of the sequences within the border regions, gene transfer may not only take place but also may induce changes in the genome sequence of the new organism. This is a consequence of the re-arrangements and mutations resulting from the interaction with the genetic material outside the border regions, e.g. the marker gene sequences, among other DNA fragments different than the desired genetic construct (Collonnier et al. 2004; Wilson et al. 2006; Windels et al. 2001).

Southern blot analyses were performed by the applicant on the Intacta RR2 Pro parental lines to confirm absence of *aadA*. However, Southern blot tests detect large-sized genetic material only, and have strong limitations for confirming absence or presence of fragmented small elements. Accordingly, they are insufficient to accurately establish the transfer of genetic material and its incorporation into and the resulting changes in the GM plant genome. Therefore, other strategies such as genome walking sequencing or next generation genetic sequencing are needed for adequate detection of the presence of antibiotic resistance proteins (Kohli et al. 2003; Wilson et al. 2004).

2.1.2 Interaction between the GM HT plant and the environment

Guiding questions:

a. Is the environment, i.e. the ecological conditions in the cultivation area, thoroughly characterized and explained?
b. Do the GM HT plant's genome, gene expression or properties change when the plant is cultivated in different places?
c. Might the metabolism, chemical composition and/or nutritional value of the GM HT plant change because of the ecological conditions in the cultivation area?
d. Might the effects of the GM HT plant on the environment or on its interaction with the environment vary, depending on conditions in the cultivation area or the surrounding area?

Summary:

- The ***characterization of the environmental and ecological conditions*** of the field trials and expected places of cultivation of Intacta™ Roundup Ready™2Pro in Brazil is not presented by the applicant.

- ***Changes in the plant genome,* cp4 epsps *gene expression or properties of the GM HT plant in relation to the place of cultivation*** are not addressed by the applicant. The evaluation includes expression levels of the protein Cry1Ac only, showing aggregated data from four field trials located at different sites. The consulted literature does not report on Intacta™ Roundup Ready™2Pro in this regard. However, it presents the results of proteomic studies of RR soybean in which significant protein expression differences have been found, particularly in relation to increased oxidative stress-related proteins in GM HT varieties.

- The ***effects of the ecological conditions at the cultivation site on the metabolism, chemical composition and/or nutritional value*** are addressed partially by the applicant through phenotypical development, chemical composition and nutritional value tests at different sites in Brazil. The applicant reports no differences in the expression of the characteristics indicated; hence, no effects linked to the ecological conditions in the cultivation area. Other studies from the literature report related effects of the technological package inherent to RR soybean, indicating the following changes: photosynthetic capacity and decrease in biomass production of Intacta™ Roundup Ready™2Pro soybean in relation to RR varieties; and decrease of the two of them in relation to conventional varieties. Other studies (see references below) conclude on differences in compositional content (in terms of nutrients and herbicides residues) between GM HT and non-GM HT soybeans.

- The ***effects of the HT plant on the environment or in its interaction with the environment depending on the conditions of the cultivated area*** is assessed by the applicant in relation to the activity of insect pests, mites and aphids in fields managed with practices rarely applied in GM HT crop cultivation (such as integrated pest management and other good agricultural practices). The applicant also evaluated the nitrogen fixation capacity of Intacta™ Roundup Ready™2Pro. From both studies, the applicant concludes that there are no differences between GM HT and non-GM HT soybean varieties. However, other studies on RR soybean indicate a decrease in nitrogenase activity and nodulation when compared to conventional varieties.

Characterization of the environmental and ecological conditions

The applicant does not present any environmental characterization of the places where field trials of Intacta RR2 Pro were conducted, nor any characteristics of the expected sites of cultivation in Brazil. The environmental characterization of the trial locations in the United States, where this GM HT variety was developed, is not presented either. The applicant only indicates the places of the corresponding field trials.[1] Basic information on the eco-regions in which they are located is described in section 3.3.3 ("Agronomic factors").

Changes of the plant genome, gene expression or properties in relation to the place of cultivation

The applicant does not address possible changes of *cp4 epsps* gene expression (pleiotropic effects) or related properties of Intacta RR2 Pro. The evaluation focuses on different

[1] Sorriso in Mato Grosso; Cachoeira Dourada in Minas Gerais; Rolândia in Paraná; and Não-Me-Toque in Rio Grande do Sul.

expression levels of the protein Cry1Ac showing only the data of the four different field trial sites (see footnote 1) presented as aggregated values. As in the case of agronomic and phenotypic studies, the aggregation of protein expression data from field sites located in different agroecosystems is not appropriate. This because possible gene expression differences resulting from the interaction of the GM plant with distinctive agroecosystems may be masked through data aggregation. Despite this, the applicant concludes that no variations on the gene expression of the GM HT soybean exist (Berger and Braga 2009).

No specific studies on Intacta RR2 Pro proteomics or further analysis of its gene expression, or properties in relation to the sites of cultivation, were found. The closest analyses are proteomic studies of other RR soybeans. For instance, a proteomic analysis of RR soybean seeds by Brandão *et al.* (2010), found a significant difference in the expression of 10 percent of the proteins analyzed, showing at least a 90 percent variation in comparison to the conventional soybean. Barbosa *et al*. (2012) also studied the proteomic differences of RR soybean seeds and found differences in the expression of stress-related proteins and enzymes when compared to non-GM varieties. They found higher production of compounds associated with lipid peroxidation and oxidative stress (i.e. malondialdehyde, MDA) in RR soybean, as well as higher activity of APX, GR and CAT enzymes related to the antioxidant defense systems against toxic oxygen intermediaries. The authors mention that the correlation between the expressed proteins and the enzyme activity is indicative of a higher level of oxidative stress in the RR soybean seeds, even in the absence of herbicides. Thus, they conclude that the inserted gene responsible for glyphosate tolerance (*cp4 epsps*) by itself is a seed stress factor, provoking changes in the activity of some enzymes as well as alterations in the proteomics of soybean seeds.

Neither the applicant, nor the literature consulted, address changes in gene expression in relation to the place of cultivation of GM HT soybeans. The study of Agapito-Tenfen *et al.* (2013) on the same issue in GM maize found differences in gene expression and protein profiles between GM insect resistant maize (MON 810) and non-GM varieties cultivated in two different sites in the Brazilian state of Santa Catarina. These differences consist of 32 proteins either present, absent, or up- or down-regulated in one of the varieties analyzed and cultivated at the different locations. The functional groups of these proteins were linked to carbohydrate and energy metabolism, genetic information processes (e.g. transcription, translation, etc.) and stress response. The largest variations were found in GM maize cultivated in different agroecosystems. With this study, the researchers demonstrated that gene and protein expression could vary between GM and non-GM varieties, and also in relation to the environmental and cultivation conditions, where GM varieties were possibly the most affected.

Effects of the ecological conditions at the cultivation sites on the metabolism, chemical composition and/or nutritional value

The applicant reports similar phenotypical development (related to plant metabolism), chemical composition and nutritional value of Intacta RR2 Pro tested at different sites in

Brazil, when compared to the GM parental lines and an unspecified conventional control. The literature does not contain information on this specific GM HT soybean.

Studies with RR soybean indicate influence on the plant nutrition from the agronomic conditions. This is a result coming from the RR soybean´s technological package, specifically concerning glyphosate applications. For instance, field research in Brazil conducted by Santos *et al.* (2007) found toxic effects on RR soybean from different commercial glyphosate-based formulations, particularly those containing isopropylamine salt (e.g. Roundup Transorb®), a common surfactant used in Roundup®. They determined that applications of glyphosate-based herbicides higher than 2,000 g/ha (common field application dosages) could alter the RR soybean plant nutritional status due to deficiencies in nitrogen, calcium, magnesium, iron and copper absorption. This is consistent with other studies also carried out in Brazil. Zobiole *et al.* (2009) reports a significant nutritional decrease (in terms of macro and micronutrients) in leaf tissues, and in photosynthetic parameters with glyphosate use, either as one or multiple sequential applications in both GM and near-isogenic HT soybean varieties. The authors also report that regardless of glyphosate applications, concentrations of macro and micronutrients in the plant shoots and biomass production were lower in RR varieties, probably due to the decrease in the photosynthetic parameters.

Follow-up research was conducted by Zobiole *et al*. (2011) on GM HT soybean, single-stacked RR varieties (denominated as RR1 by the authors) and Intacta RR2 Pro (denominated as RR2, genetic trait MON87701 x MON89788). These researchers compared the soybean cultivation performance on different soil types, finding lower nutrient accumulation, nodulation, and shoot and root biomass in plants that received higher glyphosate rates and late applications. They also report decreases in macro and micronutrients accumulation (except of nitrogen and potassium) in RR2 in comparison to RR1. In another study, the same group of researchers indicate higher nutrient content and dry matter in conventional soybean than in glyphosate-treated RR soybean (Zobiole *et al*. 2012). Complementary to previous studies, Bøhn *et al*. (2014) found different nutritional content in soybean grains from different production systems. The details of the findings are presented in section 3.1.2 (“Food safety”).

The studies described above indicate that the ecological and cultivation conditions could induce changes in the gene expression and plant characteristics, resulting at the same time in changes on the metabolism, chemical composition and/or nutritional value of the GM plant. Based on this, EFSA specifies that in comparative assessments the "consideration of management is essential since interactions between the events on biota may occur even if the products of the genetic modification themselves do not interact directly” (EFSA Panel on Genetically Modified Organisms (GMO) 2011, p. 14). Yet, the applicant did not address such crop management considerations.

Effects of the GM HT plant on the environment or on its interaction with the environment, depending on conditions in the cultivation or the surrounding area

The applicant assessed ecological interactions with Intacta RR2 Pro at four different locations in Brazil (see footnote 1) with regard to defoliating and sucking insects; insects that attack the green beans; striped mites and aphids. The experiments were managed according to integrated pest management (IPM) and good agricultural practices (GAP). No significant differences were found in pest incidences, with the exception of Lepidopteran insects (the species were not specified by the applicant). The conclusion drawn from these experiments is that there is no difference in ecological interactions between Intacta RR2 Pro, its parent lines and conventional soybean (Berger and Braga 2009). An important aspect to note in these experiments is that the IPM and GAP management applied in the field trials is rarely implemented in GM HT cultivation. Based on this, the results reported may change if re-evaluated according to real farming conditions.

The applicant also evaluated the nitrogen fixation capacity of Intacta RR2 Pro by appraising the number of nodules (nodulation) and joint weight of nodules (on dry weight basis) in the legume-rhizobium relationship and the soybean nitrogen fixing symbiotic bacteria, *Bradyrhizobium japonicum,* at field trials managed according to IPM and GAP. Measurements were taken from ten plants, and no differences in nodulation and biomass of nodule were reported (Berger and Braga 2009). Important limitations of these studies include the application of uncommon GM HT crop management (i.e. IPM and GAP) and the use of a very limited sample size. Further research would be required on this issue.

Part of the literature consulted reports differences in nodulation from mycorrhizal and rhizobial colonization among different soybean varieties (both GM and non-GM). Powell *et al.* (2007) and Zablotowicz and Reddy (2004) indicate a decrease in nitrogenase activity (related to *B. japonicum)* due to glyphosate inhibition in RR soybean. Zobiole *et al.* (2011) report a comparative decrease in nodulation in Intacta RR2 Pro soybean in relation to RR1 also as result of glyphosate applications. However, the relationship and impacts between nitrogen-fixing microorganisms and GM HT crops is not fully clear, particularly in contrast with the well-established impact of IR crops (e.g. Bt corn) in this regard (for instance by Castaldini *et al.* 2005; and Cheeke *et al.* 2012). Additional research is needed on the combinatorial effects of Intacta RR2 Pro, considering possible insertion and expression effects of the *cp4 epsps* and *cry1Ac* genes.

Further information on the effects of the GM HT plant concerning its technological package is provided in sections 2.2.4 (“Soils”), 2.2.5 (“Water”) and 2.2.6 (“Energy”).

2.1.3 Gene flow

Guiding questions:

a. Is there a risk of vertical gene transfer to other species?
b. Is there a risk of horizontal gene transfer to other species?

Summary:

- The applicant indicates no ***risk on vertical gene transfer*** based on a one-season study of cross-pollination and dissemination frequency of the *cp4 epsps* gene. The literature reviewed reports low levels of vertical gene transfer, possibility of transgene introgression and likelihood of hybridization, depending on the specific ecological conditions (i.e. simultaneous flowering and presence of adequate pollinators). Pollination may be a relevant factor of vertical gene transfer in soybeans. Studies in the United States estimate that 10 percent of soybean production depends on insect-mediated pollination, thus demonstrating its ecological and economic importance.

- The applicant reports no occurrences, detection or possibility of ***horizontal gene transfer*** from Intacta™ Roundup Ready™2Pro. This issue is still debated in the literature in terms of its occurrence, relevance and also of the methodological limitations of its assessment.

- Another important path in gene flow is ***seed-mediated gene mobility and physical commingling***. Volunteer GM HT crops and socio-economic factors (particularly among small-scale farming who depend on rented machinery) are relevant in GM soybean seed-mediated gene flow.

Risk of vertical gene transfer to non-GM plants of the same or related species

Soybean is an autogamous—meaning self-fertilizing—plant with low levels of cross-pollination. The applicant reports a rate of natural outcrossing ranging from <0.5 - 1 percent (Berger and Braga 2009). A similar rate of self-pollination is reported by Nakayama and Yamaguchi (2002).

In relation to vertical gene transfer from GM to non-GM soybean species, neither the applicant nor the literature consulted report enough empirical work on Intacta RR2 Pro. The applicant reports a one-season study on vertical gene flow carried out on RR soybean in different locations in Brazil.[2] The results include low levels of cross-pollination, resulting in a low dissemination frequency of the *cp4 epsps* gene. Other studies of unspecified GM soybean also indicate a low possibility of cross-pollination between GM and non-GM soybean varieties: a rate of 0.52 percent at one meter of separation between the two, and zero percent at a distance of ten meters (Abud *et al.* 2007). Based on these low rates of vertical gene transfer, Stewart *et al.* (2003) concludes that the possibility of transgene introgression in soybean is very low and that there is no molecular evidence of it.

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[2] These locations are: in 2005/2006 in Sao Luis Gonzaga in Rio Grande do Sul and Rondonópolis in Mato Grosso; in 2006/2007 in Ponta Grossa in Paraná and Barreiras in Bahia; in 2007/2008 in Ponta Grossa and Lodrina in Paraná, and Sorriso in Mato Grosso.

However, introgression between different soybean varieties (either cultivated to wild or GM to non-GM) may not be completely negligible. Contrary to Stewart *et al.* (2003), other researchers describe the possibility of soybean outcrossing as an evolution path of certain varieties (i.e. Hymowitz 1970), and increased likelihood of hybridization (a process that may result from consecutive back-crossings). This may take place under conditions of simultaneous flowering and the presence of adequate pollinators (e.g. honeybees and bumblebees). Hence, rates of self-pollination actually depend on ecological factors (Nakayama and Yamaguchi 2002). For instance, Morse and Calderone (2000) calculated that in the United States 10 percent of soybean production depends on insect-mediated pollination, of which honeybees are responsible for 50 percent. This level of pollination is relevant not only ecologically, specifically in terms of increased potential for vertical gene flow, but also economically. The authors estimated the value of insect pollination of soybean at approximately USD 835 million for 2003. Given the increase in soybean market prices, this value might be even higher now.

Horizontal gene transfer (HGT)

The applicant reports no occurrences or detection of HGT in soybean in general. As for Intacta RR2 Pro, the applicant indicates that the ecological conditions for DNA availability, release from the donor organism and incorporation into the recipient cell genome in a functional form are unlikely to materialize. Accordingly, the applicant concludes that there is a low possibility of HGT from, or related to, this GM HT crop.

In general, the occurrence of HGT from GM plants to other organisms is still debated. Latham and Steinbrecher (2004) analyze and report upon several studies indicating that HGT and the resulting creation of new microorganisms (e.g. viruses) from GM plants and other GMOs is inevitable, and that their consequences cannot be predicted.

Conversely, Nielsen and Townsend (2004) denote that HGT from GM plants happens at low rates and would add little risk to them. However, new GM plants with traits for the production of chemicals, pharmaceuticals and vaccines would require higher attention. The authors therefore add that the focus on HGT rates of occurrence is not relevant and that HGT research should "shift to identification of bacterial genetic composition and environmental conditions that facilitate positive selection of bacterial transformants carrying horizontally acquired transgenes" (p. 1113), among other factors. Conversely, Heinemann and Traavik (2004) indicate that the actual HGT studies ignore the fact that microbes may have an environmental impact at frequencies much lower than that estimated by risk assessments, and that the current methods of HGT frequency appraisal are insufficiently sensitive to monitor its evolution.

Seed-mediated gene flow

The mobility and physical commingling of seeds are important paths of gene flow (Heinemann 2007). It can be facilitated by natural processes (e.g. biotic vectors such as animals or abiotic vectors such as wind transportation), as well as via anthropogenic processes, as indicated below. An important factor contributing to seed-mediated gene flow is the emergence of volunteer GM HT crops, which appear in several cases due to socio-economic drivers.

Volunteer GM HT crop. According to the applicant, the possibility of Intacta RR2 Pro becoming a volunteer GM HT crop is negligible given the results obtained from analyses on vigor, germination, dormancy, morphology and viability of pollen of this GM HT variety. A similar conclusion was drawn after monitoring a one-season trial (May to September 2008) simulating the incorporation of residual Intacta RR2 Pro seeds after harvesting and counting volunteer GM HT soybean. From both experiments, the applicant indicates limited tolerance of Intacta RR2 Pro to adverse environmental conditions. The overall conclusion provided is that the introduction of GM traits does not alter the germination potential or confer competitive advantage, more invasive potential or higher fitness to this GM HT variety (Berger and Braga 2009). However, this research was conducted but for a very short period of time, and its results may need further evaluation during consecutive growing seasons as a proxy to a real farming context.

The literature consulted does not contain any other studies on Intacta RR2 Pro. However, field observations in GM soybean producing countries reveal volunteer RR soybean (Catacora-Vargas 2007). GM seed developers also acknowledge other volunteer GM HT crops (e.g. canola, corn and wheat) and recommend specific practices for their management (CropLife Canada 2012).

Although the applicant indicates that Intacta RR2 Pro soybean is unable to become a weed (see 2.1.1 "Characterization of the GM herbicide tolerant plant"), it recommends—in the case that Intacta RR2 Pro becomes a volunteer GM HT crop—that control strategies are based on the use of highly toxic herbicides (such as atrazine, dicamba, fluroxypyr and paraquat) (Berger and Braga 2009). More information on this issue is addressed in section 2.2.3 ("Resistance of other plants to the herbicide").

Socio-economic factors of gene flow. Gene flow is not restricted to naturally occurring reproductive processes, but also includes human intervention and management (Heinemann 2007). Short-term rotations; the use of machinery by multiple farmers; seed residues in transportation and storing containers; and even farmers exchanging seeds are some of the possible socio-economic factors that can influence seed-mediated gene-flow of GM crops (for the latter, see van Heerwaarden *et al.* 2012 with regard to GM maize). The commingling of GM and non-GM RR soybean has been observed particularly in small-scale production systems that rely on rented machinery for sowing and rented trucks for harvest transportation (Catacora-Vargas 2007), resulting in the increased geographical dissemination of GM seeds beyond farm borders.

2.1.4 The preservation of biological diversity

Guiding questions:

a. Might the cultivation of the GM HT plant have health effects (toxic, immunological, including allergic or anti-nutrient effects) that are acute; chronic or long-term; and/or that lead to a change in the viability, fertility and development rate of non-target organisms, i.e. wild populations of:
- mammals?
- birds?
- amphibians/reptiles?
- insects (herbivores, predators, pollinators and decomposers)
- red-listed species?
- prioritized species?

b. Have the conclusions in the previous point been drawn on the basis of exposure to:
- plant material from the GM HT plant?
- the protein expressed by the inserted gene, after extraction from tissues from the GM HT plant?
- the protein expressed by the inserted gene in the organism it is obtained from?

Summary:

- The applicant reports no adverse effects from Intacta™ Roundup Ready™2Pro on ***health, fertility and development of non-target organisms***. This conclusion is drawn from very limited field observations on this GM HT crop´s effects on insects and small invertebrates, and does not include the impacts of the co-technology: application of glyphosate. Hence, only the effect of the GM plant material is evaluated in terms of effects on non-target organisms. The literature reports both absence and materialization of negative impacts on non-target organisms from the genetic modification in GM HT crops. As for the technological package inherent to GT soybean, the literature indicates adverse effects on biodiversity at landscape and species level due to the disappearance of natural habitats around GM crop fields and the negative impacts of glyphosate on wildlife, respectively.

- The ***material used for assessing exposure*** is the plant version in field studies carried out by the applicant and other researchers in the literature. The applicant´s laboratory tests are based on the bacterial version of the protein.

Health, viability, fertility and development effects on non-target organism

Intacta RR2 Pro was developed to control: (i) a broad spectrum of weeds through the expression of the CP4 EPSPS protein to tolerate glyphosate-based herbicide applications, and (ii) economically important soybean Lepidopteran insect pests such as velvetbean caterpillar moth (*Articarsia gemmatalis*), soybean looper (*Pseudoplusia includens*) and secondary pests like soybean shoot moth (*Crocidosema aporema*) and sunflower looper (*Rachiplusia nu*) through the expression of the Cry1Ac protein.

With regard to the effects on non-target organisms, the applicant reports the results from field observations (on insects and other small invertebrates) in relation to the ingestion of Intacta RR2 Pro and Cry1Ac, and laboratory observations (on mice, bird and chicken) from Intacta RR2 Pro ingestion. Mice and chicken studies on oral toxicity were conducted for food safety and animal welfare tests (see the corresponding sections 3.1.2 "Food safety" and 3.2 "Animal welfare"). However, the applicant also reports them as part of the studies on non-target organism. Besides the latter, the tests reported by the applicant are as follows:

- Evaluation of the impacts on non-target insect organisms on two lines (line 6 and 8) of field trial plots, which were intended for the collection of non-target organisms. Arthropod abundance was analyzed in four different locations (see footnote 1) in two growing seasons (2007/2008 and 2008/2009). In this field test, Intacta RR2 Pro was compared to its GM parental lines and to unspecified conventional varieties. The applicant reports no difference among the varieties observed in terms of the abundance and distribution of insects, except in the target pests of Cry1Ac, concluding that the control of economically important insect pests is efficient. Similar tests were carried out in the United States by the applicant, concluding that there is an absence of possible adverse effects on non-target organisms (either symbiotic, predatory, pollinating, parasitic or competing) from Intacta RR2 Pro soybean.

- Field tests with MON 87701 are also reported using mice; a bird species (bobwhite quail); soil decomposers (two collembolans, *Folsomia candida* and *Xenylla grisea,* and the earthworm *Eisenia fetida*); and four beneficial insects (the honey bee *Apis millifera,* the parasitoid wasp *Pediobius foveolatus,* the spotted lady beetle *Coleomegilla maculata* and *Orius albidipennis*). The applicant reported no observed effects on these species, concluding no adverse impacts differing from conventional crops or IPM on the wide range of non-target organisms (either abundant or endangered species) due to the ingestion of Cry1Ac.

The results of the applicant's observations are very restricted in terms of area (e.g. impacts on non-target insects only in two designated lines of field trials) and in time of study (two seasons of surveillance). Additionally, the conventional counterparts of the GM parent lines are excluded. Moreover, the effects on non-target organisms from the HT inserted gene and the inherent glyphosate-based herbicide application are not addressed.

The literature retrieved does not report the effects on biological diversity due to Intacta RR2 Pro or equivalent stacked GM soybean varieties. Comparative information in relation to the HT trait relates to RR soybean, about which the information is very restricted with regard to the effects of the genetic modifications. Part of the available literature indicates no adverse effects on biodiversity from RR and GM crops. For instance, Carpenter (2011) concludes that current commercialized crops have few negative impacts on biodiversity. This conclusion derives from an analysis of peer-reviewed literature on the biodiversity impacts of GM varieties at the crop, farm and landscape levels in terms of environmental and managerial factors inherent to GM crops. Jasinski *et al*. (2003) is an example of an empirical study indicating no adverse effects on biodiversity, reporting no significant differences in the populations of green lacewing (*Chrysopa* spp.) when comparing RR and non-RR soybean. Similar conclusions are drawn by Bitzer *et al.* (2002), who mention no short-term effect on the abundance of springtail from GM HT soybean varieties and their associated weed management systems.

Another part of the literature indicates adverse effects from RR soybean on biodiversity at the landscape and species level. At the landscape scale, Bindraban *et al.* (2009) denote the disappearance of natural habitats surrounding GM fields since GM crops encourage large-scale farming, as any other industrialized agricultural system does. The consequence is the reduction of landscape complexity and functional biodiversity. This is consistent with Grau *et al.* (2005), who report on increased deforestation with the introduction and expansion of RR soybean in Argentina, and Catacora-Vargas *et al.* (2012) who show an increased area of RR soybean cultivation in relation to forest area. At the species level, most of the adverse effects of RR soybean relate to the herbicide glyphosate (see section 2.2.2 "Effects of altered spraying regime"). For example, Cuhra *et al.* (2014) describe the decrease of life cycle fitness of water flea (*Daphnia magna*) when fed with RR soybean in comparison to conventional and organic soybean feed.

Material used for assessing exposure

The field trials reported by the applicant and other researchers in the literature on non-target organisms are based on exposure to the plant material from the GM HT plant. Studies performed on digestibility were conducted with the recombinant bacterial version of the protein. However, plants and bacteria differ in their post-translational processing of proteins, which justifies the need to use the plant protein version. The concept of equivalence between these two versions of the protein is usually demonstrated by structural analysis only (e.g. molecular weight and amino acid sequence) (Raybould *et al*. 2013) and not, for instance, based on their folding and possible new or different active sites.

2.1.5 Comparison with control plants

Guiding questions:

a. Has the GM HT plant been compared with its closest genetic relative under the same ecosystem conditions?
b. Have the characterization and comparative investigations been made with GM HT plants that
  − have been sprayed with the herbicide(s) that they are modified to tolerate?
  − have been exposed to predators or other biotic or abiotic stress factors?

Summary:

- In different agronomic and environmental studies, the applicant does not distinguish among Intacta™ Roundup Ready™2Pro, its parental GM lines, conventional cultivars for developing such GM parental lines, and conventional varieties for ***comparing with the closest genetic relative***. Inappropriate arithmetical aggregation of the results data from different test sites was performed. There are also significant variations in the sites and temporal scales of research. These variations make it difficult to achieve a consistent and integral analysis and comparison of the results. To a certain extent, they also are misleading in relation to some findings reported by the applicant.

- The applicant does not indicate whether the field trials included real farming conditions for ***research under managerial and biotic/abiotic stress factors***. Some of the ecological tests are in fields managed under integrated pest management and good agricultural practices, approaches rarely applied in commercial GM HT crops cultivation.

Comparison with the closest genetic relative under the same ecosystem conditions

The comparisons described among soybean varieties are, in general terms, methodologically inconsistent. In the different agronomic and environmental studies reported, the applicant fails to distinguish among the following genetic material: (i) Intacta RR2 Pro, (ii) its GM parental lines (MON 89788 expressing the *cp4 epsps* gene and MON 87701 expressing the *cry1Ac* gene), (iii) the conventional cultivars used for developing the GM parental lines (A3244 for MON 89788 and A5547 for MON 87701), and/or (iv) conventional varieties (several times unspecified). Furthermore, in several tests the conventional cultivars are not included. Since the conventional counterparts of the GM parental lines are available, their exclusion contradicts the EFSA guidelines on selection of the comparators for biosafety research (see EFSA Panel on Genetically Modified Organisms (GMO) 2011).

Additionally, the sites of different tests vary. While the majority of the studies were carried out in four specific locations (see footnote 1), others were carried out in different places (see footnote 2). Moreover, deliberate arithmetical aggregation of the results was performed on data from different ecosystems, potentially masking the differences of each site of study. For instance, the results of the protein expression tests held in the Midwest of the country (Cachoeira Dourada in the State of Minas Gerais and Sorriso in the State of Mato Grosso) were aggregated, as were the data from the Southern Region (Não-Me-Toque in the State of Rio Grande do Sul and Rolândia in the State of Paraná). Considering that the ecological conditions of each site are significantly different, the data aggregations applied by the applicant become erroneous, particularly in light of other studies that found important gene-expression changes in GM cultivars produced in different locations within the same region (e.g. Agapito-Tenfen *et al.* 2013). Finally, some of the observations were carried out in one growing season, while others were carried out over two or three.

Overall, the choice of comparators in several tests (sometimes using the GM parental lines only, sometimes using their conventional counterparts as well); the location of the field trial; and the temporal scale of the different studies are not substantiated by the applicant. These methodological variations—in terms of the genetic material used as a comparator, as well as the geographical and temporal research scales—make it difficult to achieve a consistent and integral comparison of the different parameters appraised and the results obtained. To a certain extent, they are also misleading in relation to some of the findings reported by the applicant since specific effects may be masked by inconsistent use of comparators and data aggregations. The confusing results are particularly due to comparative tests that include GM varieties only, without the conventional counterpart as a control, potentially resulting in an absence of significant differences since the GM comparators involve similar genetic modifications and, accordingly, similar possible impacts among themselves.

Research under managerial and biotic/abiotic stress factors

The applicant does not clearly report whether the laboratory studies included plant material produced under real field conditions, or if the field trials where the plant material came from were managed according to the actual commercial production practices. Additionally, some environmental studies were carried out in test plots managed under IPM and GAP, which are uncommon in real agroindustrial commercial soybean cultivation circumstances. The biotic and abiotic stress factors, and the conditions of the controlled field trials, were also not described.

2.2 The herbicide

2.2.1 Characterization of the herbicide

Guiding question:

What are the mechanisms by which the herbicide(s) function?

Summary:

Glyphosate is a systemic, non-selective herbicide that inhibits the shikimate pathway, which is responsible for the synthesis of essential aromatic amino acids, whose absence results in plant necrosis.

The characteristic of glyphosate tolerance of Intacta RR2 Pro results from the insertion of the gene *cp4 epsps* from *Agrobacterium* sp. The general mode of action of glyphosate is the inhibition of the activity of the enzyme 5-enolpyruvyl shikimate 3-phosphate synthase (EPSP), responsible for triggering the shikimate pathway, which only takes place in plants, fungi and bacteria. The shikimate pathway is responsible for the synthesis of essential aromatic amino acids (triptophan, phenylalanine and tyrosine) and critical plant metabolites. The lack of biosynthesis of those aromatic amino acids and derived proteins (due to the blockage of the shikimate pathway) results in rapid plant necrosis. Due to the high solubility of glyphosate, it is easily up-taken and transported through the plant´s fluids; this defines its systemic property on a broad spectrum of weeds (Székács and Darvas 2012). A detailed description of the biochemistry of the glyphosate´s mode of action is found in Alibhai and Stallings (2001), Jaworski (1972) and Sprankle *et al*. (1975).

2.2.2 Effects of an altered spraying regime (change in frequency, concentration, quantity and type of herbicide)

Guiding questions:

a. The preservation of biological diversity
   i. Might the cultivation of the GM HT plant cause health effects (toxic, immunological, including

allergic or anti-nutrient effects) that are acute; chronic; late-onset or long-term and/or lead to a change in the viability, fertility and development rate of non-target organisms, i.e. wild populations of:

− mammals
− birds
− amphibians/reptiles
− insects (herbivores, predators, pollinators and decomposers)
− red-listed species
− prioritized species?

ii. Might cultivation of the GM HT crop lead to a change in the biodiversity of weeds and animals (vertebrates and invertebrates)?

iii. Might cultivation of the GM HT crop harm microflora and microfauna in the soil?

iv. Might the herbicide(s) or degradation product(s) thereof affect the growth cycle or division/proliferation of eukaryotic cells, and in such cases, how?

v. Might the herbicide(s) or degradation product(s) thereof have a hormone-mimicking or hormone-inhibiting effect?

vi. How long and in what concentrations do(es) the herbicide(s) and degradation products remain in plant tissue and different soil types?

b. Does cultivation of the GM HT crop result in a change in the timing of the herbicide application?

c. Does cultivation of the GM HT crop increase the risk of herbicide drift, and thereby also the risk that non-GM crops in surrounding areas may be unintentionally affected?

d. Does cultivation of the GM HT crop lead to an increased/decreased use of herbicide?

e. Does cultivation of the GM HT crop lead to the use of herbicides with more/less adverse effects than previously?

f. Might unexpected combinatory effects such as additive or synergistic effects occur when more than one herbicide is used in the same area?

Summary:

- The applicant does not address possible effects of glyphosate or its metabolites on the ***preservation of biological diversity***, including non-target organisms.

- Reported ***health effects on non-target organisms*** relate to direct exposure to or ecosystem changes derived from glyphosate applications (either as an active ingredient or as a commercial formulation). The literature reports adverse effects on vertebrate and invertebrate organisms exposed to a range of concentrations, from low to maximum doses, registered at field usage. These studies also indicate negative impacts from lethal and sub-lethal, as well as short and long-term, exposure. The types of adverse health effects observed in non-target organisms, including beneficial organisms, are behavioral, reproductive, developmental, morphological and physiological.

- ***Changes in biodiversity of plants and animals*** also relate to direct glyphosate exposure and derived changes in habitats and ecosystems. As for plants, intense applications of commercial formulations of glyphosate have exerted a selection pressure resulting in the emergence of GR weeds, which change the flora composition due to their competitiveness, perennial characteristics and faster dissemination in comparison to other plat species. In Brazil, seven GR weeds have been confirmed. As for animals (vertebrates and invertebrates), changes in their biodiversity is linked to the behavioral, reproductive, developmental, morphological and physiological changes triggered by glyphosate exposure, as well as ecosystem changes such as modifications in the environment´s profile of toxic substances, variations in food availability and alterations of the associated species.

- The applicant reports no adverse effects on ***soil microflora and microfauna*** from Intacta™ Roundup Ready™ 2Pro based on results from a one-season experiment, which did not consider glyphosate applications. The literature points out to different results, ranging from beneficial effects on soil microbiology, to no effects and adverse impacts. Regarding the latter, adverse effects have been observed through direct glyphosate exposure and changes in the soil ecosystem derived from the emergence of GR weeds.

- Part of the literature indicates adverse ***effects on the growth cycle or division of eukaryotic cells*** of non-target organisms, including humans, resulting from exposure to glyphosate, its metabolites (e.g. AMPA) and adjuvants present in commercial formulations. Some of the reported cellular effects from acute and chronic exposure include: biochemical changes; cellular atrophy and hypertrophy; cytotoxicity; genotoxicity; apoptosis; necrosis; and nucleus alterations (including nucleus absence).

- ***Hormone mimicking and hormone inhibiting effects*** from glyphosate and glyphosate-based commercial formulations are reported in vertebrate (including humans) and invertebrate organisms. Some of the described effects include changes in hormone-synthesis enzymes; hormone signaling and receptors; hormone imbalance; modifications in growth and reproductive development; and fecundity and fertility.

- Glyphosate and AMPA ***residues in plant tissue and soil*** vary in terms of concentration and degradation time. Residues in plants change according to the agricultural management and sampling period, while accumulation of residues and time of degradation in soils depend on agroecological and biological conditions (particularly soil microbiota) and the chemical characteristics of the commercial formulations. A general breakdown time of half of the glyphosate applied is 2 to 215 days. However, a study found glyphosate and AMPA residues in soils one year and five months after the last application. The general half-life time reported for AMPA is 60 to 240 days.

- ***Changes in the timing of the herbicide applications*** vary. In the short term, the adoption of GT crops simplifies the weed management by reducing the number of applications and types of herbicides used, thereby making the timing of the application flexible. Upon emergence of GR weeds over the medium- and long-term, the complementary herbicides used to manage them lead to more applications at specific times in order to avoid damage to the GM HT crop.

- ***Herbicide drift on non-GM crops*** and other organisms is a generalized phenomenon due to the physical characteristics of the droplets, resulting in extremely reduced efficacy to reach their target organisms (0.1 percent of the total volume applied) and a wide droplet spread (up to 800 meters). Herbicide drift rises in agroindustrial production due to the applications in extensive areas. As for GT soybean, glyphosate drift risk increases because of the expansion of the crop and, accordingly, the use of the inherent herbicide in high volumes encouraged by its decrease in price. Glyphosate drift can damage non-GT crops and plants since it is a systemic, broad-spectrum herbicide. Also, some studies indicate that it can change the population dynamic of the GM crop itself, in terms of gene flow and fitness.

- The applicant indicates a potential decrease in the ***use of herbicides*** with the adoption of Intacta^{TM} Roundup Ready^{TM}2Pro. However, no empirical data is provided. The experience and research gathered indicates that changes in herbicide use with GT crops vary over time. In the short-term at the farm level, these crops have resulted in a reduction of the volume and types of herbicides applied. However, at a large scale, the aggregate volumes of glyphosate increased due to their expansion, especially soybean. In the long term, the appearance of GR weeds eventually also increases the volume, types and toxicity of herbicides used.

- Adoption of glyphosate-tolerant soybean results in ***changes in the use of herbicides with a different profile of effects.*** During the first period of adoption, several toxic herbicides were replaced by glyphosate (category III with LD_{50} from 1,500 to 10,000 ppm), given its systemic and broad-spectrum characteristics. In the medium- and long-term, the emergence of GR weeds resulted in the use of more complementary toxic herbicides (e.g. glufosinate, dicamba, 2,4-D and paraquat with lower LD_{50}).

- ***Unexpected combinatorial and synergistic effects from multiple herbicide applications*** in Intacta^{TM} Roundup Ready^{TM}2Pro cultivation is not addressed by the applicant. The literature contains very limited information in this regard. The few examples found indicate changes adversely affecting development and reproduction as well as survival capabilities of non-target organism.

Preservation of biological diversity

The applicant does not address the effects of glyphosate, the herbicide inherent to Intacta RR2 Pro, or of its metabolites and commercial formulations on non-target biodiversity.

Generally speaking, agrochemicals such as synthetic herbicides have different levels of toxicity on non-target organisms, whether cultivated, domesticated or wild species. The exposure to these chemicals occurs through different means such as direct applications, pesticide drifts, accumulation of residues in the ecosystem and release of bound residues, usually from soils (Finizio *et al.* 1998; Scholtz and Bidleman 2006).

The following section reports on information from the literature related to glyphosate and its commercial formulations.

Health effects on non-target organisms. Although glyphosate is considered a low toxicity herbicide (WHO 2006), a growing body of literature reports adverse health effects on non-target organisms via two pathways: (i) direct exposure and (ii) ecosystem changes derived from glyphosate applications.

Some examples of studies that report on the health effects on non-target organisms derived from direct exposure to glyphosate are:

- Paganelli *et al*. (2010) carried out embryological research on the effects of low doses (1/3000 - 1/5000 dilutions) of glyphosate and glyphosate-based herbicides in the development of African clawed frogs (*Xenopus laevis*) and chicken embryos. The treated clawed frog embryos (with pure glyphosate or the commercial Roundup® Classic formulation) presented, *inter alia*, highly abnormal cephalic and neural crest development or damage, as well as a severe suppression of the primary neuron formation. Chicken embryos exhibited a reduction of the optic vesicles, and microcephaly, among other effects. The authors conclude direct impacts of glyphosate (as the active ingredient and as a commercial formulation) on the morphogenesis mechanisms in vertebrate embryos, which may also be relevant to human populations exposed to this herbicide.

- Brodeur *et al.* (2011) observed reduced body conditions and different levels of malformation and enzymatic alterations in different frog species (terrestrial fossorial *Rhinella fernandezae*, terrestrial *Leptodactylus latinasus*, semi-aquatic *Leptodactylus ocellatus* and arborescent *Hypsiboas pulchellus*) located in proximity to RR soybean fields in Argentina. The authors indicate that the cause was the sub-chronic exposure to several pesticides, among them glyphosate.

- Lanctôt *et al*. (2014) report effects on wood tadpole frogs (*Lithobates sylvaticus*), and conclude that glyphosate-based formulations (Roundup WeatherMax®) can modify the mRNA profiles, and hence alter hormonal response during metamorphosis. The authors recommend further studies to better understand this effect.

- Uren Webster *et a*l. (2014) investigate the reproductive effects of Roundup Ready® and glyphosate in zebra fish (*Danio rerio*) with a 21-day exposure to 0.01, 0.5, and 10 mg/L (glyphosate acid equivalent) compared to a control group. The highest concentration tested showed a reduction in egg production, higher early stage embryonic mortality and premature hatching. These effects were significantly different than the control from ten days of exposure, and increased along the time of observations. Ovarian abnormalities were also detected in 50 percent and 63.6 percent of females exposed to 10 mg/L of Roundup Ready® and glyphosate, respectively, compared to 9.1 percent of females with ovarian abnormalities in the control group. The authors conclude that high concentrations of glyphosate have toxic reproductive effects on zebra fish.

- Other studies on the effects of glyphosate on different fish species, such as piava (*Leporinus obtusidens*) (Glusczak *et al*. 2006) and silver catfish (*Rhamdia quelen* Teleostei) (Glusczak *et al.* 2007) indicate that glyphosate could alter the acetylcholinesterase (the enzyme that degrades the acetylcholine neurotransmitter) activity in the brain, as well as hepatic metabolic parameters, among other biochemical modifications.

Glyphosate can also lead to changes in biodiversity and in the health of non-target organisms through the modifications of their ecosystem, either by changing the profile of existing chemical residues or by modifying specific species that determine the characteristics of hosting habitats. For instance:

- Based on a literature review, Annett *et al*. (2014) report from several studies on the impacts of glyphosate on freshwater environments. They focus on cellular responses (oxidative stress, genotoxicity and acetylcholinesterase inhibition), and acute and chronic toxicity on fresh water organisms. They conclude that the increase in the use of glyphosate also results in an increased likelihood of contamination of freshwater environments, and consequently exposure to non-target organisms. Additionally, the authors indicate that surfactants used in commercial glyphosate formulations caused most of the toxic effects. However, there is no consensus on a single toxicity mechanism and it is likely that multiple pathways exist.

- Mercurio *et al*. (2014) tested coastal seawater for glyphosate and AMPA, finding moderate glyphosate persistence under light conditions and high persistence in the darkness. They indicate that limited degradation—and hence accumulation of glyphosate and AMPA residues—could be expected in seawater, where sediments containing bound glyphosate may be disseminated into wildlife and habitats of domesticated species.

- Watkinson *et al*. (2000) indicate that changes in weed populations in GM HT crops may alter the availability of important food sources, such as the plant *Chenopodium alba* for birds including the Eurasian skylark (*Alauda arvensis*). Complementary, Firbank and Forcella (2000) report as a result of glyphosate applications a decrease in creeping Thistle (*Cirsium arvense*), another bird food source.

Examples of effects on non-target invertebrate organisms are listed in the next subsection.

Changes in the biodiversity of plants and animals (vertebrates and invertebrates). Change in ***plant biodiversity*** (i.e. wild flora, called weeds) due to the cultivation of GM HT crops is broadly documented in a number of different countries (see: Duke and Powles 2009; Owen 2008; Owen and Zelaya 2005; Vila-Aiub *et al*. 2008), particularly in relation to the emergence of glyphosate-resistant (GR) weeds in glyphosate-tolerant (GT) soybean cultivation. At this point, it is important to mention that the plants called “weeds” are considered undesirable due to their interference with human activities or interests. Accordingly, the different definitions of weeds are anthropogenic. In fact, weeds are no different than other plants and are part of the global biodiversity (Pitty and Godoy 1997).

The advent of GR-weeds is inherently a change in biodiversity resulting from an evolutionary process trigged by glyphosate-related selection pressure (Vila-Aiub *et a*l. 2008). Moreover, herbicide-resistant (HR) weeds are more competitive—in some cases perennial—and expand faster than other wild flora species, directly impacting the composition of flora biodiversity (Cerdeira *et al*. 2011; Powles 2008; Vila-Aiub *et al*. 2008). In Brazil, seven GR-weeds have been confirmed (see Table 1).

Table 1. Glyphosate-resistant weeds in Brazil reported in the literature

Species	Common name	First year detected
Lolium perenne ssp. *multiflorum*	Italian Ryegrass	2003
Conyza bonariensis L. Cronquist	Hairy Fleabane	2005
Conyza canadensis L. Conquist	Horseweed	2005
Euphorbia heterophylla L.	(Mexican) fireplant	2006
Digitaria insularis L. Mez ex Ekman	Sourgrass	2008
Conyza sumatrensis Retz	Sumatran Fleabane	2010
Chloris elata Desvaux	Tall Windmill Grass	2014

Source: Heap (2014); Cerdeira et al. (2011)

Changes in the ***biodiversity of animals*** (vertebrates and invertebrates) are related to the effects of glyphosate and its commercial formulations, both on the animals' fitness for survival and on their ecosystems.

Examples of adverse effects on the survival of non-target vertebrate organisms are listed in the previous subsection. As for invertebrates, one of the few peer-reviewed articles specific to Intacta RR2 Pro was published by the developer´s research personnel affiliated with the Brazilian Federal University of Grande Dourados (UFGD), reported in Justiniano *et al*. (2014). These authors evaluate arthropods diversity, composition and population dynamics in RR1 and RR2 soybean cultivation. Based on a one-season field study, the paper concludes that RR2 soybean shelters a greater diversity of arthropods as compared to RR1 varieties. The study is limited insofar as it is restricted to short-term observations of RR soybean varieties only; no conventional counterparts or controls were included in the comparison; and the observations solely relate to insecticide applications, and accordingly, the effects of glyphosate were not tested.

In relation to the specific effects of glyphosate, part of the literature reports no adverse effects (e.g. Michalková and Pekár 2009), particularly from short-term studies (as indicated by Haughton *et al.* 2001), while a growing body of research suggests the opposite. The following are some examples of papers reporting laboratory tests that observed negative impacts of glyphosate on invertebrate organisms:

- Mirande *et al*. (2010) studied the side effects of a glyphosate-based herbicide (GlifoGlex 48®)—at the maximum concentrations registered for field use—on *Eriopis connexa* Germar, an arthropod predator of the aphid pest *Rophalosiphum padi*. The parameters evaluated were development time; weight of pupae; adult emergence; pre-oviposition period; and fecundity and fertility in females. The authors reported that the glyphosate-treated larvae molded earlier than the controls, and that there was a drastic reduction in pupae weight, longevity, fecundity and fertility. They concluded that there was an overall decrease in the reproduction capacity of *E. connexa* and deleterious effects of glyphosate on the developmental, morphological, physiological, biochemical and immunological features of this arthropod, which is considered a biological control of different aphid species attacking economically relevant crops in South America.

- Benamú *et al*. (2010) researched the sub-lethal effects of commercial formulations of glyphosate (GlifoGlex 48®) on an orb-weaver spider (*Alpaida veniliae*) abundant in soybean fields. The spiders were collected from a GM HT soybean field and exposed to glyphosate via ingestion of glyphosate-treated preys. They found no difference in mortality (direct lethal effect), but found negative sub-lethal changes expressed in: (i) web building: females exposed to glyphosate built irregular webs and started the building activities 17 days later than the control, (ii) development of ovaries and egg sacs: females exposed to glyphosate had abnormal ovaries with limited oocyte development and the ovi-position of abnormal eggs was significantly higher from them, (iii) fecundity and fertility: glyphosate showed negative effects on the number of eggs laid and hatched, and (iv) significantly slower development time of progeny—and hence, longer—in eggs laid from glyphosate-exposed females. The authors established that lethal impact evaluations are insufficient for a proper characterization of glyphosate effects on species performance and biodiversity, and sub-lethal impacts need to be assessed.

- Complementing the previous study, Griesinger et. al. (2011) characterized the effects of glyphosate on the natural chemical-mediated communication of arthropods, which is critical to their ability to interact with their environments, set a developmental trajectory, avoid predation, find food, identify social groups and assess mates. The researchers evaluated the effects of airborne exposure to glyphosate-based herbicides (Buccaneer®, registered under the Environment Protection Agency (EPA) of the United States as Roundup® II) on the ability of male wolf spider (*Pardosa milvina*) to find females. Among their results, they report that females surrounded by glyphosate formulations were slower to attract males than those surrounded by water, and in the presence of female pheromones, males were more likely to search for females in control areas than in the areas treated with glyphosate. The authors concluded that glyphosate-based herbicides appear to be chemical information disruptors affecting the ability of male wolf spiders to detect or entirely respond to female signals.

- Schneider *et a*l. (2009) carried out a lifetime study on the ecotoxicological effects of glyphosate on green lacewing (*Chrysoperla externa*), a beneficial and biological control organism in the Neotropical Region. *C. externa* was exposed to the commercial glyphosate-based formulation GlifoGlex 48® via ingestion of contaminated preys. In the short term, glyphosate did not induce any adverse effects. However, in the long-term it resulted in the following negative impacts related to development, fertility and demography: (i) significant reduction in fecundity and fertility, (ii) lengthened adult pre-reproductive period, (iii) the majority of *C. externa* glyphosate-treated eggs were abnormal, smaller and dehydrated in comparison to the control, becoming black two days after oviposition, and (iv) development of tumors among adults (more severe in females) in the abdominal region 20 days after emergence. The authors indicate that these observed effects may result in long-term detrimental effects on *C. externa* population.

Effects on soil microflora and microfauna. The applicant indicates no interference in the dynamic of soil microorganisms from Intacta RR2 Pro. This conclusion is drawn from an experiment consisting of incorporating this GM HT crop's biomass into the soil to evaluate the formation of bacteria, fungi and actinomycete colonies in four testing locations (see note 1) during the 2007/2008 growing season. In this experiment Intacta RR2 Pro was compared with its GM parental lines and an unspecified conventional control. The applicant did not describe either the management (past and current) of the soils serving as place of study nor the presence of any historical factors (e.g. previous applications of agrochemicals and for how long time) that could influence the soil microorganisms' diversity and abundance. Since the applicant restricts the analysis to the effects of the proteins related to the genetic traits inserted, it may be assumed that the impacts of the technological package of Intacta RR2 Pro were not evaluated.

The literature reports dissimilar effects of glyphosate applications on soil microflora and microfauna, as follows:

- *Beneficial impacts*. For example, Busse *et al*. (2001) found increased microbial activity from glyphosate applications in ponderosa pine plantations. The authors attribute the utilization of glyphosate as available carbon substrate as the driving cause of this process.

- *No effects on soil microbiology*. For instance, Nakatani *et al*. (2014) studied the soil microbial changes from application of commercial formulations of glyphosate on different soybean varieties (GT and near isogenic cultivars). They appraised soil microbial biomass-carbon and nitrogen availability, as well as enzyme activities at six sites in Brazil during two growing seasons. In this study, no changes in the parameters characterized were found. In another example, Weaver *et al*. (2007) compared the microbial community and mineralization activity in soils before and after glyphosate applications using soybean soil samples from a location in the United States. They reported no differences in soil microbiology; however, a possible aspect influencing these results is that the fields of study received an application of the herbicide paraquat one to four days before soybean planting in order to eliminate weeds before the field trial. The possible effects of paraquat on soil microbiology may have been a factor masking possible glyphosate effects.

- *Adverse effects* via two pathways: (i) direct impact from glyphosate exposure, and (ii) soil ecosystem change through GR weeds. For instance, in relation to direct exposure to glyphosate, Druille *et al*. (2013) evaluated the effects of glyphosate on arbuscular mycorrhizal fungi in boreal forest soil samples. They observed a decrease in spore viability and root colonization in the glyphosate-treated soil, resulting in alteration of the symbiotic functionality of these fungi, particularly in relation to nutrient exchange. Tanney and Hutchison (2010) report an inhibition of pigmentation and sporulation of soil borne micro-fungi; significant reduction in the colony diameters; and a decrease in the number of fungi colony-forming units at 1, 5 and 50 mg/L doses of glyphosate application respectively. Another study was carried out by Zobiole *et al.* (2011) consisting of greenhouse evaluations of root microbial interactions of RR1 and RR2 soybean after glyphosate applications at field production doses. They describe a drop in shoot and root biomass, as well as a reduction in the population of the following bacteria: (i) fluorescent pseudomonads in the root system, which are important multi-functional bacteria for producing different secondary metabolites in plants; (ii) manganese-reducing bacteria; and (iii) indoleacetic acid producing bacteria that function as growth enhancers. The authors conclude that glyphosate negatively impacted the interactions of soil microbial groups, their corresponding biochemical activity and, as a result, root growth. All these changes hold potentially unfavorable effects on plant growth and yield capacity.

In relation to soil ecosystem changes, Kremer (2014) analyzes the potential effects of GR weeds on the biological and ecological soil properties based on previous works on crops, weeds and invasive plants. The author concludes that the root exudates from persistent weeds, such as HT like GR *Amaranthus* and GR *Chenopodium*, exert a selective biochemical pressure on specific soil microbial groups that are beneficial to weeds, but not necessarily to crops. This can result in important changes in symbiotic and associative microbial interactions, particularly for arbuscular mycorrhizal fungi, which are important for nutrient and water uptake as well as for protection against plant pathogenic microorganisms. Accordingly, glyphosate applications may result in the selection of soil microbial groups that could play a role in nutrient immobilization, or in the emergence of opportunistic pathogens.

Effects on the growth cycle or on the division of eukaryotic cells. The literature contains information from in vivo and in vitro research, indicating adverse effects on growth cycles and on the division of eukaryotic cells from exposure to glyphosate, its metabolites (e.g. AMPA) and adjuvants present in commercial formulations (i.e. Cassault-Meyer et al. 2014; Clair et al. 2012; Gasnier et al. 2009; Mesnage et al. 2013a). The corresponding findings point out that glyphosate—as active ingredient or commercial formulation—and its metabolites are toxic to non-target cells beyond plants and other organisms (such as fungi and bacteria) that carry out the shikimate pathway, the target biochemical process inhibited by glyphosate.

The information presented in section 2.1.4 ("Preservation of the biological diversity") and section 3.1.2 ("Food safety") specifically related to glyphosate provides examples of the possible adverse effects on humans and other species linked to eukaryotic cell damage from glyphosate exposure. The following are some complementary examples.

Related to glyphosate as active ingredient and its metabolites:

- Benachour and Séralini (2009) tested—on human placenta, embryonic kidney and neonate cells—the mechanism of cellular action of glyphosate, its main metabolite AMPA and four glyphosate-based commercial formulations (Roundup®) containing the adjuvant POEA (polyethoxylated tallowamine). They found that glyphosate as an active ingredient and at commercial formulations causes cellular death. In the case of Roundup®, it produced total cellular death within 24 hours of exposure via the inhibition of mitochondrial enzymes. It also resulted in necrosis through the release of enzymes damaging the cell membrane. Moreover, Roundup® formulations induced apoptosis manifested as DNA fragmentation, nuclear shrinkage and nuclear fragmentation. The endothelial cells of the umbilical vein cord proved particularly sensitive to Roundup® exposure.

- Mañas *et al*. (2009) observed significant genotoxicity caused by AMPA, expressed in DNA damage to hepatic cells, and clastogenic effects in human lymphocytes after 4 and 48 hours of *in vitro* exposure, respectively. Clastogenic effects refer to induced mutagenesis resulting in chromosome disruption or breakage, leading at the same time to deletion, addition or rearrangement of chromosomal sections.

- Monroy *et al*. (2005) describe dose-dependent cytotoxicity and genotoxicity in normal and fibro-sarcoma human cells resulting from acute and chronic exposure to glyphosate as active ingredient. They indicate morphological DNA damage, particularly in cells exposed to high glyphosate concentrations (above DL_{50} of pure glyphosate), which significantly decreased their survival (up to 80 percent).

Related to glyphosate-based commercial formulations:

- Shiogiri *et al*. (2012) studied the effects of acute toxicity of Roundup Ready® in a Neotropical fish (*Piaractus mesopotamicus*). They observed moderate to severe liver damage, resulting in different stages of liver dysfunctions. The detected changes in hepatic cells were dose dependent, and specifically induced cytoplasmic vacuolization, lipid accumulation, glycogen depletion, cellular hypertrophy, nuclear and cellular membrane alterations deriving in cellular deformations, nucleus alterations (hypertrophy or atrophy) and absence of the nucleus.

- Mesnage *et al*. (2013) evaluated the cellular toxicity effect of one of the main adjuvants used in commercial glyphosate formulations: polyethoxylated tallowamine POE-15. They compared glyphosate as active ingredient with POE-15 and ten glyphosate-based commercial herbicides available on the market. Upon occupational concentration exposures, they concluded that POE-15 and, accordingly, glyphosate-based formulations containing this adjuvant, act as cellular membrane and mitochondrial disruptors, inducing higher rates of necrosis (cellular death caused by external-factors). In comparison, glyphosate induces more apoptosis (genetically regulated cellular death produced by several interrelated processes that result in cellular collapse).

- Kim *et al*. (2013) obtained similar findings when comparing the effects of glyphosate as active ingredient and polyethoxylated tallowamine TN-20, a common surfactant in commercial formulations. They report increased mitochondrial damage and induced apoptosis and necrosis in cells exposed to commercial formulations containing this surfactant, in comparison to pure glyphosate.

The literature also reports adverse effects on prokaryotic non-target cells exposed to glyphosate, its metabolites and its commercial formulations (Clair *et al*. 2012; Bonnet *et al*. 2007).

Hormone mimicking and hormone inhibiting effects. Different studies indicate that glyphosate and glyphosate-based formulations cause hormone-related disruption effects in vertebrate (including humans) and invertebrate organisms. Some examples in vertebrates include:

- Richard *et al*. (2005) studied human placental cells and aromatase (the enzyme synthesizing estrogen) and observed that glyphosate and its commercial versions (Roundup®) have endocrine effects at concentrations ten times lower than used in agriculture. They observed that these specific glyphosate-based formulations altered the activity of aromatase and mRNA levels, and interacted with the active site of the enzyme (in its purified version). Among other observations, the researchers describe a significant increase (40 percent) of estrogen synthesis after one hour of exposure to Roundup®, and subsequently a severe decrease at 18 hours of exposure due to a reduction in aromatize activity. Among others, they also report a two-fold decrease in the availability of placental cells when exposed to Roundup® in comparison to glyphosate-treated cells.

- Motivated by studies on the glyphosate effects on aromatase activity and serum testosterone concentrations, Romano *et al*. (2012) researched the effects of gestational maternal glyphosate exposure on the reproductive development of male mice off-spring. They observed an early onset of puberty; a disturbed masculinization process; behavioral changes; and histological and endocrine alterations in reproductive parameters.

- Howe *et al*. (2004) indicate that disruption of metamorphosis processes in frogs exposed to glyphosate-based herbicides containing POEA might be related to a disruption in hormone signaling, caused by an increase in the transcript level of a specific thyroid hormone receptor.

As for invertebrates, Mirande *et al*. (2010) found a reduced fecundity and fertility in glyphosate-treated *Eriopis connexa* (a beneficial coleopteran), among other changes. They identified hormonal disruptions by glyphosate as a possible cause.

Residues in plant tissue and soil. There is high variation in the concentration values of glyphosate, metabolites and residues, as well as their time of degradation in exposed plant material and soils.

Regarding residue concentration, Arregui *et al*. (2003) did an early assessment by measuring glyphosate and AMPA deposits in tissues of soybean cultivated in experimental plots in Argentina. The samples were taken after harvesting in three consecutive seasons (from 1997 to 1999) and showed a range of 0.3 - 5.2 mg/kg of glyphosate residues and 0.3 - 5.7 mg/kg of AMPA in leaves and stems. In grain samples, these values ranged from 0.1 - 1.8 and 0.4 - 0.9 mg/kg, respectively. In another recent residue assessment study carried out by Bøhn *et al*. (2014), a much higher concentration was found in harvested grains in the United States: 0.4 - 8.8 mg/kg (mean of 3.26) of glyphosate, and from 0.7 - 10 mg/kg (mean of 5.74) of AMPA.

Regarding the ***time of degradation in soils***, glyphosate is a compound that strongly binds to different soil types. Microbial processes are primarily responsible for degrading it into AMPA (Battaglin *et al*. 2014). Accordingly, changes in soil microbiota could significantly affect glyphosate breakdown.

Persistence of glyphosate and other chemicals is measured in terms of the degrading time for half of the substance applied, DT_{50}, which varies substantially according to the agroecological and biological soil conditions, as well as the characteristics of the commercial formulation. For instance, Battaglin *et al*. (2014) reports a general glyphosate DT_{50} of two to 215 days. Kools and Roovert (2005) indicate a DT_{50} of five to 23 days based on a study in The Netherlands. They specify the presence of heavy metal content as the main factor affecting glyphosate degradation in the studied soils. Trewavas and Leaver (2001) identified the average half-life of glyphosate as 47 days; while the European Commission reports a glyphosate DT_{50} of four to 180 days (Committee on Plant Health 2002). Other studies indicate even larger periods of glyphosate persistence in soils. This was so in the case of Simonsen *et al*. (2008), who analyzed soil samples from fields in Denmark. They extracted significant amounts of glyphosate and AMPA one year and five months after the last glyphosate application, as well as from soil samples incubated for six months. The authors mention that fertilized soils (with synthetic nitrogen, phosphorous and potassium) have a higher risk of leaching aged glyphosate and concentrate greater amounts of AMPA residues. As for AMPA soil DT_{50}, Battaglin *et al*. (2014) indicate a range of 60 to 240 days. Mamy *et al*. (2005) detected different concentrations of glyphosate and AMPA after 140 days of application and five months after the incubation of soil samples taken from experimental plots in France. They indicate that glyphosate exhibited degradation by mineralization pathways and that it is less persistent in soils than AMPA.

Besides plant tissue and soil, glyphosate deposits are also found in water and sediments. Section 2.2.5 (“Water”) provides examples of this.

Changes in the timing of herbicide applications

The applicant does not address possible changes in the timing of glyphosate applications with the adoption of Intacta RR2 Pro.

Generally speaking, the introduction of GM HT crops changes the management of agroindustrial production, particularly in terms of soil preparation and application of herbicides (Suárez *et al*. 2010). Regarding weed management in GT soybean, field observations indicate that the changes in timing of herbicides application occur in two scenarios: short term, and medium- to long-term.

In the short term following the adoption of GT crops, there is the possibility of glyphosate use at any time during the crop cycle, contrary to the pressure existing in non-GT crop production of applying herbicides in specific times to avoid crop damage. An example of this is the application at crop pre-emergence or at certain crop development stages with herbicides selective to narrow and broad leaf weeds, since they have different metabolism pathways (Pitty 1997).

In the medium- and long-term, the emergence of GT weeds leads to a return to the application of complementary herbicides at specific times and for specific weed types to avoid damage of the GT crop (Catacora-Vargas *forthcoming publication*). In the case of the emergence of GT narrow leaf weeds among GT soybean (which is a broad leaf crop), herbicides specific to the former are usually applied at the time of glyphosate application with no or low risk of GT soybean damage. In the case of GT broad leaf weeds, management to prevent damage on the GT soybean becomes more difficult. It may consist of spot application, if economically feasible, of a broad-leaf-specific or broad-spectrum herbicide.

Changes in herbicide drift risk to non-GM crops

Particle drift is unavoidable in all pesticide applications due to the physical characteristics: the tiny size and light weight of the micro-droplets generated by the application equipment (either handle or mechanized sprayers, terrestrial and aerial). As a result, the particles are extremely easy to transport by wind or by updrafts. As a result, pesticide drift is a widespread phenomenon. For instance, Pimentel (1995) calculated that approximately 25 percent of sprayed pesticides reach the target area, and in perfect environmental conditions this rate of efficiency may increase to 50 percent. Moreover, only 0.1 percent of the applications make contact with the target organism. The author reports that pesticide drift can reach 1.5 to 800 meters from the source of application over other crop fields, natural habitats, human settlements, etc.

Herbicide drift increases in agroindustrial mono-cropping systems due to the large quantities applied in extended areas. Accordingly, the expansion of GT soybean—motivated mainly by management simplification and the reduction in the price of glyphosate after the expiration of the corresponding patent in 2000 (Binimelis *et al*. 2009; Catacora-Vargas *et al*. 2012)—has increased the risk of herbicide drift on non-GM crops and in surrounding areas. This was identified as one of the main adverse effects from GM HT crop adoption (Knezevic 2007).

The risks of glyphosate drift relate not only to the physiological damage of non-GT crops, but also to other changes in plant population dynamics, due to its non-selective and broad spectrum characteristics. In this regard, Londo *et al*. (2010), using the case of GM HT canola, point to potential changes in gene-flow processes between related crops (both GM and non-GM) and weeds as a result of the alterations in the fitness of plants exposed to glyphosate drift. The authors based this conclusion on the observed decrease in the reproductive fitness of non-GM HT canola in comparison with GM HT varieties, when exposed to glyphosate drift. They indicate that these glyphosate-driven changes may influence the flow of transgenes through hybridization and introgression processes, and thereby increase their presence in the seed bank. With regard to GM HT-soybean production in South America, it is important to acknowledge that the possible risks generated by glyphosate drift may be low, since soybean is a self-pollinating species and wild species or landraces are non-existing in the region. However, further research is needed on the effects of glyphosate drift from GM HT soybean fields.

Changes in herbicide use

The applicant refers in general terms to the possibility of a decrease in the use of pesticides (both herbicides and insecticides) with the adoption of Intacta RR2 Pro. However, no empirical data is provided to support these assertions.

The literature indicates that the pattern of herbicide use with the adoption of GM HT crops varies over time. In the short-term, during the first years of the introduction of GM HT varieties at the farm level, a reduction in the number of applications is generally seen: from multiple applications to (usually) two. Also, the reduction of different types of herbicides is observed: from pre- and post-emergence herbicides specific to narrow and broad leaf weeds, to a broad-spectrum and non-selective weed killer: glyphosate (Bonny 2011; Cerdeira *et al*. 2007; Graef 2009; Nelson and Bullock 2003). Cerdeira and Duke (2006) provide an example of the aggregate replacement of herbicides in the United States during the first years of GT crop adoption. They mention that 3.27 million kg of different herbicides were replaced by 2.45 million kg of glyphosate. However, other authors contradict this early trend, indicating that the actual aggregate application of glyphosate-based herbicides in GT soybean in the United States was two- to ten-fold higher than non GT-soybean (Benbrook 2001), given the increase in area under GT soybean cultivation. Consistent with the latter, Vila-Aiub *et al*. (2008) indicate that the vast adoption of GT soybean together with no-till practices has amplified the accumulated volume of glyphosate used. This is also expressed by Pengue (2005a), who reports that the change in herbicide regime from GT-soybean adoption in Argentina, resulted in an increase in glyphosate use from one million liters at the time of adoption in 1996 to 160 million liters in 2004.

In the medium- and long-term, when the emergence of GR weeds takes place, the number and concentration of glyphosate applications increases as an initial measure to control such weeds. Then, it is followed by the use of complementary and more toxic herbicides that are applied together with glyphosate (if there is chemical compatibility) or in separate applications (Barnett *et al*. 2013; Beckie *et al*. 2006; Bonny 2011; Binimelis *et al*. 2009; Boerboom and Owen (ed) 2007; Catacora-Vargas *forthcoming publication*; Pengue 2005a; Tuesca *et al*. 2007). See next subsection for additional information on changes in herbicides use.

Changes in the use of herbicides with different profiles of effects

In the first period of adoption of GT soybean, multiple herbicides are replaced by glyphosate, which is considered to be a low-toxic herbicide classified under category III with an oral LD_{50} (based on active ingredient) from 1,500 to 10,000 mg/kg in small and medium sized mammals (WHO 2006). In the medium- and long-term, at the time of emergence of GR-weeds, the management commonly applied by producers includes more toxic herbicides capable of eliminating such resistant weeds. The most frequently used are the following:

- Glufosinate (oral LD_{50} from 1,500 - 2,000 mg/kg in rats)
- Dicamba (oral LD_{50} from 757 - 3,000 mg/kg in small mammals)
- 2,4-D (oral LD_{50} from 320 - 1000 mg/kg in small mammals)
- Paraquat (oral LD_{50} from 20 - 150 mg/kg in rats)

With exception of paraquat, tolerance to these herbicides is being inserted in the new generation of crops tolerant to multiple herbicides to simplify the management of GR weeds (for reference see, Behrens *et al*. 2007 and Krieger 2011).

Unexpected combinatorial and synergistic effects from multiple herbicide applications

The applicant does not address possible combinatorial or synergistic effects from multiple herbicide applications (e.g. glyphosate and insecticides) with the adoption of Intacta RR2 Pro. In general, the literature and actual empirical research on the effects of multiple or mixtures of herbicides applications are scarce. An example is Helmer *et al*. (2014) who studied caged honeybees (*Apis mellifera*) at field-realistic doses of atrazine, metolachlor and glyphosate. Exposure was administered through contaminated syrup for ten days. Among other parameters, the authors measured diet-derived antioxidants such as carotenoids and retinol, which are considered essential for honeybees' larval development, vision and antioxidant capacities; and the imbalance of which may negatively affect honeybee health and, hence, populations. The authors found that honeybee extracts had decreased concentrations of carotenoids and derived substances under atrazine and glyphosate exposure, while metolachlor increased retinol content. The researchers concluded that the honeybees' carotenoid-retinoid metabolism system might be altered by sub-lethal doses of herbicides.

Another example is provided by Bicho *et al*. (2013), who researched wild lizards (*Podarcis bocagei*) exposed to herbicides applied in agricultural fields. They found several herbicide residues in the soil, including glyphosate in low concentrations. Based on histological and biochemical studies, they indicate that the mixtures of agricultural herbicides could have thyroid-disrupting effects capable of adversely impacting the reproductive system of male lizards.

2.2.3 Resistance of other plants to the herbicide

Guiding questions:

a. What are the resistance problems associated with the herbicide in the cultivation area?
b. What strategies are used to prevent the development of resistance by plants other than the HR crop (example: integrated plant protection)?

Summary:

- The ***problems associated with herbicide resistance*** relate to: (i) an increase in herbicide use (in volume, type and toxicity) and its corresponding residues in plant tissue, soil and harvest. The increase in exposure to and residues of glyphosate also rise the possibility of nutritional imbalances in soybean, and a resulting decrease in biomass production and yield, (ii) changes in local wild flora due to the emergence of GR weeds that become more competitive and dominant, which also relates to changes in the ecological functions of the wild flora, (iii) changes in the soil biota and its ecosystem functions, particularly in relation to nutrient cycling and the regulation of pathogen populations, and (iv) the appearance of volunteer GT soybean resulting in the increased use of more toxic herbicides.

- The ***strategies to prevent herbicide-resistance development*** suggested by the applicant involve weed seed bank management through adequate glyphosate management; a decrease in the selection pressure exerted by glyphosate; a reduction in the ecological adaptation of weeds; and the elimination of the selection pressure factor. With the exception of the first point, the applicant does not provide the needed insights for a feasible implementation of the strategies proposed. Another GM-based strategy promoted is the development and introduction of crops tolerant to multiple herbicides. However, given the current experience, this could have larger negative environmental and health effects than that of the current use of single HT crops. More comprehensive suggestions include ecosystem management through agroecological approaches and integrated weed management.

Problems associated with herbicide resistance

There are three main direct problems associated with herbicide resistance in weeds in GM HT crops:

- *Increase in herbicide use and residues*. A commonly observed first measure for managing GR weeds in fields is to increase the dosage of glyphosate, followed by the application of complementary (e.g. cletodim and imazetapir) and more toxic herbicides (as in the case of 2,4-D, dicamba, glufosinate and paraquat) (Barnett *et al*. 2013; Catacora-Vargas *forthcoming publication*; Graef 2009; Pengue 2005a; Tuesca *et al*. 2007). The application of complementary herbicides depends on the species of the GR weed and its distribution. For example, herbicides with modes of action that do not interfere with soybean metabolism (as in the case of imazetapir) can be applied together with glyphosate. Others that are selective to broad leaf weeds (e.g. 2,4-D) or which have a broad spectrum (e.g. certain formulations of paraquat) may be subject of localized applications (APIA 2012). A problem with this approach is the increase in the concentration of glyphosate and other complementary herbicides and their metabolites in the plant tissue, soil and harvest (for instance, see: Arregui *et al*. 2003; Bøhn *et al*. 2014; Duke *et al*. 2003; and Simonsen *et al*. 2008). Part of the cascading effect from increased glyphosate exposure to plant tissues in soybean is a reduction in the crop´s photosynthetic capacity and nutritional imbalances (Zobiole *et al*. 2009; 2011; 2012), which directly affect biomass production and yield. Additional information on the changes in herbicides use and residues is provided in sections 2.2.2 ("Effects of altered spraying regime") and 3.1.2 ("Food safety").

- *Changes in local wild flora*. The emergence of GR weeds inevitably modifies the composition of wild flora in agricultural fields by providing a competitive advantage to species that have evolved to resist the herbicide, which eventually become dominant under the agricultural management applied to the GT soybean (Beckie *et al*. 2006; Graef 2009; Owen 2008; Powles 2008; Vila-Aiub *et al*. 2008). This change in wild flora is expressed as a modification in the weed species composition and distribution, biomass and seed production, as well as the soil seed bank (Cerdeira and Duke 2006; Firbank *et al*. 2006; Graef 2009; Owen and Zelaya 2005). A second level of effects deriving from the emergence of GR weeds includes the modifications in some ecosystems functions, such as the maintenance of microhabitats to host natural enemies (M. A. Altieri 1999); provision of food to different herbivores (e.g. birds) (Watkinson *et al*. 2000); and to ensuring a cycle of nutrients sustained by specific soil biota related to particular weed species (Kremer 2014).

- *Changes in soil biota and ecosystem functions*. Soil biota can be affected by direct applications of herbicides (either inherent to the trait inserted—in this case glyphosate—or complementary herbicides used to manage GR weeds) and also by their residues and metabolites. Some changes in soil biota could impair ecosystem functions, such as nutrient fixation and cycling (for instance, see: Druille *et al*. 2013 in relation to the effects on arbuscular mycorrhyzal fungi). At the same time, HR weeds have the capacity to change the soil ecosystem, since different weed species are related to specific root biota. For example, Kremer's (2014) study (described in more detail in section 2.2.2 "Effects of altered spraying regimes") observed that the GR weeds *Amaranthus* and *Chenopodium* exert a selective biochemical pressure on particular soil microbial groups, which may influence nutrient cycling and the emergence of pathogens.

Problems with herbicide resistance not only relate to the resulting changes in weeds, but also to the crop in which the resistance trait was inserted. This is expressed as volunteer GM HT crops.

- *Volunteer GT soybean.* Both the applicant and part of the literature on GT soybean (Beckie *et al*. 2006; Bindraban *et al*. 2009) indicate a very low possibility of Intacta

RR2 Pro becoming a volunteer GM HT crop. However, field observations reveal different levels of GT soybean volunteerism in different countries (e.g. Catacora-Vargas 2007). This is consistent with Cerdeira and Duke (2006), who indicate that GT crops have a greater potential of becoming volunteers, particularly in comparison to conventional varieties. Given the limited dormancy of soybean seeds (Beckie *et al*. 2006), the main factor that leads to the emergence of a GT soybean as volunteer in crops is harvest residues in the soil and in harvesting equipment (Catacora-Vargas 2007). Since glyphosate tolerance prevents its control with this herbicide, if GT soybean volunteer requires management, usually mechanical or chemical approaches are applied (Beckie *et al*. 2006). In the case of the latter, this is done by spot applications of highly toxic herbicides, such as paraquat (Cerdeira *et al.* 2007; Catacora-Vargas *forthcoming publication*).

Strategies to prevent the development of herbicide-resistance

The applicant makes the following suggestions as strategies to avoid HR weeds with the adoption of Intacta RR2 Pro: (i) weed seed bank management through the adequate use of glyphosate, (ii) a decrease in the selection pressure exerted by glyphosate through applications of adequate concentration and timing, together with other management practices (not specified by the applicant), (iii) a reduction in the ecological adaptation of weeds (the applicant does not specify any recommended approach for this measure), and (iv) the elimination of the selection pressure factor (Berger and Braga 2009). Given that the selection pressure is exerted by the constant application of glyphosate on GT crops, which is inherent to the GM trait inserted in the Intacta RR2 Pro soybean, the latter proposed by the applicant (i.e. elimination of the selection pressure, meaning glyphosate) seems unrealistic, even if referring to herbicide rotation.

One proposed genetic modification-driven strategy is the development of GM crops tolerant to multiple herbicides (for instance, see: Behrens *et al.* 2007; Krieger 2011). Yet, based on current experiences, such a strategy will cause more severe problems of weed resistance; increased herbicides use in terms of volume, type and toxicity; and consequently, greater negative impacts on the environment (Mortensen *et al.* 2012), food quality, food safety and even field occupational health (Binimelis *et al.* 2009; Bøhn *et al.* 2014).

Other strategies to prevent the development of HR weeds are:

- *Agroecological approaches* for agroecosystem management to control weeds via physical interference and allelopathy (referring to the reduction or inhibition of competition among plant species upon the release of biochemical substances, known as allelochemicals) (Pitty and Godoy 1997). A practical example of this method was developed in Brazil by using different cover crops together with organic conservation tillage (Altieri *et al*. 2011).

- *Integrated weed management*. Davis *et al*. (2009), for instance, applied crop rotation, cover crops, non-residual glyphosate-based herbicides and applications previous to sowing in order to control GR horseweed (*Conyza canadensis*). With these practices, the authors succeeded in reducing the presence of this GR weed in the seed bank and, indirectly, avoiding the further emergence of other GR weeds. Other examples and analyses of IPM of GR weeds are provided by Mortensen *et al*. (2012).

2.2.4 Soils

Guiding questions:

a. Does cultivation of the GM HT plant lead to more/less soil erosion?
b. Does cultivation of the GM HT plant lead to a higher/lower soil pH?
c. Does cultivation of the GM HT plant lead to a change in the nutrient composition of the soil?

Summary:

- The applicant does not address changes in ***soil erosion*** due to the adoption of Intacta™ Roundup Ready™2Pro. No-till soil preparation introduced in the cultivation of HT crops is intended to contribute to the simplification of weed management, yet this practice is originally designed for soil conservation. The soil conservation purpose of no-till can be fulfilled when integrated with the use of cover crops, multiple-year crop rotations and the reduced use of heavy machinery. None of these practices are currently applied in GT soybean production. Another factor limiting the soil conservation aspect of no-till is the emergence of GR weeds.

- Neither the applicant nor the literature report on possible ***soil pH*** changes with Intacta™ Roundup Ready™2Pro adoption. Considering that soil pH is impacted by several managerial and ecological factors, it is likely that the agroindustrial soil management of GT soybean will impact pH soil levels, particularly in naturally fragile soil regions. Further research on this issue is needed.

- The applicant does not address possible ***changes in soil nutrient composition*** with the adoption of Intacta™ Roundup Ready™2Pro. The agroindustrial management of soybean together with the physiological characteristics of this crop (high nutrient demand and uptake) results in severe depletion of soil nutrients. For instance, in Argentina, soybean is related to the extraction of about 1,75 million metric tons of nutrients. Glyphosate-related changes in soil biota linked to the nutrient cycle also impact the soil's nutritional composition.

Soil erosion

The applicant does not address issues related to changes in soil erosion with the adoption of Intacta RR2 Pro.

The intensive production system that characterizes agroindustrial production, of which GM HT crops are a part, has resulted in soil erosion and fertility depletion (Pengue 2005b). No-till agriculture—introduced as a complementary mechanical approach for simplifying weed management in GM HT cropping—was originally intended to reduce soil erosion. However, in the context of GM HT crop production, two factors prevent no-till from achieving its soil conservation goals: the agronomic management practices in GM HT cropping and the emergence of GR weeds. First, no-till is capable of achieving its conservation purposes when combined with cover crops and multiple-year crop rotations, together with a decrease in the use of heavy machinery (Díaz-Zorita *et al.* 2002; Domínguez *et al*. 2010; Lal 2009). Given that these conditions are not fulfilled in GT soybean production (or any agroindustrial crop), it is very unlikely that soil erosion will decrease. Second, under the current practice, no-till results in an increase in the weed population, particularly of perennial species. Hence, the soil management applied to GM HT crops is suitable for the emergence of GR weeds, the control of which is incompatible with no-till soil preparation and sowing under the conventional agroindustrial system (Owen and Zelaya 2005; Price *et al*. 2011).

Changes in soil pH

The applicant does not address possible changes in the soil pH due to Intacta RR2 Pro cultivation. Likewise, the literature does not report specifically on this issue, neither in relation to this particular crop, other GM HT variety nor with regard to glyphosate. However, it is reasonable to expect changes in soil pH values in areas of large-scale GM HT soybean production, considering that soil properties and fertility are the results of a number of interrelated managerial and ecological factors (M. Altieri 1999; Bot and Benites 2005; Hamza and Anderson 2005), that the management applied to GM HT crops relies on heavy application of synthetic inputs and intensive consecutive cultivation (Catacora-Vargas *forthcoming publication*), and that interrelated changes in weed populations from cropping of GM HT varieties also change the soil biota (Kremer 2014). This is particularly applicable to fragile soils, such as the ones existing at the Brazilian Cerrado region, characterized as being one of the oldest and least fertile in the world (Landers 2001), and where the largest expansion of Brazilian soybean is taking place (Bourscheit 2012; Pirauí Cremaq 2010). About 50 percent of the current soybean produced in Brazil comes from the Cerrado region, and approximately 70 - 75 percent is GM HT (ABRANGE 2012). Nonetheless, specific monitoring and research on soil pH alterations is still needed.

Photo: saratm #43350607 (dollarphotoclub.vom)

Changes in soil nutrient composition

The applicant does not address possible changes in soil nutrition resulting from the cultivation of Intacta RR2 Pro. The applicant only presents a study comparing MON 87701 (expressing the protein Cry1Ac) with the conventional cultivar used for the corresponding genetic modification (A5547) and four other unspecified commercial soybean varieties, reporting no negative changes in the carbon and nitrogen cycle. However, it is not clear if the study was restricted to the effects of the insect resistance trait or whether it included the effects of the technological package (i.e. glyphosate applications) as well.

As indicated previously, the intensive system employed by the agroindustrial production of soybean—both conventional and GM varieties—inevitably results in the depletion of soil nutrients. Soybean is a particularly nutrient-demanding crop and efficient up-taker, requiring several nutrients for proper development (i.e. nitrogen; phosphorus; potassium; iron; molybdenum; calcium; magnesium; and sulfur) (Mueller *et al*. 2010; Iowa State University Extension 2007). These factors create a significant volume of soil nutrients removal by soybean. For instance, Pengue (2005a) calculated that, in Argentina, about 1.75 million metric tons of nutrients are extracted annually by soybean crops, phosphorus being the main nutrient depleted.

Besides direct nutrient uptake, in the specific case of GT soybean, soil fertility could be also negatively influenced by glyphosate-related changes in the soil microbiota linked to nutrient cycling (Druille *et al*. 2013; Kremer 2014).

More comprehensive studies regarding the effects of agroindustrial soybean production and GM HT varieties are still missing in the literature.

2.2.5 Water

Guiding questions:

a. Might cultivation of the GM HT crop change the water-spraying regime so that water sources and groundwater become polluted by "new" proteins and residues of herbicide or degradation products thereof?
b. Does the cultivation of the GM HT crop reduce evapotranspiration as a result of less tilling?

Summary:

- Knowledge of ***water pollution with GT related protein and herbicides*** is still limited. Glyphosate and AMPA residues and effects in water are variable. Part of the literature points to a possible microbial disturbance at glyphosate concentrations as low as 1 mg/L. Studies also indicate a potential drift, leaching, accumulation and persistence of glyphosate in water bodies, and from there, to the dissemination of glyphosate across the environment, leading to the exposure of non-target organisms.

- Empirical information is needed on ***changes in soil evapotranspiration*** with the adoption of Intacta™ Roundup Ready™2Pro soybean and HT crops in general. Considering the current management of GM HT crops, it could be anticipated that evapotranspiration is not reduced.

Water pollution with GM HT-related proteins and herbicides

No empirical studies were found on the evaluation of the presence of proteins or genetic components of the Intacta RR2 Pro or GT soybean genetic cassette in water.

In relation to residues from herbicides, in the 2003/2004 growing season, Peruzzo *et al*. (2008) assessed the concentration of glyphosate in a river located north of the province of Buenos Aires, Argentina. They found a concentration of glyphosate ranging from 0.10 to 0.70 mg/L, which significantly increased after rainfall due to high water solubility of glyphosate, drift and leaching from the river basin. Although these concentrations are low, based on empirical research, Stachowski-Haberkorn *et al*. (2008) indicate that glyphosate concentrations of about 1 mg/L can have an aquatic (e.g. marine) microbial disturbance effect.

From 2001 to 2010, Battaglin *et al*. (2014) did an extensive assessment in 38 states of the United States. They collected thousands of environmental samples (in water and sediments) finding, on average, glyphosate and AMPA residues in about 40 and 55 percent, respectively, of them. The authors report a high frequency of glyphosate and AMPA in soils and sediments, ditches, drains, precipitation waters, rivers and streams; and a lower frequency in lakes, ponds, wetlands, soil water and groundwater. The corresponding median and maximum values of glyphosate and AMPA concentrations in all samples were <0.02 and 476 µg/L, and 0.04 and 397 µg/L. Battaglin *et al*. (2014) indicate that the concentrations found were below human and wildlife concern, yet they also point out that chronic sub-lethal effects are still uncertain.

Other field studies referenced by Mercurio *et al*. (2014) on glyphosate residues in water report concentrations of 54 mL/L in Australia and 40.8 mL/L in Canada, where concentrations as high as 1,700 mL/L were also found. It is probable that not all these concentrations result from applications in GT soybean cultivation. Nonetheless, they indicate the elevated capacity of accumulation of glyphosate in water. Mercurio *et al*. (2014) also report on an empirical study in which they found that aquatic DT_{50} of glyphosate varies in relation to temperature and light exposure. They determined a DT_{50} of 47 days at 25°C at low light, 267 days in darkness at the same temperature and a longer DT_{50} (315 days) in the dark at 31°C. AMPA varied inversely in relation to glyphosate concentration, since it is the main glyphosate metabolite. The ecotoxicological implications of these findings is that glyphosate could be more persistent under low light conditions, which characterize deep waters, thus increasing the potential exposure to deep-water and non-target wildlife.

All these studies reveal a high mobility and accumulation of glyphosate and its metabolites in the environment.

Changes in soil evapotranspiration

No information was found on the effects of no-till in GM HT crops on evapotranspiration. As indicated before, soil and water conservation properties of no-till are achieved under specific management conditions, i.e. combined with cover crops; multiple-year crop rotations; reduction in the application of synthetic agrochemicals; and limited used of heavy machinery (Domínguez *et al.* 2010). These conditions are not fulfilled in agroindustrial GM HT soybean production. Consequently, the GM HT soybean management contributes to soil deterioration, and given that part of the growing season soils are uncovered, it may be anticipated that evapotranspiration is not reduced. However, empirical research on real field conditions is still lacking.

2.2.6 Energy

Guiding question:

Is there an increase or decrease in the energy consumed in connection with cultivation of the GM HT plant, measured by means of life cycle analysis of the full production and harvesting cycle?

Summary:

There was no information found in the applicant´s report or in the literature regarding the ***energy*** impacts of the adoption of Intacta^{TM} Roundup Ready^{TM}2Pro soybean. The few studies found contain contradicting information. On one hand, some studies report less energy consumption and better environmental and health performance in GT soybean production. On the other hand, other studies report that agroecological, organic and even conventional non-GM soybean production systems are more efficient with regard to energy; renewable and natural resource use; and the use of other materials when compared to GT varieties.

Neither the applicant nor the literature consulted contains information on changes in energy consumption from Intacta RR2 Pro cultivation. Empirical data regarding the effects on energy use derived from GM HT crops, and specifically GT soybean, are limited. Cerdeira and Duke (2006) indicate that in GT cultivation one of the main positive environmental impacts is a reduction in use of fossil fuels resulting from more simplified crop management. They base their assertion in part on the work presented by Bennett *et al*. (2004), who analyze herbicide regimes applied to conventional and GM HT sugar beet production in the UK and Germany by calculating the following energy, climate and health-relevant parameters: energy requirements, global warming potential, ozone depletion, ecotoxicity, acidification, nitrification, summer smog, release of toxic particles and carcinogenic potential. They conclude that these parameters have much lower values in GT cropping than in conventional production systems. However, this research has the following flaws: the methodology applied for each type of calculation is unspecified; the characteristics of the production systems compared are not indicated; and it is unclear the time scale used for both the production systems and the evaluations reported. Moreover, although the study refers to its findings as a “life-cycle analysis,” it only focuses on herbicide application at the crop production stage.

Another energy analysis was done by Ortega *et al*. (2005) on soybean production in Brazil. They applied an "emergy" approach—an ecological method consisting of the conversion of all forms of energy, materials and human services into equivalents of solar energy. Based on previous empirical data, they analyzed four types of farming systems in Brazil: (i) agroecological, production systems based on the enhancement of ecological synergies and positive interactions, (ii) organic, related to the substitution of synthetic inputs for biological ones, (iii) conventional, based on the application of high-external and -synthetic input technological packages, and (iv) no-till GM HT crop production. They accounted for the inputs used and outputs per hectare for one year in each system, organized as renewables, non-renewables, materials, services and products. Based on this, they calculated the emergy yield ratio (EYR) as an indicator of efficiency of each purchased input. In general, EYR values were higher in agroecological (3.69) and organic (3.24) systems in comparison to conventional (2.17) and no-till GM HT production systems (1.37). This was mainly due to the use of local resources in the first two cultivation schemes. In terms of the renewability of resources, the values were 73 percent for agroecological systems, 69 percent for organic systems, 27 percent for no-till GM HT crops and 19 percent for conventional. Values of were as follows: 0.07 for conventional, 0.10 for no- till GM HT cropping, 0.02 for organic and 0 for agroecological. The main factors in their calculations were the high use of fossil fuel energy in industrial systems (conventional and no-till GM HT-based). The authors conclude that agroecological systems showed the best performance in terms of the use of renewables and natural resources, reduction in soil erosion, externalities and the use of off-farm materials (related to the system self-reliance).

2.2.7 Climate

Guiding question:

Do the greenhouse gas emissions associated with the cultivation of the GM HT plant, as measured by life-cycle analysis of the full production and harvesting cycle, increase or decrease?

Summary:

It was not found in the applicant´s report or the revised literature information on ***climate change*** impacts from the adoption of Intacta^{TM} Roundup Ready^{TM}2Pro. Several statements are made on the climate-friendly characteristics of GT and HT crops; however, empirical data is still missing. The information provided in sections 2.1.4; 2.2.2; 2.2.6 and 3.1.1 of this report are useful in analyzing the potential effects of GT soybean on climate change. In this analysis, the fact that this crop is part of the agroindustrial production model should be considered, since a number of climate effects are related to it.

The applicant does not address the potential changes on greenhouse gas (GHG) emissions or other climate change factors resulting from the cultivation of Intacta RR2 Pro. The literature approaches these issues on GM HT crops from two perspectives: calculation of direct GHG emissions and carbon sequestration, and indirect impacts from deforestation and land use change.

Research on actual estimations of GHG and carbon sequestration is rather limited. The main scholarly work on these issues are the reports by Brookes and Barfoot (2013) and Barfoot and Brookes (2014). By using the same methodology and, essentially, the same sources of secondary information, these authors calculated the accumulative reduction of GHG emission in terms of carbon dioxide savings. These estimations consider the decreased fuel consumption resulting from the reduction in machinery use and pesticides applications with the adoption of GM crops. The authors report cumulative carbon dioxide savings of about 14 million kilograms from 1996 - 2011 (Brookes and Barfoot 2013), and approximately 17 million kilograms from 1996 - 2012 (Barfoot and Brookes 2014). In addition, for the same periods they estimated soil carbon sequestration levels (due to no-till practices) of 170,961 million tons and 203,560 million tons, respectively.

Some limitations of these estimations are that: (i) with the exception of few sources of secondary information, they are based on non-GM crop related agriculture and general analyses of no-till impacts, (ii) the sources of information report on data from at least six years ago, without considering the current changes in machinery use and pesticide applications due to the GR weed emergence, and (iii) other changes in natural resources, such as soils, deriving from intensive agroindustrial production systems and affecting the efficacy and application of no-till practices are not considered. Moreover, the estimations on soil carbon sequestration may be overestimated due to methodological drawbacks in the empirical research. According to Baker *et al.* (2007), positive impacts on soil carbon sequestration from no-till agriculture may be the result of methodology sampling bias. This is given that samples are often taken from 30 cm soil depth or less, when studies applying deeper sampling found no carbon sequestration difference and even higher in conventional tilling. Based on this, the authors conclude that there is not yet compelling information that no-till favors soil carbon sequestration.

Indirect impacts on climate change from GM HT soybean production relate to deforestation and land use change. GM HT crops in general and GM HT soybean in particular show a remarkable increase in production area (Pirauí Cremaq 2010) accompanied by deforestation, land use change and crop substitution (Catacora-Vargas *et al.* 2012). This crop expansion has two main drivers: (i) the simplification of the agronomic management with GM HT soybean due to its technological package, and the resulting cultivation in areas where it was not feasible in the past (Oyhantçabal and Narbondo 2010), and (ii) the high prices of soybean in the international market (IMF 2012). Complementary analysis is presented further in this report.

In conclusion, while a number of statements are made with regard to the decrease of climate change impacts with the adoption of GM HT crops—i.e. reduction in the use of heavy machinery, drop in fossil fuel use, reduced tillage, decrease in greenhouse gases emissions and airborne herbicides associated with shrinking herbicide use—the empirical data supporting such assertions remain quite scarce.

The studies described and analyzed in sections 2.2.6 ("Energy"), 2.1.4 ("Preservation of biological diversity"), 2.2.2 ("Effects of the altered spraying regime"), and 3.1.1 ("Food security”) provide further insights into the potential impacts of GT soybean cultivation on climate change. These sections also analyze the contradictions—particularly in the long-term—between climate-friendly statements and empirical research reported in the literature. In this analysis, it is important to consider that GM HT crops are part of agroindustrial production systems practiced at different scales, from small to large. Accordingly, a significant part of the climate-change related effects of GM HT crops are linked to the agricultural system in which they are embedded.

III Social and economic sustainability

3.1 *The right to sufficient, safe and healthy food*

3.1.1 Food security

Guiding questions:

a. Does the GM HT plant contribute to reduced/increased input factors per production unit?
b. Does the yield per unit area increase/decrease?
c. What is the purpose of the GM HT plant? Will it be used for food, feed, biofuel or material?

Summary:

- The applicant does not provide information on changes in ***input factors*** with the adoption of Intacta™ Roundup Ready™2Pro or its parental GM lines. The main changes reported in the literature in relation to GM HT and GT soybean are: a decrease in the volume of herbicides applied during the first years of adoption, and an increase in the medium- and long-term; reduced access to non-GM seeds, particularly for small-scale production systems; and a decrease in labor requirements.

- The applicant does not provide information on changes in ***yield*** of Intacta™ Roundup Ready™2Pro. In another document, the applicant reports an increase in yield as a result of plant protection against pests. However, other empirical studies on RR soybean found minimal or no increase in yield when compared to conventional varieties. There are also reports on potential adverse effects of glyphosate application on GT soybean photosynthesis capacity, nutrient update and plant toxicity, all of which negatively influence yield.

- Intacta™ Roundup Ready™2Pro is approved for multiple ***purposes*** ranging from free registration to consumption.

- ***Crop replacement*** and ***production intended for local markets*** are additional issues that impact food security. Long-term observations indicate that local crops are replaced by GM HT soybean due to its easy management and high selling price in commodity markets. The majority of the soybean produced in Brazil (60 percent in 2012) is destined for export or biofuel production.

Changes in input factor per production unit

In the context of this report, *input factor* refers to the materials (e.g. agrochemicals and seeds); workforce (i.e. labor); energy; and capital used for producing an area unit. Input factor has a direct relationship with: (i) production costs—the more external inputs needed, the more expensive the system turns out to be, (ii) sustainability of the production system in relation to the availability of inputs and their impacts on the environment (including the society), and (iii) farmers' self-reliance—the greater the use of internal inputs, the more autonomous the production system becomes.

Soybean field spraying in spring
Photo: sima #66704257 (dollarphotoclub.com)

The applicant does not provide information on changes in input factors per production unit, or yield per unit area due to the cultivation of Intacta RR2 Pro or its GM parental lines. Likewise, the literature accessed does not include any study on these aspects for this specific GM variety. The following summarizes the relevant information found from different sources in relation to GM HT and RR soybean.

A main change in inputs with GM HT cultivation is ***herbicides***; both the herbicide directly related to the genetic modification (i.e. glyphosate-based formulations) and complementary weed killers. Barfoot and Bookes (2014) report that since the adoption of RR soybean in Brazil (in 2003), the use of glyphosate as an active ingredient had decreased by 220 g/ha. Yet, due to changes in the availability and price of this herbicide, together with changes in GR weeds and adoption of no-till, in 2012 the use of glyphosate on RR soybean increased, applied at an average rate of 2.91 kg/ha in comparison to 2.68 kg/ha in conventional varieties (a difference of 230 g/ha). Barfoot and Bookes (2014) also indicate a rise of 13 percent in glyphosate active ingredient use in RR soybean in relation to conventional production, but a decrease of 11 percent in the environmental load (measured as Environmental Impact Quotient, EIQ) given the lower toxicity profile of glyphosate when compared to other herbicides. The limitation of the latter is that the estimation of EIQ is based on the active ingredient, without considering the adjuvants present in the commercial formulations of glyphosate, which may be more toxic than the active ingredient itself (Mesnage *et al*. 2013; Surgan *et al*. 2010).

Another study from Argentina carried out by Qaim and Traxler (2005) report field findings from the first three years of RR soybean adoption. From the 1995/1996 growing season to that of 2000/2001, the use of glyphosate in the country increased 11-fold. In the 2000/2001 agricultural season approximately 65 percent of this herbicide was applied to soybean. The increase in the volume of glyphosate used is explained by the expansion of GM HT soybean, since 87 percent of the total soybean in Argentina in 2000/2001 was planted with RR varieties (Catacora-Vargas *et al*. 2012). Qaim and Traxler (2005) also mentioned that, on average, the number of herbicide applications in RR soybean increased in the period analyzed. Similarly, based on data from the United States Department of Agriculture (USDA), Benbrook (2012) reports an rise of approximately 9 percent from 2000 to 2006 in the volumes of glyphosate applied in GM HT soybean in the United States, totaling a cumulative value of 53.4 percent in the indicated period.

Concerning complementary herbicides, GT crops involve weed management systems that overemphasize the use of this weed killer, causing an accelerated appearance of GR weeds due to the ecological selection pressure it exerts (Owen 2008) (see section 2.2.3 "Resistance of other plants to the herbicide" for further details on reported GR weeds). As a result, in the mid and long term, glyphosate has limited efficiency, requiring the application of complementary herbicides such as 2,4-D, glufosinate and dicamba (Behrens *et al*., 2007; Krieger, 2011; Wright *et al*., 2010) (see section 2.2.2 "Effects of and altered spraying regime" and section 2.2.3 "Resistance of other plants to the herbicide" for further details). The need for complementary herbicides in this type of production system has become the justification for developing GM crops that are tolerant to multiple weed killers (Green *et al*., 2008) and which may lead to an increased demand for even more toxic herbicides (Binimelis *et al*. 2009). Farmer testimonies in Brazil on the use of larger volumes of herbicides [*personal communication*] are consistent with data from the Latin American soybean producing countries showing the ascending trend of weed killer volumes used, complementary to glyphosate (Catacora-Vargas *et al*. 2012).

The availability of and access to ***seeds*** (mainly determined by increased costs) is another important change with regard to the inputs used in GM HT crop cultivation. Sections 3.3.2 ("Developments in costs and incomes for farmers in the short term") and 3.3.4 ("Right to seeds") provide information on this topic.

Another important change regarding inputs used in GM HT crop production is the decrease in labor requirements and, accordingly, the decrease in rural and on-farm job opportunities (See section 3.4.3 "Employment").

Changes in yield

The Intacta RR2 Pro applicant indicates on its website that the GM traits (i.e. herbicide and insect tolerance) contribute to increased yields as a result of the trait effects in protecting plants against pests. Accordingly, the inserted characteristics become a factor of secondary order for yield increase (Monsanto, 2014).

In line with this view, in its latest report on GM crops performance, PG Economics—a private advisory and consulting firm that provides services to the agriculture and natural resource-based industries—suggests that there is an increase in the yields of GM HT and insect-resistant crops. No specific information on yields is provided for RR soybean in Brazil (see: Brookes and Barfoot, 2014). Similarly, another report prepared by a private Brazilian consulting firm specializing in agribusiness studies reports a cumulative national increase in yields with the adoption of RR soybean, indicating further expected increases with the adoption of Intacta RR2 Pro. Yet, neither specific data supporting these statements nor the original data upon which the projections are based are provided (see Attie *et al*., 2012[3]).

A recent publication by Klümpler and Qaim (2014) on a meta-analysis of GM crop performance also indicates an increase in yields with the adoption of GM HT crops. However, another extensive review of the literature (Catacora-Vargas *et al. forthcoming publication*) found that an important portion of the empirical studies showing positive impacts on yield were performed during the first years after the adoption of GM HT crops, when the short-term positive impacts are clear; however, they start to fade in the medium term. Conversely, a long-term (1991 to 2010) and large-scale appraisal of soybean yields in Brazil, based on data reported by official sources and institutions specialized on this crop, indicates that after the introduction of RR soybean, yield rates did not increase and remained highly variable (Catacora-Vargas *et al*. 2012).

No scientific publications were found in the literature that address yield changes related to Intacta RR2 Pro in Brazil or in any other country. A proximate technical study was done by Zobiole *et al*. (2011), who found that high and late application of glyphosate in this soybean variety—a common practice among farmers—decreases photosynthesis. A complementary study by the same group of researchers found higher nutrient accumulation and dry matter (grain weight without moisture) in conventional soybean in comparison to RR soybean. The authors indicate that this is a result of the possible reduction in the nutritional efficiency in RR soybean resulting from glyphosate applications (Zobiole *et al*., 2012). Additionally, Santos *et al*. (2007) note that—from a comparison between different glyphosate formulations—there is a toxic effect on soybean plants caused by the adjuvant isopropylamine salt. All these studies also observed less foliar development and biomass production, changes that can negatively affect the yields. In line with this, Areal *et al*. (2012) report, from a meta-analysis (of both journal articles and institutional reports), yield changes ranging from negative to slight increases in GM HT soybean production in different countries (mainly Canada and the United States). They point out that there is no difference in yield, and that the performance of GM HT crops over conventional is doubtful.

Empirical research relevant to assessing specifically RR soybean yields in Brazil is surprisingly scarce. Ludwig *et al*. (2010) found different responses in yields of RR soybean varieties to glyphosate and fungicide applications. However, the research was carried out only among RR varieties without non-GM comparators. Melhorança Fliho *et al*. (2010) carried out an evaluation of production characteristics between different varieties of soybean (both RR and conventional). They observed that glyphosate applications higher than 1,800 g/ha decrease yields in RR varieties. This implies that there is a high probability of RR soybean not reaching optimal yield rates, since field observations showed that the common glyphosate application varies from 1,500 - 2,500 g/ha. Similarly, Santos *et al*. (2007) compare the effects of different glyphosate applications on RR soybean, concluding that, in order to avoid decreased nitrogen, calcium, magnesium, iron and copper plant intake, as well as plant intoxication, this herbicide should not be applied at high concentrations. The first three nutrients

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[3] This is a summary report of the findings of a longer document containing, according to the authors, the details of the data collected. The original report was not found at the online source indicated by the consulting firm.

(nitrogen, calcium and magnesium) are involved in photosynthesis and, accordingly, changes in their plant intake could influence biomass production and yield. The empirical research by Santos *et al*., however, covered only one growing season.

Purpose of the GM HT plant

Based on the Technical Opinion issued by the Brazilian CTNBio, the commercial release of Intacta RR2 Pro is intended for free registration[4], use, assays, tests, sowing, transport, storage, marketing, consumption, release into the environment, disposal and any other activities related to this soybean variety and its progenies (CTNBio, 2010). Despite this regulatory statement, the free use of GM seeds is limited (see section 3.3.4 “Right to seeds” and section 3.6 “Plant genetic resources for food and agriculture”). As indicated in the following subtitles, soybean production is mostly intended for export and biofuel production.

Crop replacement

An important characteristic of the management of GM HT crops is the simplification of agricultural activities, particularly when combined with no-till soil management (Bonny 2011; Brookes and Barfoot 2009; Fernandez-Cornejo *et al*. 2005). As previously mentioned, this facilitates the expansion of GM HT crops to large areas where cropping was not formerly feasible (Oyhantçabal and Narbondo 2010). Together with the high market price of soybean (IMF 2012), this results in the further expansion of soybean production to maximize income opportunities. One of the consequences of these factors is substitution of local crops by GM HT soybean. This process has been documented in Argentina (Pengue 2005b) and in other soybean-producing countries of South America. In the case of Brazil, the exceptional expansion in soybean production area (approximately 67 percent between 2001 and 2010) in comparison to other relevant crops (e.g. maize with a 4 percent increase in production area and beans with a 0.3 percent for the same period of time) reveals that the expansion of soybean is dominant (Catacora-Vargas *et al*. 2012). Additionally, the literature indicates that the replacement of crops intended for local markets impacts food security in the medium- and long-term. This is caused by the simplification of diets (Heinemann 2009) as result of the reduction in the availability of local crops and their increased purchase.

Production intended for local markets

The majority of the soybean (both GM and non-GM) produced in the Americas is destined for international trade, with Brazil being one of the major players. In 2012, based on FAOSTAT and USDA data, Brazil exported approximately 60 percent of its soybean production, mainly to Europe (Hamburg port) and China (Shanghai port) (FAOSTAT 2014a; FAOSTAT 2014b; Salin 2014). The remaining percentage is used mostly for biofuel production. According to Lapola *et al*. (2010), the largest volumes of Brazilian biofuel are derived from soybean, followed by sugar cane.

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4 The corresponding Technical Opinion does not specify what is meant by ”free registration”.

3.1.2 Food safety

Guiding questions:

a. Will the content and quantity of herbicide residues (active ingredients in the herbicide) in food increase/decrease?

b. Will intake of products from the GM HT plant have health effects (toxic, immunological, including allergic or anti-nutrient effects) that are acute; chronic; long-term; and/or lead to a change in metabolism and fertility?

c. Have the conclusions in the previous question been drawn on the basis of exposure to

- − plant material from the GM HT plant?
- − the protein expressed by the inserted gene, after extraction from tissue from the GM HT plant?
- − the protein expressed by the inserted gene in the organism it is obtained from?

Summary:

- The applicant does not provide information on the ***content and quantity of herbicide residues in food*** from the regular cultivation of Intacta™ Roundup Ready™2Pro. A recent study indicates that there is a presence of glyphosate and AMPA residues in RR soybean grains, compared with conventional and organic soybean. Based on the common GT soybean management, another study projects significant increases in glyphosate (five to ten times) and AMPA (ten to 25 times) residues in the harvested grain in comparison to conventional and organic harvests.

- Neither the applicant nor the available literature report any study on ***health effects resulting from the intake*** of products derived from Intacta™ Roundup Ready™2Pro. The applicant carried out research and a literature review on the individual proteins inserted in the GM HT crop analyzed here. However, no combinatorial effects of CP4 EPSPS and Cry1Ac proteins were studied. The applicant concludes that there are no potential adverse health effects and that possible changes in health status are negligible. However, the applicant provides in its report a methodological description and a set of raw data insufficient for a comprehensive evaluation of the robustness and adequacy of the tests carried out and the conclusions presented. This becomes an important limitation, particularly in light of other scientific studies that report severe chronic disorders from the consumption of RR crops due to glyphosate residues. Research on the effects of glyphosate and glyphosate-based commercial formulations found cellular and endocrine disruption, reproductive disorders, carcinogenic and teratogenic impacts, among other effects, at lower rates of exposure than regular agricultural use and the maximum residue limits established by United States and European Union regulations. Research also indicates that the adjuvants commonly used in the commercial glyphosate-based formulations (e.g. POE-15) influence the toxicity of the herbicides more than glyphosate as the active ingredient itself. In relation to compositional studies, a research found a higher content of saturated fatty acids in GM HT soybean.

- The applicant's conclusions regarding the possible health effects of Intacta™ Roundup Ready™2Pro are based on ***exposure*** to the bacterial protein version of the parental GM lines. Other studies (e.g. soil tests) use the plant material of RR soybean.

Content and quantity of herbicide residues in food

Health effects of herbicides in food, particularly the ones related to GM HT crops—glyphosate in the case of the crop analyzed in this report—have been subject of scientific and regulatory controversy (see Antoniou *et al.* 2012). Despite the fact that part of the scholarly (e.g. De Roos *et al.* 2005) and regulatory literature (e.g. BfR 2014; GTF 2013) indicates no adverse effects from exposure and intake of glyphosate, a large body of the research point out the contrary as described in a later section.

In relation to Intacta RR2 Pro soybean, neither the applicant nor the literature provides any information on the content and quantity of herbicide residues in food derived from this GM HT crop. The literature reports some relevant information concerning RR soybean. This is the case of a study by Bøhn *et al*. (2014), who compare the presence of glyphosate and AMPA residues in yield from different soybean production systems in the United States. In this analysis, no residues of glyphosate or AMPA were found in conventional and organic soybean, while the minimum and maximum values of residue concentration were 0.4–8.8 mg/kg for glyphosate and 0.7–10.0 mg/kg for AMPA in RR soybean harvests. Regarding glyphosate, the residue concentrations found were below the current European Union standards (20 mg/kg). However, the authors note that this threshold was increased (from 0.1 mg/kg in 1999) as a pragmatic response to expanded use and subsequent rise in residues of glyphosate in soybean grains, rather than on basis of new evidence of decreased toxicity.

The work of Bøhn *et al*. (2014) is consistent with earlier research done by Arregui *et al*. (2004). They found glyphosate and AMPA residues in leaves and grains of RR soybean cultivated in Argentina. These authors indicate that the more the glyphosate applied during the cultivating cycle (particularly during flowering), the higher residue concentration in the grains.

Given the common management of RR soybean, in which glyphosate is applied later in the growing season as a desiccant for homogenizing the maturation in order to facilitate mechanical harvest operations, an increase in the grain´s glyphosate residue concentration from five to ten times, and ten to 25 times for AMPA, is expected (Duke *et al*., 2003).

Health effects resulting from the intake of products derived from GM HT plant

The applicant reports no potential adverse effects from the consumption of Intacta RR2 Pro soybean. This conclusion is drawn from the following tests:

- *Effects in the human and animal food chain due to the ingestion of individual CP4 EPSPS and Cry1Ac proteins*. Conclusions on their safety derived from their historical use of the donor organisms, *Agrobacterium* sp. and *Bacillus thuringiensis*, respectively, as well as the trajectory of GM crops containing the genes that synthesize the corresponding proteins. Analysis of exposure thresholds reveled no adverse effects, according to the applicant. Additionally, the dossier indicates the possibility of low/no risks due to: (i) fast digestibility of both proteins in the gut fluids, particularly in mammals, resulting in negligible allergenic potential, and (ii) the absence—in relation to the proteins in question—of similar amino acid sequences to either known allergens or toxic proteins that may have adverse effects in mammals. The applicant concludes that Intacta RR2 Pro is safe based on the tests applied to its GM parental lines. Yet, the potential safety effects were studied in separate assays for each protein, not both ingested at the same time. Hence, combinatorial effects of Intacta RR2 Pro were not assessed.

- *Margins of exposure*. Margins of exposure of Cry1Ac and CP4 EPSPS in human diets (from soybean flour, grains and milk) were calculated using the parameters of the USDA Continuing Survey of Food Intakes by Individuals. The applicant does not specify the parameter for comparison.

- *Absorption of exogenous proteins*. Based on a literature review, the applicant claims that there is a minor possibility that absorption of the exogenous proteins present in Intacta RR2 Pro—intact and in sufficient quantities to enter animal fetal circulation—may occur. Upon the results of compositional analyses carried out by the applicant, it is also indicated that this GM soybean has a negligible capacity to produce toxins or metabolites with adverse effects on humans or animals. Based on the substantial equivalence approach—and the similarities between Intacta RR2 Pro, the GM parental lines and conventional varieties—the applicant concludes that there is no possibility of toxicity of the inserted traits.

- *Acute toxicity.* Studies of acute oral toxicity were carried out for both Cry1Ac and CP4 EPSPS using the crystalized bacterial versions, since they are considered by the applicant to be equivalent to the versions of the proteins produced in the GM parental lines. With regard to Cry1Ac, it was concluded that given the structural and functional similarities and high rates of fast digestibility, it is not likely that it will cause acute oral toxicity. As for CP4 EPSPS, it is characterized as safe by the applicant due to a lack of structural similarities with the possible toxic proteins in soybean that act as allergens or anti-nutrients (such as lecithin and protease inhibitors). Confirmation tests (the experimental design of which were not described) were carried out on mice, concluding that: (i) no statistical differences were found among the subjects compared, (ii) there is high digestibility of the inserted protein, (iii) there is an absence of structural similarity with toxins and allergens (according to the results of bioinformatics analysis), and that (iv) these proteins represent a small portion of the total grain protein intake. The applicant concludes that no acute oral toxicity or allergenic potential of the protein were found and that Intacta RR2 Pro is safe as food and feed.

It is difficult to assess the robustness and adequacy of the results reported because the methodologies applied and the majority of the raw data are not provided. An evident drawback of the safety assessments carried out by the applicant is the use of the bacterial version of the protein. As indicated before, equivalence between the bacterial and the plant version of proteins occurs only at a structural but not necessary at a processing level, since plants and bacteria have dissimilar post-translational protein processing mechanisms

(Raybould *et al*. 2013). Accordingly, different results than those reported may be obtained if the plant version of the protein is tested.

Contrary to the findings reported by the applicant, other studies point to health effects from the intake of GM HT plants resulting from the genetic modification and effects from the inherent herbicide. The next paragraphs indicate some of the findings described in the scientific literature in relation to this.

Potential chronic disorders from consumption of GM RR crops. A life-cycle study of mice fed with RR maize (testing Roundup Ready® treated and non-treated GM maize) and exposed to Roundup Ready® showed the following hormone and sex-dependent chronic disorders: (i) internal organ defects, such as kidney deficiencies in males and females, and liver congestions and necrosis in males at an incidence rate of 2.5 - 5.5 times higher than the control, (ii) nephropathies in males with an occurrence 1.3 - 2.3 times higher than the comparator, (iii) increased mortality in females up to 2 - 3 fold, and (iv) a higher incidence of tumors (mammary tumors in females, while in males tumors tended to be four times larger and palpable 600 days earlier than in controls). The authors conclude that the chronic health effects may result from the non-linear endocrine disrupting effects of Roundup Ready®, the overexpression of the EPSPS protein inserted, or the mutational effects and metabolite impacts of the GM HT maize studied (Séralini *et al*. 2014). Although this research was the subject of a large controversy and much scientific disagreement, its main findings are relevant to illustrate the importance of carrying out long-term feeding studies.

Health effects from glyphosate and glyphosate-based commercial formulations. Dallegrave *et al*. (2003) report teratogen effects of glyphosate and Roundup Ready® formulations—such as those commercialized in Brazil—causing 50 percent mortality in rats during laboratory studies when exposed to the highest concentration tested (1,000 mg/kg), and alteration in skeletal development from 500 mg/kg exposure. Some authors indicated that Dallegrave *et al*. (2003) experimental rates were particularly and unrealistically high (Aris and Leblanc 2011). Hence, in another study, Dallegrave *et al*. (2007) analyzed reproductive effects of glyphosate—in a commercial formulation available in Brazil—using much lower exposure rates (50, 150 and 450 mg/kg). The results indicate adverse reproductive effects on male offspring, expressed in alterations of sperm (decrease in tail and daily production, and increase in abnormal sperm production), among other effects.

Richard *et al*. (2005) report that glyphosate toxicity to human placental cells exists within 18 hours of exposure at lower rates than agricultural use. Complementarily, Benachour and Séralini (2009) and Gasnier *et al.* (2009) point out that Roundup Ready® is an endocrine disruptor at concentrations lower than the maximum residue limit (MRL) according to United States and European Union regulation. Gasnier *et al*. (2009) established that glyphosate-based herbicides are carcinogenic, mutagenic and reprotoxic due to their human cell effects at sub-agricultural doses (endocrine disruption starting at 0.5 ppm, cytotoxicity at 10 ppm and DNA damage at 5 ppm). Aris and Leblanc (2011) found residues of glyphosate in 5 percent of non-pregnant women participating in a study intended to determine GM HT-plant herbicide residues in blood. Research by Cassault-Meyer *et al*. (2014) evaluated the acute exposure to Roundup® on male rats in terms of endocrine and testicular functions. They observed alterations in aromatase mRNA levels in at least 50 percent of the treated rats. They also described increased abnormal sperm morphology, among other effects, although sperm concentration and motility were normal.

***Health effects from adjuvants present in the commercial glyphosate-base formulations*.** Complementary to the above-cited studies, the literature indicates that there is a link between surfactants used in commercial formulations of Roundup Ready®, and effects on human development and reproduction (Williams *et al*. 2012). For instance, the following findings were observed: an increase in glyphosate´s teratogen behavior (Antoniou *et al*. 2010); increased placental cell toxicity; and higher bioavailability and bioaccumulation (Richard *et al*. 2005). Additionally, Mesnage *et al*. (2013) applied a compositional and toxicity comparison among nine glyphosate-based formulations, POE-15 (polyethoxylated tallowamine, also known as POEA, the most common adjuvant used), pure glyphosate, and an herbicide formulation without glyphosate. They report that POE-15 had the most toxic effects in human cells, causing damage on cellular respiration and membrane integrity, and developing cellular necrosis. The effects were observed at 1 to 3 ppm of POE-15, which is the range of environmental and occupational doses. Gasnier *et al*. (2009) state that the toxic effects of glyphosate-based formulations are more influenced by the adjuvants than by the concentration of glyphosate itself.

Changes in composition. Bøhn *et al*. (2014) applied a compositional comparative study between organic, conventional and RR soybean. In the GM HT soybean grains analyzed, higher content of saturated fatty acids was found (e.g. palmitic acid) than that recommended for consumption due to possible health effects.

Material used for assessing exposure

The applicant´s laboratory tests and safety conclusions regarding Intacta RR2 Pro are based on exposure to the crystalized bacterial versions of the proteins obtained from *Agrobacterium* sp. (for CP4 EPSPS expression), and *Bacillus thuringiensis* (for Cry1Ac expression). For agronomic and compositional analysis, they used plant material (both leaf and grain samples) from Intacta RR2 Pro or RR soybean (MON 89788).

Photo: Andrea Wilhelm #68776995 (dollarphotoclub.com)

3.1.3 Food quality

Guiding questions:

a. Does the GM HT plant yield better/poorer nutrition in terms of composition, quantity and energy content?
b. Does the GM HT plant have properties that make the crop last better/more poorly during storage?
c. Does cultivation of the GM HT plant yield greater/less benefits for the consumer?

Summary:

- The applicant reports no ***compositional differences*** between Intacta™Roundup Ready™2Pro, their parental GM lines and conventional varieties, concluding that there is a substantial equivalence with comparable soybean varieties and therefore no alteration in food safety. No other study on this GM HT variety was found. A recent related research indicates nutritional differences between RR, organic and conventional soybean, specifically higher total protein, microelements and sugar content found in organic varieties, and higher saturated fat in RR soybean.

- Neither the applicant nor the literature consulted report any ***changes in storage properties*** or ***benefits to consumers*** of Intacta™ Roundup Ready™2Pro. In relation to the latter, this GM HT variety corresponds to the first generation of GM crops, which was intended to create value among producers.

Compositional analysis and changes in nutritional content

The applicant concludes that there is no difference in the compositional analysis of Intacta RR2 Pro in comparison to its conventional counterparts. This conclusion is based on assessments of: (i) MON 87701 (one of the GM parental lines tolerant to insects) and the conventional cultivar A5547, (ii) MON 89788 (the other GM parental lines tolerant to herbicides) and the conventional cultivar A3244, and (iii) MON 87701 x MON 89788 (Intacta RR2 Pro). The applicant presents results on its analysis of fiber, ash, carbohydrates, lipids, proteins, fatty acids, vitamins, isoflavonoids, antinutrients and humidity, indicating that no significant compositional differences exist among Intacta RR2 Pro, its parental and conventional lines (not specified). Additionally, the applicant concludes that all these varieties are substantially equivalent and do not alter food safety.

The study by Bøhn *et al.* (2014) on the compositional difference between GM HT, conventional and organic soybean yields found major differences between harvest from GM HT and organic production systems. This research showed that organic soybean had higher total proteins, microelements (in five of the seven microelements characterized) and sugar content, as well as lower levels of total saturated fat. With regard to the latter, RR soybean had higher palmitic acid content, usually recommended for limited consumption only. No other compositional studies were found in the literature on the GM HT soybean analyzed in this report.

Changes in storage properties

The modification does not relate to storage properties, hence the applicant does not provide information on possible changes in this regard, and no complementary information was found on this topic.

Changes in the benefits to consumers

No information is reported regarding benefits to consumers, neither by the applicant nor in the literature consulted. However, the traits inserted correspond to the first generation of GM crops, which focused on agronomic properties intended to benefit producers.

3.2 Animal health and welfare

Guiding question:

Feed quality: Do the products of the GM HT plant improve/detract from feed quality?

Summary:

- The applicant reported no changes in the ***feed quality and weight gain efficiency*** of animal feed with Intacta™ Roundup Ready™2Pro. The applicant also reports no adverse effects on animal nutrition and health based on feed, toxicity and digestibility trials.

- At the time of the review, no other studies were found on ***animal welfare*** when fed with Intacta™ Roundup Ready™2Pro. However, a comparative assessment of pigs fed with insect resistant maize and HT soybean reports significant disorders, particularly in relation to internal organ health among animals fed GM-diets.

Based on the following tests, the applicant indicates that there are no changes in feed quality and weight gain performance, and hence no possibility of adverse effects on animal nutrition and health:

- *Chicken feed tests for evaluating weight gain*. A study with farmed chicken was carried out for 42 days to compare the nutritional value of diets based on Intacta RR2 Pro, conventional soybean A5547 and diets derived from six other conventional cultivars. The study included a previous analysis of pesticide residues and mycotoxins. The performance parameters assessed were weight gain and body weight per unit of feed weight consumed. The applicant concludes that there was an absence of biological differences in the performance of chickens fed with the different diets, and that the GM and non-GM diets are nutritionally equivalent.

- *Animal toxicity feed trial.* Based on a 90-day animal feed trial, the applicant indicates that exogenous proteins in the Intacta RR2 Pro soybean are structurally and functionally unrelated to toxic proteins, and therefore are exempted from pharmacologically active substances with possible adverse effects on the welfare of animals and their offspring.

- *Digestibility tests.* The applicant reports that digestibility of Cry1Ac in simulated gastric and intestinal fluid is fast and highly efficient, and that the stable fragments do not show a reaction with antibodies specific to Cry1Ac, discarding the possibility of allergenic potential. The applicant mentions that this protein is safe to use. However, since the details on the peptide fragments are not provided, it is difficult to assess whether antibodies could be triggered or not. Similar tests to Cry1Ac were carried out for the protein CP4 EPSPS with identical conclusions. Additionally, it is stated that there is no evidence that the proteins inserted are mutagenic, teratogenic or carcinogenic.

No other studies were found on Intacta RR2 Pro used as animal feed. Perhaps the closest research is that carried out by Carman *et al*. (2013), in which they report a comparative assessment of biological effects of a GM-based diet (a mixture of insect-resistant corn with GM HT soybean) and non-GM diets in commercially raised pigs. They report the following differences in relation to the control:

- Increase in uterus weight by 25 percent in GM-fed pigs
- Stomach inflammation in 41 percent of the animals fed GM diets, showing an increase in inflammation rates of 4 and 2.2 times in males and females, respectively, compared to those fed non-GM diets
- GM-fed animals had an average of 16 percent lower concentration of GGT (a blood substance linked to liver health)

Carman *et al*. (2013) conclude that substantial differences on health effects were observed when feeding animals with GM and non-GM feed. Further analysis will be needed to determine to which extent the characterized health impacts relate to the genetic modification introduced in the GM feed and to herbicide residues.

3.3 Living conditions and profitability for farmers who cultivate GM HT plants, in the short term (less than five years) and in the long term (more than 20 years)

3.3.1 Health and safety

Guiding questions:

a. Will any changes in the use of the herbicide affect the health of the farmers/farmworkers positively/negatively?

b. Will farmers/farmworkers be given HES training and access to protective equipment and the information they need in order to use the herbicide(s) that is/are to be used with the GM HT plant?

Summary:

- Information related to the ***effects on farmers' and farmworkers' health due to changes in herbicide*** use with the adoption of Intacta™ Roundup Ready™2Pro soybean is not presented by the applicant nor reported in the literature. Studies mostly refer to RR soybean, indicating either a decrease in herbicide exposure during the first years of adoption or an increased use of glyphosate and other complementary, more toxic herbicides in the medium- and long-term due to the appearance of weeds resistant to glyphosate.

- ***HES systems*** are implemented mostly on voluntary basis. There is limited enforcement of safety regulations by pesticides manufacturers or importers. In practice, the burden of safety and responsibility rests on the producers. The use of basic or insufficient protection equipment during pesticides applications is common.

Effects on farmers' and farmworkers' health due to changes in herbicide use

The applicant does not include information about this topic, and no specific empirical studies in Brazil were found regarding Intacta RR2 Pro soybean. The literature points out two different possible effects on farmers and farmworkers in relation to GM and GM HT crops. First and generally speaking, the studies conclude that there is a decrease in exposure to pesticides with the adoption of GM crops (Brookes and Barfoot 2014; Burachik 2010; Qaim and Traxler 2005). However, these analyses are based on short-term empirical data calculating or modeling the EIQ. As indicated in section 3.1.1 ("Food security"), this approach has significant limitations since it quantifies exposure to the active ingredient only; yet, farmers and farmworkers are exposed to glyphosate´s commercial formulations that have a much greater risk factor since include other toxic substances (Surgan *et al*. 2010) resulting from the toxicity of the adjuvant (Mesnage *et al*. 2013). Moreover, such calculations do not consider: (i) the context of more permissive regulations of developing countries, resulting in the use of more toxic commercial formulations (Qaim 2009), and (ii) the fact that poorer farmers tend to purchase cheap pesticides, which may be formulated with old or banned chemicals (Areal *et al*. 2012). Accordingly, EIQ-based studies require complementary research based on the commercial formulations and real conditions of

Photo: sima #28249118 (dollarphotoclub.com) herbicide applications.

Other effects described in the literature relate to the increased exposure to glyphosate and complementary herbicides a few years after the adoption of GM HT crops (Bonny 2011). This is the result of decreased glyphosate efficacy as illustrated by the appearance of GR weeds (Catacora-Vargas *et al*. 2012; Waltz 2010). The applicant suggests the application of highly toxic herbicides (e.g. atrazine, dicamba, fluroxypyr and paraquat) for controlling GR weeds. The increased application of such herbicides can escalate the exposure to harmful chemicals for farmers, farmworkers and communities located near GM HT soybean cultivation. Such changes can have important public health implications (for instance, see Barry *et al*. 2012; Corasaniti *et al*. 1998; Kuter *et al*. 2007; and Sun *et al*., 2014).

The increase in volume of toxic pesticides used is not unique to GM HT crops, but rather is implicit to the agroindustrial technological package they are part of. However, losses of efficacy in weed control in GM HT crops perpetuate high pesticide exposure in agroindustrial systems.

Health, environment and safety (HES) systems and access to protective equipment

In Brazil, as in most other developing countries, HES systems are implemented on a voluntary basis in agricultural management. There is limited enforcement of regulations imposed on pesticides manufacturers or importers. In practice, the burden of safety and responsibility rests on the user.

The management of pesticides (e.g. containers) varies according to the production scale. Usually, large-scale production systems implement at least minimum safety measures, such as special pesticide storage rooms. This is less common in small-scale production systems, where pesticide containers are often stored inside houses, or left in the field. Farmers use basic or insufficient protective equipment when manually applying pesticides in small-scale production systems. In large-scales schemes, a common argument is that there is no need for protective measures since applications are done from machinery cabins [*personal communications and field observations*]. No official reports or formal research was found on this topic.

3.3.2 Contacts and network conditions

Guiding question:

Are there restrictions on access to seed, the right to terminate contracts, or on information about seeds, spraying schedules and prevention of resistant weeds?

Summary:

Restriction on ***access to seed*** is determined by legal excludability through patents, contracts, seed prices and local seed availability. According to farmers' testimonies, large-scale production systems benefit from more flexibility regarding termination of contracts and also receive, in general, more training and information on the technology related to GM crops.

The conditions for accessing seeds of Intacta RR2 Pro are described in section 3.3.4 ("Right to seeds") and 3.8 ("Freedom to choose agricultural systems in the future"). According to farmers' testimonies [*personal communication*], the right to terminate contracts, seed access privileges and technical support on the herbicide regime and management of resistant weeds is provided to large-scale farming systems, but not to the small-scale ones. Further research is needed on these issues.

3.3.3 Developments in costs and revenues for farmers in the short term (less than five years) and in the long term (more than 20 years)

Guiding questions:

a. Will farmers' costs for input factors increase/decrease?
b. Will the GM HT plants reduce the need, in the short and/or long term, for other input factors such as production plans, spraying programs, work input, and machinery and equipment?
c. Will any resistance problems increase in the future, and in the event, reduce profitability in the long term?

Summary:

- The applicant indicates that there are production and economic advantages with the adoption of Intacta™ Roundup Ready™2Pro. However, no data supporting such statements is presented. The literature contains information on ***changes in costs for input factors*** in relation to RR soybean. On one hand, decreased costs are registered in the first years of adoption due to the reduction in herbicide use (before the emergence of GR weeds), machinery operation and labor requirements. However, the technology fees on seeds increase costs. Nutritional inefficiency imposed by glyphosate applications, according to recent research, calls for new fertilization programs. Currently, another cause of increase in production costs is the high prices of land (both for lease and purchase). In Brazil, Intacta™ Roundup Ready™2Pro soybean seeds have a cost at least five times higher than RR soybean due to royalty fees.

- Current experiences on RR soybean indicate that ***changes in the need for other factor inputs and weed resistance*** determine a decrease in ***profitability*** in the long term. The higher need for herbicides (in terms of both volume and type) for controlling HR weeds, and for producing multiple-herbicide tolerant crops reduces the profitability in the long term, while non-GM soybean producers receive higher payment from GM-free export markets. Research on the increased nutritional inefficiency of the second generation of HT soybean also indicates that there is a need for more input factors (fertilizers) in the medium- and long-term.

Changes in costs for input factors

The applicant indicates that Intacta RR2 Pro is a variety that will eliminate or reduce the use of agrochemicals, improve the current cultural practices and increase efficiency in the production system of soybean and the productive potential of the crop. However, no empirical data supports these statements.

Specific studies on this topic in Brazil are limited. The following paragraphs provide insights as to the experience of RR soybean in Brazil and other countries.

A comparative study conducted by the Ministry of Agriculture, Livestock and Fisheries of Argentina and the Inter-American Institute for Cooperation on Agriculture (IICA) reports that the production costs of soybean in Brazil during the 2010/2011 growing season varied from USD 543 to 614/ha, the highest in South America in comparison to the major soybean producing countries (i.e. Argentina, Paraguay, Uruguay and Bolivia). The distribution of costs among different production items is relatively similar when comparing GM and conventional soybean production systems, with RR soybean having the lowest production costs during the first years after adoption (USD 15-30/ha less compared to conventional systems) (MAGP and IICA 2013).

Different researchers agree that during the first years of production of RR soybean, the efficacy in weed control—before the emergence of GR weeds—results in decreased production costs due to the reduction of herbicide applications (for instance, see: Areal *et al*. 2012; Pengue 2005a; and Qaim 2009). Additionally, there is also a decreased cost of machinery operation, fuel and labor requirements (Acosta Reveles 2008; Gianessi 2008; Qaim 2009). See section 3.4.3 ("Employment") for additional information on changes in labor costs. Another factor for the decrease in herbicide costs is the reduced price of glyphosate-based formulations due to its patent expiration (Brookes and Barfoot 2014; Qaim and Traxler 2005).

The initial drop in weed control costs are countered by the increase in seed prices resulting from royalty payments. A meta-analysis of related studies done by Areal *et al.* (2012) indicate a general rise in the price of GM seeds ranging from USD 2.6 to 16.3/ha, noting that this tendency is less clear in GM HT crops because of the limited research despite their level of adoption. Further information on seed prices is provided in section 3.4.4 ("Ownership rights").

Research by Zobiole *et al*. (2012) found reduced nutritional efficiency in RR2 in comparison to RR1 varieties, and of these two when compared to conventional soybean. These findings indicate that—under the conventional agroindustrial production systems—there is more of a need for the application of fertilizers; hence, a possible increase in production costs, to compensate for the nutritional imbalances that appear to result from the extensive glyphosate use.

Changes in land access and prices significantly increase the costs of GM HT soybean production. The expansion of this GM crop over new agricultural lands and forests (Pengue 2005b), together with high soybean selling prices (IMF 2012), have caused a parallel process of over-demand for land for soybean cultivation and ascending land prices (Catacora-Vargas *et al*. 2012).

The price of seeds of Intacta RR2 Pro soybean is five times higher than RR soybean due to the increase in the royalty fees applied (Rural BR 2013). Section 3.4.4 (“Ownership rights”) provides further details.

Short and long-term need for other input factors and changes in weed resistance and profitability in the long-term

On the whole, although production costs for RR soybean are reported to be lower than conventional soybean in the short term, in the long run the overall production expenses for RR soybean show to be higher (USD 25 - 45 per hectare more). This is mainly due to the need for complementary herbicides to glyphosate for controlling emerging HR weeds (for further details on the management of GR weeds, see section 2.2.2 “Effects of an altered spraying regime” subtitle “Changes in the timing of herbicide application”, and section 2.2.3 “Resistance of other plants to the herbicide”). Conversely, the costs of conventional production have remained relatively constant, particularly with the decreased price of pesticides, according to MAGP and IICA (2013). This source also indicates that the maximum difference in costs between GM and conventional soybean is 15 percent. Contrary to these numbers, the study concludes that GM soybean production in the region is 15 percent cheaper than conventional production.

In line with these studies, glyphosate weed resistance emergence (see section 2.2.3 “Resistance of other plants to the herbicide”) results in the need for more herbicides in terms of quantity, type and toxicity. For instance, in Argentina, from 2005 to 2010 the volume of paraquat applied increased by 45 percent (Catacora-Vargas et al. 2012). Authors such as Bonny (2011), Bryant *et al*. (2003) and Pengue (2005b) provide a contextual analysis of the augmented use of herbicides due to weed resistance emergence, which implies increased expenses in weed management. Binimelis *et al*. (2009) state that, with the new stacked-GM HT soybean varieties exhibiting tolerance to multiple herbicides, the use of herbicides will constantly grow. Examples of such multiple GM HT soybean varieties are Genuity® Roundup Ready™ 2 Xtend™ (tolerant to glyphosate and dicamba) and DAS 444Ø6-6 (tolerant to glyphosate, 2,4-D and glufosinate ammonium), all tolerant to herbicides that are either strictly regulated or not permitted to use in Europe, with the exception of glyphosate.

As indicated previously, the decreased nutritional efficiency (expressed as the reduced capacity of nutrient uptake) of RR soybean in general—and particularly of Intacta RR2 Pro—may impose the need in the agroindustrial systems for more fertilizers to compensate for such nutritional deficits in the medium- and long-term (Zobiole *et al*. 2012).

In relation to profitability and revenue, during the 2013/2014 growing season, conventional soybean growers obtained higher payment for GM-free soybean destined for Europe. The bonus price paid to GM-free soybean growers represented 10 to 15 percent of regular producers income, resulting in a gross income advantage of USD 300 per hectare (CIB 2014).

3.3.4 Agronomic factors

Guiding question:

What sort of cultivation conditions, soil types and technological standards have the GM HT plant varieties been developed for?

Summary:

Intacta™ Roundup Ready™2Pro was originally developed in the United States. However, the specific ***cultivation conditions and soil types*** are not reported. Field trials in this country and in Brazil were carried out in different eco-regions; hence, it is inferred that it is adapted to a wide range of agroecological conditions (including soil types). As for ***technological standards***, since GM HT soybean was developed mostly for agroindustrial mono-cropping systems, it is highly reliant on external and synthetic inputs, as well as on heavy machinery.

Cultivation conditions and soil types

The specific cultivation conditions and soil types for which Intacta RR2 Pro was developed are not reported by the applicant. Based on the locations of the growth and yield tests that the applicant carried out in the United States, it can be inferred that it was adapted to a wide range of agroecological conditions, although one of the Intacta RR2 Pro parental lines (MON 87701) was developed from a cultivar adapted to the Mid-South and the East Coast of this country. In the United States, Intacta RR2 Pro tests were held in 12 different states[5] from the Midwest and South regions, located in forest and herbaceous ecotypes. The testing sites represent temperate and tropical thermo-types, ranging from semi-arid to ultra-hyper humid, and from hyper-arid to ultra-hyper humid iso-bioclimates, respectively (USGS/US Department of the Interior 2014).

In Brazil, the applicant reports that field tests were conducted on phenotypic and agronomic characteristics, protein expression and ecological interactions in four locations, each one in a different state.[6] No information on the soil characteristics is provided for field test site. Based on what it is reported by the applicant, two of the field trials were conducted in the Atlantic forest biome, one in the Cerrado and the other in the Amazon (South Amazon river basin) (CSR 2014).

Technological standards

Given the experience with RR soybean and the inherent application of synthetic herbicides that GM HT crops imply, Intacta RR2 Pro has been developed for agroindustrial production systems, which are characterized by mono-cropping, reliance on heavy machinery and significant use of synthetic external inputs.

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[5] Alabama, Arkansas, Georgia, Illinois, Indiana, Kansas, Louisiana, Mississippi, North Carolina, South Caroline, Texas and Virginia.

[6] Same sites indicated in footnote 1.

3.3.5 Right to seeds

Guiding question:

Does the applicant restrict the farmers' possibilities of saving, exchanging or selling seed from their own harvest?

Summary:

The applicant does not provide information concerning ***farmers´ possibilities of saving and exchanging seeds***. However, legal excludability applies in Brazil under the "Terms of Technology Licensing" as contracts signed by Monsanto and each producer at the time of purchasing the Intacta^{TM} Roundup Ready^{TM}2Pro seeds.

The applicant does not provide information on the conditions of access to, use and exchange of Intacta RR2 Pro seeds in Brazil. However, legal excludability applies in the country involving restrictions to save and re-use such seeds under the "Terms of Technology Licensing", a legally binding agreement between Monsanto and each producer purchasing their seeds. According to the Brazilian Law on Crop Protection 9.456/97, this restriction will be in effect for 15 years following the international registration of Monsanto's patent of this GM HT soybean variety (see section 3.4.4 "Ownership rights" for complementary information).

Photo: sima #29895549 (dollarphotoclub.com)

3.4 *Living conditions and profitability in the production area, in the short term (less than five years) and in the long term (more than 20 years)*

3.4.1 Health and safety

Guiding question:

Will any change in the use of the herbicide affect the health of the community positively or negatively?

Summary:

Neither the applicant nor the literature reviewed provides information on the ***effects on communal health*** due to changes in the use of herbicides arising from Intacta^{TM} Roundup Ready^{TM}2Pro adoption. Public health records from countries such as Argentina indicate a significant increase in chronic diseases in surrounding GM HT crop production areas because of the constant exposure to the agrochemicals applied to RR soybean cultivation. Crops tolerant to multiple herbicides may aggravate such exposure given the use of more toxic herbicides such as glufosinate, dicamba and 2,4-D. The lack of official and publicly available data, together with weak regulations, pose in both general and specific terms important challenges regarding public health linked to the agroindustrial production system, including GM HT crops.

The applicant does not provide information on public health issues linked to Intacta RR2 Pro adoption. No research was found on the possible effects on communal health from changes in herbicide use derived from Intacta RR2 Pro soybean in Brazil. The available information related to experiences on GM GT soybean production in Argentina and Paraguay.

Arandia (2009) and Tomei and Upham (2009) report in the community of Ituzaingó, Córdoba province in Argentina, an increase in chronic diseases resulting from the constant exposure to the agrochemicals applied in the surroundings of RR soybean cultivation. The main chronic diseases identified are cancer, respiratory dysfunction, neurological illness and newborn malformation, among others. The same authors indicate that in 2009 Ituziagó, a community of 5,000 inhabitants, registered 200 cases of cancer. The Faculty of Medical Sciences of the National University of Córdoba confirmed the consistency between the testimonies of local people and newspaper articles with clinical records from the local hospitals. Other soybean producing regions also report an increase in chronic disorders. This is the case of the Chaco area, where the neonatal service of one local hospital recorded an increase in congenital malformations of 46 cases in 1997 (the year GM HT soybean was approved) to 186 in 2008, a four-fold increase in terms of number of cases. These figures correspond to 19.1 and 85.3 malformation per 10,000 newborns in the region in 1997 and 2008, respectively (Ávila and Nota 2010).

The long-term and multiple pathways of exposure to several agrochemicals; the lack of official monitoring and data collection on pesticide applications, residues and metabolites; and the weak implementation of pesticide and biosafety regulations pose significant challenges to public health related to agrochemical use in industrial agriculture, including GM crops. The trend of cultivating GM crops with tolerance to multiple herbicides may aggravate community health problems given the increased exposure to more toxic herbicides (e.g. glufosinate, dicamba and 2,4-D).

Additional information related to on-farm herbicide applications, is described in section 3.3.1 (“Health and safety”).

3.4.2 The democratic rights and profitability of other farmers

Guiding questions:

a. Are there rules for co-existence, and are they complied with, such that it is possible to choose to cultivate non-GM, for example organic, crops instead of GM HT crops?
b. Is there a system for preventing the spread of GM HT crops to other, non-GM crops?
c. Is there a compensation system if other farmers are affected by unintentional dispersal of genes,

pollen or seed from the GM HT crop?
d. Is there a system for keeping GM and non-GM crops separate in the production and transport line and, in the event, who pays for this system?
e. Will cultivation of the GM HT crop lead to more or less problems with weeds for other farmers?

Summary:

In Brazil:

- ***Rules for co-existence*** focus on GM maize only with regard to separation distances.

- ***Systems for preventing the spread of HT crops*** are restricted to risk classification and biosafety measures for GMOs and GMO products under containment.

- ***Systems for separating GM and non-GM crops*** are implemented only by people and institutions interested in segregating non-GM produce.

- There is an absence of an official and specific set of regulations for ***compensation for unintentional dispersal of GM genes, pollen or seeds.*** Farmer testimonies indicate that large-scale farming systems have a greater possibility of compensation compared to small-scale farmers. Brazil has signed the Nagoya-Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety, but ratification is still pending.

- ***Problems with weeds for other farmers*** may increase with the Intacta™ Roundup Ready™2Pro through different pathways such as the spread of GR weeds, glyphosate drift to neighboring fields and volunteer HR weeds.

Rules for co-existence

The Brazilian Biosafety Framework on co-existence refers to GM maize only and focuses on separation distances (Normative Act No. 4 2007) without considering other relevant ecological and socio-economic factors as indicated in section 2.1.3 ("Gene flow"). Based on the field observations of this report´s author, co-existence rules are inconsistently complied with and have limited effectiveness in protecting the ability of farmers to choose among different production systems. There are no similar regulations for GM HT soy.

Systems for preventing the spread of GM HT crops

The Brazilian Biosafety Framework addresses the prevention of GM HT crop spread in general terms, prescribing risk classification and biosafety measures for GMOs and GMO products under containment. GM plants for containment must be assessed in relation to their capacity for spreading reproductive structures, including mechanisms for dispersal via air, water and soil, as well as pollen viability, pollinators and geographical location within Brazil (Normative Act No. 2 2006).

Systems for separating GM from non-GM crops

In operational terms, the measures for preventing the commingling of GM HT and non-GM HT crops, as well as the separation of production and transport lines, are implemented and financed by traders or organizations (including farmers associations) interested in segregating non-GM produce, most commonly those who sell their harvest to markets demanding for GM-free or organic soybean.

Compensation for unintentional GM-crop dispersal

Official and GMO-specific compensation systems for unintentional dispersal of genes, pollen or seeds have not been put in place in Brazil yet. The testimonies of producers dedicated to large-scale cultivation indicate that they are able to successfully access compensation for non-GM market loss through regular civil procedures. However, small-scale farmers seem skeptical of the application of such a compensation system for producers with little economic power [*personal communication*].

At the international level, in March 2012 Brazil signed the Nagoya-Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety. Ratification and entry into force are still pending (CBD 2014).

Problems with weeds for other farmers

Based on the current experience with RR soybean, the cultivation of GM HT crops such as Intacta RR2 Pro may lead to more problems with weeds for other farmers due to the natural spread of HR weeds species, volunteer weeds and drift to neighboring fields during application (Owen and Zelaya 2005; Wagner 2011). Sections 2.2.3 ("Resistance of other plants to the herbicide") and 3.3.2 ("Development in costs and revenues for farmers in the short-term") provide additional information on the potential spread and relevance of weed species expressing herbicide tolerance.

3.4.3 Employment

Guiding questions:

a. Will the cultivation of the GM HT crop create more or less employment locally and regionally?
b. Will the cultivation of the GM HT crop create more or less employment for women?

Summary:

The applicant does not provide information on the possibilities for ***job creation*** locally or regionally for either men or women with the adoption of Intacta™ Roundup Ready™2Pro. Studies on RR soybean point to a decrease in labor opportunities, the externalization of unemployment, and a reduction in small-scale and family farming as a result of the RR soybean introduction.

The applicant does not provide information on the possibilities of job creation locally or regionally for either men or women with the adoption of Intacta RR2 Pro. In the literature, the majority of the existing information reports general projections on this issue for GM HT crops and GM HT soybean in few Latin American countries.

With regard to Brazil, based on local farms "emergy" calculations, Ortega *et al.* (2005) report that there is an externalization (meaning off-loading to society) of unemployment and a rural exodus equivalent to USD 50/ha/year resulting from the adoption of GM HT soybean.

Soybean harvest
Photo: illini #19741843 (dollarphotoclub.com)

As for other countries and regions, Pengue (2005a) reports that there is a decrease in labor costs in Argentina because of the reduced labor requirements of GM HT soybean, specifically due to the simplification of weed management and labor replacement by machinery. This is consistent with the GM HT soybean analysis done by Qaim (2009), who indicate the existence of a general trend of descending labor costs in this type of crop. Additionally, Gianessi (2008) mentions that RR soybean decreases 23 percent of household labor. Both authors state that such decrease in workload results in more time available for pursuing off-farm activities. However, these analyses do not address the issue of concrete off-farm job opportunities. Correspondingly, Trigo and Cap (2003) suggested that the decrease in on-farm labor requirements in RR soybean production is compensated for by the aggregate labor demand generated by the expansion of this crop. However, there are no empirical data to prove this.

Contrary to Trigo and Cap's view, using data from the Uruguayan Directorate of Agricultural Statistics, Oyhantçabal and Narbondo (2010) observe that the expansion of RR soybean is leading to the disappearance of small-scale and family farming, including a reduction in employment and a constant decrease in the demand for permanent employment (see section 3.4.4 "Ownership rights"). This is an outcome of the decrease in labor from three hours/man/ha in conventional production, to 40 min/man/ha in RR soybean cultivation. According to these authors, given the technological package of GM RR soybean, one to three workers are needed for 1,000 hectares. They also calculated a loss of approximately 2,280 jobs for rural families in 2006 as a result of the increased area occupied by industrial RR soybean production.

Current and proposed solutions to the managerial challenges of GM HT crops, such as GR weeds, are highly technology based, relying on the use of heavy machinery and stacked GM crops tolerant to multiple herbicides. This, together with the decreasing rural job opportunities around the GM HT soybean mono-cropping (as described by the literature indicated above) indicates that, in the long-term, the possibilities for employment for small-scale farmers and landless people seems to be shrinking.

3.4.4 Ownership rights

Guiding questions:

a. Will the cultivation of the GM HT crop lead to changes in the ownership of land and/or water in the area?
b. Will the cultivation of the GM HT crop lead to changes in ownership of seed in the area?

Summary:

- The applicant does not provide information on ***changes in land and water ownership*** with the adoption of Intacta™ Roundup Ready™2Pro. Related information is found on RR soybean. Historically, soybean has expanded rapidly and dominates the arable land of soybean producing countries in South America. This imposed a high land demand pressure expressed as increased prices (for both selling and renting) in soybean cultivation areas, to the detriment of producers with low investment capacity. The result is an intense land concentration of large-scale industrialized agricultural systems.

- ***Water ownership*** may not be affected with the adoption of Intacta™ Roundup Ready™2Pro since the majority of soybean production in South America is based on rainfall.

- ***Seed ownership*** is negatively affected by patents on Intacta™ Roundup Ready™2Pro and the corresponding technology fees, resulting in increased prices for GM seeds (e.g. an approximate rise of USD 52/ha for Intacta™ Roundup Ready™2Pro).

The applicant does not provide information regarding potential changes in the ownership of land, water or seeds with the adoption of Intacta RR2 Pro. Most of the following information is drawn from the literature on RR soybean in Brazil and neighboring countries.

Ownership of land and water

Land. Since the early 1990s, soybean has been a dominant crop in Brazil. This trend continued after the approval of GM varieties. Since the authorization of RR soybean in 2003, this crop occupies more than 30 percent of the national arable land, reaching its maximum-recorded area in 2005 at 38 percent of the area dedicated to agriculture (of which 24 percent was GM HT soybean). These figures reached 33 percent of the arable land under by soybean production in 2011, of which 74 percent was GM HT. Generally speaking, this soybean dominance exerts pressure on the demand for land and land prices. No information was found which related to changes in the prices of land in soybean producing areas of Brazil. However, a close example is Uruguay, where the value of land dedicated to soybean cultivation increased from USD 25/ha in 2001, for both renting and purchasing, to UDS 125/ha for renting and USD 2,500/ha for purchasing in 2010. The increase in land prices has become a driver of land concentration among those with high investment capacity, to the detriment of farmers practicing small-scale cultivation (less than 50 hectares). In 2006, for instance, 5 percent of Brazilian soybean producers managed 59 percent of the total area dedicated to soybean, managing areas larger than 500 hectares; while 73 percent of farmers with plots smaller than 50 hectares occupied 15 percent of the soybean land (Catacora-Vargas *et al*. 2012).

Water. Information on water ownership related to Intacta RR2 Pro was not assessed. However, in the majority of cases, it is probable that water is not a source of tension since soybean production in South America is rain fed.

Ownership of seeds

Access and use of Intacta RR2 Pro is negatively affected by patents and corresponding technology fees, since these impose restrictions to farmers and trading companies.

In the past, RR soybean royalties in Brazil included the payment of the technology fee by farmers (approximately USD 9/ha) and a post-planting charge (2 percent royalty on subsequent RR soybean plantings) (Gazeta do Povo 2013). These fees were eliminated for RR soybean in July of 2013 when the Third Chamber of the Superior Court of Justice of Brazil rejected Monsanto´s petition to extend the patent of this GM variety in light of the Brazilian Law on Crop Protection 9.456/97. This Law determines that a crop variety loses its private protection rights and enters the public domain 15 years after its patent registration. The international patent on RR soybean expired the 31st of August of 2010, twenty years after its was registered. As for Intacta RR2 Pro, the royalty involves the technology fee (approximately USD 52/ha paid when purchasing the seed), which is 5 times higher than the fee previously applied to RR soybean (Rural BR 2013).

In relation to traders, as per July 2014, a dispute on royalty payments continued among Monsanto and the Brazilian Association of Vegetable Oil Industries (ABIOVE, according to its name in Portuguese), which gathers the largest grain traders, such as ADM, AMAGGI, Bunge, Cargill and Louis Dreyfus (Bonato and Stauffer 2014).

3.4.5 Monitoring

Guiding question:

Will the GM HT crop lead to a greater or reduced need for surveillance of land, water and the environment around the field?

Summary:

Monitoring of GM crops is generally very limited in Brazil. Based on this, it is not clear if the adoption of Intacta™ Roundup Ready™2Pro implies a greater or reduced need for monitoring. The applicant presented a monitoring plan focused on training, the appraisal of farmer perception, literature review and customer care. These activities exclude information on what, how and where to monitor the environmental and socio-economic effects of Intacta™ Roundup Ready™2Pro.

The monitoring of currently approved GM crops is limited in Brazil. In light of this, it is difficult to assess whether Intacta RR2 Pro soybean implies a greater or reduced need for the surveillance of land, water and the ecosystems surrounding producing fields.
As a requirement of Brazilian regulation, the applicant presented a monitoring plan that the applicant itself will implement. This plan includes the general monitoring of human health and the environment. The key aspects of monitoring are not specified, and socio-economic and ethical issues are not considered. The three main activities proposed and approved for monitoring are: (i) training on the appropriate use of the production technology related to Intacta RR2 Pro through field-trips, information events (e.g. congresses, meetings, etc.) and agricultural fairs, (ii) the use of questionnaires after the harvesting period to appraise farmers' perceptions of the trends and variations in the technology's performance and impacts, particularly with regard to pests and control methods, (iii) a monthly review of the published literature in indexed scientific journals in order to follow-up the advances in GM crop monitoring, and (iv) customer care consisting of a toll-free number. According to the monitoring plan, findings and outcomes will be presented in an annual report to the corresponding biosafety authorities (Berger and Braga 2009). In the case of identifying any unforeseen effects, the applicant has the duty to inform the Brazilian CTNBio within 30 days of observing such effects (Normative Act No. 9 2011).

Based on the "Guidance on Risk Assessment of Living Modified Organism" prepared and currently being discussed under the United Nations Cartagena Protocol on Biosafety (CBD, 2012) and monitoring reports (e.g. Quist 2013), the plan presented by the applicant excludes the following information, which would allow for an adequate monitoring of Intacta RR2 Pro: (i) a choice of indicators and parameters for monitoring (i.e. "what to monitor?"), (ii) a discussion of monitoring methods (i.e. "how to monitor?"), including the establishment of baselines, reference points, and the duration and frequency of monitoring, and (iii) choice of monitoring sites (i.e. "where to monitor?").

3.4.6 Ecosystem functions

Guiding question:

Will the GM HT crop affect ecosystem functions in a manner that yields a positive or negative economic effect?

Summary:

The applicant does not addresses possible ***effects on ecosystem functions*** from the adoption of Intacta™ Roundup Ready™2Pro. The available information refers to the ecological impacts of agroindustrial systems including soybean, either in general terms or specific to GM HT varieties. Given that GM HT soybean production follows expanding mono-cropping and agroindustrial production, GM HT crops may negatively influence ecosystem functions such as nutrient and water cycling, as well as the regulation of insect populations and local weather. In agronomic terms, these effects are expressed as nutritional imbalance and pest emergence, both of which have negative economic impacts, either as lower yields or higher input investments.

In this report, *ecosystem function* is understood to mean the biological, geochemical and physical processes that take place in the interface between biotic and abiotic components of an ecosystem, enabling the regulation of ecological processes, provision of habitat to different species, delivery of ecological services and cultural value. Biodiversity plays a key role in securing the functions of an ecosystem (Rockström *et al*. 2009), as well as the socio-ecological resilience of agricultural systems (Nicholls *et al.*, 2013; Perfecto *et al*., 2009).

The applicant does not address effects on ecosystem functions stemming from the adoption of Intacta RR2 Pro. The analysis presented below is based on the impacts of agroindustrial production systems in general, of which GM HT cropping and the ecological impacts of RR soybean are a part.

GM HT crops are produced through agroindustrial approaches, implying the establishment and expansion of monocrops together with the intense use of synthetic inputs. In the medium- and long-term, the cultivation of GM HT varieties involves both biodiversity compositional modifications and loss, including deforestation and fragmentation of natural habitats (deepening the trend of biodiversity erosion) and soil degradation. These processes negatively influence important ecosystem functions, such as nutrient and water cycling, as well as regulation of insect populations and local weather patterns, among others. In agronomic terms, these changes are expressed as: (i) plant-soil nutritional imbalances, due to disruption in the natural nutrient cycle, and deterioration of the biological, physical and chemical soil properties, and (ii) emergence of pests reaching a point of economic loss, due to the population changes resulting from the application of synthetic inputs and the elimination of microhabitats that serve as a refuge for natural pest enemies. Over time, the end outcome is a decrease in yields unless additional inputs of synthetic fertilizers and pesticides are applied in order to artificially compensate for the ecological imbalance and impaired plant physiology. However, this synthetic input-based strategy also implies the continued deterioration of the ecosystem (Altieri 1999; Altieri and Nicholls 2003; Lin 2011; Perfecto *et al.* 2009; Tilman *et al*. 1994; Zobiole *et al*. 2012).

Large-scale soybean field
Photo: Dustan Kostic #52112230 (dollarphotoclub.com)

The ecological effects described in sections 2.1.2 (“Interactions between the GM HT plant and the environment”), 2.1.4 (“Preservation of biological diversity”), 2.2.3 (“Resistance of other plants to the herbicides”), 2.2.4 (“Soils”), 2.2.5 (“Water”), 2.2.6 (“Energy”) and 2.2.7 (“Climate”) are relevant to ecosystem functions, and have also possible economic impacts of regarding yields and production costs.

3.5 Rules for herbicides

Guiding questions:

a. Is/are the herbicide(s) to which the GM HT plant is tolerant prohibited or permitted for restricted use in Norway because it is/they are a hazard to health or the environment?
b. Does/do the herbicide(s) to which the GM HT plant is tolerant have the same effects in the cultivation country as in Norway?
c. Is/are the herbicide(s) on lists of herbicides that should be prohibited, in international agreements?
d. What sort of rules does the production country have for the use of herbicides, and are these rules enforced?

Summary:

- Glyphosate is ***approved in Norway*** in 26 commercial formulations. Under European Union regulations, a new glyphosate assessment is planned for 2015. However, in Norway there is a planned five-year renewal of approval of the current commercial glyphosate-based formulations that will expire in 2016.

- Glyphosate is expected to have the ***same type of effects in Norway as in countries cultivating GT crops,*** but there may be important variations in their specific materialization caused by the agricultural characteristics, biodiversity, ecosystems and socio-economic conditions in Norway compared to the countries cultivating GM HT soybean.

- The herbicide inherent to Intacta™ Roundup Ready™ Pro (glyphosate) is currently ***authorized*** worldwide for agricultural purposes. In the European Union, it is allowed under the Commission Directive 2001/99/EC of 20 November 2001.

- International and national ***rules for the use of herbicides*** apply in Brazil. Internationally, Brazil ratified the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade, and the Stockholm Convention on Persistent Organic Pollutants. At the national level, there are specific regulations concerning herbicide management. However, these regulations are only partially implemented.

Status of the authorization of glyphosate in Norway

In Norway, glyphosate is approved as an active ingredient and in commercial formulations. In the Norwegian market, there are 26 glyphosate-based commercial formulations allowed, of which 12 are for home use (gardens). In compliance with European Union regulations, a new assessment of glyphosate approval is planned for 2015. Under the Norwegian Food Safety Authority, a five-year renewal of the existing permit of glyphosate and its 26 commercial formulations is expected. The current permits will expire in 2016 (Mattilsynet 2012; 2014).

Foreseen effects of glyphosate in Norway

In principle, glyphosate has comparable ecological effects in Norway as in the GM HT soybean cultivation countries if similar production systems are implemented (see the corresponding sections under 2.2 "The herbicide"). However, specific variations due to different ecological conditions, with possible different effects on biodiversity and wildlife composition, could be expected. For instance, Eggestad *et al*. (1988) studied the habitat preference of black grouse (*Tetrao tetrix*) upon habitat exposure to glyphosate in Southeast Norway. Their results indicate that black grouse cocks and hens avoided the glyphosate-sprayed area for up to two years after the application. After four to six years, hens were once again found in the area. The study indicates that glyphosate applications are generally unfavorable to cocks. Further studies on the ecological implications of these behavioral changes are needed.

Status of the authorization of glyphosate worldwide

As per September 2014, the use of glyphosate is allowed worldwide for agricultural purposes. The World Health Organization (WHO) classifies glyphosate and its metabolite AMPA as low-toxicity pesticides, based on a risk assessment carried out in 2003 (WHO 2006). Glyphosate as an active ingredient is allowed in the European Union according to the Commission Directive 2001/99/EC of 20 November 2001, which amended Annex I to the Council Directive 91/414/EEC concerning the placing of plant protection products on the market by including glyphosate as an active substance for herbicide use only (European Commission 2001). The corresponding toxicological report was presented in 2002 (Committee on Plant Health 2002). The WHO toxicity classification and the European Union authorization of glyphosate are based on toxicological studies of this herbicide as active ingredient and not of the actual composition of commercial formulations. As a result, the safety statements reported by the WHO and the European Commission differ from some of the latest published research on the health implications of glyphosate and adjuvants (additional information on ecological and human health effects of glyphosate is available in section 2.2.2 "Effects of the altered spraying regime", 2.2.4 "Soil", 2.2.5 "Water" and 3.1.4 "Food safety").

Rules for the use of herbicides in Brazil

In May 2004, Brazil ratified the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade (UNEP and FAO 2010), and the Stockholm Convention on Persistent Organic Pollutants (UNEP 2014).

At national level, the main legal instruments that apply to herbicide regulation in Brazil are:

- Law No. 7802, issued on July 11, 1989, which regulates the research, experimentation, production, packaging and labeling, transportation, storage, marketing, commercial advertising, use, import, export, waste disposal and packaging, registration, classification, control, inspection and surveillance of pesticides, their components and related products; including other measures.

- Law No. 9974, issued on June 6, 2000, which amends Law No. 7802 of July 11, 1989.

Other instruments are:

- Normative Act No. 48, issued on July 7, 2008 (which modified Act No. 10 of February 22, 2008), that provides administrative procedures for toxicological re-evaluations of products based on active ingredients that present health concerns.

- Normative Act No. 216, issued on December 15, 2006 (which modifies the Act No. 216 of December 15, 2006), that provides for studies of pesticide residues in vegetable products and wild mushrooms submitted by the applicants and holders of the corresponding record.

- Normative Act No. 119, issued on May 19, 2003, which creates the Program on Analysis of Pesticide Residues in Food (PARA, according to its name in Portuguese).

The implementation of such regulations has some limitations, which is illustrated by the 2012 surveillance of PARA on pesticides residues in food. Nationally, 29 percent of the samples taken were unsatisfactory, mostly due to the use of unauthorized pesticides (PARA 2013).

3.6 Plant genetic resources for food and agriculture

Guiding questions:

a. Will the GM HT crop be cultivated in an area defined as a center of origin or center of diversity for the corresponding non-GM crop?
b. Are there wild relatives of the GM HT plant in Norway or in the country of cultivation?
c. Is the GM HT crop available for further plant breeding?

Summary:

- Soybean´s ***center of origin and genetic diversity*** is Far East Asia. Wild relatives are not found in Brazil or Norway.

- The ***availability for further plant breeding*** of Intacta™ Roundup Ready™2Pro is subject to legal excludability through intellectual property rights and contracts with the developer.

Possible cultivation in the center of origin or center of genetic diversity

Soybean has its center of origin in China and its center of genetic diversity is in the whole Far East Region (Hymowitz 1970). Wild relatives or local races are not found in Norway or in the countries currently with the largest cultivation areas, including Brazil.

Availability of the GM HT crop for further plant breeding

The current patent on Intacta RR2 Pro imposes legal excludability from farmers and anyone other than the company Monsanto, the developer. Despite of this, the authorization of GM HT soybean in Brazil indicates free access and use GM plant material only for educational and non-commercial research purposes, as indicated in the following section 3.7 ("Independent risk assessment").

3.7 Independent risk assessment

Guiding question:

Is the GM HT crop available for independent risk research?

Summary:

Under Brazilian law, GM plant material is available to third parties for educational and non-commercial research purposes. However, all the safety studies presented on Intacta™ Roundup Ready™2Pro by the applicant were carried out by the developer's own personnel in its own research facilities.

In Brazil, under Law 9.279 issued on May 14, 1996 (article 43, paragraphs II, IV and VI), third party institutions and individuals (those who have not signed a technology license with the GM plant's developers) are allowed to buy seeds for educational and non-commercial research purposes (National Congress of Brazil 1996).

In general, intellectual property rights and confidential business information impose major limitations for independent research and monitoring, quality assurance and transparency of the data presented by applicants (Nielsen 2013). For instance, the biosafety dossier on Intacta RR2 Pro presented by the applicant to the Brazilian authorities indicates that such a document is subject to intellectual property rights in favor to *Monsanto do Brazil Ltda*. Yet, as indicated in the preface, the dossier was accessed under the Federal Brazilian Law No. 12.527 of November 11, 2011 (which aims at facilitating the transparent management of the information relevant to the public).

In the case of Intacta RR2 Pro, safety studies were carried out by the applicant's own personnel in their own research facilities. For instance:

- The verification of PCR tests was carried out and documented by Monsanto Biosafety Regulatory Sciences' Product Characterization Technology Center. The resulting raw data was stored in Monsanto Regulatory Archive in St. Louis, Missouri, United States. The statistical analyses were performed by Monsanto Statistics Technology Center.

- Agronomic, phenotypic and environmental studies (e.g. arthropod abundance) were held in the *Monsanto do Brasil Ltda*. experimental stations.

- The studies on seed vigor, germination and dormancy were carried out at the Purity Laboratory of Monsanto in Uderlândia, Minas Gerais, Brazil.

- Pollen morphology and viability research was carried out at the Experimental Research Station of Monsanto in Rolândia, Paraná, Brazil.

- Allergenic potential was assessed using Monsanto´s internal database of allergens to compare the similarity of CP4 EPSPS with other proteins.

Subsequently, independent research institutes have not been involved in the safety assessment of Intacta RR2 Pro soybean.

3.8 Freedom to choose a different agricultural system in the future

Guiding question:

How does cultivation of the GM HT crop affect the possibility of changing in the future to other agricultural systems, such as organic farming or farming without GMOs?

Summary:

Freedom of choice between different agricultural systems in the Brazilian context is influence by seed availability and the likelihood of effective co-existence. Farmers report that there is a decrease in the variety of available seeds, particularly non-GM. Effective co-existence is more challenging for small-scale producers than the large-scale ones, due costs and their limitations for implementing measures that prevent seed commingling.

Based on field observations in Brazil, the possibility of changing to an agricultural system other than GM HT production would be determined by seed availability, the possibility of co-existence and segregation regimes.

Seed availability. Commercial retailers of agricultural inputs, including those belonging to farmers' cooperatives, determine the pool of seeds available during each agricultural season. Only self-supplied farmers are independent from variations in commercial seed availability. Small-scale farmers indicate that there is a decrease in seed variety and a lack of non-GM seeds [*personal communication*]. There are no studies on the Brazilian context in this regard. However, the farmers testimonies are consistent with Hilbeck *et al*. (2013), who report a decrease in the diversity of commercially available seeds in the European countries that had adopted GM crops.

Likelihood of effective co-existence. Effective co-existence is highly influenced by the scale of production. Small-scale farmers have fewer possibilities of real co-existence given the difficulties of implementing separation barriers (e.g. tree division barriers) and the lack of their own machinery. Based on field observations, rented machines, mainly sowers, harvesters and transportation trucks, can be important sources for GM and non-GM seed commingling, making segregation very expensive and difficult to achieve. Additional information is provided in section 2.1.3 (“Gene flow”).

IV Final comments

According to the Brazilian Normative Act No. 02 passed on November 27, 2006, Intacta RR2 Pro is classified under Risk Class 1 (low individual risk and low collective risk) (CTNBio 2006). According to the applicant, this is because it contains genetic material from donor and receptor organisms that do not cause harm to human and animal health, and therefore it does not cause adverse effects in crops and the environment (Berger and Braga 2009). Contrary to this assertion, the literature provides indications of harmful and adverse effects to the environment and to health (both animal and human), as well as to socio-economic conditions, particularly over the medium- and long-term.

The literature related to the biosafety of GM HT plants has a number of important limitations making a comprehensive analysis of GM plants' sustainability difficult. These are:

- *Methodological limitations of studies indicating positive results*. The trend of using a reduced and repetitive set of indicators is noticeable in an important portion of scholarly work reporting beneficial effects of GM plants, e.g. compositional analysis based on substantial equivalence in the case of research on potential health effects, and change in production costs and yield in the case of socio-economic aspects. A deeper analysis of some of these studies (Catacora-Vargas *et al. forthcoming publication*) reveals other methodological limitations, among them the lack of consideration of possible long-term effects. Therefore, the large number of studies indicating positive impacts of GM crops becomes less important as their methodological robustness may be questioned.

- *The majority of the research compares GM-based agriculture to conventional industrial production systems*. This imposes difficulties for comparative analysis due to two important factors. First, significant differences are masked given the similarity between GM and non-GM conventional industrial systems in terms of their technological package and their ecological, social and economic effects. Second, the gaps of knowledge on other production systems (e.g. IPM, organic and agroecological approaches) are not addressed.

- *Strong focus on single-trait GM HT plants*. As a result, most of the information available relates to single GM HT events and not to stacked ones. Accordingly, the literature rarely addresses the combinatorial and additive effects of multiple-trait GM crops.

- *Common overlooking of the inherent technological package and its application in real field conditions.* Examples of this limitation are a number of studies that do not consider the applications of the herbicide inherent to the genetic modification, and analysis of the herbicide based on the use of active ingredient and not of the commercial formulations used during field production.

These, among other methodological limitations (i.e. inconsistent selection of control, see more in section 2.1.5 “Comparison with controlled plants”), are present in the dossier of Intacta RR2 Pro submitted to the Brazilian authorities. These limitations partially explain the kinds of findings reported by the applicant: all of them showing no possible adverse effects in contrast to a significant body of literature.

Tables 2, 3 and 4 summarize the findings reported in the dossier on Intacta RR2 Pro elaborated by the applicant and the reviewed literature, organized according to the guiding questions of the NBAB to assess the sustainability of GM HT plants (see Annex). As shown in the Tables, the information on Intacta RR2 Pro as a stacked event is scarce from both the dossier and the literature. The majority of the findings are on Roundup Ready® soybean.

Given the limited research on stacked GM HT crops such as Intacta RR2 Pro, the biosafety regulatory framework faces the challenge of having to make decisions under conditions of incomplete knowledge. The findings in this report clearly reveal that more empirical research is still needed on the multiple dimensions of sustainability of GM HT plants, including independent research regarding possible adverse effects at ecological, socio-economic and human health, particularly of multiple-trait GM varieties.

Table 2. Summary of the reported information on "Environmental sustainability and ecology" of the genetically modified plants, according to the NBAB guiding questions.

Criteria	Specific effect	Applicant			Other sources			Applicant			Other sources		
		Type of impacts reported * / ** Intacta™ Roundup Ready™ 2 Pro soybean						Type of impacts reported Roundup Ready™ soybean					
		(+)	(-)	NR	(+)	(-)	NR	(+)	(-)	NR	(+)	(-)	NR
Characterization of the GM HT plant	Genotypic and phenotypic characterization	--	--	--	--	--	--	--	--	--	--	--	--
	Stability of the genome, genetic expression and properties												
	Substantial equivalence to the unmodified parent												
	Inclusion of antibiotic resistance marker genes	NO										YES	
Interaction between the GM HT plant and the environment	Characterization of the environmental and ecological conditions	NO				YES					YES	YES	
	Changes in the plant´s genome, genetic expression and properties												
	Effects of the ecological conditions on the chemical properties												
	Effect of the GM HT plant on the environment												
Gene flow	Risk of vertical gene transfer	NO						NO			NO	YES	
	Risk of horizontal gene transfer	NO									NO	YES	
	Seed-mediated gene flow											YES	
Biological diversity	Effects on the health of non-target organism												
	Material used for exposure	BACT						BACT			BACT PLT	BACT PLT	
Control plants	Comparison to the closest genetic relative	NO						YES			YES NO	YES	
	Comparison under the glyphosate application	NO				YES		NO			YES NO	YES	
	Comparison under biotic and abiotic stress factors												

* The colors represent the relative availability of information on the corresponding topic. It does not give any indication on the amount of information available.

** (+) = Positive effect; (-) = Negative effect; NR = Not reported; Dashed line = Not applicable.

Table 3. Summary of the reported information on "Environmental sustainability and ecology" of the herbicide, according to the NBAB guiding questions.

Criteria		Specific effect	Applicant			Other sources			Applicant			Other sources		
			Type of impacts reported */** Intacta™ Roundup Ready™ 2 Pro soybean						Type of impacts reported Roundup Ready™ soybean					
			(+)	(-)	NR	(+)	(-)	NR	(+)	(-)	NR	(+)	(-)	NR
Mechanism by which the herbicide functions			--	--	--	--	--	--	--	--	--	--	--	--
Effects of altered spraying regime	Preservation of the biological diversity	Health effects on non-target organism												
		Changes in biodiversity of plants and animals												
		Effects on soil microflora and microfauna												
		Effects on growth cycle or division of eukaryotic cells												
		Health effects from the intake of products from the GM HT plant												
		Hormone mimicking and inhibiting effects												
		Glyphosate and metabolite residues in plant tissue and soil												
		Changes in the timing of herbicide application												
		Herbicide drift on non-GM crops												
		Changes in the volume of herbicides used												
		Changes in the profile of herbicides used												
		Emergence of unexpected combinatorial or synergistic effects												
Resist-ance		**Problems associated with herbicide resistance**												
		Strategies to prevent herbicide-resistance development	--	--	--	--	--	--	--	--	--	--	--	--
Soil		**Effects on soil erosion**												
		Effects on soil pH												
		Changes in soil nutrient composition												
Water		**Water pollution with new proteins and herbicides**												
		Changes in soil evapotranspiration												
Energy / Changes in the amount of energy consumed														
Climate / Emission of greenhouse gases														

* The colors represent the relative availability of information on the corresponding topic. It does not give any indication on the amount of information available.

** (+) = Positive effect; (-) = Negative effect; NR = Not reported; Dashed line = Not applicable.

Table 4. Summary of the reported information on "Environment, economic and social sustainability", according to the NBAB guiding questions.

Criteria		Specific effect	Applicant			Other sources			Applicant			Other sources		
			Type of impacts reported */** Intacta™ Roundup Ready™ 2 Pro soybean						Type of impacts reported Roundup Ready™ soybean					
			(+)	(-)	NR	(+)	(-)	NR	(+)	(-)	NR	(+)	(-)	NR
Right to sufficient, safe and healthy food	Food security	Changes in input factors per production unit												
		Changes in yield												
		Purpose of the HT plant	--	--	--	--	--	--	--	--	--	--	--	--
	Food safety	Content and quantity of herbicide residues in food												
		Health effects from the intake of products from the GM HT plant												
		Material used for assessing exposure	--	--	--	--	--	--	--	--	--	--	--	--
	Food quality	Changes in nutritional content												
		Changes in storing properties												
		Changes in benefits to consumers												
Animal welfare / Changes in the feed quality														
Living conditions & profitability (Farmers)	Health safety	Effects on farmers' and farmworkers' health from changes in herbicides												
		HES training and access to protective equipment												
	Contacts and framework conditions / Restriction on access to seeds and information													
	Cost and revenues	Changes in costs for input factors												
		Changes in the need for other input factors												
		Changes in weed resistance and profitability in the long term												
	Agronomic factors / Cultivation conditions that GM HT plant was developed for		--	--	--	--	--	--	--	--	--	--	--	--
	Right to seeds / Restrictions to save, exchange and sell seeds													
Living conditions & profitability (Production area)	**Health and safety /** Effects on communal health from changes in herbicides													
	Employ ment	Changes in local and regional employment												
		Changes in employment for women												
	Owners' rights	Changes in ownership of land and water												
		Changes in ownership of seeds												
	Surveillance / Changes in the need for surveillance													
	Ecosystem functions / Changes in ecosystem functions, yields and economy													

* The colors represent the relative availability of information on the corresponding topic. It does not give any indication on the amount of information available.

** (+) = Positive effect; (-) = Negative effect; NR = Not reported; Dashed line = Not applicable.

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Annex – Sustainability questions for applicants as defined by the NBAB

Table A1. Sustainable development environment/ecology questions (related to the HT plant).

Criteria	Specific effect
1. Characterization of the GM HT plant	a. Has the HT crop been thoroughly genotyped and phenotyped?
	b. Are the genome, gene expression and properties of the HT crop stable over time and through several generations?
	c. Is the HT crop substantially equivalent to the unmodified parent plant with the exception of the inserted gene and the protein it expresses, and does the answer apply irrespective of cultivation site and conditions?
	d. Is the HT crop resistant to more than a one herbicide?
	e. Does the HT crop have of a gene for resistance to antibiotics?
2. Interaction between the GM HT plant and the environment	a. Is the environment, i.e. the ecological conditions in the cultivation area, thoroughly characterized and explained?
	b. Do the HT plant´s genome, gene expression or properties change when the plant is cultivated in different places?
	c. Might the metabolisms, chemical composition and/or nutritional value of the HT plant change because of the ecological conditions in the cultivation area?
	d. Might the effects of the HT plant on the environment or its interaction with the environment vary, depending on the conditions in the cultivation area or the surrounding area?
3. Gene flow	a. Is there a risk of vertical gene transfer to other species?
	b. Is there a risk of horizontal gene transfer to other species?
4. Preservation of biological diversity	a. Might the cultivation of the HT plant have health effects (toxic, immunological, including allergic, or anti-nutrient effects) that are acute; chronic or long-term; and/or lead to a change in the viability, fertility and development rate of non-target organism, i.e. wild populations of: • Mammals • Birds • Amphibians/reptiles • Insects (herbivores, predators, pollinators and decomposers) • Red-listed species • Prioritizes species?
	b. Have the conclusions of 4a been drawn on the basis of exposure to: • Plant material from the HT plant? • The protein expressed by the inserted gene, after extraction from tissues from the HT plant? • The protein expressed by the inserted gene in the organisms it is obtained from?
5. Comparison with control plants	a. Has the GM HT plant been compared with its closest genetic relative under the same ecosystem conditions?
	b. Have the characterization and comparative investigations been made with GM HT plants that • Have been sprayed with the herbicide(s) that they are modified to tolerate? • Have been exposed to predators or other biotic or abiotic stress factors?

Table A1. Continuation (questions related to the herbicide).

<table>
<tr><th colspan="2">Criteria</th><th>Specific effect</th></tr>
<tr><td colspan="2">6. Characterization of the herbicide</td><td>What are the mechanisms by which the herbicide(s) function?</td></tr>
<tr><td rowspan="11">7. Effects of altered spraying regime (change in frequency, concentration, type of herbicide)</td><td rowspan="6">a. Preservation of biological diversity</td><td>i. Might the cultivation of the HT plant cause health effects (toxic, immunological, including allergic, or anti-nutrient effects) that are acute; chronic or long-term; and/or lead to a change in the viability, fertility and development rate of non-target organism, i.e. wild populations of:
• Mammals
• Birds
• Amphibians/reptiles
• Insects (herbivores, predators, pollinators and decomposers)
• Red-listed species
• Prioritizes species?</td></tr>
<tr><td>ii. Might cultivation of the HT crop lead to a change in the biodiversity of weeds and animals (vertebrates and invertebrates)?</td></tr>
<tr><td>iii. Might cultivation of the HT crop harm microflora and microfauna in the soil?</td></tr>
<tr><td>iv. Might the herbicide(s) or the degradation product(s) thereof affect the growth cycle or division/proliferation of eukaryotic cells, and in such case, how?</td></tr>
<tr><td>v. Might the herbicide(s) or degradation product(s) thereof have a hormone-mimicking or hormone-inhibiting effect?</td></tr>
<tr><td>vi. How long and in what concentrations do(es) the herbicide(s) and degradation products remain in plant tissue and different soil types?</td></tr>
<tr><td colspan="2">b. Does cultivation of the HT crop result in a change in the timing of herbicide application?</td></tr>
<tr><td colspan="2">c. Does cultivation of the HT crop increase the risks of herbicide drift, and thereby also the risk that non-GM crops in surrounding areas may be unintentionally affected?</td></tr>
<tr><td colspan="2">d. Does cultivation of HT crop lead to increase/decrease use of herbicide?</td></tr>
<tr><td colspan="2">e. Does cultivation of the HT crop lead to the use of herbicides with more/less adverse effects than previously?</td></tr>
<tr><td colspan="2">f. Might unexpected combinatory effects such as additive or synergistic effects occur when more than one herbicide is used in the same area?</td></tr>
<tr><td rowspan="2">8. Resistance of other plants to the herbicide</td><td colspan="2">a. What are the resistance problems associated with the herbicide in the cultivation area?</td></tr>
<tr><td colspan="2">b. What strategies are used to prevent the development of resistance in plants other than the HT crop (example: integrated plant protection)?</td></tr>
</table>

Table A1. Continuation (questions related to the herbicide).

Criteria	Specific effect
9. Soil	a. Does cultivation of the HT plant lead to more/less soil erosion?
	b. Does cultivation of the HT plant lead to higher/lower soil pH?
	c. Does cultivation of the HT plant lead to a change in the nutrient composition of the soil?
10. Water	a. Might cultivation of the HT crop change the water spraying regime so that water sources and groundwater become polluted by “new” proteins and residues of herbicide or degradation products thereof?
	b. Might the cultivation of the HT crop reduce water evaporation as a result of less tilling?
11. Energy	Is there an increase or decrease in the energy consumed in connection with cultivation of the HT plant, measured by means of life cycle analysis of the full production and harvesting cycle?
12. Climate	Do the greenhouse emissions associated with the cultivation of the HT plant, as measured by life cycle analysis of the full production and harvesting cycle, increase or decrease?

Table A2. Sustainable development economy and society questions

Criteria		Specific effect
1. Right to sufficient, safe and healthy food	**1.1. Food security**	a. Does the HT plant contribute to reduce/increase input factors per production unit?
		b. Does the yield per unit area increase/decrease?
		c. What is the purpose of the HT plant–will it be used for food, feed, biofuel or material?
	1.2. Food safety	a. Will the contents and quantity of herbicide residues (active ingredients in the herbicide) in food increase/decrease?
		b. Will intake of products from the HT plant have health effects (toxic, immunological, including allergic, or anti-nutrient effects) that are acute; chronic; long-term; and/or lead to a change in metabolism and fertility?
		c. Have the conclusions in 1.2b been drawn on the basis of exposure to: • Plant material from the HT plant? • The protein expressed by the inserted gene, after extraction from the tissue from the HT plant? • The protein expressed by the inserted gene in the organism it is obtained from?
	1.3. Food quality	a. Does the HT plant yield better/poorer nutrition in terms of composition, quantity and energy content?
		b. Does the HT plant have properties that make the crop last better/more poorly during storage?
		c. Does cultivation of the HR plant yield greater/less benefits for the consumers?
2. Animal health and welfare	**1.4. Feed quality**	Do the products of the HT plant improve/detract from feed quality?
3. Living conditions and profitability for farmers who cultivate HR plants, in the short term (less than 5 years) and in the long term (more than 20 years)	**3.1. Health and safety**	a. Will any changes in the use of the herbicide affect the heath of the farmers/ farm workers positively/negatively?
		b. Will farmers/farm workers be given HES training and access to protective equipment and the information they need in order to use the herbicides(s) that is/are to the used with the HT plant?
	3.2. Contacts and framework conditions	Are the restriction on the access to seed, the right to terminate contracts, or on the information about seeds, spraying schedules and prevention of resistant weeds?
	3.3. Development cost and incomes for farmers in the short-term (less than 5 years) and in the long term (more than 20 years)	a. Will farmers´ costs for input factors increase/decrease?
		b. Will the HT plants reduce the need, in the short and/or long term, for other input factors such as production plan, spraying program, work input and machinery and equipment?
		c. Will any resistance problems increase in the future, and in the event, reduce profitability in the long term?
	3.4. Agronomic factors	What sort of cultivation conditions, soil types and technological standards has the HT plant variety been developed for?
	3.5. Right to seeds	Does the applicant restrict the farmers´ possibilities of saving, exchanging or selling seeds from their own harvest?

Table A2. Continuation (questions related sustainable development economy and society)

Criteria		Specific effects
4. Living conditions and profitability in the production area, in the short term (less than 5 years) and the long term (more than 20 years)	**4.1. Health and safety**	Will any change in the use of the herbicide affect the health of the community positively or negatively?
	4.2. The democratic rights and profitability of other farmers	a. Are these rules for co-existence, and are the complied with, such that it is possible to choose to cultivate non-GM, for example organic, crops instead of HT crops?
		b. Is there a system for presenting the spread of HT crops to other non-GM crops?
		c. Is there a compensation system if other farmers are affected by unintentional dispersal of genes, pollen or seed from the HT crop?
		d. Is there a system for keeping GM and non-GM crops separate in the production and transport line and, in the event, who pays for this system?
		e. Will cultivation of the HT crop lead to more or fewer problems with weeds for other farmers?
	4.3. Employment	a. Will cultivation of the HT crop create more or less employment locally and regionally?
		b. Will the cultivation of the HR crop create more or less employment for women?
	4.4. Owners' rights	a. Will the cultivation of the HR crop lead to changes in ownership of land and/or water in the area?
		b. Will the cultivation of the HR crop lead to changes in ownership of seed in the area?
	4.5 Monitoring	Will the HT crop lead to greater or reduced need for monitoring of land, water and the environment around the field?
	4.6. Ecosystem functions	Will the HT crop affect ecosystem functions in a manner that yields a positive or negative economic effect?
5. Rules for use of herbicides	a. Is/are the herbicides(s) to which the HT plant is resistant prohibited or permitted for restricted use in Norway because it is/they are a hazard to health or the environment?	
	b. Does/do the herbicide(s) to which the HT plant is resistant have the same effects in the cultivation country as in Norway?	
	c. Is/are the herbicide(s) on lists of herbicides that should be prohibited, in international agreements?	
	d. What sort of rules does the production country have for the use of herbicides, and are these rules enforced?	
6. Plant genetic resources for food and agriculture	a. Will the HT crop be cultivated in an area defined as a center of origin or center of diversity for the corresponding non-GM crop?	
	b. Are there will relatives of the HR plant in Norway or the country of cultivation?	
	c. Is the HR crop available for further plant breeding?	
7. Independent risk research	Is the HT crop available for independent research?	
8. Free choice of agricultural systems in the future?	How does cultivation of the HT crop affect the possibility of changing in the future to other agricultural systems, such as organic farming or farming without GMOs?	