General Conclusions

Principal Herbicide-Tolerant Plant Varieties (HTVs) Currently on the Market and Their Status

This ESCo is focused on the **agronomic trait of herbicide tolerance (HT)**. Introduced into a plant variety, this trait makes possible the post-emergence use of the associated herbicide, that is to say, its use on a standing crop and any weeds growing within that crop. While the breeding technique used to develop the HTV has little effect on its use in the field, it will determine the type of herbicide with which the crop variety is associated as well as the variety's regulatory status.

Breeding Techniques, Species and Herbicides Involved

The HTVs currently on the market worldwide have been developed by a variety of methods:

– so-called "traditional" selection, using natural genetic variability (the identification of spontaneous mutations followed by their integration into the genome of the cultivated plant *via* sexual crossing);
– mutagenesis, which involves additional variability (mutations induced by physical or chemical treatments);
– transgenesis, permitting the insertion into the genome of the cultivated plant of a gene of interest obtained from another organism, especially when the two species are too genetically distant for direct crossing.

HTVs are available for the following major field crop species: maize, soybeans, cotton, oilseed rape, sunflower, sugar beet, wheat, rice, and chicory/endive.

The herbicides associated with HTVs belong to various herbicide classes, which is to say they possess various modes of action – the target enzyme an herbicide

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inhibits in the plant is characteristic of its class. The spectrum of activity is also variable: some herbicides are selective, which is to say they are effective on certain botanical groups only (as with classes A, B, and C, for example); other herbicides, known as non-selective, are effective on all plant species, whether wild or cultivated (classes G and H).

Selective vs Non-selective Herbicide Tolerance

A global survey of herbicide-tolerant varieties currently on the market and in cultivation for major field crop species reveals two basic HTV groups according to the selectivity of the herbicides with which they are associated:

- **tolerance to a non-selective herbicide,** introduced into the majority of HTVs currently in cultivation, especially in North and South America (maize, soybeans, oilseed rape, sugar beet). Developed in the mid 1990s, varieties currently on the market falling into this first group are exclusive transgenic. This includes the varieties marketed within the Roundup Ready® (RR®) range, tolerant to glyphosate (class G), and the Liberty Link® (LL®) range, tolerant to glufosinate (class H) – the latter being more recently developed and less widely cultivated;
- **tolerance to a broad-spectrum but still selective herbicide.** Most varieties carrying this trait have been obtained by traditional selection or by mutagenic techniques. This includes a wide range of crops tolerant to class B herbicides (notably the Clearfield® range) and maize varieties tolerant to class A herbicides. Relatively rare at the global level, some of these varieties are cultivated in France (maize and sunflower); others are currently pending listing on the French Official Catalogue (sunflower and oilseed rape).

The correlation currently observable between the breeding technique used to obtain the HT trait and the selectivity of the herbicide associated with it results from the link between utilisable genetic resources and utilisable breeding techniques. Although spontaneous mutations conferring these forms of resistance exist in plants for all herbicide classes, such mutations appear at frequencies and with associated physiological costs (detrimental to the plant) that are highly variable according to the class. Only resistances identified in cultivated species or their interfertile relatives can be used for variety improvement using traditional selection methods or mutagenesis (the case with resistance to classes A, B and C). This has not been the case with resistances to glyphosate and to glufosinate, which were first identified in microorganisms and then introduced into plants through the use of transgenic techniques.

More recently, varieties tolerant to two different herbicide classes have appeared on the market. These are obtained by transgenesis, and can associated two selective herbicides, two non-selective herbicides, or one selective and one non-selective herbicide.

HTV Breeding Techniques and Varieties' Regulatory Status

In Europe, a new variety must be listed on the official national catalogue of a member-state prior to being offered for sale within the European Union. In order to obtain such a listing, it must satisfy certain criteria, including both agronomic and technological qualities. Directive 2001/18/EC, relating to the deliberate release of GMOs into the environment, specifies supplementary requirements for varieties considered to be genetically modified. Among the techniques currently used for the breeding of HTVs, mutagenesis and transgenesis are defined by this directive as creating GMOs, although only those varieties obtained by transgenesis fall within the directive's sphere of application, making them subject to a preceding environmental and health impact review and to obligatory labelling of any resulting food or feed products.

This distinction between two different statutory types of GMO is questioned by some observers, who regard the cultivation of HTVs obtained by mutagenesis as a way of side-stepping the regulations intended to limit the growing of GM crops in Europe. This point of view has found expression in a number of crop-destruction protests targeting HT sunflowers obtained by mutagenesis.

This distinction between HTVs obtained by mutagenic vs. transgenic techniques is likewise threatened by the development of new breeding technologies, more or less close to commercial application, which blur the line between the two methods. These new technologies are prompting a review at the EU level as to the eventual regulatory status of varieties so obtained. Two of these techniques – based on homologous recombination, permitting the insertion of a new gene or a targeted point mutation of the genome – have resulted in new HTVs in the laboratory. Plants resulting from these techniques carry no molecular trace of the modification other than the modified sequence itself, and thus cannot be distinguished on this basis from plants obtained by traditional selection.

Dynamics of HTV Development

Rapid Adoption at the Global Level

The dynamics of HTV adoption has only been well documented for RR® varieties, the spread of which has been the focus of a number of studies, notably in the United States. Their adoption has been particularly widespread and rapid: in less than 10 years, HTVs have come to occupy 80 % of total crop area for cotton and soybeans; for sugar beet, HTVs reached 98 % of total crop area in 2 years.

Quantitative and qualitative data on the adoption of varieties tolerant to a selective herbicide are, by contrast, much less numerous and have mainly been conducted by the companies responsible for their development. In the one documented case in which such varieties were approved for use at the same time as varieties tolerant to a non-selective herbicide (for spring oilseed rape in Canada), their adoption seems to have been limited, with RR® and LL® varieties rapidly securing the larger part of the market.

Factors Influencing HTV Adoption by Farmers

Several technical advantages of HTVs are advanced by biotechnology and seed companies and disseminated by agricultural advisory services. In some cases, experiments have made it possible to test the validity of these claims under real conditions. Various bibliographic sources suggest four main advantages expected from HTV use, although it is not possible to evaluate their relative importance in adoption decisions:

- an **increase in the range of weeds controlled** relative to the selective herbicides typically used in non-HT systems, and notably an effectiveness on species botanically close to crop species, on invasive species and on parasitic species;
- a **facilitation of farmers' work** *via* a reduction in the number of herbicide treatments, the flexibility gained through post-emergence application and by the discontinuation of ploughing or even of all soil tillage, as favoured by the HT strategy;
- a **reduction in the quantity of herbicides** used, made possible by the substitution of a single molecule in the place of multi-herbicide programs and the tailoring of quantities applied to weed conditions actually present;
- a **securing of weed control** and a reduction in the risk of yield losses caused by weed competition.

In the field, surveys conducted among farmers who have adopted HTVs are the only means of identifying, *a posteriori*, the motivations prompting the decision. The only available studies were conducted among North American farmers growing RR® HTVs. Initial studies showed that the anticipated yield gains which could *a priori* explain the HTV choice were only confirmed in situations in which pre-existing weed-control challenges were impacting yields. Later studies on reasons for HTV adoption suggest two main conclusions:

- while the price of HT seed, which is considerably higher than that of non-HT seed, may act as a brake on adoption, **savings in weed-control costs** (both direct – the price of herbicides – and indirect – increased flexibility and time savings), at least in the short term, represent a major motivation for adoption;
- a **strong correlation** is observed **between HTV adoption and reduced tillage**. The joint implementation of these two techniques is likewise favoured in regions subject to erosion where the shift to no-till is encouraged by financial incentives (for example in the United States). The commercial success of RR® HT soybeans in Argentina is explained by the possibility of using HTVs to facilitate the successful introduction of direct seeding practices.

Commercial Strategies of HTV Developers

In terms of the commercial presentation of HTVs, the strategies of the biotechnology companies involved have mostly been studied on a theoretical basis and in the context of the North American market. In contrast to the European legislative framework, which specifies intellectual property protections for plant varieties *via* a Plant Breeders' Right (PBR), potentially in conjunction with a patent on a specific genetic trait, American law allows for the patenting of varieties themselves; the patent has been widely adopted by biotechnology firms since it offers a stronger protection than those provided for by a PBR.

The coupled utilisation of an HTV and its associated herbicide has created a link on the market between demand for seeds and demand for herbicides. The companies that have developed HTVs are in most cases agro-chemical companies that either already possessed or recently acquired a seed-breeding arm. Nevertheless, in order to expand the sales of their herbicides, these companies generally have an interest in granting non-exclusive licenses to other seed companies for use of the HT trait.

When a company loses its monopoly on an herbicide molecule (as with glyphosate, the patent for which expired in 2000), it will retain the possibility of making profits on sales of the HT seed. In order to "preserve" its market share for the herbicide, it may be tempted to favour so-called "linked" sales (sales of seed conditional upon purchase of the herbicide); but such practices are in violation of market competition laws. Companies thus tend to limit competition from generic herbicides by marketing special commercial formulations and/or offering users of the HT trait stronger performance guarantees when using "their" herbicide.

Effects on Weed Flora, the Durability of the HT Innovation and Changes in Herbicide Use

The adaptation of weed flora under the selection pressure exerted by the herbicide leads to the appearance and spread of weeds that are not or are no longer destroyed by the herbicide, thus rendering the HT strategy less effective or even inoperable. The evaluation of this phenomenon thus constitutes a major question for this ESCo. This is not a "theoretical" risk: it has been confirmed by field studies, and it has led to an increase in herbicide consumption on HTVs in the United States. The scale and rapidity of these phenomena vary according to agronomic conditions.

Phenomena of Weed Flora Adaptation and Their Consequences for the Durability of the HT Strategy

The space opened up by the elimination of weeds sensitive to an herbicide will be rapidly colonized by other species. While this phenomenon of **"floral shift"** is clear with selective herbicides, which by definition are not active against all botanical families, it obtains as well for non-selective herbicides, the application of which will select for species naturally less sensible or which develop after the period of effectiveness of the herbicide.

The **spontaneous development of resistant plants** is a general phenomenon, known for all herbicide classes and identified in some 200 plant species to date. The appearance and expansion of such resistances in not an outcome specific to cultivation of HTVs, but it may be amplified by the conditions of herbicide use in HT systems.

This effect is clear in America in the case of glyphosate, where the rapid and massive adoption of RR® varieties (on which the herbicide is used at lower doses than for other uses) marked the beginning of the development of resistant weeds as an effect of the selection pressure exerted.

For certain classes of selective herbicides, prior large-scale use has led to the development of numerous resistant mutants even before introduction of the HTV. The phenomenon is particularly distinct for classes A, B and C, which made possible the development of HTVs based on the selection of these spontaneous mutations. Varieties tolerant to these selective herbicides, under conditions of massive use, are thus those which will be most rapidly confronted by the appearance of resistant weeds, and even more so if the herbicides involved are already widely used in cropping systems other than those making use of the HTV. No-till, often associated with HTVs, will likewise contribute to the selection of spontaneous resistance by favouring the development of weeds.

Diffusion of the HT trait may occur *via* seeds produced by the HTV and its offspring, and *via* pollen liable to fertilise non-HT cultivated plants or related interfertile wild plants. The risks of diffusion of the HT trait are thus above all a function of the cultivated species and its geographic area of cultivation, which will determine the survival of volunteers, the establishment of so-called feral populations (beyond field margins) and the presence of interfertile weeds. Maize, without related species in Europe and volunteers of which will not survive the winter, is thus much less of a concern than oilseed rape.

The question of gene flow between the crop and related weed species is crucial to the durability of the HT strategy. Gene flow of this sort has been shown for sugar beet, rice, sunflower, oilseed rape and wheat. This renders transfer of the HT trait into related weed species inevitable. Difficult weed-control situations due to weeds botanically closely related to and interfertile with the crop, which make HTV adoption particularly attractive, are thus also those presenting the strongest likelihood of acquisition of the HT trait by the targeted weed.

Various biological (for example, the use of asynchronous flowering periods) or biotechnological (for example, insertion of the transgene into chloroplast DNA, which is not transmitted by pollen) options have been proposed in order to reduce the probability of transfer of the tolerance gene by sexual reproduction; there is however no way to entirely eliminate such transfer.

Changes in Herbicide Use

In the short term, the substitution of a single, broad-spectrum herbicide in place of an herbicide program including several selective compounds may potentially lead to a reduction in quantities used to achieve the same level of effectiveness. In the case of tolerance to a non-selective herbicide (glyphosate), *a priori* evaluations as well as statistical data confirm this short-term reduction in quantities of herbicides used.

However, recent studies conducted in the United States show that the difference in herbicide consumption between RR® and non-HT crops, initially in favour of the HTVs, reverts within a few years to being unfavourable for soybeans and cotton. This increase over time in quantities of herbicides used on HTVs is explained by the **recourse to supplementary herbicide treatments:** an increase in application rates and/or the number of treatments of glyphosate in response to the phenomenon of floral shift, followed by recourse to complementary herbicides, notably against species that have become resistant to glyphosate.

Crop protection practices associated with the adoption of varieties tolerant to a selective herbicide are poorly documented at the global level. With these varieties, moreover, a reduction in herbicide use is not necessarily the object in view: the HTV may simply make it possible to complement standard pre-emergence weed control in order to manage a specific problem.

The ability to apply herbicides post-emergence makes it possible at least in theory to adapt weed-control treatments to the weeds actually present, and hence to avoid some treatments or to limit them to infested areas – thus enabling a reduction in quantities used per hectare. No such instances of the elimination of treatments within certain field areas are cited in the literature. The desire to eradicate potentially resistant individuals as soon as they appear leads on the contrary to technical guidelines for a reinforced use of herbicides (at full strength on the entire field and its borders).

Awareness of the risks of resistance development has now led seed breeders and agricultural advisors to recommend immediate adoption of the **preventive use of several herbicide compounds**, whether in combination or successively – a strategy the interest of which has been established by theoretical studies. In order to facilitate the combined use of multiple herbicides, biotechnology companies are now developing varieties combining multiple tolerances – to glyphosate and to one, or even two, other herbicide modes of action. Treatment strategies featuring a mixture of two herbicide classes are inappropriate when resistance to one of the classes already exists among the target flora present, however (the mixture leading to the acquisition of double resistance among individuals already possessing single resistance).

By offering the possibility of using a single herbicide, glyphosate-tolerant varieties enable a simplification of weed control in the short-term. Recurrent use of the same molecule within a rotation, however, leads to floral shifts and the appearance of resistant weeds. These emergent problems have altered the effectiveness of the HT strategy, requiring recourse to complementary weed control solutions, a return to programs involving several applications and an eventual increase in tonnages of herbicides used relative to the initial savings. This increase in quantities applied is amplified by an awareness of the risk of development of resistant weeds, prompting a preventive use of herbicide combinations. As they have been utilised up to this point, HTVs thus fit into – and reinforce – tendencies toward the increased use, both preventive and curative, of multiple herbicides in combination with a reduction in other weed control methods, whether mechanical (soil tillage) or agronomic (rotations).

Effects on the Environment

Impacts on Non-target Organisms and on Biodiversity

The potential environmental impacts of HTV use may *a priori* be caused by the varieties themselves and/or by the herbicides with which the varieties are associated. HTVs being used in conventional agricultural systems, their advantages and disadvantages should be evaluated with reference to conventional systems not using HTVs. Insofar as HTV adoption is combined with other changes in cropping systems – or even production systems – it is at these levels that an evaluation of their impacts is relevant.

No impact of cultivated HT plants themselves has been shown, but little research has been done in this direction; the few studies that have been conducted on bees, in HT oilseed rape crops, examined only a fraction of the possible effects.

A single research program, conducted in England, has sought to compare conventional and HT crops in real agricultural conditions. It found that biodiversity impacts appear to be linked first of all to the effects of chemical weed control: a reduction in weed flora will have direct consequences on associated fauna and hence repercussions for the rest of the food chain. The effects of HTV adoption on biodiversity thus depend on the efficacy of the associated weed-control program compared to that of previous weed-control methods – which in general are found to be less effective, except for maize (prior to the banning of atrazine).

Chemical Contamination of Water and Soil Resources

Once again, methodological difficulties not specific to HTVs are encountered in all attempts to predict water contamination: notably, uncertainty as to the fate of the molecule (persistence, transfer into aquatic environments) due to the variability of transport and transformation phenomena as a function of soil characteristics (pH, percent organic matter, etc.) and climatic conditions; and a lack of or difficulty of access to the analytical data produced by companies in connection with their product approval requests.

Degradation and transport reactions of herbicide molecules in the environment depend on the physical and chemical properties of each molecule: data established for one molecule cannot be extrapolated or generalised to the class to which the herbicide belongs. The two best documented cases, and for which follow-up field studies as well as laboratory data exist, are atrazine and glyphosate. While glyphosate is said to be only weakly persistent in the environment, it and especially its principal degradation product (AMPA) are among the most frequently detected pesticides in French water supplies.

The most significant environmental argument in favour of HTVs is said to be a reduction in quantities of herbicides used: the analysis (cf. *supra*) of actual weed-control practices (in the United States) reveals the limits of this claim. The second argument advanced in favour of HTVs is that they permit the replacement of older, more toxic compounds by new molecules with more favourable eco-toxicological profiles. While results of eco-toxicity tests conducted in the laboratory are difficult to extrapolate for field conditions, data on the persistence of glyphosate call this assessment into question when degradation products are taken into account. The principal effect linked to HTV adoption appears to be the increased use of the same molecules (over larger areas), leading automatically to higher levels in water resources and increasing the risk of reaching or surpassing regulatory limits for drinking water.

Furthermore, evaluation of the environmental impacts of HTV use is confronted by other classic methodological questions and challenges (as noted for example in the ESCos on “Pesticides” and “Agriculture and Biodiversity”), which the present ESCo was unable to treat in exhaustive fashion: the impacts of herbicides on non-target organisms and on biodiversity more generally (the question of toxicity for humans not falling within the domain of this ESCo), the fate and transport of pesticides in the physical environment, comparative evaluation of the impacts of different systems, the relevance of the indicators utilised, etc.

HTV Cultivation in France

While analysis of the North American context has demonstrated the risks associated with large-scale cultivation of specific types of HTVs, these results are not directly transposable to the French context. The implications of a potential widespread adoption of HTVs in France must be considered with regard to the current characteristics of French cropping systems, but also with regard to current agronomic trends that may either favour HTV adoption or influence its outcomes. These outcomes will likewise depend on the types of HTVs in question.

The social context (the public perception of biotechnological innovations) and the regulatory framework likewise differentiate the American and European cases. In America, GMOs have not encountered significant opposition, and transgenic HTVs have been in use for 15 years or more. In Europe, on the other hand, and particularly in France, the social context is not favourable to GMOs, and the controversy they have provoked has led to the establishment of regulatory procedures governing their prior evaluation and use, and has limited for the time being the number of transgenic varieties included in the European and French catalogues. Thus, two transgenic HT maize varieties are listed on the European catalogue; in France, their cultivation is not prohibited by a moratorium but the herbicide to which they are tolerant is not approved for this use. The only HTVs grown or in the application process are thus non-transgenic. Nevertheless, they too are the focus of public protests, including several crop-destruction demonstrations targeting HTVs obtained by mutagenesis. It should be noted that although cultivation of transgenic HTVs is not currently practiced in Europe, nor is it envisioned in the short term, it cannot be excluded from a longer view.

The French Agronomic Context

With the exception of the "Agricultural Practices" surveys conducted by the statistical service within the Ministry of Agriculture, the significant lack of data with regard to farmers' practices makes possible only a very general and imperfect view of the current situation and its patterns of change. Broad tendencies can nevertheless be described, based on these surveys, on more qualitative analyses and on the application of agronomic reasoning (with the caveat that further speculation must await the results of the 2011 survey).

The textbook examples offered by North and South America illustrate a strong link between HTV adoption and the abandonment of mouldboard ploughing. Agricultural systems in North and South America are based on very short rotations, moreover, or even continuous cropping, which are likely to exacerbate weed-control challenges. This context contributes to the widespread, even near-universal adoption of HTVs. In France, no-till is still a minority practice but it is expanding (in 2006, 34 % of fields planted to major field crops were in no-till; over 50 % for farms of greater than 300 ha). Rotations and cropping patterns remain relatively diversified; however, the tendency is toward a simplification and shortening of rotations, including the planting of fewer spring crops within winter crop rotations.

HTV Use and Issues of Concern

The principal specificity of the French/European situation resides in the fact that, for the time being at least, only varieties obtained by traditional selection or by mutagenesis are cultivatable. These are tolerant to a selective herbicide (class B), and may appear to offer a solution to weed-control challenges encountered in the most important crop species grown in France, notably those due to weeds closely related to and interfertile with the crop, or even belonging the same species (sugar beet, sunflower, oilseed rape); to invasive species such as ragweed; and to parasitic plants such as broomrape (sunflower). As we have seen, the risk of transfer of the HT trait into weeds belonging to interfertile species is particularly high. There exists, furthermore, a strong probability of resistance development in ragweed, a species that can spread rapidly, is already very abundant in France and one that has become resistant to class B herbicides in other parts of the world.

Moreover, class B herbicides are already widely used in cereal crops. The introduction into cereal-oilseed crop rotations of HT oilseed rape or HT sunflower will thus increase the frequency of utilisation of this herbicide mode of action in the fields involved, and consequently increase the selection pressure exerted on these weeds.

To prevent this risk of resistance development and diffusion of the HT trait, technical guidelines currently issued for cultivation of HT sunflower suggest that use of these varieties should be reserved for situations with difficult weed flora, and in such cases to combine several modes of action on the HT crop itself and/or at the scale of the rotation. Respect of such weed-control programs entails herbicide consumption higher than that recorded for conventional crops (in 2006).

In France the phenomena of HT trait diffusion and resistance development, as well as their repercussions on herbicide use, will depend on the conditions of HT utilisation. A limited use of HTVs in both time and space, respecting "best agricultural practices" and integrating mechanical weed management methods and diversified rotations, would serve to limit these risks and help preserve the efficacy of the HT technology over time. The question thus presents itself as to the introduction of follow-up procedures for the growing of HTV crops in accordance with such best practices. In the United States, however, survey results have shown that farmers are little inclined to adopt preventive measures to reduce the risks of resistance development when such measures run counter to the simplifications (of soil tillage and herbicide treatments) that motivated the choice of HTVs in the first place.

The first crops of sunflowers tolerant to class B herbicides (Clearfield® tolerant to imidazolinone and Express Sun® tolerant to a sulfonyl-urea) appeared in France in 2010; in 2011 they covered an area estimated at 80,000 ha (equal to more than 10 % of total sunflower acreage in France). These areas are likely to be represented in the national "Agricultural Practices" survey for 2011; they could likewise justify the creation of specific surveys to record the agronomic conditions and motivations for adoption, the crop protection practices implemented and the resulting changes in weed flora.

Annexures

Annex 1. Mission Statement for the HTV ESCo

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The post-war appearance of synthetic chemical herbicides considerably facilitated a reduction in weed competition while at the same time posing a phytotoxicity risk for the crop. The agro-chemical industry thus sought selective molecules having maximum herbicidal effect while affecting the crop as little as possible. In recent years, an alternative to the discovery of new herbicide families has been the adaptation of cultivated varieties to existing active ingredients.

The term "herbicide-tolerant varieties" (HTVs) refers to cultivated plants characterised by possession of a genetic trait for tolerance (or resistance) to one or more herbicides (the HT trait), this trait being exploited and/or claimed for the plant variety. All cultivated plant species display tolerances to herbicides active against other botanical families; the term HTV is specific to varieties possessing tolerances which are either not native to the species or which were not identified as such at the time of their listing in the Official Catalogue of Varieties of Agricultural Species. The HT character may have been introduced by use of traditional selection methods only, or by use of transgenesis or mutagenesis. In the case of genetically modified varieties (known as GMOs), herbicide tolerance may also be a trait introduced as a marker gene in order to facilitate screening for those cells having successfully incorporated the transgene; thus many GM plants possess the HT trait without being advertised as such.

In December 2009, the Ministries of Agriculture and Ecology officially requested that CNRS and INRA jointly undertake a collective scientific expertise (ESCo) on the subject of herbicide-tolerant varieties. CNRS, INRA and the requestors together agreed on the following guidelines for this undertaking.

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1. **Context and consequences**

Plant breeders propose varieties possessing tolerance to an herbicide or family of herbicides, generally of large-spectrum, with which the crop varieties can be sold in the form of an HTV-herbicide "package". The genetic HT trait seems to be attractive to farmers, in part because of its ease of use and because of the agronomic efficacy of the herbicides used with HTV crops. Breeders likewise promote an environmental advantage for HTVs, particularly if the herbicide in question has a more favourable ecotoxicological profile than the herbicides typically used, and/or based on the possibility of treating standing crops in function of the weed flora actually present – in other words to treat only as needed, and thus *a priori* to use less than would be the case with a systematic preventive treatment.

Varieties marketed with the HT trait are either transgenic varieties (not currently authorised in France), or, more recently, varieties obtained without use of transgenesis and thus excluded from the sphere of application of European Directive 2001/18/EC,[1] which specifies the approval process for deliberate release into the environment and placing on the market of genetically modified organisms (GMOs).

These non-GMO HTVs are a focus of current public policy review and evaluation, for a number of reasons:

- first, in response to applications for inclusion on the Official Catalogue of Varieties of Agricultural Species, the Ministries of Agriculture and of the Ecology are seeking further information with regard to these varieties' genuine long-term benefits, as well as their compatibility with the objectives of the Ecophyto 2018 plan for the reduction of pesticide use in France and other current environmental policies;
- furthermore, these non-transgenic HTVs have begun to attract public opposition – individuals and groups opposed to GMOs suspecting that they represent an intentional avoidance of GMO regulations – as well as questioning and discussion among farmers. At the same time, the rapid advance of new techniques of mutagenesis and high-speed genome sequencing (making it possible to identify mutations affecting a specific gene) are rendering these breeding strategies of increasing interest to seed developers;
- finally, France seeks to take a leadership role in discussions surrounding the possible evolution of European regulations governing evaluation of non-GMO varieties obtained by techniques such as mutagenesis.

Nevertheless, it seems preferable to not restrict this ESCo to varieties obtained by mutagenesis. In the first place, many review studies of the impacts of HTVs have been conducted either on or in relation to GMOs.

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[1] Directive 2001/18/EC covers the following breeding techniques: transgenesis, cisgenesis and cell fusion techniques by means of methods that do not occur naturally. Excluded from its field of application are techniques making use of genetic modifications but resulting in non-GM organisms, such as mutagenesis and the fusion of plant cells from organisms that can exchange genetic material through traditional breeding methods.

Moreover, a number of agronomic and environmental risks potentially associated with herbicide tolerance (difficulties in managing HTV volunteers, increased use of a given herbicide leading to increased risks of pollution and the appearance and spread of resistant weeds, for example) are independent of the breeding technique used to develop this trait. Such risks arise more generally from the use of traits for tolerance to one or more herbicides and from the herbicides themselves.

2. **Objectives and focus of the expertise**

The preceding analytical elements have led to the definition of the following subject focus for this expertise: **the genetic trait of herbicide tolerance (for an herbicide to which the botanical family is normally sensitive), regardless of the breeding technique used to develop this characteristic; and an analysis of the various consequences of this trait's use.**

The objective of this ESCo is to provide to the public authorities, and to society at large, a critical review of the state of available scientific knowledge in order to identify the specific questions posed by HTVs and to evaluate their advantages and disadvantages in agronomic, environmental and socio-economic terms (not including potential impacts on human health).

More precisely, the objective is to assemble and analyse the available information in order to establish:

– a summary of peer-reviewed scientific studies relating to the impacts of HTVs already in cultivation;
– a review of useable methods and protocols for the prior evaluation and subsequent monitoring of such varieties;
– a diagnosis of knowledge gaps and controversies within this material and an identification of evaluation methodologies requiring the development and establishment of new research.

This ESCo will thus cover all HT varieties, whatever the breeding technique used for their development, for annual cultivated species of temperate climates (grain, oilseed and field-scale vegetable crops). Particular attention will be paid to species for which applications for inclusion on the French Catalogue of Varieties of Agricultural Species are pending: maize, oilseed rape, sunflower, sugar beet, soybeans, endive and chicory.

3. **Questions and scientific themes**

The analysis of the available scientific literature will seek to identify and evaluate potential impacts linked to the production and utilisation of HTVs. These impacts – agronomic, environmental and socio-economic – may result directly from use of the HTV and its associated herbicide, or they may be more indirect; for example, changes in agricultural practices and production choices induced or enabled by HTVs. The impacts may vary according to the characteristics of the HTVs, their associated herbicides and the cropping systems involved. Criteria potentially determining different levels of risk include the following: the **characteristics of the herbicide** (mode of action, eco-toxicity, etc.), the **biochemical toler-**

ance mechanisms (how the plant neutralises the effects of the herbicide), the **breeding techniques** used to obtain the HTV (currently in use or in development), the **biological characteristics of the cultivated species** under consideration, its **technical and economic characteristics** (position and function within crop rotations, total crop area, use, agro-economic importance, etc.), the **herbicide application methods** (spraying equipment, etc.), the **characteristics of the cropping and production systems** within which the HT crop is included, and the **conditions of HTV diffusion** (commercial marketing campaigns, regulatory frameworks, conditions of sale, etc.).

Principal Types of Impact

The goal is to extract key elements from the scientific literature so as to make possible an evaluation of whether the benefits claimed for HTVs are real, sustainable, technically possible and readily obtained under field production conditions; whether they are countered or cancelled out by indirect effects; and whether the risks cited appear to be scientifically founded. The ESCo will take care to consider all possible impacts of HTV use, including those figuring within contemporary debates as well as those identified by the research community. These impacts are to be studied at multiple temporal and spatial scales, in order to take into account cumulative and/or secondary effects linked to the widespread use of HTVs and/or of their associated herbicides.

Agronomic Impacts (on Cropping Systems and for Farmers)

Impacts to be considered include:

- in the short term and at the field level: changes in weed management strategies and crop management strategies, as well as changes in the cropping system more generally (length of rotation, soil tillage practices, etc.);
- in the medium term and at the landscape level: potential effects of the "success" of HTVs, resulting in the development of rotations (and thus agricultural regions) featuring a reduced number of crops, varieties and/or herbicide molecules.

  The following impacts will be given particular attention:

- Impacts on <u>pesticide use</u>: changes in weed control strategies for the HT crop, both in terms of use of the associated herbicide and potential recourse to complementary herbicides (in order to manage volunteers and resistant weeds), and with regard to other pesticides (changes in cropping systems associated with HTVs potentially favouring pests and disease, thus making supplementary crop protection treatments necessary).
- Impacts on the <u>genetic quality of varieties and the genetic performance of crops</u>: the ESCo will examine the risk of genome perturbations caused by the introduc-

tion of the HT trait into the plant, perturbations that may not be detected during the selection phase but may appear in the form of phenotype consequences in the medium term, such as an increased vulnerability to pests or to abiotic stresses. At the global level, the ESCo will evaluate risks linked to high levels of crop genetic homogeneity, which may compromise the sustainable use of HTVs.
- Impacts on production (quantity and quality of the primary product).

Impacts on the Environment

- Environmental contamination by herbicides: the ESCo will examine the specific conditions of herbicide use in the case of HTV crops that may influence the risk of contamination of various environmental components, whether biological or physical.
- Two environmental components are of particular interest: agricultural soils and water resources, both surface and subsurface. The atmospheric component will also be examined, although the bibliographic resources in this area are *a priori* meagre.
- Potential effects on biodiversity in agricultural areas:
  - effects of HTV use on crop genetic diversity, due to a potential reduction in the number of varieties in cultivation;
  - effects of the herbicide on in-field non-crop flora (diversity and abundance of weed flora, impacts in terms of herbicide resistance);
  - effects of HTV use on fauna associated with the crop and/or its weeds (wild pollinators, honeybees, mammals);
  - effects of HTV dispersal on wild biodiversity; the potentially invasive nature of HTV populations (long-distance dispersal as a function of seed-dispersal capacity and the capacity for population maintenance by vegetative propagation, etc.);
  - the transfer of tolerance into other closely related plant species (weeds or species belonging to semi-wild areas).

Consequences for energy consumption, water management and greenhouse-gas emissions linked to changes in agricultural practices associated with HTV use (the elimination of ploughing, for example), if such analyses are possible in light of the available scientific literature.

Socio-Economic Impacts (for Farmers and Other Agri-Food System Actors)

If relevant scientific studies exist, the ESCo will also examine the economic effects of HTV use at the farm scale (impacts on the organisation of farmers' work, production costs, risks of dependence on the biotechnology company holding rights to the variety, etc.), at the level of the production sector (effects on market segmentation, etc.), and at the level of the seed and agrochemical sectors.

Conditions likely to influence these effects include the governance of this domain: legislation regulating the market introduction and use of these products and these varieties, the intellectual property regimes adopted, political leadership, etc.

The perception, by various actors, of innovation in the context of HT trait use will likewise be examined, insofar as the academic literature and data available on this topic are applicable to the French context. The perception of GMOs as such will not form part of the range of topics covered, however; this ESCo being limited in this regard to perception of the HT trait.

Annex 2. HRAC Classification of Herbicides According to Site of Action

HRAC group	Mode of action	Family – principal molecules currently used with HTVs	
A	Inhibition of acetyl CoA carboxylase (ACCase) (lipids biosynthesis)	Aryloxyphenoxy-propionate (FOPs)	Phenylpyrazoline (DEN)
		Cyclohexanedione (DIMs) **– cycloxydim, sethoxydim**	
B	Inhibition of acetolactate synthase ALS (acetohydroxyacid synthase AHAS) (amino acids biosynthesis)	Sulfonylurea **– chlorsulfuron, tribenuron-methyl**	Pyrimidinyl(thio) benzoate
		Imidazolinone **– imazamox**	Sulfonylaminocarbonyl-triazolinone
		Triazolopyrimidine	
C1	Inhibition of photosynthesis at photosystem II	Triazine **– atrazine**	Uracil
		Triazinone	Pyridazinone
		Triazolinone	Phenyl-carbamate
C2		Urea	Amide
C3		Nitrile **– bromoxynil**	Phenyl-pyridazine
		Benzothiadiazinone	
D	Interference with electron flow at photosystem I	Bipyridylium	
E	Inhibition of protoporphyrinogen oxidase (chlorophyll biosynthesis)	Diphenylether	Triazolinone
		Phenylpyrazole	Oxazolidinedione
		N-phenylphthalimide	Pyrimidindione
		Thiadiazole	Other
		Oxadiazole	
F1	Bleaching: inhibition of carotenoid biosynthesis	Pyridazinone	Other
		Pyridinecarboxamide	
F2		Triketone	Pyrazole
		Isoxazole	Other
F3		Triazole	Urea
		Isoxazolidinone	Diphenylether

(continued)

HRAC group	Mode of action	Family – principal molecules currently used with HTVs	
G	Inhibition of EPSP synthase (amino acids biosynthesis)	Glycine **– glyphosate**	
H	Inhibition of glutamine synthetase (photosynthesis)	Phosphinic acid **– glufosinate-ammonium**	
I	Inhibition of dihydropteroate (DHP) synthase	Carbamate	
K1	Microtubule assembly inhibition	Dinitroaniline	Benzamide
		Phosphoroamidate	Benzoic acid
		Pyridine	
K2	Inhibition of mitosis/ microtubule organisation	Carbamate	
K3	Inhibition of cell division	Chloroacetamide	Tetrazolinone
		Acetamide	Other
		Oxyacetamide	
L	Inhibition of cell wall (cellulose) synthesis	Nitrile	Triazolocarboxamide
		Benzamide	Quinoline carboxylic acid
M	Uncoupling (membrane disruption)	Dinitrophenol	
N	Inhibition of lipid synthesis – not ACCase inhibition	Thiocarbamate	Benzofuran
		Phosphorodithioate	Chloro-carboniccacid
O	Action like indole acetic acid (synthetic auxins)	Phenoxy-carboxylic-acid **– 2,4-D**	Quinoline carboxylic acid
		Benzoic acid **– dicamba**	Other
		Pyridine carboxylic acid	
Z	Unknown	Arylaminopropionic acid	Organo selenate
		Pyrazolium	Other

Table based on HRAC (www.hracglobal.com) and Schmidt 1997

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