GURTs: No Case for Field Trials

Federation of German Scientists, Curitiba, March 2006

No peer-reviewed scientific data has been published on GURTs since 2000 that would alter the assessment and implications of CBD decision V/5-III, recommending that field trials not take place before "appropriate scientific data can justify such testing". In particular, there is an absence of:

- **Evidence that the components of GURTs (individually and in combination) perform with the degree of reliability and accuracy required for a stable and reliable GURT;**
- **Evidence concerning impacts on the environment, biodiversity, and human health;**
- **Evidence that v-GURTs applications to be used for bio-confinement will not allow gene flow to occur, via seed or pollen.**

Unless reliable data from greenhouse trials, published in the peer-reviewed scientific literature, can show the existence of a complete GURT and its reliable and stable performance, and address outstanding issues, there is no reason for Parties to the CBD to consider field trials or case-by-case risk assessment.

Genetic use restriction technologies (GURTs) aim to restrict the use of genetic material and their related traits. Though they can be applied to plants and animals alike, this briefing only addresses GURTs in relation to plants.

Inserted into a plant by means of genetic engineering, GURTs are designed to provide external control over genes or traits.

GURTs are a special class of genetic engineering technologies that are characterised by "gene switches", which respond to external inducers, such as chemicals. Genes functionally linked to the switch mechanism can be turned on or off at will through the application of an inducer, such as a chemical compound sprayed on the plant. Alternative systems to chemical inducers are being developed (e.g. plant varieties with "inducer lines").

GURTs have been sub-divided into two categories, namely T-GURTs and V-GURTs. As is discussed below, the dividing line is not always clear or easily drawn and the molecular mechanisms used for either T- or V-GURTs are principally the same and exchangeable.

Trait-related GURTs (T-GURTs) are designed to restrict the use of particular traits, such as herbicide tolerance, insect resistance, special oil content, nutritional components, defence mechanisms, drought and stress tolerance, industrial or pharmaceutical compounds, flowering, ripening or female and male fertility. Unless chemical inducers are applied at the appropriate developmental or growth stages, the trait remains either switched on or off, or might be removed altogether from the plant's genetic material. The use of the trait is thus bound to the application of specific chemicals, e.g. through spraying the crop or seed coating. Unless the chemical is bought and applied, the quality for which the seed has been purchased does not materialise. In the case of seed coating, the quality will not materialise in saved and replanted seed, but only in newly bought seed.

Varietal GURTs (V-GURTs) are designed to restrict the use of the genetic material and germplasm of an entire plant variety. Here, the switch mechanism allows for external control over the capacity of a plant to successfully reproduce. Designed as a *Technology Protection System* (TPS),[1] it prevents farmers from growing crops from saved seeds or from using the variety for breeding purposes, thereby protecting a company's "intellectual property." The target genes of the switch mechanism here are genes involved in the formation of the embryo, the germination of seeds or the development and growth of seedlings to mature plants. V-GURTs are generally characterised by giving rise to either sterile seed or non-germinating seed, and are commonly referred to as *terminator technology*.

Depending on the design, the external application of chemicals would either trigger or prevent the

[1] Delta & Pine Land refers to their GURTs design as "Technology Protection System".

capacity of seed to germinate or to grow into a mature plant.

Common to all V-GURTs designs to date is the production of viable pollen, capable of out-crossing into nearby fields of related plants and to wild relatives. Seeds fertilised by such pollen, irrespective of whether sterile or not, would contain all the transgenes of the V-GURTs plant, and potentially their products. Hence, out-crossing would give rise to contamination.

Molecular mechanism behind GURTs

A number of different designs of GURTs and their molecular components have been described in patents and patent applications. Whilst most designs are applicable for both T-GURTs and V-GURTs, some patents place particular emphasis on inducible seed sterility or solely V-GURTs.[2]

Both T- and V-GURTs are constructed in the same way on the molecular level, and there is no clear distinction between them. In general, as shown in **Figure 1,** GURTs are composed of four main elements, namely: the target gene (D), the promoter of the target gene (C), the trait activator gene (B) and the gene switch (A), which itself is usually composed of three elements.

The Target gene, also referred to as the *Gene of Interest*, is the gene (or its RNA or protein) for which the switch mechanism is ultimately meant. Depending on the trait, the gene may, for example, code for an agronomic trait (e.g. pest resistance) or for a disrupter cell-toxic protein[3], e.g. the cell lethal compounds ribosome inhibitor protein (RIP) or barnase ribonuclease.

The Promoter of the target gene is chosen according to when and where the target is to be expressed. The use of the LEA promoter (late embryogenesis abundant), for example, will lead to the expression of the gene at the late seed setting stage. If combined with a disrupter gene, the trait will be seed sterility (V-GURTs); if combined with a gene for an extractable compound, such as an industrial substance, the trait will be, for example, that of industrial oil production (T-GURTs).

The Trait Activator gene stands between the gene switch and the target gene. It is turned on or off by the gene switch and will in turn activate or deactivate (or repress, or counteract) the target gene. If, as in the Delta & Pine Land V-GURT design, the target gene is kept inactive through a blocking sequence placed between promoter and disrupter gene, the block can be removed by a recombinase enzyme, which acts like a pair of molecular scissors. In this case the trait activator gene will be a recombination enzyme. The same trait activator gene can, on the other hand, also be used to deactivate a trait like insect resistance by removing the promoter or parts of the target gene. In this case, the absence of the application of a chemical inducer will result in the loss of the agronomic trait (T-GURTs).

The Gene Switch is commonly made up of three elements, a promoter, an enabler gene, and a second promoter. The latter is directly linked to the trait activator gene. Depending on the specifics of the gene switch, application of a chemical will either result in activation or deactivation of the whole cascade of gene interactions. In Delta & Pine Land's V-GURT design, the treatment of seeds with an inducing chemical will trigger a cascade resulting in sterile seed production. In contrast, the Syngenta V-GURT design requires the application of chemicals for the production of fertile seed and only the absence of the inducing chemical will result in sterile seed production.

Some gene switches are made up of five elements, whilst the "plant-derived safener system" requires only one.

In rare cases (according to patent applications), the gene switch is directly linked to the target gene, with the trait being activated directly at the point of chemical induction.

Alternatives to the chemical induction system are being developed, in which the inducer resides in a second plant line (the "inducer line" or "activator line") that can be crossed into the first plant line containing the trait or gene of interest. In this system, F1 hybrid seeds produced from a cross between inducer and maintainer line would result in plants with an activated GURT system.

GURTs formula

As shown in **Figure 1**, the general formula for GURTs is (A) gene switch, (B) trait activator gene, (C) promoter of target gene and (D) target gene. Thus

[2] The first V-GURTs or terminator patent was granted in March 1998 (US 5723765) to the Seed Company Delta & Pine Land (DPL) and the US Department of Agriculture (USDA). Other V-GURTs designs include those detailed in WO 9744465 (Monsanto), and EP 065820731 (Syngenta).

[3] Other disrupter proteins put forward in GURTs patents include adenine nucleotide translocator (ANT), beta-tubulin, and invertase.

A+B+C+D = GURTs,

with A=a1+a2+a3. Accordingly, any combination is possible, e.g. A3+B1+C2+B5.

For example, in the Delta & Pine Land design[4] in its preferred embodiment, V-GURT = A (tetracycline inducible gene switch) + B (CRE/lox recombination system) + C (late embryogenesis abundant promoter) + D (cell toxic disrupter protein RIP).

In the Monsanto design[5], V-GURT = A (any inducible gene switch) + B (restorer) + C (constitutive promoter) + D (germination inhibitor).

Consequently, on the molecular level, no clear distinction can be drawn between V-GURTs and T-GURTs, as it depends on the combination of different individual elements used.

TV-GURTs

As seen in different patents, some GURTs are designed to produce extractable compounds in plants. As many of these are harmful to plant growth or development, production of these compounds throughout the life of the plant is not optimal. To expose the plant to the negative effects of these compounds as late as possible, the genes for such compounds are linked to seed specific promoters, with the seed becoming the factory to produce the compounds. As a direct consequence, the seed will often no longer be able to germinate. Whilst designed as a T-GURT, the outcome is also a V-GURT, i.e. seed sterility.

Reliability and performance

To date, no functional V-GURT has been detailed in the peer-reviewed scientific literature. Whilst there is mention of greenhouse trials by one company (Delta & Pine Land), no results have so far been published in any form. Thus, GURTs remain purely a design, lacking the data vital to judge whether indeed GURTs would work once engineered into plants. However, some components of GURTs are the subject of increased research. The data published to date do not provide evidence that the components, individually or in combination, perform with the degree of reliability or accuracy required for a stable and reliable GURT. The evidence is rather to the contrary.

Unless reliable data from greenhouse trials, published in the peer-reviewed scientific literature, can show the existence of a complete GURT and its reliable and stable performance, there is no reason to consider field trials. Furthermore, data also has to be provided that field trials will not pose a risk to the environment, biodiversity, and human health or have negative socio-economic or cultural impacts.

With regards to the use of V-GURTs for bio-confinement, data from greenhouse trials over a number of growing cycles will have to provide the evidence that gene flow can not occur either via seed or via pollen.

As no data of a functional and reliable GURT have been made available for thorough assessment by either the scientific community, the CBD or its relevant bodies, a debate regarding field trials can only be seen as hypothetical at this point.

Finally, given the lack of reliability of the individual components of GURTs, there is little ground to presume that any GURT, especially V-GURT, will offer reliable performance over numerous growing seasons. Indeed, as stated by the US National Academy of Science in 2004, no methods of biological containment can offer complete containment, given the state of knowledge to date. [6]

Consequently, any field trials of this technology would invariably lead to gene flow out of the system, with potentially adverse biological consequences. Considering all the above points, no provision should be made for allowing field trials at this stage.

[4] Delta & Pine Land patent US 5723765

[5] Monsanto holds the WIPO patent WO 9744465

[6] National Research Council (2004). Biological Confinement of Genetically Engineered Organisms. National Academy Press, Washington, D.C.

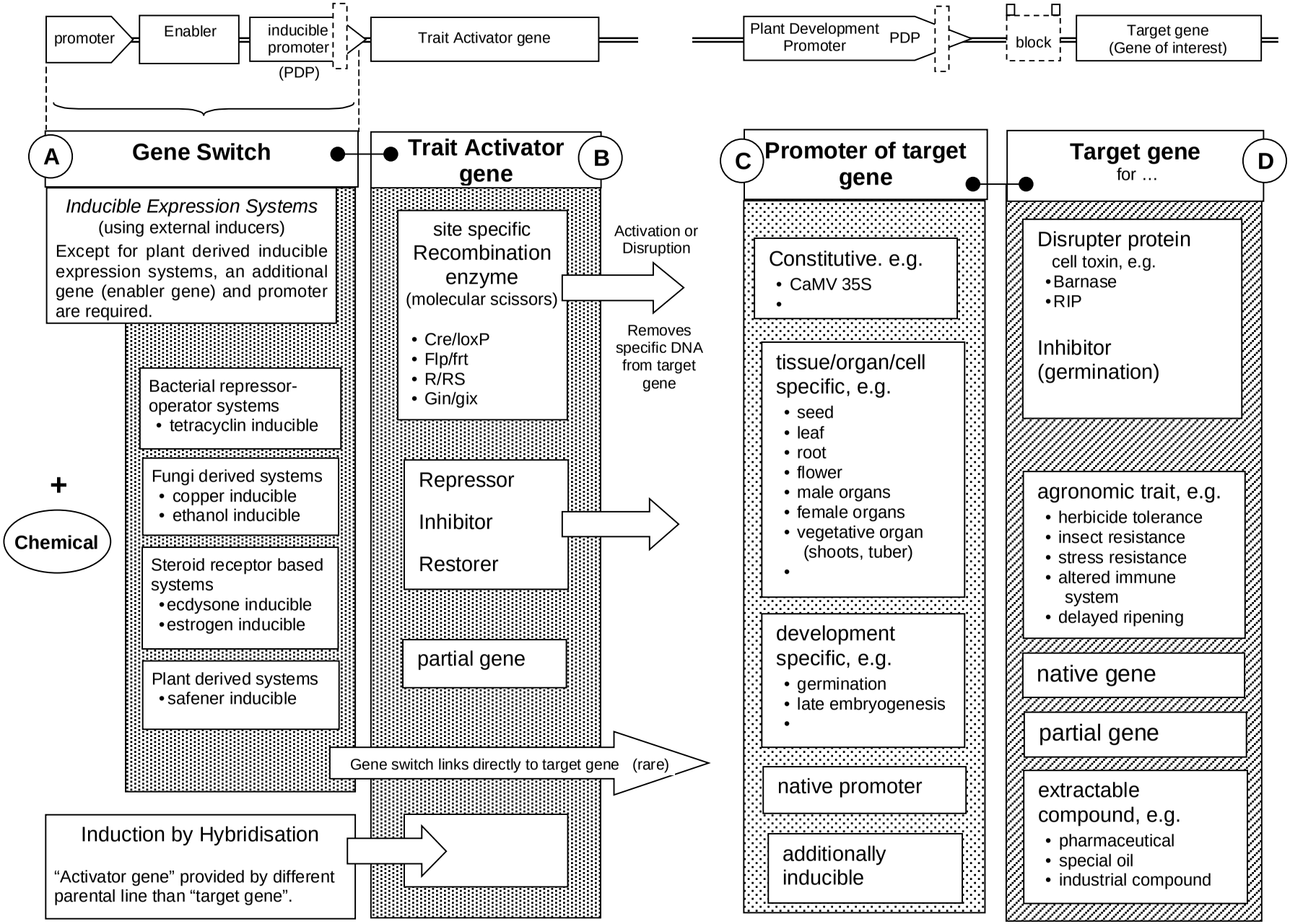

Figure 1: The molecular components of GURTs (for details see text)
Federation of German Scientists and EcoNexus (info@econexus.info)