Document Title

Potential influence of the genetically modified EPSPS expressing genes and introduction of the pat or bar genes on the metabolism of spirotetramat in cotton and soybean compared to the wild-types

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Request from EFSA:

"...EFSA notes that some residue trials on soya and cotton have been performed with genetically modified crop. The applicant should therefore provide evidence that the genetically modification does not alter metabolism in the modified crop. If in the genetically modified crop a gene is inserted that conveys active ingredient resistance due to pesticide metabolism, additional studies are required elucidating the metabolism in genetically modified crops."

Answer from BCS:

Some of the supervised residue trials in cotton and soybean were conducted with genetically modified plants being resistant towards the herbicide "Roundup", which contains the active ingredient glyphosate (Roundup-Ready plants). Some cotton trials were conducted with glufosinate-resistant plants Liberty-Link plants.
In this statement, the mode of action of the herbicides and mechanism of herbicide resistance is explained, followed by the consideration about an influence of genetic modification on the metabolism of spirotetramat.

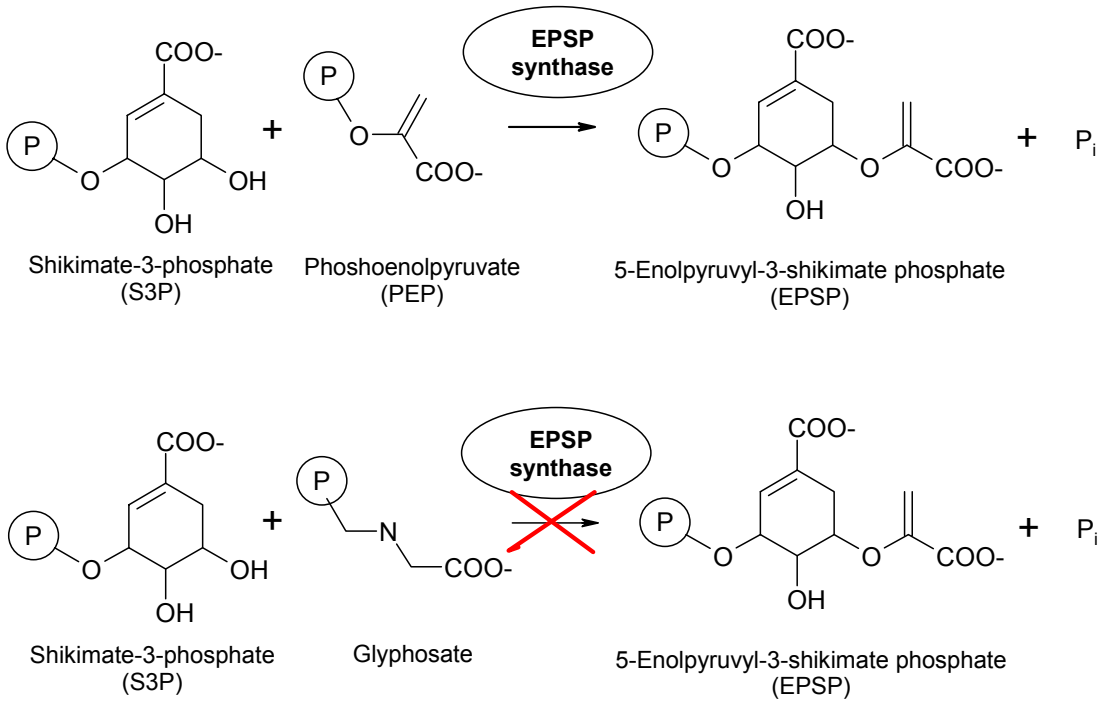
Glyphosate resistant plants

Glyphosate is a non-selective herbicide. Its primary mode of action is the inhibition of the plant enzyme 5-enolpyruvyl-3-shikimate-phosphate synthase (EPSPS), an enzyme in the shikimate pathway. This metabolic pathway produces aromatic amino acids in plants (i.e. phenylalanine, tyrosine, and tryptophan). These aromatic acids serve as precursors for numerous secondary plant products such as anthocyans, lignin, growth promoters, growth inhibitors, and phenolics, as well as proteins. The EPSPS enzyme converts phosphoenolpyruvate (PEP) and shikimate-3-phosphate (S3P) to 5-enolpyruvyl-3-shikimate phosphate (EPSP) (see Figure 1). The EPSPS enzyme is encoded in the nuclear genome and is localized in the plastids.

Glyphosate competitively inhibits the enzyme with respect to phosphoenolpyruvate with a Ki of 1 µM (Duke, 1988). Glyphosate structurally resembles the PEP oxonium ion with the exception that the phosphor in its phosphonate moiety is not attached to oxygen as in PEP but to a carbon atom. This allows glyphosate to bind very efficiently to the enzyme, but it cannot take part in the enzymatic reaction since the carbon-phosphor bond lacks the high energy of the oxygen-phosphor bond (P-C 513.4 kJ mol-1, P-O 596.6 kJ mol-1, CRC, 1986-1987). The glyphosate molecule locks the active complex of the EPSPS enzyme and its substrate, and the glyphosate inhibitor remains bound to the enzyme (Schönbrunn et al., 2001). In this inhibitory mechanism the enzymatic reaction is completely blocked and neither the S3P substrate nor the inhibitor is further processed by the enzyme.

Blockage of the EPSPS enzyme results in a disruption of the shikimate pathway and consequently in a massive accumulation of shikimate in affected plant tissue. The in vivo activity of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase, an earlier enzyme in the shikimate pathway, is also increased as a result of glyphosate inhibition of EPSPS (Pinto et al., 1988). Glyphosate is the only compound known to inhibit EPSPS sufficiently to be a valuable herbicide.

Figure 1: Inhibition of the EPSPS mediated reaction by the glyphosate molecule



Glyphosate tolerant crop varieties have now been developed by introduction of an alternative EPSPS expressing gene into the plant's genome. The resulting EPSPS enzymes, i.e. a CP4 EPSPS version in Roundup-Ready plants from Monsanto, or a 2mEPSPS version from BCS, have a significantly lower affinity to glyphosate, but no altered functionality in synthesizing aromatic amino acids when compared to the wild-type crops.

In an alternative approach to render the plant tolerant to the glyphosate, a copy of an enzyme that metabolizes this herbicide is introduced. Two different activities are currently used in this strategy: the glyphosate oxidoreductase (GOX) that is degrading glyphosate to non-active AMPA (aminomethyl-phosphonic acid) and the glyphosate acetyl transferase (GAT) that is catalyzing the acetylation of the herbicide and thus inactivating the phytotoxic effect (Franz et al., 1997).

In Roundup-Ready plants production of the genetically modified EPSP synthase is implemented resulting in glyphosate tolerance by production of an EPSPS enzyme that is not inhibited by glyphosate while its enzymatic activity remained virtually unchanged. As this modified enzyme is not involved in the co-metabolism of xenobiotica the metabolic transformations of other herbicides, e.g. Spirotetramat, remains unchanged compared to the wild-type species.

Glufosinate resistant plants

Glufosinate is a non-selective post-emergence herbicide that controls weed by irreversibly inhibiting the enzyme glutamine synthetase which plays a primary role in the plant metabolism. This enzyme incorporates glutamate and ammonia to form the amino acid glutamine as shown in the following schema (Figure 2).

As glufosinate is a structurally analogous compound to glutamate it competes during the glutamine synthetase reaction.

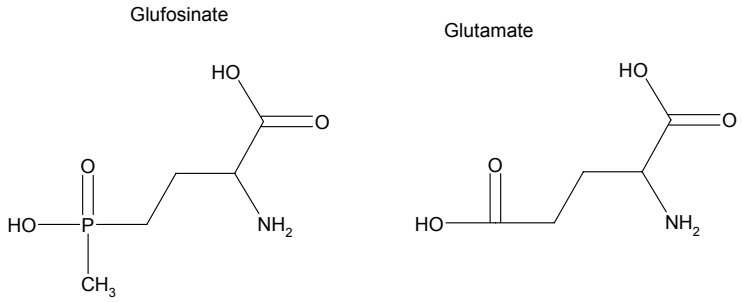
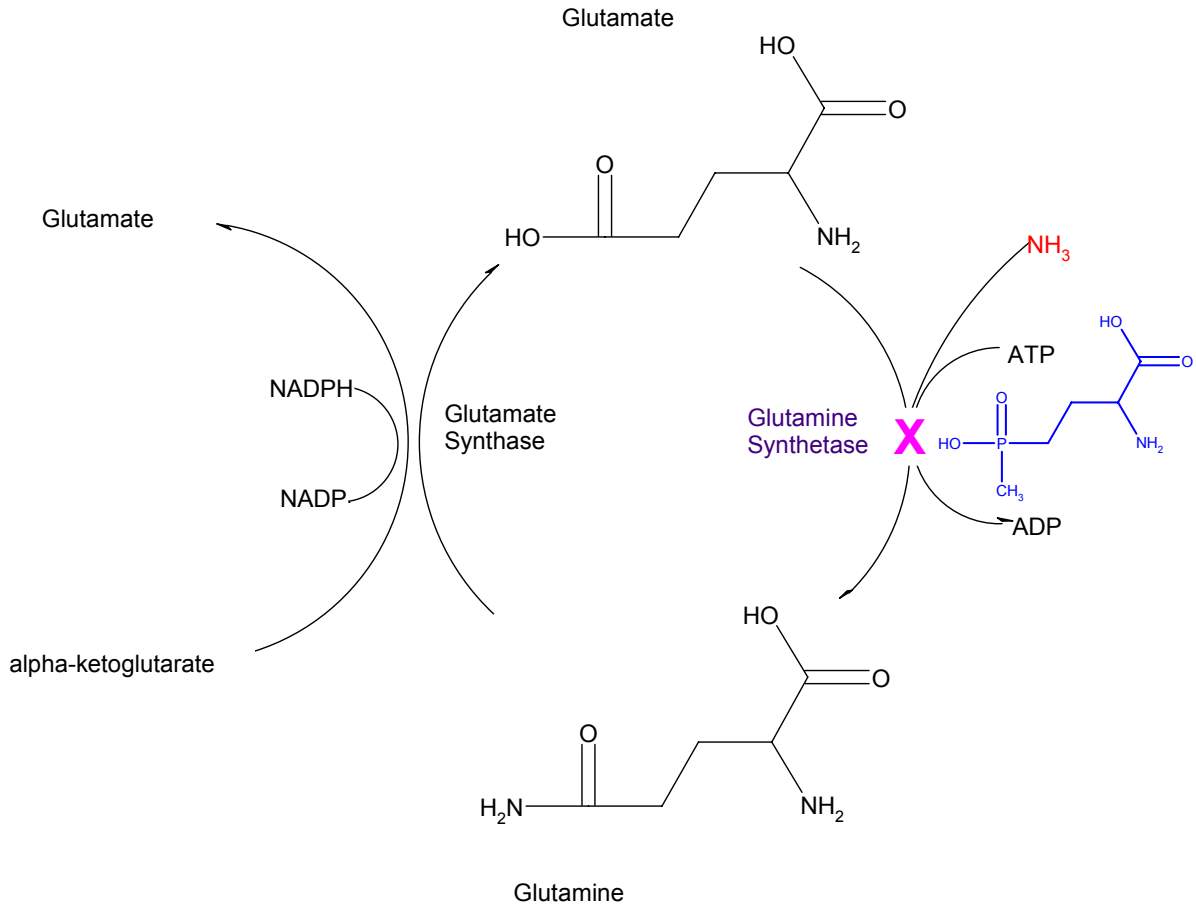


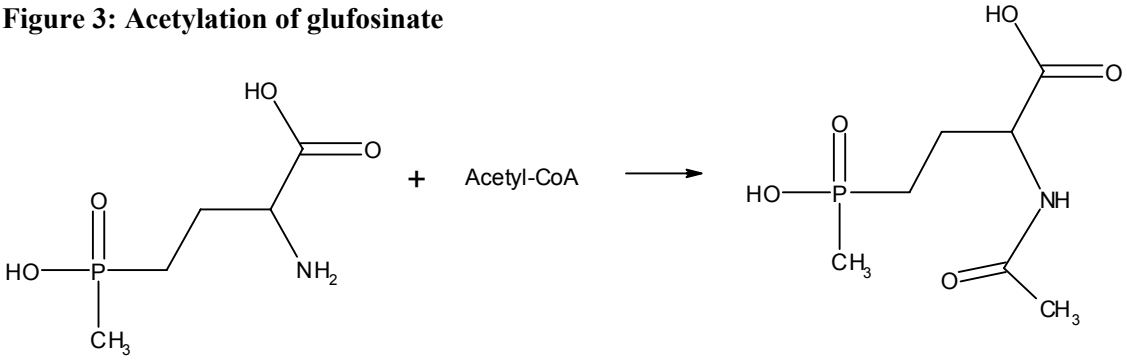
Figure 2: Inhibition of the glutamine synthetase by glufosinate



Glutamine synthetase contained in leaves and roots having different isoforms that show different sensitivities to glufosinate. The inhibition of glutamine synthetase is manifested by ammonia accumulation, inhibition of amino acid synthesis and inhibition of photosynthesis.

Glufosinate tolerance is conferred to plants by incorporation of either the pat (phosphinothricin acetyl transferase) or the bar (bialaphos resistance) gene, both of which code for enzymes that inactivate glufosinate by acetylation (Liberty Link plants). Both, the pat and bar enzymes are similar in their ability to selectively acetylate glufosinate but not other amino acids (Figure 3).

Figure 3: Acetylation of glufosinate



Glufosinate **N-Acetyl-Glufosinate**

The acetylation of glufosinate by pat and bar-gene-expressed acetyl transferase enzymes is very specific to glufosinate. Xenobiotic molecules with a different structure, e.g. spirotetramat, are metabolically not changed if compared to the wild-type species.

Influence of a genetic modifications of the EPSPS expressing and pat or bar genes on spirotetramat metabolism

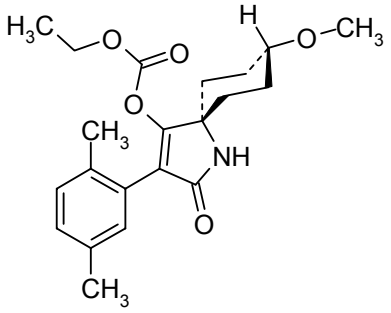
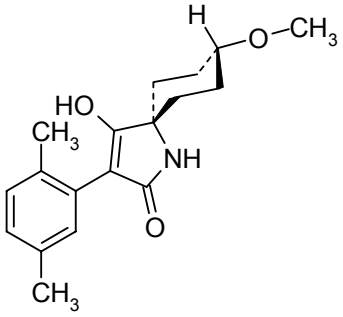
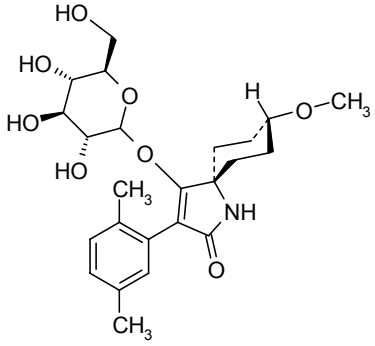
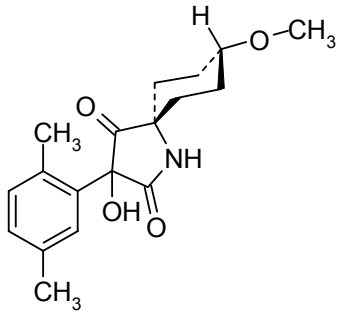
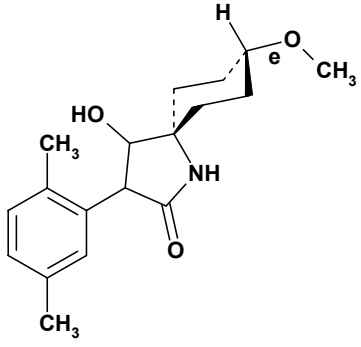
The enzymes responsible for the glyphosate and glufosinate tolerance in plants exhibit very specific reactions that do not influence the metabolism of other xenobiotica.

EPSP synthase exclusively appears in the shikimate pathway. A production of the genetically modified version of this enzyme in Roundup-Ready plants results in the same biochemical synthesis reactions as observed in the wild-type species and does not have any influence on spirotetramat metabolism.

The acetylation of glufosinate by genetically modified bar and pat gene-expressing enzymes in Liberty-Link plants is also considered as a very specific process. Other amino acids are not acetylated (Wehrmann et al., 1996). As spirotetramat and its metabolites in plants are structurally completely different from glufosinate (Figure 4), the enzyme acetylating glufosinate would not alter metabolism of spirotetramat.

Considering the specifity of the enzymes and the structure of their substrates, there is no evidence that the genetical modification in Roundup-Ready or Liberty-Link soybean or cotton plants could have an influence on the metabolic transformation of spirotetramat.

Figure 4: Structures of spirotetramat and its plant metabolites:

Spirotetramat	Spirotetramat-enol	Spirotetramat-enol-glucoside
		
Spirotetramat-ketohydroxy	**Spirotetramat-monohydroxy**	
		

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