TESTBIOTECH Background 17 - 12 - 2020

Synergistic effects between Bt toxins and plant constituents (proteinase inhibitors, PI) multiply toxicity of GE plants

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Relevant findings show that the selectivity and efficacy of Bt toxins produced in GE plants can be influenced by many co-factors (see, for example, Then, 2010; Hilbeck & Otto, 2015). One crucial impact factor are protease inhibitors (PI), which delay the degradation of Bt proteins and thereby enhance their toxicity. In many of its comments on EFSA opinions, Testbiotech has highlighted this flaw by referring, for example, to Pardo-López et al. (2009). However, EFSA has never provided a detailed response.

Testbiotech is now aware of several other publications confirming this gap in risk assessment that EFSA has constantly ignored or denied: as Monsanto already showed in the 1990s, maize, cotton and soybeans produce protease inhibitors (PI), which considerably enhance the toxicity of Bt proteins in plants. In the presence of PIs, Bt toxin will degrade much more slowly than in isolation. This results in a much higher toxicity of the Bt toxin (if it is taken up together with the plant tissue) compared to the isolated toxin (MacIntosh et al., 1990; Zhao et al., 1999; Zhang et al., 2000; Gujar et al., 2004; Zhu et al., 2007; Pardo-López et al., 2009; Ma et al., 2013; Mesén-Porras et al., 2020). The effects described indicate, for example, a 20-fold higher toxicity of Bt proteins if produced in the plants and taken up with PIs (MacIntosh et al., 1990).

Therefore, any risk assessment that does not take a combination of plant material and the Bt toxin into account is not reliable and systematically underestimates the risks. As can be concluded from the EFSA opinions, most of the data on the toxicity of Bt proteins are based on tests using isolated Bt toxin.

In summary, the evidence for enhanced toxicity of Bt proteins produced in maize, cotton and soybeans was published by Monsanto 30 years ago (MacIntosh et al., 1990) and has since then been confirmed in multiple studies. Crucially, EFSA has never assessed this crucial aspect in any of its opinions.

It is known from scientific publications that co-factors which enhance the toxicity of the Bt proteins can also impact their selectivity (for overview see Then, 2010): if synergistic or additive effects occur that increase efficacy of the Bt toxin, its selectivity may be decreased and a wider range of non-target organisms may become susceptible. In addition, there has never been any systematic research into these combinatorial effects. There are just a few publications available which indicate effects on non-target insects from protease inhibitors combined with Bt toxins (Babendreier et al., 2005; Liu et al., 2005a; Liu et al., 2005b; Han et al., 2010).

The synergistic effects described by MacIntosh et al. (1990), Zhao et al. (1999), Zhang et al. (2000) Gujar et al. (2004), Zhu et al. (2007), Pardo-López et al. (2009), Ma et al. (2013), Mesén-Porras et

al. (2020) causing higher toxicity of the Bt toxins are also relevant in risk assessment in regard to the immune system: the combination with protease inhibitors is likely to be associated with a delay in the degradation of the Bt toxins after consumption. This delay in degradation extends the exposure of the intestinal immune system to Bt toxins and may trigger or enhance chronic inflammation and allergies.

In this context, it is relevant that Bt toxins produced by plants can indeed survive digestion to a much higher degree than has been assumed by EFSA. Chowdhury et al. (2003) and Walsh et al. (2011) showed that when pigs were fed with Bt maize, Cry1A proteins could frequently and successfully still be found in the colon of pigs at the end of the digestion process. This means that Bt toxins are not degraded quickly in the gut and can persist in larger amounts until digestion is completed; therefore, there is enough time for interaction between various food compounds. In addition, a study testing corn with a combination of Bt toxins (Cry1Ab and Cry34Ab1) indicates health impacts in rats (Zdziarski et al., 2018). Currently, around 40 events that produce Bt toxins are already authorised for import, many of them producing several Bt toxins in combination.

In conclusion, these findings show new evidence that safety of Bt plants has not been demonstrated as required by EU regulation. Therefore, no further market authorisations for Bt plants should be granted in the EU. Instead, gaps and flaws in current risk assessment must be closed and existing market approvals re-examined.

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