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Commentary

Assessment of potential adjuvanticity of Cry proteins

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ABSTRACT

Genetically modified (GM) crops have achieved success in the marketplace and their benefits extend beyond the overall increase in harvest yields to include lowered use of insecticides and decreased carbon dioxide emissions. The most widely grown GM crops contain gene/s for targeted insect protection, herbicide tolerance, or both. Plant expression of *Bacillus thuringiensis* (*Bt*) crystal (Cry) insecticidal proteins have been the primary way to impart insect resistance in GM crops. Although deemed safe by regulatory agencies globally, previous studies have been the basis for discussions around the potential immuno-adjuvant effects of Cry proteins. These studies had limitations in study design. The studies used animal models with extremely high doses of Cry proteins, which when given using the *ig* route were co-administered with an adjuvant. Although the presumption exists that Cry proteins may have immunostimulatory activity and therefore an adjuvanticity risk, the evidence shows that Cry proteins are expressed at very low levels in GM crops and are unlikely to function as adjuvants. This conclusion is based on critical review of the published literature on the effects of immunomodulation by Cry proteins, the history of safe use of Cry proteins in foods, safety of the *Bt* donor organisms, and pre-market weight-of-evidence-based safety assessments for GM crops.

1. Introduction

Microbial products derived from various strains of the common soil bacterium *Bacillus thuringiensis* (*Bt*) have been used as insecticides since the 1930s (Ibrahim et al., 2010). No harmful or adverse effects have been demonstrated after occupational exposure to *Bt* products, and no adverse effects have been reported in the consumer population exposed to these products in the form of spray residues on conventional or organic crops (WHO, 1999). The crystal proteins that confer insecticidal properties to *Bt* sprays are

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Abbreviations

APC	antigen-presenting cell
BSA	bovine serum albumin
Bt	*Bacillus thuringiensis*
Cry	Crystal
DON	deoxynivalenol
EFSA	European Food Safety Authority
Fb_1	fumonisin B_1
GM	genetically modified
GMO	genetically modified organism
HbsAg	hepatitis B surface antigen
HOSU	history of safe use
ig	intragastric
IgA	immunoglobulin A
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
in	intranasal
ip	intraperitoneal
LPS	lipopolysaccharide
Maalox	magnesium-aluminum hydroxide suspension
PPB	parts per billion
PPM	parts per million
US	United States

highly specific to a subset of immature insects and have been widely used in genetically modified (GM) crops to confer insect protection. The mammalian and environmental safety of Cry proteins have been well studied and they are as safe in *Bt* spray products used in conventional and organic farming and forestry as they are in *Bt*-based GM crops (Federici and Siegel, 2007). Extensive uses of Cry protein-containing products and safety studies have provided robust evidence of vertebrate safety. Interestingly, this safety profile has prompted some preliminary investigation of the potential for Cry proteins, like Cry1Ac, to act as medically safe vaccine adjuvants in animals (Jarillo-Luna et al., 2008; Moreno-Fierros et al., 2003; Rojas-Hernandez et al., 2004; Vazquez et al., 1999). Although the experimental design has been questioned, these studies concluded that Cry proteins can act as adjuvants.

Adjuvants are commonly used to enhance the efficacy of vaccines by inducing a stronger and more durable immune response (Brunner et al., 2010). Typically, Cry protein studies investigating adjuvanticity used classic vaccination strategies, which focus on immunizing by systemic exposure to both the antigen and adjuvant. This contrasts with the oral exposure scenarios of *Bt*-sprayed crops or novel proteins in GM foods. Nevertheless, the interpretation of Cry protein studies investigating adjuvanticity of Cry proteins has led to postulation of adjuvanticity for Cry proteins in humans and, by extension, possible enhancement of immune responses to dietary components (EFSA, 2009). In this review, we will discuss the use of Cry proteins in agriculture, the safety of Cry proteins, and the literature concerning potential adjuvanticity of the Cry proteins.

2. History of safe use of the *Bt* microorganism and Cry proteins

Bacillus thuringiensis is a common bacterium present in soils, on grains, and in environmental habitats including the phylloplane and water (Martin and Travers, 1989). Not only is *Bt* found in the environment, it is also found in many animals including voles, deer, rodents, and insectivorous mammals, as well as in processed food products, such as pasta, bread and other foods that contain flour (OECD, 2007). Numerous *Bt* strains produce insecticidal Cry proteins or inclusion bodies that are effective in controlling certain species of insect pests (Aronson and Shai, 2001). *Bt* biopesticides have been adopted for use in commercial agriculture, forestry, and mosquito control (OECD, 2007). Because of their robust insecticidal activity, preparations of whole *Bt* organisms have been used by farmers since the 1920s, being sold commercially in France (as Sporine) as early as 1938, and registered as an insecticide in the United States (US) beginning in 1961 (Ibrahim et al., 2010). As of 2011, more than 100 microbial *Bt* products have been registered to provide effective control of insect pests (US-EPA, 2011). Despite the years of occupational exposure from aerial application of *Bt* microbial sprays to forests and agricultural crops, only one scientific report describes potential respiratory or dermal sensitization to *Bt* pesticides (Bernstein et al., 1999). The report describes skin prick reactivity and antibody responses to *Bt* spray formulations in farm workers, but these responses did not correlate well with exposure and do not appear to be directed against the spore or delta endotoxin component of the products. The lack of toxicity of *Bt* spores has been established by other studies: one clearly demonstrated that human acute oral exposure to live spores (1 g/day for 5 days) resulted in no toxic effects, nor did inhalation of 100 mg of Btk powder daily for 5 days (Fisher and Rosner, 1959), Another showed a lack of correlation between the presence of *Bt* in feces collected from greenhouse workers after exposure and any gastrointestinal ill effects (Jensen et al., 2002). Based on these and additional studies, the World Health Organization and others have concluded that *Bt* sprays containing Cry proteins have demonstrated no adverse health effects in workers who apply them, nor in the general public in the areas where the sprays are applied (WHO, 1999) (Federici and Siegel, 2007). Because of their demonstrated safety, *Bt* formulations are often sprayed on crops such as broccoli, tomatoes, cucumbers, cauliflower, and lettuce plants for insect control just prior to harvest (Frederiksen et al., 2006). These crops are eaten raw and typically with only minimal washing, meaning humans have been safely consuming *Bt* spores and Cry proteins (Federici and Siegel, 2007). Thus, in over 75 years of commercial use as biopesticides, *Bt* insecticidal proteins have been used and consumed safely with no reported adverse effects on human health or the environment (McClintock et al., 1995; Siegel, 2001).

The first GM crop generated by engineering the plant genome to express an insecticidal Cry protein was approved in the US in 1995. The safety of the plant-expressed Cry proteins in GM crops was supported by the evidence from decades of safe use of these same proteins in microbial sprays. Modification of plants to express specific insecticidal proteins is a safe and highly effective method for insect control for which safety has been confirmed through mammalian and environmental safety assessments (Betz et al., 2000). In addition, GM crops expressing Cry proteins have been grown since 1995 with no documented reports of adverse health effects (AMA, 2012; Betz et al., 2000; Key et al., 2008; Toxicology, 2003; US-EPA, 2005).

Cry proteins expressed in GM crops have been evaluated in accordance with internationally recognized guidelines for assessing potential allergenicity and toxicity (Codex, 2009; FAO/WHO, 2001). The results indicate that Cry proteins show no relevant amino acid