Appendix Three

# Potential Human Health Risks from Bt Plants

BT PLANTS are those that have been genetically engineered to express an insecticidal toxin. The insecticidal toxins are produced from genes of the *cry* (for crystal) family, which are actually found on mobile genetic elements called plasmids. There are many different genes in this family. The plasmids are usually isolated from the bacterium *Bacillus thuringiensis* (or Bt), but these plasmids are not necessarily only found in *B. thuringiensis*.

There are uncertainties about the effects of *cry* toxins on mammals and humans (Box A3.1). Very few have been tested for their effects on humans (Tayabali and Seligy, 2000). Some Cry proteins are cytotoxic to human or mouse cells, but surprisingly not to insects (Ito et al., 2004; Vázquez-Padrón et al., 2000). Moreover, the toxicity was cell-type specific, meaning that if the wrong kind of cellular tissue culture is used in the assay, toxicity may be underestimated. Some Cry proteins are even being considered for use as new chemotherapy agents due to their ability to kill certain kinds of human cells (Akiba et al., 2004; Kim et al., 2000). Cry toxin proteins may also stimulate an immune response leading to the need to test them as allergens.

> Assessment of the immunotoxicological effects of GMOs has mainly focused on the allergenic potential of genetically modified proteins whereas general immunotoxicological investigations of whole GMOs are not described in the literature…This finding, together with the findings of a Bt-specific IgE response in humans working with Bt pesticides reported by Bernstein et al. (1999, 2003) and Doekes et al. (2004) highlights the importance of evaluating the sensitization of consumers, especially atopic fieldworkers, of "foreign" proteins or GM food prior to their introduction to world market (Kroghsbo et al., 2008, p. 32).

Historically, *B. thuringiensis* strains have been isolated based on their toxicity to target insects. This has given rise to the common claim that their Cry toxins are essentially specific to insects (Betz et al., 2000). Recent screens of *B. thuringiensis* have not been restricted to that criterion. These screens are finding many strains with the parasporal inclusions characteristic of the crystal proteins, but with no detectable toxicity to insects.

> [These] observations suggest that *B. thuringiensis* as a species is not characterized by insecticidal activity of parasporal inclusions. This raises a question of whether noninsecticidal inclusions have any as yet undiscovered biological activity (Kim et al., 2000, p. 16).

**Box A3.1: A new trend in food safety findings?**

Some studies have found no particular toxicity or threat of either *B. thuringiensis* or Cry toxins to human health (Monsanto review published under Betz et al., 2000; He et al., 2008; DuPont/Dow/Pioneer study published under Malley et al., 2007). However, of late significantly different studies have been indicating otherwise. These studies differ in a number of ways, including that they are among the first to use the whole GMO as the source of the test rather than a surrogate source of Cry toxin (from laboratory bacteria or *B. thuringiensis* itself), proper statistical methods or animals at developmentally important stages or under stress, when important but not acute toxic effects would be most easily detected in short-term experiments.

In May of 2007, French researchers published their reanalysis of Monsanto data and concluded that there were indications of liver/kidney toxicity in rats fed Bt corn MON863, saying that "with the present data it cannot be concluded that GM corn MON863 is a safe product" (Seralini et al., 2007, p. 596). This conclusion was rejected but not invalidated by various food safety regulators (Terry, 2007). The importance of this study was its ability to show how poor the designs of industry studies have been and that, when proper statistical analyses are used, previously undetected toxic effects can sometimes be revealed.

Likewise, in March of 2008 Turkish researchers reported liver "[g]ranular degeneration level in 10% of examined sections was maximum (level 4) in Group III [fed on Bt GM corn] while no degeneration was observed at level 4 in Groups I and II" (Kiliç and Akay, 2008, p. 1166) which were fed on a standard diet or the isogenic conventional corn. In this case the researchers did not feel that their statistically significant results indicated "severe" effects on the health of rats. However, few foods would be expected to cause severe effects. The importance of long-term studies is to reveal chronic and sub-chronic effects.

In July of 2008, Austrian researchers found significant effects on mice fed a diet that contained a stacked Bt GM corn variety, called NK603 x MON810, when these rodents were under reproductive stress, with effects revealed by the third litter from the same breeding parents. In addition to some effects on kidneys, the researchers concluded that "multi-generation studies, especially based on the [reproductive assessment by continuous breeding (RACB)] design are well suited to reveal differences between feeds. The RACB trial showed time related negative reproductive effects of the GM maize under the given experimental conditions. The RACB trial with its specific design with the repeated use of the parental generation is a demanding biological factor for the maternal organism" (Velimirov et al., 2008, p. 4).

In November of 2008, Italian researchers concluded that "the consumption of [Bt] MON810 maize…induced alterations in intestinal and peripheral immune response of weaning and old mice. Although the significance of these data remains to be clarified to establish whether these alterations reflect significant immune dysfunctions, these results suggest the importance of considering the gut and peripheral immune response to the whole GM crop, as well as the age, in the GMO safety evaluation" (Finamore et al., 2008, p. 11537).

There are two reasons to draw attention to *B. thuringiensis*. First, there has been a change in the environmental interface between *B. thuringiensis* or its *cry* genes (in plants) and other bacteria. Second, there has been a dramatic change in the environmental interface between *B. thuringiensis* and humans in the last few decades. These changes are to the concentration of human exposure to the bacterium and/or its toxins, and the variety of ways in which we are being exposed.

### *A change in* B. thuringiensis*'s environment*

Worldwide at the turn of the century, 13,000 metric tons of *B. thuringiensis* were annually produced in fermenters (Anonymous, 1999). Corn and cotton engineered with *cry* genes covered a claimed 114 million ha in 2007 (Youngsteadt and Stokstad, 2008), an area of the Earth's surface over 4 times the size of the country of New Zealand (26.8 million ha). Particular strains and alleles of *cry/cyt* genes are being selectively and significantly amplified by an intervention that takes them outside their natural ecological context. Human enrichment of these sequences provides unprecedented opportunity for recombination with many uncharacterized homologues in the environment. Moreover, transgenic *cry* genes have a different DNA sequence (de Maagd et al., 1999), making the range of possible recombination products different from simple amplification of the same genes.

### *A change in our environment*

The scale of human exposure to *B. thuringiensis* and its toxins also increases with commercial production of *B. thuringiensis* products and transgenic crops. Traditionally, *B. thuringiensis* might have been ingested because it is common on grains and in soil that might adhere to raw foods. We may have breathed it in with blowing dust or become infected in a scraped knee. The quantity of *B. thuringiensis* from these sources is likely extremely low, however. Although it is very difficult to find studies that quantify vegetative *B. thuringiensis* or spores in soil, my best estimate is that the soil burden is usually under 1,000 spores/g. From a variety of studies it appears that the detection limit varies from 1,000-100,000 spores/g soil, and most sampling regimes fail to detect *B. thuringiensis* in all soil, grain and water samples that they test (e.g., Apaydin et al., 2005; Martin and Travers, 1989; Quesada-Moraga et al., 2004). A study in New Zealand reported a detection limit for one strain of Bt at 1,000 spores/g soil, and detected none in eight samples (Anonymous, 2003).[1] From the available data, an estimate of 1,000 spores/g would probably err on the generous side and be within an order of magnitude of the correct figure.

Routine exposure by ingestion of natural soil is unlikely to be significant. No disease was established in human volunteers fed $3 \times 10^9$ spores per day for days or rats fed $2 \times 10^{12}$ spores per kg (discussed in Drobniewski, 1994).[2] The minimum dose of *Bacillus cereus*

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[1] It was not possible to tell from this study whether they failed to detect any *B. thuringiensis* or failed to detect the particular target strain.

[2] I have switched to scientific notation because the numbers get exceedingly large and the nomenclature for naming large numbers can vary by country. For those unfamiliar with scientific notation, the exponent is the number of zeros, so $10^3 = 1{,}000$.

estimated to cause disease is $10^5$ cells or spores (range 200-$10^8$) (Schoeni and Lee Wong, 2005). *B. cereus* and *B. thuringiensis* are unreliably distinguishable on the genetic level, meaning that if *B. thuringiensis* were capable of causing disease, it would probably be no more pathogenic than *B. cereus*. The best available extrapolation from these data indicates that a minimum of 200 g of soil enriched for an unknown variant of *B. thuringiensis* would have to be consumed for the minimum disease-causing exposure.

After aerial spraying of Bt over the city of Auckland, the New Zealand Ministry of Agriculture and Forestry detected between $10^4$ and $10^6$ spores of Bt/g soil (up from $<10^3$ before spraying), and this population was stable for the next two years of testing. That experiment demonstrated that a single bout of *B. thuringiensis* spraying could reduce the accidental minimum ingestion of soil from 200 g to as little as 2 g, within close range of the average, and probably within normal extremes, of the daily amount consumed by some adults[3] (Davis and Mirick, 2006).

*B. thuringiensis* is also found in our drinking water. Studies of *B. thuringiensis* loads in drinking water in Japan found on average 0.45 (and up to 8) colony forming units/ml (Ichimatsu et al., 2000). I could find no data on *B. thuringiensis* loads in drinking water of developing countries or in freshwater available to livestock in either developed or developing countries. Since run-off into water supplies is an obvious route to concentrating spores, this should be an area of investigation.

Aerial spraying and airborne soil also present *B. thuringiensis* in aerosol form. The concentration of Cry toxins in crop plants presents another important exposure mechanism, as do high concentrations of *B. thuringiensis* on milled material. During food preparation, for example, corn flour becomes airborne and is inhaled. Even less is known about the long-term effects of breathing *B. thuringiensis* and its toxins, especially their potential to induce allergic responses.

The yield of Cry protein ranges from $1.7 \times 10^{-7} - 7 \times 10^{-7}$ μg/spore produced in a fermenter (Ghirbi et al., 2005). Fermenter yields could be as much as 10-100 times the yield in nature. Using fermenter numbers would be conservative because they overestimate the historical exposure to Cry protein. Using these conservative figures, the Cry toxin load in soil at the *B. thuringiensis* detection limit (1,000 spores/g) is $1.7 \times 10^{-4} - 7 \times 10^{-4}$ μg/g. At the highest estimate of Cry protein in soil (0.7 ng/g) and at the highest average daily ingestion of soil by an adult (625 mg), the maximum dietary exposure to Cry protein would be $4 \times 10^{-10}$ g/day.

Although Cry toxin concentrations vary considerably among cultivars, the average American eating the commercial corn producing at the low end of the scale (MON810 at 0.29 μg/g) would consume 10 μg of Cry protein per day, the equivalent exposure to eating 14 kg of soil (Table A3.1). Under more likely concentrations of Cry protein of 10-100-fold less (that is, amounts in nature and not in a fermenter), the equivalent amount of soil would be over 1 metric ton. Cry toxin reaches concentrations as high as 115 μg/g in commercial GM corn (Table A3.2). At this concentration, the corn component of a normal

[3] In a survey of 19 families, the average daily intake of soil varied from 37-207 mg for children, to 23-625 mg for adults.

**Table A3.1: Kilograms of soil that would need to be eaten for equivalent Cry exposures from Bt maize[1]**

| µg Cry/g seed | estimated amounts (ng) of Cry/g soil | | | |
|---|---|---|---|---|
| | **0.02** | **0.05** | **0.2** | **0.7** |
| 0.29 (MON810) | 500 | 200 | 50 | 14 |
| 1.4 (BT11) | 2,500 | 1,000 | 250 | 71 |
| 20 | 36,000 | 14,400 | 3,600 | 1,030 |
| 40 | 72,000 | 28,800 | 7,200 | 2,060 |
| 115 | 207,000 | 82,800 | 20,700 | 5,910 |

[1] Based on average American consumption of corn; see Table A3.2.

**Table A3.2[1]: Soil and transgenic corn mass equivalents of Cry toxin**

| Plant | Toxin in µg/g seed (range) | Consumed Cry (µg/day)[2] | New global Cry load in human food[3] | Equivalent necessary soil mass[4] |
|---|---|---|---|---|
| BT11 | 1.4 | 50 | $2.7 \times 10^3$ metric tons | $3.9 \times 10^{12}$ metric tons[4] |
| MON810 | 0.29 (0.19-0.39) | 10 | | |
| cry1F (Herculex?) | 93 (71-115) | 3,300 | | |
| MON863 | 67.5 (49-86) | 2,400 | | |
| Average | 40.5 | 1,500 | | |

[1] Clark et al. (2005). [2] Based on FAOSTAT, 2003 annual consumption data (36g/day USA) and product as sole source. [3] Based on an average of 40.5µg Cry/g seed, 4.41 metric tons of grain/ha[5] and 15 million ha of *B. thuringiensis* corn in 2004 (Clark et al., 2005). [4] Assuming 1,000 spores/g soil and $7 \times 10^{-7}$µg Cry/spore.

American diet could contain up to 4,140 µg of Cry protein. This translates into an equivalent soil consumption of 6-600 metric tons per person per day. 600 tons of soil is the amount carried by approximately 10 standard-sized railroad boxcars.

Mexicans and Africans eat significantly more maize per capita than do Americans and New Zealanders (Table 4.5). The proportion of daily protein from maize for an African is 40 times that for New Zealanders. Some individual statistics are even more profound. In Malawi 55% of daily protein comes from maize whereas New Zealanders get only

[4] Based on figures from Heinemann and Traavik (2004), this amount of soil would fill a train 60 billion standard US boxcars long.

[5] http://www.fas.usda.gov/wap/circular/2005/05-09/Wap%2009-05.pdf

0.5% of their daily protein from maize (FAOSTAT, 2008). If all the maize consumed in Malawi and New Zealand were Bt, then those in Malawi would be exposed to 15 times more Bt on average from ingestion, and potentially far more from inhalation. A protein or amino acid-based food hazard is a quantitatively different risk for Mexicans and Africans than it is for Americans and New Zealanders because of different exposures.

Pre-market acute toxicity studies are not the same as chronic studies and do not anticipate the safety of new varieties, toxins and novel forms of toxins.

> The introduction of new varieties and toxin mixtures, such as those derived from recombinant techniques, should not be assumed safe on the basis of previous work and should be carefully evaluated (Drobniewski, 1994, p. 106).

Where previous studies may match most closely, such as in the concentrations of toxin or bacteria used, they still differ in that they use only a very select group of strains and toxins and each is produced under conditions that differ from exposures that might arise outside of the laboratory.

Moreover, I am unaware of any commercial Bt crop that has been subjected to allergenicity testing using inhalation exposure, the way humans are expected to be exposed when they handle flours for cooking or breathe in pollen. A study from 1959 found no evidence of disease in 18 human volunteers who inhaled *B. thuringiensis* spores, but later studies could not exclude disease in three people exposed to aerial spraying (NPTN, 2000). Chronic exposures to *B. thuringiensis* and its toxins could easily be overlooked without concerted efforts to monitor them. Disease could also be more likely among the immunocompromised, which, because of both AIDS and malaria (Drobniewski, 1994), is an increasingly common predisposition.

When testing rats for an immune response to a variety of Bt rice, researchers found low or no response from oral exposure, but a high response from inhalation exposure. The route of exposure was even capable of eliciting an immune response in control groups kept in the same room but not fed the experimental rice. The control animals developed anti-Cry antibodies (Kroghsbo et al., 2008).

> Surprisingly, an antigen-specific antibody response was also detected in the control groups kept in the same room in both the 28- and 90-day study with Bt toxin and PHA-E lectin. As the nasal and bronchial mucosal sites are potent sites for induction of an immune response, the results may be explained by inhalation of particles from the powder-like non-pelleted diet containing PHA-E lectin or Bt toxin, thereby inducing an anti-PHA-E or anti-Bt response (Kroghsbo et al., 2008, p. 31).

In summary, there is a conspicuous absence of research on Cry protein toxins as either toxins or allergens in human food plants, both on the unique ways that they may be expressed in plants and on the unique context and concentration in which we are exposed to them through food (see an extended discussion on the effects of cooking in Chapter Four).

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