

Innspill til EFSA GMO Extranet søknad EFSA/GMO/NL/2009/70

28. april 2010

C.03

The *nptII* expression cassette in maize line MON 87460 contains the coding sequence of the *npt II* gene from *E. coli*, which is flanked by two functional *loxP* sites. The *loxP* recombination site is recognized by the P1 bacteriophage Cre recombinase.

The Cre/*loxP* site-specific recombination system has been applied in various plant species for marker gene removal, and according to the applicant the *lox-P* sites in MON 87460 were inserted to facilitate the potential excision of the *nptII* cassette, specifically using Cre recombinase. However, referring to the lack of safety concerns around the NPTII protein, the applicant concludes that it was unnecessary to excise the *nptII* gene using the *loxP* sites that flank this gene. The applicant is therefore asked to clarify why the *loxP* sites have been added to MON 87460.

D.01

The ability of MON87460 to tolerate drought stress is conferred by expression of the *cspB* gene from *B. subtilis*. CspB belongs to the bacterial cold shock protein (CSP) family, which is a group of small proteins characterised by the presence of a highly conserved RNA-binding sequence identified as cold shock domain (CSD). According to the applicant, the CspB protein is suggested to function as a “RNA-chaperone”. CSPs recognize single stranded polynucleotides without apparent sequence specificity and facilitate the initiation of translation by destabilizing non-productive secondary structures in mRNA under environmental stress.

Since the exact mechanism of drought tolerance in MON87460 maize remains to be defined, and due to the apparent absence of binding sequence specificity indicating that plant CSD-containing proteins could be involved in a more general response to stress by binding RNAs, we would like the applicant to discuss the possibility that other physiological processes in the plant may be affected by the expression of the *cspB* gene.

D.06

The microbial flora in the gastrointestinal tract of cattle may contain bacteria harbouring the bacteriophage P1 and the Cre-recombinase. Aminoglycoside antibiotics are to some extent used to treat gastrointestinal infection in cattle, and therefore the presence of these antibiotics will make a selective pressure which will facilitate a transfer of the *nptII* cassette to gastrointestinal bacteria.

Since the *lox* sites and intervening sequences are present in the GM variety, the putative horizontal spread of the transgenes in the intervening sequence has to be evaluated.

D.07.08

In reference to 7.8.4, 7.10.2, MSL0021408-2008 and WI-2007-064 the objective of the 42-days study and the 90 days study was to evaluate the nutritional value and potential health effects of broiler and rats fed diets containing processed corn grain from MON 87460 compared to grain from a conventional control variety. However, it is not evident from the dossier whether corn from MON 87460 plants exposed to stress conditions has been used in these studies. The Norwegian GMO Panel is of the opinion that the 42 days broiler study and the 90 days rat study also should include feed with grain from MON 87460 plants produced under water-limited conditions.

The applicant is also asked to clarify why the acute toxicity test in mice (with pure protein) has been carried out with a low protein dose (4.7 mg/kg bodyweight). According to the OECD guideline 401 "Acute oral toxicity", a limit test at one dose level of at least 2000 mg/kg bodyweight may be carried out. The Norwegian GMO Panel is of the opinion that the applicant, in order to exclude any acute health effects of CspB protein, should have performed an acute toxicity test on mice with at least 2000 mg/kg bodyweight with purified CspB protein.