
FOR THE RECORD – SCIENCE: THE RISK OF ANTIBIOTIC RESISTANCE MARKER GENES IN GENETICALLY MODIFIED PLANTS

BACKGROUND

The current uses of antibiotic resistance genes as marker genes in genetically modified (GM) plants have been reviewed and approved by the U.S. Food and Drug Administration and have been incorporated into numerous safety assessments of GM crops globally. The issue of antibiotic resistance gene safety has been the subject of two earlier assessments by the European Food Safety Authority (EFSA): (1) the scientific opinion of April 2004 on the use of antibiotic resistance marker genes (ARMG's) in genetically modified plants¹ and, (2) the scientific statement of March 2007 on the safe use of the *nptII* ARMG in GM plants² following an opinion of the European Medicines Agency (EMA)³. In 2009 EFSA issued a new Consolidated Opinion on this topic⁴, clarifying, reviewing and reinforcing the previous assessment positions.

Considering the number of pending applications or renewals concerning GM plants containing an ARMG and the GM plants already placed on the market, it is important to avoid any ambiguity on this issue.

CONCERN

The major concern related to ARMG presence in GM plants may be expressed as follows: ARMG's (used for easy detection of transformed (plant) cells and still present in the final GM plant) may be transferred to receptor bacteria which subsequently may interfere with antimicrobial chemotherapy leading to reduced treatment options for the control of infectious diseases. Although the transfer of an intact, functional gene has never been demonstrated, this may theoretically happen either in fields where GM plants are grown or in the intestinal tract when portions of the GM plant are consumed as food or feed.

SUMMARY CONCLUSION

The risk for human health and the environment associated with the marketing of GM plants containing an ARMG is negligible.

¹ http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620772328.htm

² http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620775641.htm

³ <http://www.emea.europa.eu/htms/human/opiniongen/list.htm> (for the opinion see under the year 2007)

⁴ Statement of EFSA on the consolidated presentation of opinions on the use of antibiotic resistance genes as marker genes in genetically modified plants.

http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902604575.htm

KEY MESSAGES

Scientific facts

Transfer of an ARMG from bacterium to bacterium is highly probable

- Bacteria have the ability to exchange genetic information within bacterial populations (within and between species). ARMG's are naturally present in bacteria, and were present long before humans started to use antibiotics to fight bacterial infections;
- Antibiotic resistance is genetically coded and several mechanisms of action exist that confer resistance to a given antibiotic, such as "efflux pumps" (which remove antibiotics from the cell), antibiotic-modifying or degrading enzymes (the type used in GM plants), and reduction of antibiotic binding capacity to cell targets;
- ARMG's in bacteria are often located on mobile genetic elements (plasmids or transposons), which increase the likelihood of horizontal gene transfer (HGT). In addition, high mutation rates support the prevalence of natural resistance rates in bacteria;
- HGT among bacteria is tremendously boosted under selective conditions. Environments where antibiotics are present will produce higher rates of resistant bacteria since there will be selection for bacteria that contain an ARMG. This occurs irrespective of the presence of GM plants that contain ARMG's);
- In the absence of antibiotics, there is no selective pressure and the prevalence of antibiotic-resistant bacterial populations in that environment will be low.
- Although variable, prevalence of antibiotic resistance among naturally occurring bacteria is high, especially in comparison to the rate of transfer from plants to bacteria, which has never been demonstrated to occur in a natural environment;
- Two criteria were used to guide the initial choice of ARMG's used in transgenic plants: high incidence in nature, and conferring resistance to antibiotics with limited use in clinical medicine. The ARMG's currently present in GM plants broadly fulfill both criteria.
 - *nptII* :
 - *nptII* is present on various transposons and plasmids and resistance to antibiotics inactivated by npt-II is common (due to *nptII* and to other resistance factors).
 - *nptII* has little or no impact on critical uses of antibiotics. *nptII* affects the following antibiotics:
 - Kanamycin: important in tuberculosis treatment. However, clinical resistance in tuberculosis is mediated by chromosomal point mutations. Hence, *nptII* has no role to play.
 - Neomycin: topical use at extremely high concentrations where resistance conferred by *nptII* is not relevant.
 - Gentamycin (Gm): *nptII* only inactivates Gm-B, while commercially available gentamycin is a blend of Gm-A and Gm-C.
 - Geneticin: not in clinical use.
 - *aadA*:
 - *aadA* is present on various transposons and plasmids
 - *aadA* – effects on antibiotic use
 - Streptomycin: infrequent treatment of gonorrhoea. Clinical resistance is primarily mediated by mutations. Hence, *aadA* has a negligible role.
 - Spectinomycin: limited clinical use.

Meaningful transfer (transfer of an intact, functional gene within a non-sterile actual environment) of an ARMG from plant to bacterium is highly unlikely

- HGT from plants to bacteria has never been demonstrated in a natural environment and is generally acknowledged to be very rare.
- Major barriers for HGT from plants to bacteria to occur include:
 - Free DNA in the environment is unstable, resulting in a relative absence in the environment of long, stable DNA molecules containing the complete ARMG sequence.
 - Any ARMG DNA present must compete for uptake with random pieces of plant genomic DNA and any other DNA in the environment. Since the ARMG is only a tiny portion of the plant genome (far less than 1%), this competition is an impediment to transfer;
 - Demonstrable transfer in the laboratory requires very specific conditions and cannot be demonstrated if these are not met:
 - A damaged or incomplete copy of the ARMG must already be present in the receiving bacteria (homologous recombination);
 - The environment should be otherwise sterile (free of bacteria other than the experimental target strain)
 - Antibiotics must be present to create a selective environment.
 - Bacterial regulatory sequences are required to obtain stable protein expression. The ARMG coding sequence is usually under the control of a plant regulatory sequence which functions poorly if at all in the bacterial cell.
- The transfer rate from plants to bacteria (which is low enough that it has never been demonstrated in the natural environment) is clearly far lower than the likelihood of gene transfer among bacteria or the likelihood of a bacteria becoming resistant by mutation.

Conclusions

- Antibiotic resistance is widespread in bacterial populations, supported by the exchange of plasmids and other resistance determinants as well as high mutation rates. Through the process of horizontal gene transfer, all bacteria in a given environment have access to the same virtual gene pool constituted by the genomes of the individual bacteria (which are a natural source of ARMG's). This is exemplified by the emergence of multi-drug resistant bacterial strains in clinical settings during the last 50 years, which occurred in the absence of any GM plant containing an ARMG.
- The critical question is whether the marketing of GM plants containing an ARMG will lead to an increase in antibiotic resistance in bacterial populations as a result of horizontal gene transfer.
- Any risk should be assessed relative to the resistance determinants already present in bacterial populations and the rate at which such resistance can transfer among bacteria or arise by mutation.
- Based on the information above, we can provide the following answers to the risk questions:
 - (i) the frequency of HGT from plants to bacteria is extremely low, if it occurs at all;
 - (ii) the biological impact of a potential transfer is minimal, given the high natural resistance rates and the ease with which resistance determinants are exchanged among bacteria.

Overall, the risk of diminishing therapeutic options by marketing a GM plant containing an ARMG can be deemed to be insignificant.

REFERENCES

- EFSA. 2007. Opinion of Scientific Panel on Genetically Modified Organisms on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants. June 2007: 1-7.
- Goldstein, D., Tinland, B., Gilbertson, L., Staub, J., Bannon, G., Goodman, R., McCoy, R., Silvanovich, A. 2005. Human Safety and GM Plants: A Review of Antibiotic Resistance Markers and Future Transformation Selection Technologies. *Journal of Applied Microbiology*. 99: 7-23.
- EFSA GMO and BIOHAZ Units. 2009. Consolidated Presentation of the Joint Scientific Opinion of the GMO and BIOHAZ Panels on the “Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants” and the Scientific Opinion of the GMO Panel on “Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants”. EFSA-Q-2009-00589 and EFSA-Q-2009-00593: 107 pages.