



COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE AND COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE

Presence of the antibiotic resistance marker gene *nptII* in GM plants for food and feed uses

Background

In a letter dated 25 January 2007, Mr Robert Madelin from the Directorate General for Health and Consumer Protection of the European Commission informed the EMEA that the Commission was considering the authorisation for the placing on the market of the genetically modified potato line EH92-527-1 which contains a genetic modification that harbours an *nptII* gene as a selectable marker. This gene codes for neomycin phosphotransferase, an enzyme that generally confers resistance to several antibiotics, the most relevant ones being kanamycin, neomycin, paromycin, butirosin, gentamicin B and geneticin. In its opinion on the application for placing on the market of the genetically modified potato EH92-527-1, the GMO Panel of the European Food Safety Authority (EFSA) concluded that the use of *nptII* as a selection marker does not pose a risk to the environment or to human and animal health.

As indicated in his letter, the Commission representative is seeking confirmation from EMEA as to whether, notwithstanding the WHO classification, the current or possible uses of the antibiotics for which the *nptII* gene confers resistance are in line with the opinion of the GMO Panel of EFSA indicating that these antibiotics have no or only minor therapeutic relevance in human medicine and only restricted use in defined areas of veterinary medicine and, if not, whether the current or possible medicinal uses might have an impact on the conclusions of the EFSA GMO Panel.

In his response letter dated 6 February to Mr Robert Madelin, Dr Thomas Lönngrén, the executive director of the EMEA commented that the scientific committees of the EMEA would be able to comment comprehensively in relation to the issue of possible uses of these antibiotics in human and veterinary medicine. However, it was felt to be more appropriate for EFSA to possibly reconsider the conclusions of the panel in view of any new information provided by the EMEA scientific committees.

CHMP and CVMP overall conclusions

The following is based on the assumption that the substrate specificity of the product of the *nptII* gene used as a marker in the potato in question is restricted to neomycin and kanamycin (and geneticin), as stated in the opinion published in the EFSA journal (2004) 48:1-18., and does not extend to gentamicin. This point is important due to different medical uses of these antibiotics.

1. Human medicines considerations

The therapeutic relevance of kanamycin and neomycin in human medicine has been addressed by the GMO panel of EFSA. The EMEA/CHMP can add the following points to that opinion. In summary these points consider a more long-term view recognising the potential development in the aminoglycoside class indicating that the role of these medicinal products might become increasingly relevant.

- As indicated in the report from the EFSA, Neomycin is indicated in important clinical conditions such as for example in hepatic encephalopathy.

- Notwithstanding the EFSA opinion, aminoglycosides is a class of antibiotics that has become increasingly important in the prevention and treatment of serious invasive bacterial infections in humans. This is because gram-negative bacteria (and tuberculosis bacteria) are becoming resistant to other classes of antibiotics. Consumption data from Sweden for example show a use of about 5.7 DDD (Defined daily doses) per 1000 inhabitants, or 0.02 DDD/1000 inhabitants and day in 2006, which indicates a 25% increased usage from 2002. In France the consumption is about 0.13-0.15 DDD/Day/1000 inhabitants and relatively stable since 2002¹. Similar figures come from Estonia (personal communication).
- Although it is recognised that this marker gene only codes for resistance to kanamycin and neomycin the clinical/public health implications of this may not always remain the same. It is true that aminoglycosides and especially kanamycin and neomycin are used relatively infrequently and that the potential impact of this resistance gene therefore appears less relevant, at least in a short-term perspective. However, that situation may change as new chemical entities similar to kanamycin and neomycin could be developed. New chemical entities similar to kanamycin and neomycin could have other properties in relation to, for example, absorption from the gastrointestinal tract and with regard to side-effects. They thus have the potential to become extremely important to treat otherwise multi-resistant gram-negative infections and Tuberculosis.
- Aminoglycosides such as kanamycin are currently recommended for treatment in multidrug resistant tuberculosis (MDR-TB). Drug resistance in TB is part of the explanation for the resurgence of TB. WHO estimates that eight million people get TB every year. In the absence of an effective therapy, infectious MDR-TB patients will continue to spread the disease, producing new infections with MDR-TB strains. Until we introduce a new drug with demonstrated activity against MDR strains, this aspect of the TB epidemic could begin to explode at an exponential level (from the Global Alliance for TB Drug development (<http://www.tballiance.org>)).
- In Estonia, Kanamycin was very recently introduced in the TB program (personal communication).
- The EMEA included kanamycin for potential use in MDR-TB in the case of bioterrorism (<http://emea.europa.eu/pdfs/human/bioterror/11.Otherbacterial.pdf>)

2. Veterinary usage consideration

The use of neomycin and kanamycin is currently limited, but this does not equate to “minor therapeutic importance”. Importance is not measured by the quantity used, but rather relates to the need for the antibiotic and what alternatives exist, if any. As resistance continues to increase to the alternative drugs, the importance of neomycin and kanamycin and future derivatives of these drugs can be expected to increase, e.g. therapy of neonatal diarrhoea in piglets and treatment of multi-resistant enteric gram negative infections. Aminoglycosides, as a group, are a class of antibiotics critically important for veterinary medicine and animal production. The following aminoglycosides are part of veterinary medicines for food producing species in the EU; apramycin, dihydrostreptomycin, gentamicin, kanamycin, neomicin, paromomycin, spectinomycin and streptomycin.

3. Overall conclusions from Human and Veterinary

The Committees therefore concluded that neomycin, and kanamycin, are of importance for veterinary and human use and that their current and potential future use cannot be classified as of no or only minor therapeutic relevance.

The Committees considered that their area of competence does not extend to a detailed consideration of the likelihood of transfer of antibiotic resistance genes from plant material to bacteria of man and animals and they are not therefore in a position formally to comment on the EFSA conclusions that the likelihood

¹ Information obtained from Strama (www.strama.se) and Afssaps (personal communication).

and significance of such an event are extremely low. However, some observations on this issue and information on the use of the relevant antibiotics in animals and man are provided in Annex 1 and 2.

Annex 1: Comment and information on the use of aminoglycoside antibiotics provided by the Scientific Advisory Group on Antimicrobials of the CVMP

EFSA has evaluated the safety of the use of *nptII* as a marker gene in a modified potato line and has previously evaluated more generally the use of antibiotic resistance marker genes. EFSA considers that the use of *nptII* as a marker gene does not pose a risk for the environment or to animal, based on the following:

1. the probability of gene transfer from the GM plant to bacteria is possible but very low under natural circumstances

SAGAM noted that, transfer of *nptII* from plants to bacteria has been shown (Tepfer D, Garcia-Gonzales R, Mansouri H, Seruga M, Message B, Leach F, Perica MC. Homology-dependent DNA transfer from plants to a soil bacterium under laboratory conditions: implications in evolution and horizontal gene transfer. *Transgenic Res.* 2003 Aug;12(4):425-37.)

2. the gene *nptII* that is used as a marker gene confers resistance to neomycin, kanamycin and geneticin only, and not to gentamicin (otherwise normally expected for *nptII*).

This is crucial as the importance of gentamicin not only in human medicine, but also in veterinary medicine (e.g. for horses) is undisputed, and the following arguments refer to kanamycin and neomycin only.

In the opinion from 2004² (page 7), reference is given to two reviews from 1993 and 1994. The reference has not been located, and the CVMP/SAGAM cannot therefore assess the scientific basis for this point, nor the independence of the authors. *The following is based on the assumption that the substrate specificity of the product of the nptII gene used as marker in the potato in question is really restricted to neomycin and kanamycin (and geneticin)*

3. the use of kanamycin and neomycin in veterinary medicine is limited to certain conditions (enteritis in pigs and calves)

It is true that the main use of neomycin or kanamycin is for treatment of enteritis in young animals. This is one of the most common indications for use of antimicrobials in food-producing animals. According to DANMAP 2005, aminoglycosides were used orally in water for treatment of pigs in a quantity that corresponds to about 13% of the in water medication given to pigs. This use is mainly neomycin, thus the use is limited but not minor. Also, one should consider the alternatives; if neomycin is not used for these indications, other antibiotics like fluoroquinolones would need to be used.

In the CVMP/SAGAM opinion, neomycin and kanamycin are valuable antibiotics for treatment of, e.g. E. coli associated enteric diseases in food-producing animals. The extent of use of neomycin/kanamycin to treat that condition will vary between countries, depending on what is available and on the occurrence of resistance to alternative antimicrobials.

4. from point 3 & 4, the conclusion that neomycin & kanamycin is of "minor therapeutic importance" is drawn

The terms "infrequent use" and "limited indications" cannot be equated with "minor therapeutic importance". The use may remain infrequent, but the *importance* of use of neomycin/kanamycin for decolonisation/decontamination may well increase as a consequence of increasing problems with multiresistant or panresistant (ESBL producing) gram-negatives and of multiresistant staphylococci. This has already happened to colistin, long thought to be an old obsolete toxic drug with some minor

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http://www.efsa.europa.eu/etc/medialib/efsa/science/gmo/gmo_opinions/384.Par.0001.File.dat/opinion_gmo_05_en1.pdf

uses in veterinary medicine. Colistin is today a life saving last resort treatment for infections with multiresistant Gram-negative bacteria in human hospitals.

5. neomycin/kanamycin resistance is already widespread

Occurrence of resistance to neomycin and kanamycin varies substantially between countries and bacterial species. Information on occurrence in *Salmonella* and *E. coli* is available in the EFSA Zoonosis report, 2005³ “annex level 3”.

Importance of use in veterinary medicine of neomycin/kanamycin in some countries of the European Union

The enclosed compilation is not exhaustive and comprises answers from experts on antimicrobial resistance and not necessarily that of the National Authorities.

Maximum Residue Levels (MRL) are available for the substances neomycin and kanamycin (<http://www.emea.europa.eu/index/indexv1.htm>), indicating that applicants consider them of sufficient value to apply for an MRL to permit their use in veterinary medicinal products for food producing species:

France: neomycin is used regularly for treatment of diarrhoea in pigs and is not a minor drug. The level of resistance varies between 5 and 20 % in *E. coli* samples in the French monitoring programme (see FARM report⁴). Sales of aminoglycosides in 2005 in France were 77.8 Tonnes (5.89% of total sales)⁵.

Denmark: the use of aminoglycosides in piglets and calves in Denmark is almost exclusively restricted to neomycin.

Germany: the following number of products are licensed:

Gentamicin: Veterinary medicine 25
Kanamycin: Veterinary medicine 1
Neomycin: Veterinary medicine 30

The Netherlands: neomycin is used for oral treatment of enteritis in pigs and calves, for Bovine Respiratory disease by injection in combination with benzylpenicillin and for local treatment of mastitis (always in combined with a beta-lactam) and in eye and ear ointments. Specifically the parenteral use of neomycin-penicillin is substantial.

Spain: different Marketing Authorisations for veterinary medicinal products from different Laboratories, which include gentamicin or neomycin in its composition, are available. No kanamycin, neither paromycin, butiroxin nor geneticin is available.

Sweden: The above-mentioned aminoglycosides are not authorised for animals in Sweden.

In Ireland kanamycin is used in the treatment of mastitis in cows.

OIE list of Veterinary Critically Important Antimicrobials (VCIA)

Reference is made on the request from the Commission to the WHO list of critically important antimicrobials. We would like to highlight that kanamycin and neomycin are included in the OIE list of VCIA. For aminoglycosides the following is indicated:

“Importance of aminoglycosides

³ http://www.efsa.europa.eu/en/science/monitoring_zoonoses/reports/1277.html

⁴ FARM 2003-2004, French antimicrobial resistance monitoring in bacteria of animal origin, afssa

⁵ http://www.anmv.afssa.fr/documents/rapport2005_final-antibio.pdf

The diseases controlled by aminoglycosides, either alone or in combination, are particularly debilitating to young animals and failure to adequately treat outbreaks would result in much suffering among affected animals. Similarly, the enteric infections affecting pigs and calves are effectively and economically treated orally with aminoglycosides, either alone or in combination. The wide range of applications and the nature of the diseases treated make aminoglycosides critically important for veterinary medicine and animal production.”

The use of aminoglycosides in routine veterinary therapy is well established as illustrated by standard text books, such as: “Antimicrobial Therapy in Veterinary Medicine, third edition, Edited by J F. Prescott et al.”

Annex 2: Information on human consumption from some EU countries

Country	Information on consumption																												
Sweden	<p>See main text</p> <p>Aminoglycosides</p> <p>2002: 4.56 ddd/1000/year; 2003: 4.78 2004: 5.16; 2005: 5.32; 2006: 5.75</p> <p>Kanamycin and Neomycin not licensed.</p>																												
France	<p>See main text</p> <p>Aminoglycosides: 2002: 0.15 ddd/1000/day; 2003: 0.12; 2004: 0.13; 2005: 0.13. Kanamycin not licensed. Neomycin licensed.</p>																												
Italy	<p>Kanamycin not licensed. Neomycin licensed. The hepatic encephalopathy indication was recognised by Italian experts.</p>																												
Germany	<p>The following number of products are licensed</p> <p>Gentamicin: Human medicine 80</p> <p>Kanamycin: Human medicine 9</p> <p>Neomycin: Human medicine 44</p>																												
Lithuania	<p>Kanamycin data are available in DDD/1000 persons/per day while Neomycin in packs only:</p> <table border="1" data-bbox="507 1193 1430 1664"> <thead> <tr> <th data-bbox="507 1193 874 1285"></th> <th data-bbox="874 1193 1050 1285">IQ</th> <th data-bbox="1050 1193 1241 1285">IQ</th> <th data-bbox="1241 1193 1430 1285">IQ</th> </tr> </thead> <tbody> <tr> <td data-bbox="507 1285 874 1352"><i>Kanamycin</i></td> <td data-bbox="874 1285 1050 1352">0.98</td> <td data-bbox="1050 1285 1241 1352">0.65</td> <td data-bbox="1241 1285 1430 1352">2.93</td> </tr> <tr> <td data-bbox="507 1352 874 1442"><i>Neomycin (sold as combination products)</i></td> <td data-bbox="874 1352 1050 1442"></td> <td data-bbox="1050 1352 1241 1442"></td> <td data-bbox="1241 1352 1430 1442"></td> </tr> <tr> <td data-bbox="507 1442 874 1509"><i>Maxitrol eye drops (in packs)</i></td> <td data-bbox="874 1442 1050 1509">2608</td> <td data-bbox="1050 1442 1241 1509">4298</td> <td data-bbox="1241 1442 1430 1509">4514</td> </tr> <tr> <td data-bbox="507 1509 874 1576"><i>Maxitrol eye ointment (in packs)</i></td> <td data-bbox="874 1509 1050 1576">834</td> <td data-bbox="1050 1509 1241 1576">2095</td> <td data-bbox="1241 1509 1430 1576">1853</td> </tr> <tr> <td data-bbox="507 1576 874 1621"><i>Polygynax</i></td> <td data-bbox="874 1576 1050 1621">8958</td> <td data-bbox="1050 1576 1241 1621">18050</td> <td data-bbox="1241 1576 1430 1621">20516</td> </tr> <tr> <td data-bbox="507 1621 874 1664"><i>Dexona eye drops</i></td> <td data-bbox="874 1621 1050 1664">17803</td> <td data-bbox="1050 1621 1241 1664">73733</td> <td data-bbox="1241 1621 1430 1664">73052</td> </tr> </tbody> </table>		IQ	IQ	IQ	<i>Kanamycin</i>	0.98	0.65	2.93	<i>Neomycin (sold as combination products)</i>				<i>Maxitrol eye drops (in packs)</i>	2608	4298	4514	<i>Maxitrol eye ointment (in packs)</i>	834	2095	1853	<i>Polygynax</i>	8958	18050	20516	<i>Dexona eye drops</i>	17803	73733	73052
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Estonia	<p>Estonia has neomycin topicals authorised (ointment, cream, eye drops, tablets for oral mucosal infections) and parenteral gentamicin as well (use in 2005 was 0,22 DDD/1000/day). Kanamycin was very recently used in the TB program and most probably still is, this gets imported for the tb program, variant of named pt basis.</p>																												