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**Memorandum**

Date: February 1, 2006

From: Division of Petition Review, Toxicology Group I

Subject: Addendum to the June 3, 2005 Final Toxicology Memorandum:
Additional toxicological evaluation of the potential allergenicity /
immunotoxicity of the *Listeria* bacteriophage, LMP-102TM.

To: Raphael Davy
CSO, DPR

Through: Catherine Whiteside, Ph.D. Catherine Whiteside
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FAP 2A4738

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In this memorandum we are providing additional details regarding the safety information that were considered in our evaluation of the potential allergenicity and immunotoxicity of bacteriophages (see DPR Toxicology June 3, 2005 T. Walker memorandum). This evaluation is being performed as part of our overall evaluation of the safety of *Listeria* bacteriophage mixtures (6 phage mixture) intended for use as an antimicrobial effective against *Listeria monocytogenes* (*L. monocytogenes*). In this evaluation we considered data from published animal studies as well as information from published clinical studies that used bacteriophages which were prepared from various sources as therapeutic treatments.

ANIMAL STUDIES:

We considered results from published acute (single-dose) and repeat-dosing (20 days) toxicity studies in which mice and guinea pigs were administered intraperitoneal injections of *Klebsiella* bacteriophages. No significant adverse effects or lasting toxicity was reported in animals of these studies as a result of treatment with purified *Klebsiella* bacteriophages. However, adverse toxicities were noted with unpurified bacteriophage preparations.¹

¹ Bogovazova GG, NN Voroshilova, VM Bondarenko, GA Gorbatkova, EV Afanas'eva, TB Kazakova, VD Smirnov, AG Mamleeva, Yul Glukharev, EI Erastova, IA Krylov, TM Ovcherenko, AP Baturo, GV Yatsyk, and NA Aref'eva. 1992. Immunobiological properties and therapeutic effectiveness of preparations from *Klebsiella* bacteriophages. The Authors; pp 30 – 33. (FAP 2A4738, Volume 12, Appendix K10, pp. 001929 – 001935)

HUMAN STUDIES:

We also considered published scientific information that was provided by the Petitioner that showed the use of bacteriophages as therapies in the treatment and the prevention of infections in humans caused by species of bacteria.

A published paper by Lazareva et al. (2001)² presented data regarding the oral administration of bacteriophages in patients with severe burns in which the bacteriophages were used to facilitate wound cleaning and reduce infection. The bacteriophages were administered as a monotherapy as well as concomitantly with antibiotic therapy. The author reported positive effects with the phage therapy, as evidenced by decreased/normalized body temperature in the treated patients and decreased numbers of microorganisms in the wound discharge of the treated patients.

In a publication by Babalova, et al. (1967)³ data were reported regarding the use of a dry polyvalent bacteriophage as an oral therapy in treatment of incidences of dysentery in children ages 6 months to 7 years of age. The study authors reported there were less frequent incidences of simple diarrhea in children receiving the phage treatment than untreated children.

Pavlenishvili and Tsertsvadze (1993)⁴ reported results that showed the combined use of bacteriophages from *Klebsiella*, *Serratia*, and *Enterobacter* were effective in the treatment and prevention of gram-negative sepsis in newborns and infants infected by these bacteria. These study authors reported that "no patients revealed toxic, allergic, or pyrogenic reactions or accessory phenomena caused by the treatment carried out by the offered scheme."

OTHER INFORMATION CONSIDERED:

Additionally, we note that the petitioner has incorporated several filtration steps in the manufacture process which would make his phage preparation a purified phage product that would subsequently reduce any potential for allergic response or immunological toxicity.

² Lazareva EB, SV Smirnov, VB Khvatov, TG Spiridonove, EE Bitkova, OS Darbeeva, LM Maiskaya, RL Parfenyuk, and DD Men'shikov. 2001. Efficacy of bacteriophage in complex treatment of patients with burn trauma. *Antibiotiki i khimioterapiya* 46(1): 10 – 14. (FAP 2A4738, Volume 12, Appendix K08, pp. 001900 – 001912)

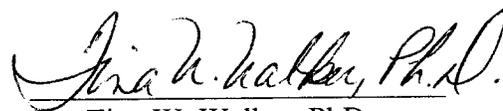
³ Babalova EG, KF Katsitadze, LA Sakvarelidze, NSh Imnaishvili, TG Sharashidze, VA Badashvili, GP Kiknadze, AN Maipariani, BND Gendzekhadze, EV Machavariani, KL Gogoberidze, EI Gozalov, and NG Dekanosidze. 1967. Prophylactic efficiency of dry dysentery bacteriophage. *Eliava Institute of Bacteriophage, Microbiology and Virology*; pp 143 – 145. (FAP 2A4738, Volume 12, Appendix K09, pp. 001920 – 001923)

⁴ Pavlenishvili JV and TD Tsertsvadze. Bacteriophagetherapy and enterosorbition in treatment of sepsis of newborns caused by gram-negative bacteria. *Tbilisi Medical Academy, Tbilisi, Georgia*; p. 104. Incomplete reference citation information. (FAP 2A4738, Volume 12, Appendix K21, pp. 002047)

We also discovered a recent 2005 scientific publication⁵ that presented a safety evaluation of a purified bacteriophage P100 preparation (P100) specific for control of *Listeria monocytogenes* in foods. These study authors presented data from an oral toxicity study in rats (5/sex/group) that were treated with doses (5×10^{11} , equivalent to 2×10^{12} phages/kg bw/d) of purified P100 over a period of 5 days. The study authors concluded there was no evidence of any abnormal changes, morbidity, or mortality in the treated rats. Based upon the results presented in this publication, the study authors concluded that purified *Listeria* bacteriophage P100 preparations were safe for consumption when used as an additive for biopreservation of foods.

CONCLUSION:

The above studies provide evidence that the oral administration of bacteriophages prepared from various bacterial sources do not pose any concerns of allergenicity or immunotoxicity when used in therapeutic applications. The data also demonstrates that the use of purified phage preparations reduces the potential for immunotoxicity or allergenic responses. Based upon the information provided in these studies and the consideration that purified phages are intended to be used in the proposed petition request, we do not expect there to be any concerns of allergenicity or immunotoxicity associated with the proposed *Listeria* bacteriophage preparation, LMP-102TM, when used as an antimicrobial against *Listeria monocytogenes* in food.


Tina W. Walker, PhD

CC: HFS-265 (Vanner, Whiteside, Walker)

⁵ Carlton RM, WH Noordman, B Biswas, ED deMeester, MJ Loessner. 2005. Bacteriophage P100 for control of *Listeria monocytogenes* in foods: Genome sequence, bioinformatics analyses, oral toxicity study, and application. *Regulatory Toxicology and Pharmacology* 43(3): 301 – 312.
<http://www.sciencedirect.com/science/journal/02732300>