

Memorandum

Date: April 11, 2005

From: Division of Petition Review
Chemistry Review Group

Subject: FAP 2A4738 (MATS#1137 M 2.3). Petition for the use of LMP-102™ - a mixture of several monoclonal bacteriophages as an antimicrobial agent in ready-to-eat meat and poultry. Submissions of 10/25/04, 1/18/05, 1/25/05, and 2/18/05.

To: Division of Petition Review, HFS-265
Regulatory Group II
Attention: R. Davy

In the memorandum dated 6/16/04, we noted a number of deficiencies regarding the chemistry-related information, which were conveyed to Intralytix in a letter dated 9/13/04. Intralytix responded to this letter in the submission dated 10/25/04. Upon reviewing this submission, we identified an additional chemistry issue, which was communicated to Intralytix in an e-mail dated 12/02/04. Intralytix responded in e-mail submissions dated 1/18/05 and 1/25/05. A subsequent request for further information (e-mail dated 2/15/05) was addressed by Intralytix in an e-mail dated 2/18/05.

Each request for additional information from the 9/13/04 letter, and 12/02/04 and 2/15/05 e-mails is provided below, followed by a discussion of Intralytix's response.

Identity

1. There is inconsistency in the active phage concentration and specification limits for total organic content (TOC). In the letter dated on 9/13/04, Intralytix was requested to clarify the information regarding the active phage concentration and the specific limit for TOC in the LMP-102™.

In the submission dated 10/25/04, Intralytix stated that the active phage concentration in the LMP-102™ preparations is in the range of 0.1 to 0.3 mg/kg (ppm) based on two independent methods. Intralytix also stated that the specification limit for TOC will be set at ≤ 36 mg/kg based on analysis of 3 lots LMP-102™ previously provided (submission dated 12/20/03). We accept Intralytix's explanation.

2. It is unclear how the data provided for the residual amino acid levels (i.e., < 100 mg/kg of amino acids) in LMP-102™ would support the claim of "no detectable amounts" of peptones, tryptone, and nuclease (DNAse 1 and RNAse A)." In the letter dated 9/13/04, Intralytix was

asked clarify this statement.

In the letter dated 10/25/04, Intralytix stated that acid hydrolysis of LMP-102™ would yield amino acids that may be derived from any of the protein components in the preparation. Intralytix states that, while amino acids levels do not directly relate to protein levels, they do serve as a reliable indicator of the presence of protein (i.e., peptone, tryptone, DNase I, and RNase A). Therefore, the sum of the mass of all amino acids will approximate the total mass of protein present. Intralytix further stated that the claim of “no detectable” proteins in LMP-102™ is based on the limit of detection (LOD) of 100 mg/kg (ppm) for the analytical methods for determining amino acids. In support, Intralytix has provided the AOAC methods for amino acids (AOAC 994.12, Appendix J02); tryptophan (AOAC 988.15 Appendix J03); and taurine (AOAC 999.12, Appendix J04). We accept Intralytix’s explanation.

3. Intralytix states that Antifoam 204 would be removed during the filtration steps. However, no data were provided to support this claim. In the letter dated 9/13/04, Intralytix was requested to provide data or a narrative that assured no detectable residues of Antifoam 204 would be present in LMP-102™.

In the submission dated 10/25/04, Intralytix responded that Antifoam 204 was regulated under 21 CFR 173.340, and that any residues of Antifoam 204 in LMP-102™ would be negligible. We considered this response to be insufficient. Therefore, in an e-mail dated 12/02/04, Intralytix was requested to provide information on the identity, analytical method, and use level of Antifoam 204, as well as residual levels of Antifoam 204 in the final product to insure compliance under §173.340. In the e-mail submissions dated 1/18/05 and 1/25/05, Intralytix stated that Antifoam 204 (also known as Industrol DF204® Defoamer, see Appendix J05), is oxirane (CAS #9003-11-6, alpha-hydro-omega-hydroxy-poly (oxyethylene)/poly (oxypropylene)), and is regulated under §173.340 and §172.808, among other regulations.

In the e-mail submission dated 1/18/05, Intralytix stated that maximum use level of Antifoam 204 in LMP-102™ is 10 mg/kg (ppm) and that the analytical method for determining Antifoam 204 in LMP-102™ is being developed. We note that the method, which is based on liquid chromatography with electrospray mass spectrometry (LC/MS), was not properly validated. In particular, the results from analyses of samples spiked at 2 different levels (10 µL/L and 4545 µL/L¹) of Antifoam 204 showed poor results, with less than 68% recovery. In the e-mail dated 2/18/05, Intralytix indicated that they are not able to validate the analytical method, nor precisely determine the concentration of Antifoam 204 in the LMP-204™ due to analytical challenges.

Our review of the pertinent regulations regarding oxirane (§173.340 and §172.808, among

¹ 50 mL of Antifoam 204 was diluted in 11L. Thus: (50 mL/11 L) (10³ µL/mL)=4545 µL/L

others) indicate that this substance is not approved for the use indicated in the subject petition. Therefore, in the e-mail dated 2/15/05, it was recommended that Intralytix use an alternative defoaming agent. In the e-mail response of 2/18/05, Intralytix stated that they will discontinue using Antifoam 204 in the manufacture of LMP-102™ and instead use dimethylpolysiloxane (DMPS) at a maximum level of 10 mg/kg in LMP-102™. DMPS is currently regulated for use as a defoaming agent in food at levels up to 10 mg/kg under §173.340. We accept Intralytix's use of DMPS at a maximum level of 10 mg/kg in LMP-102™.

We have no questions with regard to the identity and composition of the additive.

Manufacturing Process

1. In the letter dated 9/13/04, Intralytix was requested to provide further information to support the claim that low molecular weight components, such as Listeriolysin O (LLO), are removed from LMP-102™ via filtration.

In the 10/25/04 submission, Intralytix reiterated that each batch of LMP-102™ will be assayed for LLO by a standard hemolytic assay (Appendix G13), and will be reprocessed, if needed, until the level of LLO is non-detectable (LOD of 5 Hemolytic units (HU)/mL; see Specifications, below). We note that the inclusion of the specification limit of 5 HU/mL in the proposed regulation provides the appropriate assurance. We accept Intralytix's explanation.

We have no further questions regarding the manufacturing process.

Specifications

In the submission dated 10/25/04, Intralytix has proposed revised specifications for LMP-102™. These specifications are summarized in Table 1, below. Revisions from specifications previously reported (see chemistry memorandum dated 6/16/04) are noted in bold text.

Table 1. Summary of the revised specifications for LMP-102™

Quality Parameters	Method of Assay	Specification	Limit of Detection	Appendix for Analytical Method
		All component		
Identity/Enforcement	PCR*	phages detected	NA***	G-01
Potency	Spectrophotometry	OD ₆₀₀ ≤ 0.06	0.001	G-02
Listeriolysin O (LLO)	Hemolytic assay	Negative	5 HU/mL	G-13
Lytic titer		9 ± 0.5 log₁₀ PFU/mL^{○○○○}	0.5 log₁₀ PFU/mL	G-03

Endotoxin content	Std plaque formation assay LAL assay**	<350 EU/mL**	0.1 EU/mL	G-11
Bacterial sterility	USDA method Automated	No growth	< 1CFU/25g	J-07
Total organic carbon (TOC)	analyzer	≤ 36 mg/kg	0.1 mg/kg	G-21
Lead (Pb) & Arsenic (As)	ICP-OES***	Not detected	Pb < 0.5 mg/kg As < 0.1 mg/kg	G-18

* PCR=Polymerase Chain Reaction

**LAL assay=Limulus Amebocyte Lysate assay

***ICP-OES=Inductively Coupled Plasma-Optical Emission Spectrometry

°HU=Hemolytic unit

° ° EU=Endotoxin unit

° ° ° NA=not available

°°°° 9 log₁₀ PFU/mL is equivalent to 1x10⁹ PFU/mL

We have no question regarding the specifications for the additive.

Analytical Methods

1. A method for determining the level of mono/polysaccharides in LMP-102™ (stated to be < 80 ppm, see Table 1 in the 6/16/04 chemistry memorandum) was not provided. Intralytix was requested to provide this information in the letter dated 9/13/04.

In the 10/25/04 submission, Intralytix provided a description of the method ((High Performance Liquid Chromatography (HPLC) coupled to Pulsed Amperometric Detection (PAD)) for determining the level of mono/polysaccharides in LMP-102™ (Appendix J06). This response is adequate.

Estimated Dietary Intakes

i). Phage

Intralytix has stated that the "maximum surface area-to-weight ratio is 33.3 cm²/g." In the letter dated 9/13/04, we requested that Intralytix comment and clarify its calculation of the factor of 33.3 cm²/g.

In the 10/25/04 submission, Intralytix indicated that 33.3 cm²/g is intended to refer to a "typical" rather than "maximum" surface area-to-weight ratio. Intralytix also provided a supporting calculation, which is based on a 1/3 oz slice of product with a typical treated

surface area of 314 cm².² Intralytix states that the use of the typical surface area-to weight ratio mimics the "real-life situation" better than the use of the maximum surface area-to-weight ratio. While we concur with this statement, we would use the "maximum" surface area-to-weight ratio of 51.1 cm²/g (a 1/3 oz slice with maximum surface area of 478.6 cm²)³, which is presented in 10/25/04 submission as the worst-case scenario. We shall conservatively use this worst-case, maximum surface area-to-weight ratio estimate of 51.1 cm²/g, in all of our calculations.

As noted in our 6/16/04 memorandum, we do not typically report intake of ingredients added directly to food as a dietary concentration. Thus, we will not further discuss Intralytix's intake calculation that is based on dietary concentration. As noted in our 6/16/04 memorandum, Intralytix provided eaters-only food consumption data for the U.S. population (all-ages, aged 2+ years; 75.3 g/p/d (mean) and 153.2 g/p/d (90th percentile)), and for the worst-case (i.e., highest) consumers of the foods to be formulated with LMP-102TM (i.e., males aged 20+ years; 101.8 g/p/d (mean) and 203.6 g/p/d (90th percentile)). We shall use these data, together with the following assumptions to estimate the intake of phages:

- i) the maximum application rate of LMP-102TM on foods is 1mL/500 cm² (0.002 mL/cm²)
- ii) the maximum surface area-to-weight ratio is 51.1 cm²/g
- iii) LMP-102TM contains 1 x10⁹ phage/mL
- iv) Avogadro's number is 6.023 x 10²³ /mole
- v) the total mass of the phage is 5.7 x 10⁷ g/mole

The results are summarized in Table 2, below.

Table 2. Eaters-only intake of phage from LMP-102TM (µg/p/d)

Age group	Mean	90 th Percentile
2+ years (all ages)	0.73	1.4
20+ years (males)	0.95	1.9 ⁴

These intake estimates supersede those in our 6/16/04 memorandum. We note that these estimates are conservative, as they are based on the maximum use level of LMP-102TM, the

² 33.3 cm²/g = [314 cm² / (0.33 oz x 1 lb/16 oz x 454 g/lb)]

³ 51.1 cm²/g = [478.6 cm² / (0.33 oz x 1 lb/16 oz x 454 g/lb)]

⁴ Sample calculation: 1.9 µg/p/d = [(203.6 g/p/d)(51.1 cm²/g)(0.002 mL/cm²)(1x10⁹ phage/mL)(5.7 x 10⁷ x 10⁶ µg/mole)] / (6.023 x 10²³/mole)

maximum level of the phage in LMP-102™, and the assumption that all foods that are intended to be treated with LMP-102™ are so treated.

ii). Total organic solids (TOS)

1. In the letter dated 9/13/04, we requested that, in the calculation of the intake of TOS, Intralytix clarify the surface area-to weight ratio and provide explanation of the origin of the 0.4 factor for calculating TOS from TOC. We also requested that the specification limit for TOC be used in revising the calculation.

Intralytix addressed the surface area-to-weight ratio as discussed for the intake estimate of the phage, above. In the 10/25/04 submission, Intralytix provided explanation for the origin of the 0.4 factor in the calculation of TOS from TOC. TOC represents the mean carbon content of the organic components of LMP-102™, including carbohydrates, amino acids, and nucleic acids. Based on data provided previously (submission dated 12/20/03), Intralytix estimated the carbon content from these sources to be 40.2%, or 0.4.⁵ We accept Intralytix's explanation. Intralytix also notes that the revised specification limit for TOC (i.e., 36 mg/kg) is appropriate to use in estimating intake of TOS. We concur.

Intralytix has provided a revised intake estimate of TOS for males (20+ years) at the 90th percentile using the typical surface area-to-weight ratio (33.3 cm²/g) and the revised specification limit for TOC (36 mg/kg). However, this estimate was reported on a dietary concentration basis, and is not appropriate, as discussed for intake estimate for the phage, above. Therefore, we shall calculate the intake of TOS using the eaters-only food consumption data and the following assumptions:

- i) the maximum application rate of LMP-102™ on seafood is 1 mL/500 cm² (0.002 mL/cm²)
- ii) maximum surface area-to weight ratio is 51.1 cm²/g
- iii) the specification limit for TOC in LMP-102™ is 36 mg/kg
- iv) the level of TOS in LMP-102™ is 26 µg/mL⁶ (see submission dated 10/25/04)

The results are summarized in Table 3, below.

⁵ The carbon content of carbohydrates (40.1%), amino acids (45.6%), and nucleic acids (34.9%) has been reported in Tables 20-22 of the submission dated 12/20/03. Thus: $40.2\% = (40.1\% + 45.6\% + 34.9\%) / 3$

⁶ As noted in the 6/16/04 chemistry memorandum, $TOS = TOC \times 0.4$. Therefore, using the specification limit for TOC: $TOS = 36 \text{ mg/kg} \times 0.4 = 90 \text{ mg/kg}$; less 64 mg/kg "identifiable TOS" (galactosamine, glucosamine, galactose and mannose), or $90 \text{ mg/kg} - 64 \text{ mg/kg} = (26 \text{ mg/kg}) \times (10^3 \text{ µg/mg}) \times (1 \text{ kg}/10^3 \text{ g}) \times (1 \text{ g/mL}) = 26 \text{ µg/mL TOS}$

Table 3. Eaters-only intake of TOS from LMP-102™ (mg/p/d)

Age group	Mean	90 th Percentile
2+ years (all ages)	0.20	0.41
20+ years (males)	0.27	0.54 ⁷

These intake estimates supersede those in our 6/16/04 memorandum.

iii). Inorganic substance

Intralix has stated that inorganic substances (e.g., derived from salts, buffers and acids) will comprise less than 1% of LMP-102™. They estimate the intake of these substances to be 0.01 x 25 g = 25 mg/p/d. However, no explanation is provided for the origin of the 25 g. We requested clarification in the letter dated 9/13/04.

In the 10/25/04 submission, Intralix noted that there is an error in the initial calculation, and recalculated the intake of inorganic substances for males (20+ years) at the 90th percentile to reflect the maximum daily consumption of foods and the maximum application rate of LMP-102™ on foods. However, in this calculation, Intralix used the typical surface area-to-weight ratio of 33.3 cm²/g. Therefore, we shall calculate the intake of inorganic substances using the eaters-only food consumption data and the following assumptions:

- i) the maximum application rate of LMP-102™ on foods is 1mL/500 cm² (0.002 mL/cm²)
- ii) the maximum surface area-to-weight ratio is 51.1 cm²/g
- iii) the specific gravity of LMP-102™ is 1.005 g/mL
- iv) Inorganic substances are 1% of the product (see submission dated 12/20/03)

The results are summarized in Table 4, below.

Table 4. Eaters-only of inorganic substances from LMP-102™ (mg/p/d)

Age group	Mean	90 th Percentile
2+ years (all ages)	77.3	157
20+ years (males)	105	209 ⁸

⁷ Sample calculation: 0.54 mg/p/d=(203.6 g/p/d)(51.1 cm²/g)(0.002 mL/cm²)(26 µg/mL)(10⁻³ mg/µg)

⁸ Sample calculation: 209 mg/p/d=(203.6 g/p/d)(51.1 cm²/g)(0.002 mL/cm²)(1 g/100 g)(1.005 g/mL) (10³ mg/g)

These intake estimates supersede those in our 6/16/04 memorandum.

iv). LLO

In the letter dated on 9/13/04, we requested that Intralytix revise their estimation of intake of LLO using the LOD of 5 HU/mL as the worst-case estimate of LLO in LMP-102™, and using an average body weight of 60 kg instead of 70 kg.

In the 10/25/04 submission, Intralytix agreed to use the LOD of 5 HU/mL for LLO in LMP-102™ and the 60 kg body weight, and provided a revised calculation for males (20+ years) at the 90th percentile. However, this calculation was on a dietary concentration basis and will not be considered further for reasons discussed above. We have calculated the intake of LLO using the eaters-only food consumption data and the following assumptions:

- i) the maximum application rate of LMP-102™ on foods is 1 mL/500 cm² (0.002 mL/cm²)
- ii) the maximum surface area-to-weight ratio is 51.1 cm²/g
- iii) the LLO content in LMP-102™ is below the limit of detection (LOD; 5.0 HU/mL)

The results are summarized in Table 5, below.

Table 5. Eaters-only intake of LLO from LMP-102™ (HU/p/d)

Age group	Mean	90 th Percentile
2+ years (all ages)	39	78
20+ years (males)	52	104 ⁹

These intake estimates supersede those in our 6/16/04 memorandum.

v). Antifoam agent

Intralytix did not provide an intake estimate for the antifoam agent, DMPS. Therefore, we estimated the intake of DMPS using the eaters-only food consumption data and the following assumptions:

⁹ Sample calculation: $104 \text{ HU/p/d} = (203.6 \text{ g/p/d})(51.1 \text{ cm}^2/\text{g})(0.002 \text{ ml/cm}^2)(5 \text{ HU/ml})$

- i) a maximum level of 10 mg/kg DMPS in LMP-102™
- ii) the specific gravity of LMP-102™ is 1.005 g/mL
- iii) the maximum application rate of LMP-102™ on foods is 1mL/500 cm² (0.002 mL/cm²)
- iv) the maximum surface area-to-weight ratio is 51.1 cm²/g

The results are summarized in Table 6, below.

Table 6. The eaters-only intake of DMPS from LMP-102™ (mg/p/d)

Age group	Mean	90 th Percentile
2+ years (all ages)	0.08	0.16
20+ years (males)	0.11	0.21 ¹⁰

vi). Summary

A summary of estimated intake of the components of LMP-102™ is provided in Table 7, below.

Table 7. Summary of eaters-only estimated intake of the components of LMP-102™

	Males (20+ years)		All ages (2+ years)	
	mean	90 th percentile	mean	90 th percentile
Phage (µg/p/d)	0.95	1.9	0.73	1.4
TOS (mg/p/d)	0.27	0.54	0.20	0.41
Total inorganic substance (mg/p/d)	105	209	77.3	157
LLO (HU/p/d)	52	104	39	78
Antifoam agent, DMPS (mg/p/d)	0.11	0.21	0.08	0.16

These intake estimates supersede those in our 6/16/04 memorandum. Estimates for males (20+ years) represent the worst-case scenario because this population group is the highest consumer of the foods to be treated with LMP-102™. All estimates are conservative as they are based on the maximum use levels of LMP-102™, the maximum level of the component in LMP-102™, and the assumption that all foods that are intended to be treated with LMP-102™ are so treated.

¹⁰ Sample calculation: 0.21 mg/p/d = (203.6 g/p/d)(51.1 cm²/g)(0.002 mL/cm²)(10 mg/kg)(1kg/10³g)(1.005 g/mL)

Proposed Regulation

We conveyed the following comments in the letter dated 9/13/04:

1). Paragraph (a) (1) states the additive consists of an aqueous solution of one or more phages. All of the information in the petition is for a product consisting of six component phages only. We recommend that the phrase "one or more" be changed to "six". We also recommend that the ATCC number be specified for each individual phage indicated.

Intralix proposed revised wording for the regulation (10/25/04 submission, Appendix A01). The revised wording modified the identity (paragraph (a)(1)) to state that the additive consists of an aqueous solution of six phages effective against *L. Monocytogenes*. However, in paragraph (a)(2), Intralix has proposed that the ATCC numbers of the six phages should not be specified, but rather that the phages have had ATCC numbers assigned. This permits flexibility use any combination of six phages that shall meet all of the specifications in the proposed regulation. We concur with Intralix's revised wording for paragraph (a).

2). Specifications for the phage component and mean phage titer of the LMP-102™ product have not been provided. We believe that these specification limits are necessary to appropriately identify the product. The wording should be revised to include the specification limits.

Intralix's proposed revised wording (10/25/04 submission, Appendix A01) specifies the mean phage titer (1×10^9 PFU/ml), and potency test ($OD_{600} \leq 0.06$). This wording is adequate.

3). the conditions of use (paragraph (c)) should also indicate that "Current good manufacturing practice is consistent with direct spray application of LMP-102™ at a rate of approximately 1 mL/500 cm² product." We believe this is necessary to ensure appropriate use of the LMP-102™.

Intralix's proposed revised wording (10/25/04 submission, Appendix A01) specifies the revised use conditions (approximately 1 mL/500 cm² product). This wording is adequate.

We also note the following:

i). the paragraph (b)(8), the specification limit for TOC has been revised from not more than 50 mg/kg to not more than 36 mg/kg (see point 1, Identity, above).

ii). with regard to the incorporation by reference in paragraph (b) of the regulation: (a) the

wording should be consistent with that currently in use regarding the availability of the documents that are incorporated by reference; (b) reference to "Intralytix" should be deleted; and (c) the information regarding incorporation by reference should be added to paragraph (b)(5) and (b)(7) for consistency.

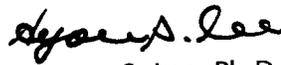
We have no questions regarding the chemistry-related issues in the proposed regulation. However, we note that the microbiologist may wish to address the adequacy of the microbiological aspects of the specifications.

Conclusion

Intralytix has provided adequate responses to the chemistry-related deficiencies identified in the letter dated 9/13/04 and the e-mails dated 12/02/04 and 2/15/05.

Based on Intralytix's responses, we have revised the intake estimates for the components of LMP-102™ (Table 7). These estimates supersede those in our 6/16/04 memorandum.

We have no further questions. The petition is suitable for regulation with respect to the chemistry-related information.


Hyoungh S. Lee, Ph.D.

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