

ORIGINAL SUBMISSION

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IVY ANIMAL HEALTH, INC.  
8857 BOND STREET • OVERLAND PARK, KANSAS 66214  
PHONE: 913-888-2192 • FAX: 913-888-3007

March 23, 2009

Dr. Robert L. Martin, Ph.D.  
Division of Biotechnology and GRAS Notice Review (HFS-255)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
5100 Paint Branch Parkway  
College Park, MD 20740

Dear Dr. Martin,

Enclosed is the claim of exemption from premarket approval requirements for colicin E1 to be used as a processing aid to control *Escherichia coli* in meat products. The notification has been prepared to be consistent with the proposed regulations, 21 CFR 170.36.

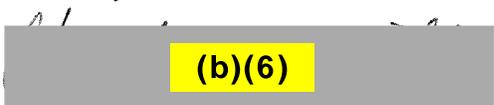
The summary of our request is as follows:

Notifier	Substance	Intended Use	Basis
Ivy Animal Health	Colicin E 1	For use as a processing aid on fresh beef carcass, beef cuts, and ground beef components pre-grind, to control <i>Escherichia coli</i>	Scientific Procedures

We have provided one redacted copy (removal of Appendix B), for your convenience.

We appreciate the assistance of your staff in preparing this notification.

Sincerely,

 (b)(6)

Charles D. Miller, DVM, PhD

- Copies: 4 Paper copies (complete)
- 1 CD electronic copy (complete)
- 1 Paper copy (redacted)



WHOLLY OWNED DIVISIONS OF IVY ANIMAL HEALTH

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## 1. GRAS Exemption Claim

### A. Claim of Exemption From the Requirement for Premarket Approval Requirements Pursuant to Proposed 21 CFR 170.36(a)(1)

Colicin E1 has been determined to be generally recognized as safe, and therefore, exempt from the requirement of premarket approval, under the conditions of its intended use as described below. The basis of the finding is described in the following sections.

Signed,

(b)(6)

Charles D. Miller, DVM, PhD

**B. Name and Address of Notifier:**

Ivy Animal Health, Inc.  
9111 Barton St,  
Overland Park, KS 66214

**C. Common or Usual Name of the Notified Substance**

COLICIN E1

**D. Conditions of Use**

The intended use of Colicin E1 will be as a spray to control *Escherichia coli* on fresh beef carcass, beef cuts, and ground beef components pre-grind, which results in a concentration of not more than 5 mg/kg (2.27mg/lb) on cut meat and trim.

**E. Basis for GRAS Determination**

Colicin E1 has been determined to be GRAS by scientific procedures as defined by 21 CFR 170.30(b). A comprehensive literature search was utilized for this review. A summary of the safety review can be found in Appendix A.

**F. Availability of Information**

The data and all information that serve as a basis for the GRAS determination are available for the Food and Drug Administration's review and copying during reasonable business hours at the following address:

Charles Miller  
Ivy Animal Health, Inc.  
9111 Barton St,  
Overland Park, KS 66214

**2. Detailed information about the identity of the substance**

**A. Identity**

Colicin E1 (CAS Registry Number 11032-88-5) is a bacteriocin (antimicrobial peptide) produced by *E. coli* K12 W3110 which contains the plasmid pColE1-K53 (NCTC, London, England). The strain was created by a conjugation of the *E. coli* K12 strain with a natural colicin E1 producing *E. coli* strain (*E. coli* K53). Colicin E1 is characterized as a pore-forming colicin, that uses BtuB as its target receptor, and has been shown to utilize the TolCAQ translocation system. Colicin E1 is isolated from the fermentation supernatant and purified, and includes no live cells.

## B. Structural Formula for Colicin E1:

Colicin is a protein produced by and effective against some strains of *Escherichia coli*. Colicin E1 has a molecular weight of 57.3 KDa and consists of 522 Amino Acids with the sequence of:

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METAVAYYKD GVPYDDKGQV IITLLNGTPD GSGSGGGGGK GGSKSESSAA  
IHATAKWSTA QLKKTQAEQA ARAKAAAEAQ AKAKANRDAL TQRLKDIVNE  
ALRHNASRTP SATELAHANN AAMQAEAEERL RLAKAE EKAR KEAEAAEKAF  
QEAEQRRKEI EREKAETERQ LKLAEAE EK R LAALSEEAKA VEIAQKKLSA  
AQSEVVKMDG EIKTLNSRLS SSIHARDAEM KTLAGKRNEL AQASAKYKEL  
DELVKKLSPR ANDPLQNRPF FEATRRRVGA GKIREEKQKQ VTASETRINR  
INADITQIQK AISQVSNRN AGIARVHEAE ENLKKAQNNL LNSQIKDAVD  
ATVSFYQTLT EKYGEKYSKM AQELADKSKG KKIGNVNEAL AAFEKYKDVL  
NKKFSKARDR AIFNALASVK DDWAKHLDQ FAKYLKITGH VSGYDVS  
ILKIKDTGDW KPLFLTLEKK AADAGVSYVV ALLFSLLAGT TLGIWGIAIV  
TGILCSYIDK NKLNTINEVL GI
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as filed with the Protein Data Bank (EMBL-EBI Q 51626 9ZZZZ).

Colicin's tertiary structure is comprised of three globular protein domains (Cascales et al., 2007) which determine specific binding to the target bacteria, translocation across the periplasm of sensitive cells and killing of the target *E. coli* cell.

## C. Quantitative Composition

Colicin E1 is prepared in a food grade buffer to a concentration of approximately  $3.79 \times 10^{-2}$  mg/mL for application at a rate of 60 mL solution/lb of trim.

## D. Method of Manufacture

Generally, colicin E1 is manufactured using fermentation technology with isolation and removal of all live cell materials. A summary of the manufacturing information can be found Appendix B.

## E. Characteristic Properties

Colicin E1 is an antimicrobial peptide (MW: 56 kDa). Colicin E1 is characterized as a pore-forming colicin, that uses BtuB as its receptor, and has been shown to utilize the TolCAQ translocation system

## F. Content of Potential Human Toxicants for Colicin E1

Gluteraldehyde (CAS 111-30-8) is used as an inducer in the fermentation but is removed during the subsequent processing and purification of the colicin E1 protein as a water soluble extract.

## G. Specifications

An aqueous solution equal to or greater than 2500 mg/L colicin E1

Additional Specifications Tested under USP32/NF27 General Chapter Methods and Specifications

Residual solvents: USP32 <467>

Less than or equal to 10 viable total bacteria per milliliter: USP32 <61>

Absence of any undesirable organisms: No detectable live *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp. or coagulase-positive *Staphylococcus* spp. per 10 ml sample: USP32 <1111>

Less than or equal to 0.2 ppm lead USP32 <251>

### 3. Information on any self-limiting levels of use

The proposed use of Colicin E1 is as a processing aid on fresh beef carcass, beef cuts, and ground beef components pre-grind, to control *Escherichia coli*. The product will be labeled for use levels not to exceed 5 mg Colicin E1/kg beef. There are a number of factors that will provide a self limitation; 1) the product will be used under the HACCP controls of a slaughter facility, as such the plants will be required to evaluate the dose and efficacy of the product specific for that plant and will be highly controlled; and 2) the cost of the product will encourage use at the minimum effective use levels.

### 4. Detailed summary of the basis for GRAS Determination (Proposed 170.36(c)(4))

The detailed summary of the basis for the GRAS determination can be found in Appendix A, which includes the suitability summary.

Signed:

(b)(6)

Charles D. Miller, DVM, PhD

Appendices:

A: Summary of Safety and Suitability

B: Manufacturing

## **Appendix A: Information To Establish The Safety Of Colicin E1 and the Suitability of Use**

### **Introduction**

Colicins, produced by *Escherichia coli* strains, are a subset of the bactericidal proteins known as bacteriocins. Colicins exert their antimicrobial effect against *E. coli* and closely related bacteria. The first colicin was identified by Gratia in 1925 and numerous colicins produced by different strains of the enteric group of bacteria (*E. coli*, *Shigella*, and *Citrobacter*) have subsequently been characterized. The name *colicin* was coined by Gratia and Fredericq in 1946 (Cascales et al., 2007). Based on their mode of action, colicins have been classified into two major groups: enzymatic colicins and pore-forming colicins.

Colicin E1 is a pore-forming colicin that exerts its lethal effect on sensitive bacteria, primarily *Escherichia coli* (Pugsley, 1984) by: a) binding to a specific receptor on the outer membrane of the target cell; b) it is then translocated across the outer membrane and through the peri-plasm; and c) finally, is inserted in the cell membrane to form the pore or channel that leads to cell death (Lazdunski, 1988; Pugsley, 1984). Schwartz and Helinski (1971) were the first to report the physical chemical characterization of purified colicin E1. They determined that colicin E1 is a simple protein with a molecular weight of approximately 56 kDa, an isoelectric point of 9.05 and a high lysine:arginine ratio. The amino acid content of colicin E1 reported by Schwartz and Helinski was supported by the amino acid sequence predicted by the colicin E1 gene sequence determined by Yamada et al. (1982).

The production of colicins is currently thought of as a mechanism to promote the survival and genetic integrity of colicin producing *E. coli* in a mixed microbial niche. Colicin E1 is expressed under conditions such as nutrient depletion, overcrowding, osmotic shock, UV light, and/or DNA damaging antibiotics (Riley and Gordon, 1999; Spangler, et al., 1985). Under such conditions the bacteria react to the stress by initiating the SOS response (van den Elzen, et al., 1982). The LexA protein is the primary transcriptional repressor of the SOS response and colicin synthesis. Under colicinogenic conditions, LexA degradation stimulates cAMP production and the transcription of the colicin protein (van den Elzen, et al., 1982; Eraso and Weinstock, 1992; Eraso, et al., 1996).

### **Colicin E1 Prevalence**

Humans have a very long history of exposure to colicin E1. From the original studies that isolated and characterized colicins (Gratia and Fredericq, 1946), colicin-producing *E. coli* have been isolated from fecal samples of healthy humans and other animals, as well as from other environmental samples (Smarda et al., 2007; Schamberger and Diez-Gonzalez, 2002; Riley and Gordon, 1992; Obi and Campbell, 1978; Lorkiewicz et al., 1964; Smarda, 1960; Gratia and Fredericq, 1946). Estimates for the number of colicin producing *E. coli* in the colon of healthy humans have ranged from as low as 9% of the total number of *E. coli* isolated (Lorkiewicz et al., 1964) to as high as 82% of recovered isolates (Smarda, 1960). While very few studies have examined the production

of specific colicins by commensal *E. coli* in healthy humans, Gordon and O'Brien (2006) examined 266 *E. coli* isolates and found that 24% produced at least one colicin, and that 8.3% of the isolates specifically produced colicin E1. From these studies it is apparent that colicin E1 producing *E. coli* are prevalent in the gastrointestinal tract of healthy humans, and due to the highly competitive microbial environment of the gastrointestinal tract, we would expect that these *E. coli* are producing and excreting colicin E1. It is therefore anticipated that humans have always been exposed to colicin E1.

It is very difficult to estimate the amount of colicin E1 that is produced in the human intestine. However, a high estimate, utilizing numerous assumptions follows; assuming 1% of the intestinal flora is *E. coli* (Todar, 2007) and that 82% produce colicin E1 (Smarda, 1960), assuming high levels (equivalent to laboratory/production induction in a culture of 10 logs of *E. coli* per ml, partially offsetting multiple generations of intestinal *E. coli* per day) of colicin E1 are produced by the *E. coli*; an estimate based on  $10^{14}$  cells in the intestine (Steinhoff, 2005) [1% are *E. coli*, 82% produce colicin E1] could produce 24 mg colicin E1/day.

#### Naturally Occurring Colicin E1 in Food

While no research to date has examined food products for colicins, it is likely that they are present in multiple food sources. Colicin producing *E. coli* have been found as commensal organisms in the gastrointestinal tract of many food animals, such as cattle, pigs, chickens, sheep, goats, ducks and deer (Obi and Campbell, 1978; Schamberger and Diez-Gonzalez, 2002). It is therefore highly likely that the food products from these animals would also have colicin producing *E. coli* present at some level. Additionally, because the feces from these production animals are frequently used as fertilizer, it is also likely that humans are also exposed to colicins on the produce they eat. Due to the virtually ubiquitous nature of *E. coli* in food products, and the frequency of colicinogenicity seen among *E. coli* isolated from multiple environments (Smarda, 1960; Lorkiewicz et al., 1964; Gordon and O'Brian, 2006) it is expected that there has been a long history of human exposure to colicins in food.

An estimated amount of colicin E1 present in beef, utilizing numerous assumptions follows; assuming high expression of colicin E1 (equivalent to laboratory/production induction in a culture of 10 logs of *E. coli* per ml) in nature, and 10,000 *E. coli* (82% produce colicin E1) per g of beef trim (Lange, L., 2008), and 77.9 g of meat consumed per day (Based on the USDA , Economic Research Food Disappearance Database, 2006 data for beef), it is estimated that an individual could consume 0.02 ug colicin E1/person/day from beef consumption under naturally occurring conditions.

#### Food Safety of Colicin E1

The use of colicin as a biopreservative on food should not cause safety concerns. This is supported by several factors including the long history of human exposure to colicins without known adverse consequences. The other attributes of colicin E1 that support its safety for use in food include enzymatic digestibility and lack of in vitro cytotoxicity. Colicins have been shown to be very susceptible to digestion by proteolytic enzymes. By definition, colicins were identified as antimicrobial compounds that lost

their efficacy after protease treatment. Slatin et al. (1986) demonstrated that colicin E1 is sensitive to pepsin digestion, and many others have shown that colicin E1 is susceptible to trypsin digestion (Elkins et al., 1994; Murinda et al., 1996; Zhang and Cramer, 1992). In a study examining the efficacy of 24 different colicins, Murinda et al. (1996) noted that all of the colicins were highly susceptible to protease treatment and that treatment with either papain, pronase E, or trypsin eliminated the colicins' activity. Since colicin E1 is a relatively large protein (approximately 56 kDa) it cannot be absorbed intact when ingested and, due to the presence of proteolytic enzymes in the gastrointestinal tract, it is rapidly digested.

The safety of colicin E1 has been further demonstrated in two swine studies evaluating dietary inclusion of colicin E1 in the prevention of post-weaning diarrhea in pigs (Cutler et al., 2007, Cutler et al., 2008). Piglets were fed colicin E1 in doses ranging from 11 to 20 mg/kg. Pigs fed 20 mg/kg for 4 weeks, to evaluate the effect of dietary inclusion of colicin E1 alone, showed no significant differences in body weight between control and colicin E1 fed pigs at any point in the study. Twenty-four pigs, fed 0, 11 or 16.5 mg colicin E1/kg for four days, demonstrated significant differences in increased body weight gain and feed consumption for colicin treated pigs. These studies indicate that when feeding high levels of colicin E1 to monogastric species there is no negative effect on pig health status as noted by the measurements of intake and weight gain.

Additionally, colicin E1 has been shown to have little if any toxic effects on both immortalized human colon and monkey kidney cells (Murinda et al., 2003). Murinda et al. (2003) compared the cytotoxicity of colicin E1 with nisin *in vitro* in both of these cell lines. This study demonstrated that colicin E1 was less cytotoxic to both of these cell lines than nisin.

The use and consumption of colicin E1 is unlikely to allow for selection for colicin E1 resistant bacteria in the intestinal tract of humans for three main reasons; 1) it is expected that the beef or ground beef will be cooked prior to human consumption, inactivating the colicin E1 by heat 2) the colicin E1 will be denatured by the low stomach pH and 3) the colicin E1 protein will be rapidly digested by proteases.

#### Safety of Colicin E1 Production Strain

The strain utilized to produce colicin E1 is an *E. coli* K12 W3110 which contains the plasmid pColE1-K53 (NCTC, London, England). This strain was created by conjugation of the *E. coli* K12 strain with a natural colicin E1 producing *E. coli* strain (*E. coli* K53) (Pugsley, 1985). *Escherichia coli* K12 has an established safe strain lineage and is currently approved for use in the production of chymosin (55 Federal Register 10932, 1990). Subsequently, this host *E. coli* strain was also serotyped as O:H- at the *E. coli* Reference Center (Department of Veterinary Science, Pennsylvania State University, University Park) (Murinda et al., 1996).

### Use and Exposure

Colicin E1 will be used as an antimicrobial spray processing aid on pre-evisceration hot and post chill beef carcasses, primal cuts, sub-primal cuts and ground beef components pre-grind for use against certain strains of *E. coli*, including *E. coli* O157:H7. It is estimated that the directed use of colicin E1 on beef would not exceed 5 mg colicin E1 per kg of meat. Based on the USDA, Economic Research Food Disappearance Database, 2006 data for beef (77.9 grams/person/day, assuming all consumed beef was treated with colicin E1 at a maximum rate); colicin E1 estimated exposure would be 0.4 mg/person/day, as related to consumption of beef.

For comparative purposes, the potential exposure of colicin E1 is less than the estimated exposure of nisin for ready-to-eat meats of 0.6 mg/person/day (as reported in GRN 65) and cytotoxicity study results indicates colicin E1 is significantly less toxic than nisin (Murinda et al., 2003).

### Suitability of Use

The foundation of the suitability claim is based on a recent publication (Patton et al., 2008) where colicin E1 was evaluated as a potential intervention strategy for controlling *E. coli* O157:H7 contamination for beef carcasses. The authors utilized untrimmed beef round roasts that were cut into sample sizes of 5.08 by 2.52 by 5.08 cm, with an adipose layer covering an entire surface of lean beef. Samples were placed on sterile metal hooks and inoculated with *E. coli* O157:H7 at a level of 5 log CFU/ml in sterile tryptic soy broth. Colicin E1 in doses of 0, 100 ug, 500 ug, and 1 mg/ml of 10 mM Tris, pH 7.6, was sprayed on the samples in a volume of 1 ml, after allowing the inoculum 10 min to attach. Samples were evaluated at 0 and 30 min, 1, 2, 3, 4, and 5 days post-spraying at 10 degrees C for *E. coli* O157:H7 inhibition. Treating samples with 500 ug and 1 mg of colicin E1 per ml effectively inhibited *E. coli* O157:H7 growth for samples inoculated with *E. coli* WS 3331, where *E. coli* contamination was reduced by 4 and 7 log CFU/cm<sup>2</sup>, respectively, compared with the untreated control samples. For strain WS 3331, treatment with 1 mg colicin E1 per ml significantly inhibited growth of *E. coli* O157:H7 compared with the untreated control during the entire study.

This study demonstrated that colicin E1 significantly reduced *E. coli* O157:H7 on beef samples and provides the supporting data that colicin E1 would be effective as a spray intervention to pre-evisceration hot and post chill beef carcasses, primal cuts, sub-primal cuts and ground beef components pre-grind. Colicin E1 should be used at a concentration of 100 µg/ml with a total of 50 ml sprayed per 1 kg of beef trim and components. This dosage is based upon the results of Patton et al. (2008) and a few assumptions regarding beef trim. In the Patton et al. (2008), study 1 ml was sufficient to provide complete coverage for 100 cm<sup>2</sup> of beef trim, which weighed approximately 17.5 g. For recommended usage, it is assumed that an average piece of beef trim would be 150 cm<sup>2</sup> (5 cm × 5 cm × 5 cm) and would weigh 35 g. Based on these assumptions and the results of Patton et al. (2008), a total of 1.5 ml would be needed to completely cover the entire surface of an average piece of beef trim. This corresponds to a total of 42.9 ml being needed to treat 1 kg of beef trim. The 100 µg/ml dose reliably provided a minimum kill of 200 CFU *E. coli* O157:H7/cm<sup>2</sup> in Patton et al. (2008), and it would be

highly unlikely for this level of *E. coli* contamination to be present on beef trim and components, as it would correspond to 30,000 CFU/average piece or more than 850 CFU/g of beef trim and components. Therefore, it is estimated that the 100 µg/ml dose is appropriate for use. Due to potential variation of surface area and weight, an approximate 20% overage for application rate is appropriate, due to the importance of *E. coli* contamination of beef trim and components. From this we arrive at our recommended dose of 100 µg/ml with a total of 50 ml sprayed per 1kg (5 mg/kg) of beef trim and components.

### Conclusion

In summary, there are several factors that support the safe use of colicin E1 on fresh cuts of beef and ground beef. Those factors include; the long history of safe gastrointestinal exposure to this protein in both humans and other animals, the safe strain lineage of the *E. coli* K12 which produces this colicin E1, the in vitro data demonstrating that colicin E1 is less toxic than nisin, the lower intended usage rate of colicin E1 compared to nisin, and the rapid digestibility of colicin E1 by proteases or denaturation within the gastrointestinal tract. Colicin E1 suitability for use as an intervention strategy for reducing *E. coli* contamination on pre-evisceration hot and post chill beef carcasses, primal cuts, sub-primal cuts and ground beef components pre-grind, has been clearly demonstrated.

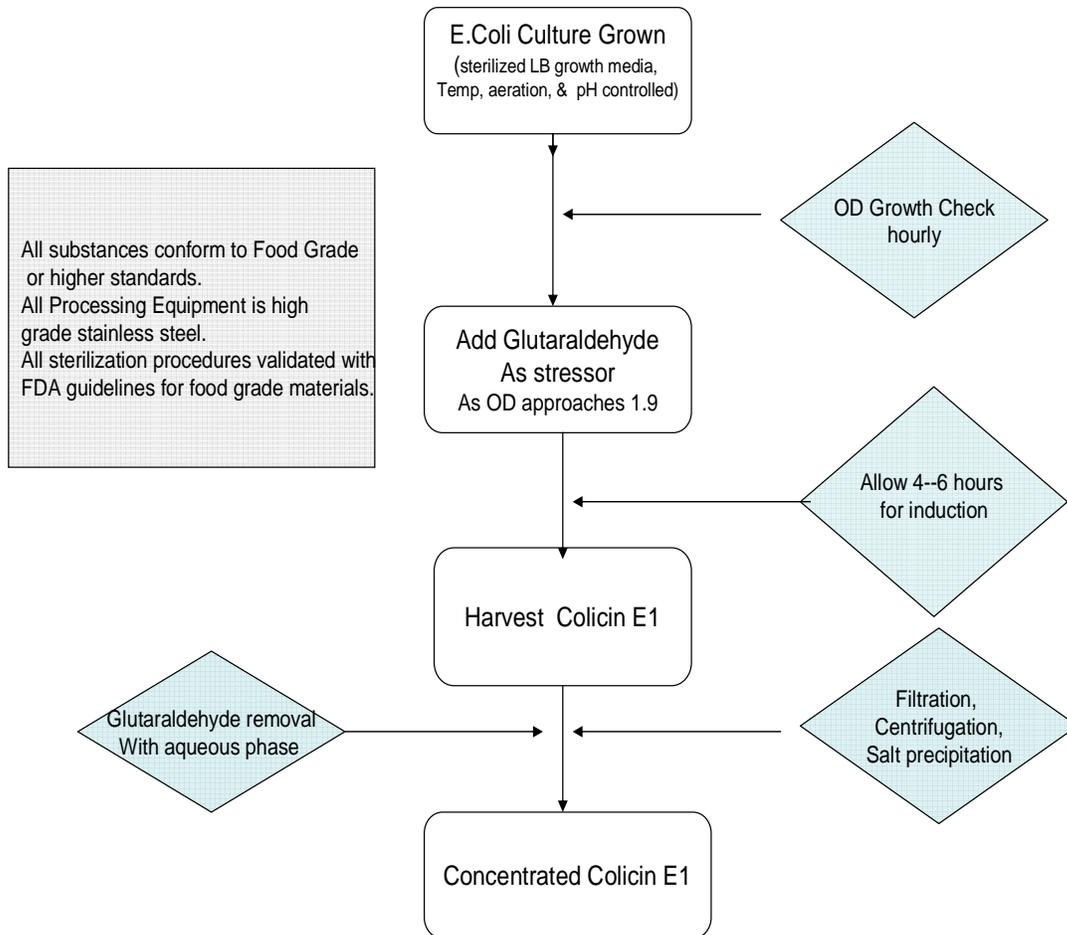
## Appendix B. Colicin E1 Manufacturing Process

### Organism Used

A non-pathogenic strain of *Escherichia coli* (*E. coli*) is used in the production of colicin E1 (*E. coli* K12 W3110 which contains the plasmid pColE1-K53 (NCTC, London, England). The strain has not been genetically modified with any artificial plasmids.

### Procedure

This *E. coli* strain naturally produces colicin E1 protein when placed under appropriate stress. *E. coli* K12 is grown in a well-known and widely used growth medium, Modified Luria Bertani (LB) broth which contains tryptone (10 g/L), yeast extract (5 g/L), and sodium chloride (10 g/L). Tryptone is a common pancreatic digest of casein. All media ingredients are food grade or higher.



The growth medium is sterilized by heat and pH is controlled with standard acids and bases. The temperature of the cultures, aeration level and pH are controlled during growth.

*E. coli* culture is grown in LB media to appropriate concentrations, after which stress is applied to the cells by introducing a small quantity of glutaraldehyde to the culture. Final glutaraldehyde concentration is approximately 0.01% for a culture with an OD<sub>600</sub> of 1.9 but can be adjusted in response to the amount of *E. coli* growth obtained to maintain a constant level of stressor to the cells.

In response to the presence of the stressor, the *E. coli* cells produce the colicin E1 translocation protein. Quasilysis of the *E. coli* cells occurs as part of this process and releases colicin E1 into the medium.

Filtration and/or centrifugation will remove unlysed cells and extraneous components of the lysed *E. coli* cells. Colicin E1 will be concentrated by centrifugation and salt participation to levels of 2500 mg colicin E1/L. Glutaraldehyde residues will be removed by aqueous solvent extraction to assure levels of less than 1 ppm. Throughout manufacture, all added substances conform to food grade or higher specifications. Processing equipment is of high grade stainless steel, food or medical grade plastics or glass. All sterilization procedures are validated as per FDA guidelines for food grade materials.

Concentrated colicin E1 solutions are obtained under strict manufacturing controls to prevent contamination by undesirable organisms and may be adjusted to high salt concentrations and/or low pH to ensure the absence of undesirable microorganisms. Each batch of colicin E1 is assayed for the concentration of colicin E1, detection of any undesirable organisms and the biological activity of the colicin E1 obtained.

### **Draft Specifications**

An aqueous solution equal to or greater than 2500 mg/L colicin E1

Additional Specifications Tested under USP32/NF27 General Chapter Methods and Specifications

Residual solvents: USP32 <467>

Less than or equal to 10 viable total bacteria per milliliter: USP32 <61>

Absence of any undesirable organisms: No detectable live *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp. or coagulase-positive *Staphylococcus* ssp. per 10 ml sample: USP32 <1111>

Less than or equal to 0.2 ppm lead USP32 <251>

## References

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# **Beef Trim Baseline Results and How FSIS Will Use Them**

**Loren Lange**  
**Deputy Assistant Administrator**  
**Office of Public Health Science**

**April 9, 2008**  
***E. coli* Public Meeting**



## Beef Trim Baseline Overview

- **Samples were collected and analyzed from December 2005 through early January 2007**
- **Design was based on getting results from 2,000 samples**
- **Samples were analyzed at four laboratories:**
  - **The three FSIS Laboratories in Athens, St. Louis, and Alameda**
  - **Food Safety Net Services, Ltd., San Antonio, TX**
- **All *Escherichia coli* O157:H7 analyses were completed by FSIS. All other analyses were conducted by Food Safety Net Services. Each sampling event required the collection of two samples.**



## Beef Trim Baseline (Overview Continued)

- **Laboratory analyses were conducted to measure the presence and quantitative level of the following bacteria:**
  - *Escherichia coli* O157:H7
  - *Salmonella* (including serotyping)
  - Generic *Escherichia coli*
  - Total coliforms
  - Enterobacteriaceae
  - Aerobic Plate Count (APC)
- **Existing FSIS laboratory methods would be used where applicable**



## **Beef Trim Baseline**

### **Sample Collection Design Issues**

- **Where**
- **When**
- **How**
- **What**



## **Beef Trim Baseline Where**

- **Samples would be collected at facilities that slaughtered cattle and boned the carcasses to produce trim.**
- **Design included collecting data on the interventions used on the slaughter line.**
- **Samples would be collected in facilities that could make adjustments to lower risk.**
- **FSIS recognized that trimmings for ground beef are also produced at other processing facilities and at retail stores.**



## **Beef Trim Baseline WHEN**

- **Two Major Options Considered:**
  - **End of Boning Line, and**
  - **after product was accepted for use in raw ground beef.**
- **Second option was selected based on data needed to update the *E. coli* O157:H7 risk assessment.**
- **Baseline population then became beef trimmings in slaughter/boning operations that had passed existing food safety systems and were available for use in raw ground beef production.**



## **Beef Trim Baseline How**

- **When study was designed, there was a wide variety of methods for sampling beef trim:**
  - **Collect Purge**
  - **Core drilling**
  - **Various amounts of surface slices**
- **FSIS had lengthy discussions with ARS and industry scientists.**
- **Decision was to use N60, a sample of 60 thin surface slices from a production lot.**



## **Beef Trim Baseline (How Continued)**

- **The number 60 is based on microbial sampling plans recommended by ICMSF<sup>1</sup> where the hazard is severe and where conditions may increase the hazard.**

**<sup>1</sup> ICMSF (International Commission on Microbiological Specifications for Foods), 2002. Microorganisms in Food, Microbiological Testing in Food Safety Management, vol. 7. Kluwer Academic/Plenum Publishers, New York. 327-330 pps.**



## **Beef Trim Baseline What**

- **The term “beef trimmings” included sub-primal cuts such as boneless chuck or parts of boneless chuck when they were being produced for use as components of raw ground beef.**
- **Study considered trying to characterize source as to fat content, but chose to direct inspectors to randomly select a sample of trimmings.**
- **The baseline study would not include samples obtained from head meat, organ meat, or Advanced Meat Recovery product, or very high fat content trimmings destined for such products as finely textured beef or partially defatted chopped beef.**

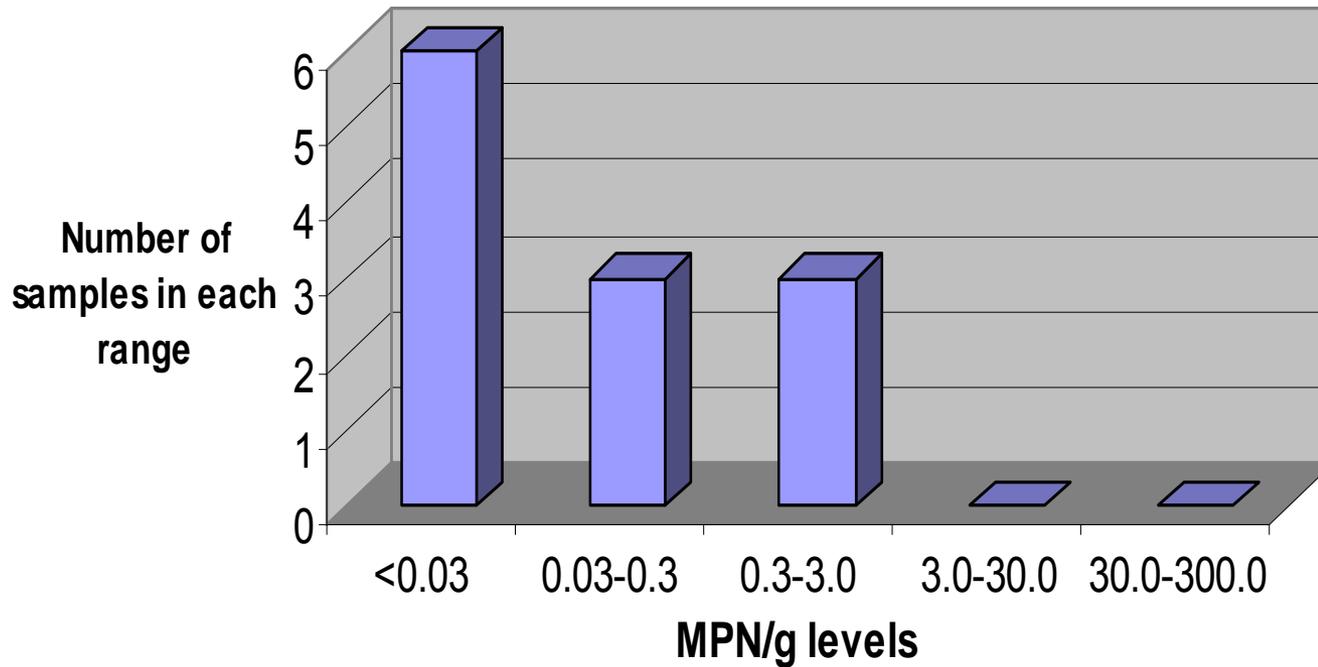


## Beef Trim Baseline Positive/Negative Results

Microorganisms	Samples Analyzed	Number Positive	Percent Positive
<b>Indicator Organisms</b>			
<i>Enterobacteriaceae</i>	1719	1015	59.0
Generic <i>Escherichia coli</i>	1719	270	15.7
<b>Pathogenic Organisms</b>			
<i>Escherichia coli</i> O157:H7	1900	13	0.68
<i>Salmonella</i>	1719	22	1.28

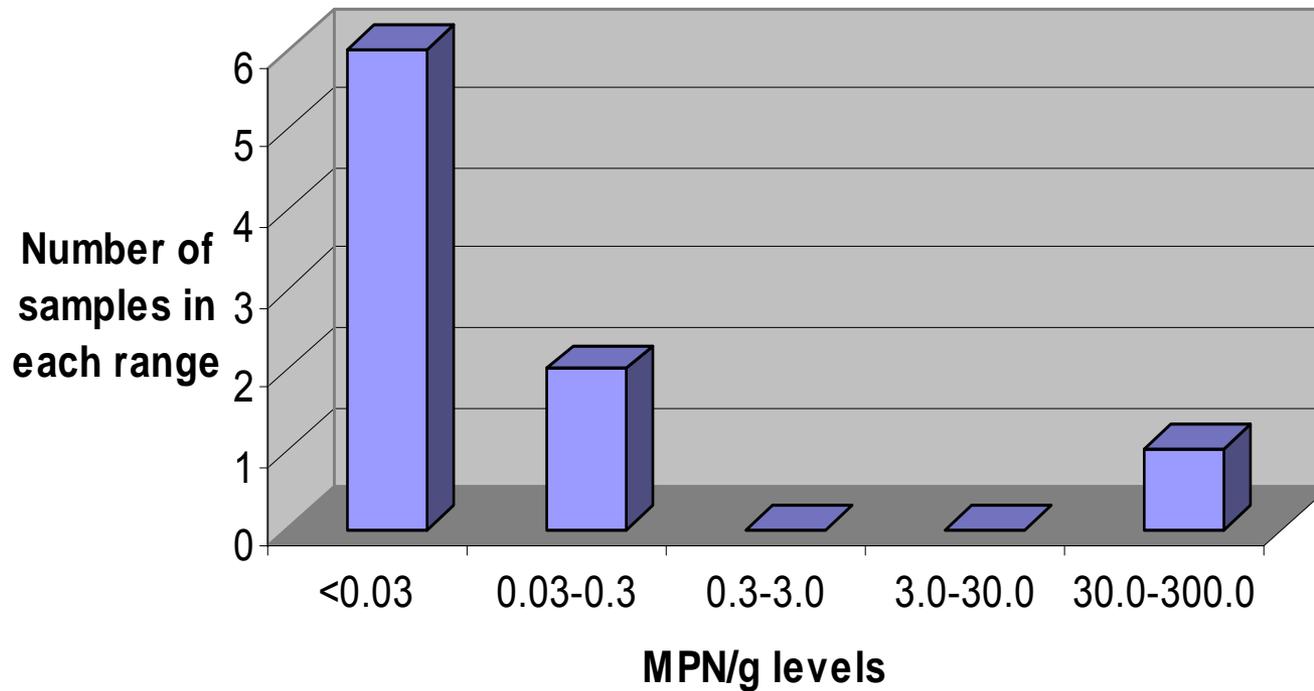


***E. coli* O157:H7 MPN ranges for beef trimmings,  
FSIS Baseline**

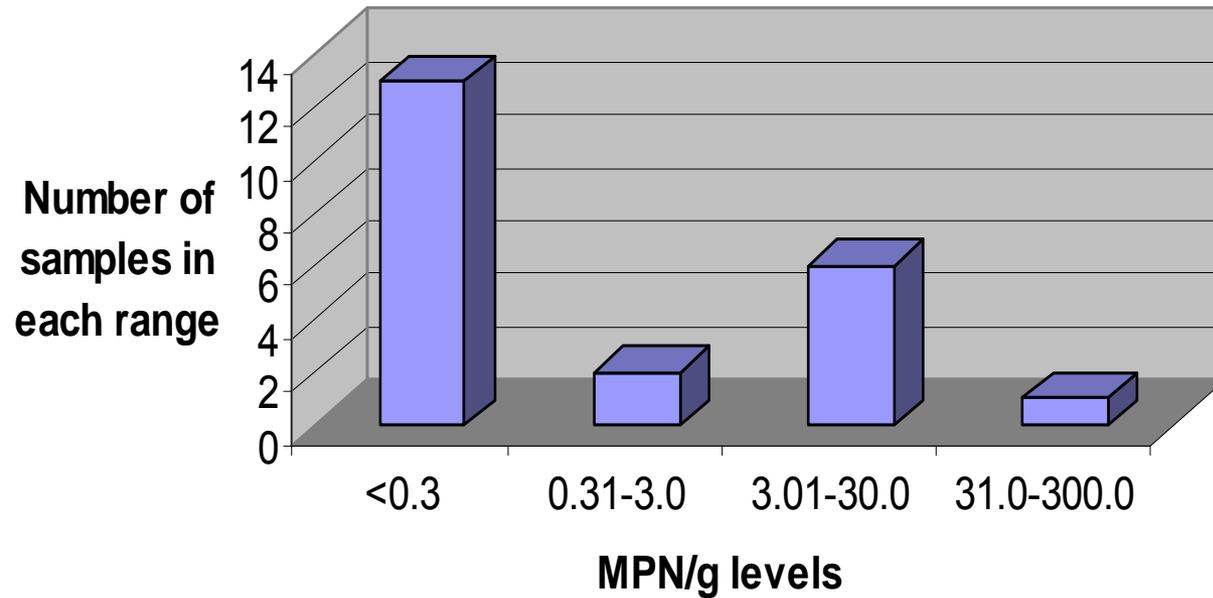




### *E. coli* O157:H7 MPN ranges for ground beef, CY2007



### ***Salmonella* MPN ranges for positive samples in beef timblings, FSIS Baseline**





**Distribution of Generic *Escherichia coli***

<b>Range, cfu/g</b>	<b>Number of Samples</b>	<b>Percent of Total</b>	<b>Cumulative Number</b>	<b>Cumulative Percent</b>
<10 <sup>0</sup>	1449	84.29	1449	84.3
10-100	239	13.90	1688	98.2
101-1,000	20	1.16	1708	99.4
1,001-10,000	10	0.58	1718	99.9
10,001-100,000	1	0.06	1719	100.0
<b>TOTAL</b>	<b>1719</b>	<b>100</b>	<b>-</b>	<b>-</b>

<sup>0</sup>Negative by Method



## **Beef Trim Baseline Next Steps**

- **Complete analysis to generate estimates of national prevalence accounting for non-responses and new producers not in the sampling frame**
- **Analyze the data collected on interventions and look for associations with bacteria levels**
- **Analyze data on pathogen levels versus indicator organisms**
- **Explore implications for policy changes**

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IVY ANIMAL HEALTH, INC.  
8857 BOND STREET • OVERLAND PARK, KANSAS 66214  
PHONE: 913-888-2192 • FAX: 913-888-3007

April 13, 2009

RECEIVED  
APR 16 2009

BY: MMW

Ms. Moraima Ramos  
Division of Biotechnology and GRAS Notice Review (HFS-255)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
5100 Paint Branch Parkway  
College Park, MD 20740

Dear Ms. Ramos,

In accordance with our April 10, 2009 telephone discussion concerning the Colicin E1 GRAS submission, please find enclosed an original and 3 copies of a modified claim of exemption from premarket approval requirements for Colicin E1 to be used as a processing aid to control *Escherichia coli* in meat products. Additionally, as discussed with you and Dr. Michael Dinovi, we understand why a redacted copy (related to manufacturing methods) will not be honored. Please dispose of the redacted copy that we previously submitted on March 23, 2009.

Thank you for your assistance in reviewing and preparing this notification.

Sincerely,

(b)(6)

Charles D. Miller, DVM, PhD

Copies: GRAS Exemption Claim  
1 original and 3 Paper copies



WHOLLY OWNED DIVISIONS OF IVY ANIMAL HEALTH

000407

## 1. GRAS Exemption Claim

### A. Claim of Exemption From the Requirement for Premarket Approval Requirements Pursuant to Proposed 21 CFR 170.36(a)(1)

Ivy Animal Health, Inc. has determined that Colicin E1 is generally recognized as safe, and therefore, exempt from the requirement of pre-market approval, under the conditions of its intended use as described below. The basis of the finding is described in the following sections.

Signed,

(b)(6)

Charles D. Miller, DVM, PhD

SUBMISSION END

## *Reference List for Industry Submission, GRN 000289*

<i>Pages</i>	<i>Author</i>	<i>Title</i>	<i>Publish Date</i>	<i>Publisher</i>	<i>BIB_Info</i>
000017 - 000088	Cascales, Eric; Buchanan, Susan K.; Duché, Denis; Kleanthous, Colin; Lloubès, Roland; Postle, Kathleen; Riley, Margaret; Slatin, Stephen; Cavard, Danièle	Colicin Biology	March 2007	Microbiology and Molecular Biology	Volume 71, Number1, pgs 158-229
000089 - 000160	Cascales, Eric; Buchanan, Susan K.; Duché, Denis; Kleanthous, Colin; Lloubès, Roland; Postle, Kathleen; Riley, Margaret; Slatin, Stephen; Cavard, Danièle	Colicin Biology	March 2007	Microbiology and Molecular Biology	Volume 71, Number 1, pgs 158-229
000161 - 000166	Cutler, S.A.; Loneran, S.M.; Cornick, N.; Johnson, A.K.; Stahl, C.H.	Dietary Inclusion of Colicin E1 Is Effective in Preventing Postweaning Diarrhea Caused by F18- Positive Escherichia coli in Pigs	November 2007	Antimicrobial Agents and Chemotherapy	Volume 51, Number 11, pgs 3830-3835
000167	Cutler, S.A.; Cornick, N.A.; Loneran, S.M.; Stahl, C.H.	Dietary Colicin E1 supplementation prevents experimentally induced post-weaning diarrhea but does not provide a growth promoting effect	2008	J. Ani. Sci.	Volume 86, Sup. 1, pg 157
000168 - 000175	Elkins, Patricia A.; Song, Ho Yeong; Cramer, William A.; Stauffacher, Cynthia V.	Crystallization and Characterization of Colicin E1 Channel-Forming Polypeptides	1994	Proteins: Structure, Function, and Genetics	Volume 19, pgs 150-157
000176 - 000184	Eraso, Jesus M.; Weinstock, George M.	Anaerobic Control of Colicin E1 Production	August 1992	Journal of Bacteriology	Volume 174, Number 15, pgs 5105-5109
000185 - 000192	Eraso, Jesus M.; Chidambaram, Monjula; Weinstock, George M.	Increased Production of Colicin E1 in Stationary Phase	April 1996	Journal of Bacteriology	Volume 178, Number 7, pgs 1928-1935

*NA- Not applicable*

<i>Pages</i>	<i>Author</i>	<i>Title</i>	<i>Publish Date</i>	<i>Publisher</i>	<i>BIB_Info</i>
000193 - 000198	Gordon, David M.; OBrien, Claire L.	Bacteriocin diversity and the frequency of multiple bacteriocin production in Escherichia coli	2006	Microbiology	Volume 152, pgs 3239-3244
000199 - 000200	Gratia, Andre; Frederico, Pierre	Diversite des Souches Antibiotiques de B. coli et Etendue Variable de Leur Champ D action	November 1945	Societe Belge de Biologie	Volume 140, pgs 1032-1033
000201 - 000212	James, Richard; Kleanthous, Colin; Moore, Geoffrey R.	The biology of E colicins: paradigms and paradoxes	1996	Microbiology	Volume 142, pgs 1569-1580
000213 - 000224	James, Richard; Kleanthous, Colin; Moore, Geoffrey R.	The biology of E colicins: paradigms and paradoxes	1996	Microbiology	Volume 142, pgs 1569-1580
000240 - 000245	Lazdunski, Claude J.	Pore-forming colicins: synthesis, extracellular release, mode of action, immunity	1988	Biochimie	Volume 70, pgs 1291-1296
000246 - 000250	Lorkiewicz, Zbigniew; Maciazek, Krystyna; Nackiewicz, Zdzislawa	The Influence of Acriflavine on Transfer of the Colicinogenic Factor	1964	Acta Microbiologica Polonica	Volume 13, pgs 273-281
000251 - 000257	Murinda, Shelton E.; Roberts, Robert F.; Wilson, Richard A.	Evaluation of Colicins for Inhibitory Activity against Diarrheagenic Escherichia coli Strains, Including Serotype O157:H7	September 1996	Applied and Environmental Microbiology	Volume 62, Number 9, pgs 3196-3202
000258 - 000264	Murinda, S.E.; Rashid, K.A.; Roberts, R.F.	In Vitro Assessment of the Cytotoxicity of Nisin, Pediocin, and Selected Colicins on Simian Virus 40-Transfected Human Colon and Vero Monkey Kidney Cells with Trypan Blue Staining Viability Assays	2003	Journal of Food Protection	Volume 66, Number 5, pgs 847-853
000265 - 000269	Obi, Samuel K.C.; Campbell, J.A.	Incidence of Colicinogenic Escherichia coli in Sheep, Goats and Cattle	1978	Zentrabl Veterinarmed B	Volume 25, Number 8, pgs 652-656
000270 - 000273	Patton, Brenda S.; Lonergan, Steven M.; Cutler, Sara A.; Stahl, Chad H.; Dickson, James S.	Application of Colicin E1 as a Prefabrication Intervention Strategy	2008	Journal of Food Protection	Volume 71, Number 12, pgs 2519-2522

*NA- Not applicable*

<i>Pages</i>	<i>Author</i>	<i>Title</i>	<i>Publish Date</i>	<i>Publisher</i>	<i>BIB_Info</i>
000274 - 000276	Pugsley, A.P.	The ins and outs of colicins. Part II. Lethal action, immunity and ecological implications	1984	Microbiological Sciences	Volume 1, Number 8, pgs 203-205
000277 - 000283	Pugsley, Anthony P.	Escherichia coli K12 Strains for Use in the Identification and Characterization of Colidns	1985	Journal of General Microbiology	Volume 131, pgs 369-376
000284 - 000291	Riley, Margaret A.; Gordon, David M.	A survey of Col plasmids in natural isolates of Escherichia coli and an investigation into the stability of Col-plasmid lineages	1992	Journal of General Microbiology	Volume 138, pgs 1345-1352
000292 - 000296	Riley, Margaret A.; Gordon, David M.	The ecological role of bacteriocins in bacterial competition	March 1999	Trends in Microbiology	Volume 7, Number 3, pgs 129-133
000297 - 000301	Riley, Margaret A.; Tan, Ying; Wang, Jinping	Nucleotide polymorphism in colicin E1 and Ia plasmids from natural isolates of Escherichia coli	November 1994	Proc. Natl. Acad. Sci. USA	Volume 91, pgs 11276-11280
000302 - 000308	Schamberger, Gerry P.; Diez-Gonzalez, Francisco	Selection of Recently Isolated Colicinogenic Escherichia coli Strains Inhibitory to Escherichia coli O157:H7	2002	Journal of Food Protection	Volume 65, Number 9, pgs 1381-1387
000309 - 000318	Schwartz, Stanley A.; Helinski, Donald R.	Purification and Characterization of Colicin E	October 25, 1971	The Journal of Biological Chemistry	Volume 246, Number 20, pgs 6318-6327
000319 - 000326	Smarda, Jan; Obdrzalek, Vlastimil	Incidence of colicinogenic strains among human Escherichia coli	2001	J. Basic Microbiol.	Volume 41, Number 6, pgs 367-374
000327 - 000334	Slatin, Stephen L.; Raymond, Lynn; Finkelstein, Alan	Gating of a Voltage-Dependent Channel (Colicin E1) in Planar Lipid Bilayers: The Role of Protein Translocation	1986	The Journal of Membrane Biology	Volume 92, pgs 247-254
000335 - 000340	Smarda, Jan; Smajs, David; Lhotova, Hana; Dedicova, Daniela	Occurrence of Strains Producing Specific Antibacterial Inhibitory Agents in Five Genera of Enterobacteriaceae	2007	Current Microbiology	Volume 54, pgs 113-118

*NA- Not applicable*

<i>Pages</i>	<i>Author</i>	<i>Title</i>	<i>Publish Date</i>	<i>Publisher</i>	<i>BIB_Info</i>
000341 - 000355	Smarda, J.	Incidence and Manifestations of Colicinogeny in Strains of Escherichia coli	1960	Journal of Hygiene, Epidemiology, Microbiology and Immunology	Volume 4, pgs 151-165
000356 - 000362	Spangler, Rudolph; Zhang, Shiping; Krueger, Judy; Zubay, Geoffrey	Colicin Synthesis and Cell Death	July 1985	Journal of Bacteriology	Volume 163, Number1, pgs 167-173
000363 - 000367	Steinhoff, Ulrich	Who controls the crowd? New findings and old questions about the intestinal microflora	2005	Immunology Letters	Volume 99, pgs 12-16
000368 - 000372	Todar, Kenneth	Pathogenic E. coli	2008	Online Textbook of Bacteriology	pgs 4-8
000373 - 000389	van den Eizen, Peter J.M.; Maat, Jan; Walters, Hanneke H.B.; Veltkamp, Eduard; Nijkamp, H. John J.	The nucleotide sequence of the bacteriocin promoters of plasmids Clo DF13 and Col E1: role of lexA repressor and cAMP in the regulation of promoter activity	1982	Nucleic Acids Research	Volume 10, Number 6. pgs 1913-1928
000390 - 000394	Yamada, Mamoru; Ebina, Yousuke; Miyata, Takashi; Nakazawa, Teruko; Nakazawa, Atsushi	Nucleotide sequence of the structural gene for colicin E1 and predicted structure of the protein	May 1982	Proc. Nati Acad. Sci. USA	Volume 79, pgs 2827-2831
000395 - 000406	Zhang, Yan-Liang; Cramer, William A.	Constraints imposed by protease accessibility on the trans-membrane and surface topography of the colicin E1 ion channel	1992	Protein Science	Volume 1, pgs 1666-1676

*NA- Not applicable*

6-9-09 Correspondence from Notifier



**West-Barnette, Shayla**

**From:** Charles D Miller [MILLER\_CHARLES\_D@LILLY.COM]  
**Sent:** Tuesday, June 09, 2009 4:30 PM  
**To:** West-Barnette, Shayla  
**Cc:** K. Smedley  
**Subject:** Re: GRN 289 Teleconference with OFAS and FSIS

Dr West-Barnette,

Thank you for both your telephone call and the attached e-mail regarding a teleconference for GRN 289 with OFAS and FSIS. Following discussions with our consultant, Dr. Kristi Smedley, we would like to schedule the call for Tuesday, June 16, 2009, from 1:00 pm to 1:45 pm, Eastern Time. We will be calling in from different sites and will need direction for calling into the conference, We look forward to the teleconference call next week.

Best regards,

**Charlie**

Charles D. Miller, DVM, Ph.D  
Representing Ivy Animal Health  
Ph 317-651-9948  
Cell 317-997-2913  
Fax 317- 277-4755

"West-Barnette, Shayla"  
<Shayla.WestBarnette@fda.hhs.gov>

06/09/2009 11:29 AM

To "Charles D Miller" <MILLER\_CHARLES\_D@LILLY.COM>  
cc "McMahon, Carrie" <Carrie.McMahon@fda.hhs.gov>, "Mosley, Sylvester"  
<Sylvester.Mosley@fda.hhs.gov>  
Subject GRN 289 Teleconference with OFAS and FSIS

Dr. Miller,

As we discussed earlier, OFAS has completed our evaluation of GRN 289 (subject:colicin E1). Since the notified substance is intended for use on meat, FSIS received a copy of the notice and has evaluated the suitability of colicin E1 on meats. Both OFAS and FSIS would like to speak with you regarding their comments on the notice, mainly regarding the suitability data that you provided. I have found that the following dates and times would work best for the OFAS staff:

- 1) Tuesday, June 16, 2009, from 11:00 am to 1:45 am, Eastern Time
- 2) Tuesday, June 16, 2009, from 1:00 pm to 1:45 pm, Eastern Time

3) Friday, June 19, 2009, from 11:00 am to 11:45 am, Eastern Time

4) Friday, June 19, 2009, from 1:00 pm to 1:45 pm, Eastern Time

I understand that you will need to check the availability of yourself and the appropriate staff on your end. At your earliest convenience, please let me know which of these dates and times would work best, or if none will work for you. Also, please let me know whether you will have staff calling in from other sites. Once I receive this information, I will confirm the date and time with you and FSIS, and send you information about calling into the conference. As always, feel free to let me know if you have any questions.

Regards,

Shayla West-Barnette, Ph.D.  
Consumer Safety Officer  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
(301) 436-1262 (office)  
Shayla.WestBarnette@fda.hhs.gov

6-19-09 Correspondence from Notifier

AM



**West-Barnette, Shayla**

---

**From:** Charles D Miller [MILLER\_CHARLES\_D@LILLY.COM]  
**Sent:** Friday, June 19, 2009 1:39 PM  
**To:** West-Barnette, Shayla  
**Subject:** RE: GRN 289 Withdrawal

Dr. West-Barnette

In response to your request that we officially respond to the Agencies recommendation to withdraw GRN 289 by the close of the week, I am emailing to indicate that we need a slight extension. We have scheduled a management teleconference call to discuss this further on Monday June 22, 2009. Therefore, I will plan to respond shortly after our discussion. Thanks for your understanding.

Regards,

**Charlie**

Charles D. Miller, DVM, Ph.D  
Ph 317-651-9948  
Cell 317-997-2913  
Fax 317- 277-4755

6-22-09 Correspondence from Notifier

AM



## West-Barnette, Shayla

**From:** Charles D Miller [MILLER\_CHARLES\_D@LILLY.COM]  
**Sent:** Monday, June 22, 2009 1:32 PM  
**To:** West-Barnette, Shayla  
**Cc:** Thomas W Campi  
**Subject:** RE: GRN 289 Withdrawal

Dr. West-Barnette:

Thank you for coordinating the teleconference with FSIS and Ivy representatives last week. We briefed the Ivy Management Team today, and they requested that we revisit the withdrawal request with the OFAS staff. Our understanding from the telephone conference, is that OFAS has no further safety questions, it is also our understanding that FSIS has reviewed our laboratory research and considers this work persuasive in determining the suitability for use on carcass and primal cuts of meat. Although FSIS has requested in-plant data to confirm the suitability assessment.

We would like to discuss with OFAS, whether it may be appropriate to request a final safety determination in the form of a letter of no objection from FDA at this time, while we continue our suitability assessment under FSIS authority. As was discussed in our teleconference, FSIS will not permit the in-plant suitability research without concurrence on safety from the FDA. We have reviewed the CFSAN responses to other GRAS notifications for substances to be used on meat and poultry. In these responses often language such as to following is used:

*During its evaluation of GRN 000171, FDA consulted with the Labeling and Consumer Protection Staff of the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA). Under the Federal Meat Inspection Act and the Poultry Products Inspection Act, FSIS is responsible for determining the efficacy and suitability of food ingredients in meat and poultry products as well as prescribing safe conditions of use. Suitability relates to the effectiveness of the ingredient in performing the intended purpose of use and the assurance that the conditions of use will not result in an adulterated product, or one that misleads consumers.*

*FSIS requested that FDA advise NPC to seek regulatory guidance from FSIS, Labeling and Consumer Protection Staff, about the use of the LAB mixture in meat and poultry products. NPC should direct such an inquiry to Dr. Robert Post, Director, Labeling and Consumer Protection Staff, Office of Policy, Program, and Employee Development, Food Safety and Inspection Service, 1400 Independence Ave., S.W., Suite 602, Annex, Washington, DC 20250-3700. The telephone number for that office is (202) 205-0279 and the telefax number is (202) 205-3625.*

Therefore, we are requesting a teleconference with the OFAS staff, to discuss this option. Also, during our teleconference call you indicated that you would be providing the review/issue information provided previously by FSIS, as well as the in-house safety review. Would you please provide this information, as it may help us have a full understanding of the issues.

Please let us know if this request for teleconference with the OFAS staff will be granted. Then I will check calendars for availability and dates for our participants and coordinate with you regarding a date and time.

Best regards,

**Charlie**

Charles D. Miller, DVM, Ph.D  
Ph 317-651-9948  
Cell 317-997-2913  
Fax 317- 277-4755

"West-Barnette, Shayla"  
<Shayla.WestBarnette@fda.hhs.gov>

To "Charles D Miller" <MILLER\_CHARLES\_D@LILLY.COM>

cc

Subject RE: GRN 289 Withdrawal

06/19/2009 01:41 PM

Thank you Dr. Miller. I look forward to hearing from you then, and please let me know if you have any questions.

Shayla

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**From:** Charles D Miller [mailto:MILLER\_CHARLES\_D@LILLY.COM]  
**Sent:** Friday, June 19, 2009 1:39 PM  
**To:** West-Barnette, Shayla  
**Subject:** RE: GRN 289 Withdrawal

Dr. West-Barnette

In response to your request that we officially respond to the Agencies recommendation to withdraw GRN 289 by the close of the week, I am emailing to indicate that we need a slight extension. We have scheduled a management teleconference call to discuss this further on Monday June 22, 2009. Therefore, I will plan to respond shortly after our discussion. Thanks for your understanding.

Regards,

**Charlie**

Charles D. Miller, DVM, Ph.D  
Ph 317-651-9948  
Cell 317-997-2913  
Fax 317- 277-4755

6-24-09 Correspondence from Notifier

AM 

**West-Barnette, Shayla**

**From:** Charles D Miller [MILLER\_CHARLES\_D@LILLY.COM]  
**Sent:** Wednesday, June 24, 2009 9:48 AM  
**To:** West-Barnette, Shayla  
**Cc:** Thomas W Campi; CFRSRV@aol.com  
**Subject:** RE: GRN 289 Withdrawal

Dr. West-Barnette-

Thank you for your emails and facilitating Dr. Zeitz's participation in today's teleconference. I was in an all day meeting yesterday and apologize for not sending this response sooner. We would like to have today's conference call to better understand the OFAS safety statement that FSIS indicated they will require before an efficacy or utility study is conducted in a commercial production facility. How is this related to a Letter of No Objection? We look forward to the teleconference call at 1:00 PM. As discussed, we will plan to utilize the call in numbers of 866-213-2145, access code 6519948.

Thanks again for your help.

**Charlie**

Charles D. Miller, DVM, Ph.D  
Ph 317-651-9948  
Cell 317-997-2913  
Fax 317- 277-4755

"West-Barnette, Shayla"  
<Shayla.WestBarnette@fda.hhs.gov>

To "Charles D Miller" <MILLER\_CHARLES\_D@LILLY.COM>

cc

Subject RE: GRN 289 Withdrawal

06/23/2009 02:28 PM

Dr. Miller,

in preparation for tomorrow's teleconference about GRN 289 (subject:colicin E1), I would like to clarify a few points, and also ask a question of you to ensure that OFAS is clear regarding the letter of no objection which you would like to discuss with us.

I stated during our teleconference on June 16, 2009 that the OFAS review team did not identify any safety concerns about the intended uses of colicin E1. I would like to clarify that OFAS has no questions at this time regarding the intended uses of colicin E1, and that our safety evaluation is ongoing. Our evaluation process also includes the review of information that OFAS requests from the notifier (i.e. the notifier's responses to the reviewers' comments). The OFAS evaluation is not considered complete until all of the safety information has been reviewed and OFAS has responded to

the notifier with a letter (response letter) that has been reviewed and signed by upper management. Given that the review team has recently reviewed GRN 289 and the reviewers' comments were transmitted to you today, the OFAS evaluation of GRN 289 is still in the preliminary phase.

In your email below dated June 22, 2009, you asked to speak with OFAS regarding a final safety determination in the form of a letter of no objection from OFAS while Ivy Animal Health continues their suitability assessment under the authority of FSIS. In this request, are you asking OFAS to provide a letter of no objection to the use of colicin E1 in the in-plant studies requested by FSIS, or are you asking FDA to provide a letter of no objection to GRN 289 (i.e., a response letter). Please keep in mind that the OFAS evaluation of GRN 289 is still underway, and that the OFAS review process generally takes 180 days to complete.

I look forward to receiving clarification from you regarding the letter of no objection which you would like to discuss with us during Wednesday's teleconference. Please contact me if you have any questions.

Regards,

Shayla

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**From:** Charles D Miller [mailto:MILLER\_CHARLES\_D@LILLY.COM]  
**Sent:** Monday, June 22, 2009 1:32 PM  
**To:** West-Barnette, Shayla  
**Cc:** Thomas W Campi  
**Subject:** RE: GRN 289 Withdrawal

Dr. West-Barnette:

Thank you for coordinating the teleconference with FSIS and Ivy representatives last week. We briefed the Ivy Management Team today, and they requested that we revisit the withdrawal request with the OFAS staff. Our understanding from the telephone conference, is that OFAS has no further safety questions, it is also our understanding that FSIS has reviewed our laboratory research and considers this work persuasive in determining the suitability for use on carcass and primal cuts of meat. Although FSIS has requested in-plant data to confirm the suitability assessment.

We would like to discuss with OFAS, whether it may be appropriate to request a final safety determination in the form of a letter of no objection from FDA at this time, while we continue our suitability assessment under FSIS authority. As was discussed in our teleconference, FSIS will not permit the in-plant suitability research without concurrence on safety from the FDA. We have reviewed the CFSAN responses to other GRAS notifications for substances to be used on meat and poultry. In these responses often language such as to following is used:

*During its evaluation of GRN 000171, FDA consulted with the Labeling and Consumer Protection Staff of the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA). Under the Federal Meat Inspection Act and the Poultry Products Inspection Act, FSIS is responsible for determining the efficacy and suitability of food ingredients in meat and poultry products as well as prescribing safe conditions of use. Suitability relates to the effectiveness of the ingredient in performing the intended purpose of use and the assurance that the conditions of use will not result in an adulterated product, or one that misleads consumers.*

*FSIS requested that FDA advise NPC to seek regulatory guidance from FSIS, Labeling and Consumer Protection Staff, about the use of the LAB mixture in meat and poultry products. NPC should direct such an inquiry to Dr. Robert Post, Director, Labeling and Consumer Protection Staff, Office of Policy, Program, and Employee Development, Food Safety and Inspection Service, 1400 Independence Ave., S.W., Suite 602, Annex, Washington, DC 20250-3700. The telephone number for that office is (202) 205-0279 and the telefax number is (202) 205-3625.*

Therefore, we are requesting a teleconference with the OFAS staff, to discuss this option. Also, during our teleconference call you indicated that you would be providing the review/issue information provided previously by FSIS, as well as the in-house safety review. Would you please provide this information, as it may help us have a full understanding of the issues.

Please let us know if this request for teleconference with the OFAS staff will be granted. Then I will check calendars for availability and dates for our participants and coordinate with you regarding a date and time.

Best regards,

**Charlie**

Charles D. Miller, DVM, Ph.D  
Ph 317-651-9948  
Cell 317-997-2913  
Fax 317- 277-4755

"West-Barnette, Shayla" <Shayla.WestBarnette@fda.hhs.gov>

06/19/2009 01:41 PM

To "Charles D Miller" <MILLER\_CHARLES\_D@LILLY.COM>  
cc  
Subject RE: GRN 289 Withdrawal

Thank you Dr. Miller. I look forward to hearing from you then, and please let me know if you have any questions.

Shayla

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**From:** Charles D Miller [mailto:MILLER\_CHARLES\_D@LILLY.COM]  
**Sent:** Friday, June 19, 2009 1:39 PM  
**To:** West-Barnette, Shayla  
**Subject:** RE: GRN 289 Withdrawal

Dr. West-Barnette

In response to your request that we officially respond to the Agencies recommendation to withdraw GRN 289 by the close of the week, I am emailing to indicate that we need a slight extension. We have scheduled a management teleconference call to discuss this further on Monday June 22, 2009. Therefore, I will plan to respond shortly after our discussion. Thanks for your understanding.

Regards,

**Charlie**

Charles D. Miller, DVM, Ph.D  
Ph 317-651-9948  
Cell 317-997-2913

Fax 317-277-4755



**West-Barnette, Shayla**

**From:** Charles D Miller [MILLER\_CHARLES\_D@LILLY.COM]  
**Sent:** Wednesday, June 24, 2009 3:06 PM  
**To:** West-Barnette, Shayla  
**Cc:** Thomas W Campi  
**Subject:** RE: GRN 289

Dr West-Barnette,

Thanks you again for your coordination to arrange todays conference call. We have a greater understanding of the safety requirements and review process leading a GRAS Notification as a result of the call. I will need to consult with my management but likely will send to you via email our response to the recommendation to withdraw GRN 289 by Friday, June 26 2009. Thank you in advance for agreeing to provide summary comments of todays conference call. In addition, would you also please provide summary comments of the June 16, 2009 conference call that would also include the FSIS review comments.

Best regards

**Charlie**

Charles D. Miller, DVM, Ph.D  
Ph 317-651-9948  
Cell 317-997-2913  
Fax 317- 277-4755



**West-Barnette, Shayla**

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**From:** Charles D Miller [MILLER\_CHARLES\_D@LILLY.COM]  
**Sent:** Thursday, June 25, 2009 5:17 PM  
**To:** West-Barnette, Shayla  
**Subject:** RE: GRAS Notice No. GRN 289 Withdrawal  
**Attachments:** GRN 289 withdrawal.doc

Dr West-Barnette,

Please find attached the formal Ivy Animal Health withdrawal of GRAS Notice No. GRN 289. Thank you for all your help these past months. We look forward completing our utility (efficacy) studies and re-submitting our notice in the future. Thank you for your earlier email today indicating you will provide memoranda from the teleconferences on June 16, 2009, and June 26, 2009 as soon as they are complete.

Best regards

**Charlie**

Charles D. Miller, DVM, Ph.D  
Representing Ivy Animal Health  
Ph 317-651-9948  
Cell 317-997-2913  
Fax 317- 277-4755

6-25-09 Correspondence from Notifier Attachment

DATE: June 25, 2009

Dr. Shayla West-Barnette  
FDA/CFSAN, HFS-255  
Office of Food Additive Safety  
5100 Paint Branch Parkway  
College Park, MD 20740

ATTENTION: Dr. West-Barnette:

SUBJECT: GRN 000289 -- **Withdrawal**  
Colicin E-1, Ivy Animal Health

Based on our telephone conversation of July 16, 2009, we are requesting that GRAS Notice No. GRN 289 be withdrawn without prejudice to resubmission.

Sincerely,

Charles Miller, DVM, PhD  
Representing Ivy Animal Health