

**A GRAIN OF CAUTION:
A CRITICAL ASSESSMENT OF
PHARMACEUTICAL RICE**



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EXECUTIVE SUMMARY¹

Since 1997, California-based Ventria Bioscience has conducted outdoor field tests of rice genetically engineered with modified human genes to produce artificial versions of human milk proteins that have antimicrobial and other drug-like properties. Ventria hopes to market the rice-extracted pharmaceutical proteins for use in oral rehydration solutions to treat diarrhea, and as nutritional supplements in yogurt, granola bars, performance drinks and other products.

Ventria was blocked from growing its rice in California (2004) and Missouri (2005) by farmers and food companies concerned about contamination of their products with the company’s pharmaceutical proteins, which have *not* been approved by the U.S. Food and Drug Administration (FDA), as shown in

Table 1. Ventria recently sponsored an experiment in Peru in which its rice-grown pharmaceutical proteins were fed to Peruvian infants suffering from severe diarrhea.² Reports that two infants who participated in the experiment developed allergies, and that some parents did not give informed consent to the experiment,³ led to an official government inquiry.⁴ Ventria is now seeking permission from the U.S. Dept. of Agriculture (USDA) to grow up to 3,200 acres of its pharmaceutical rice in Kansas, which would be the world’s largest planting of a genetically engineered (GE) pharmaceutical crop to date.

So-called “pharma crops” such as Ventria’s rice represent an experimental and highly controversial application of biotechnology. Concerns that unapproved drugs

Table 1: Ventria’s Failed Attempts to Obtain FDA “Generally Recognized as Safe” (GRAS) Approval of its Rice-Derived Pharmaceutical Proteins

DATE OF PETITION	COMPOUND	INTENDED USE	STATUS AT FDA	COMMENTS
Nov. 2003	Lactoferrin (Lf) rice	None. Lf rice as contaminant; Lf rice residues after Lf extraction for human food & animal feed	BNF 082; no action ⁵ (see Appendix 1)	Ventria sought approval of Lf rice as contaminant while publicly claiming Lf rice would not contaminate food
Dec. 2004	Lactoferrin	Ingredient in foods, beverages, medical foods	GRN. #162; withdrawn on Nov. 20, 2006 ⁶	Ventria withdrew this petition because FDA indicated it would not approve lactoferrin as safe
June 2005	Lysozyme	Antimicrobial agent; ingredient in various foods	GRN #174; withdrawn ⁷	Ventria withdrew this petition in Sept. 2005 for unknown reasons
Jan. 2006	Lysozyme	Ingredient in infant formulas & pediatric oral rehydration solutions	GRN #191; no action ⁸	A 2004 National Academy of Sciences panel recommends more stringent testing of new ingredients in infant formulas

could enter the food supply were confirmed in 2002, when 500,000 bushels of soybeans contaminated with pharma corn in Nebraska had to be seized and destroyed. Unapproved genetically engineered (GE) crops have contaminated the food supply numerous times before and since. In the past year, unapproved GE rice twice contaminated commercial rice supplies, causing export market rejection and substantial loss of income to rice farmers. USDA has been heavily criticized for its failure to properly regulate or even keep track of GE and pharma crop field tests, including a scathing report from its own Inspector General in 2005. Finally, Ventria's Vice President Delia Bethell admitted that its pharma rice could enter the commercial rice supply in a 2003 petition to the FDA (see Appendix 1).

In this report, we assess the potential adverse consequences and regulatory status of Ventria's pharmaceutical proteins; critically examine the Ventria-sponsored Peruvian experiment; and briefly review the status of diarrheal morbidity, treatment and prevention. Finally, we compare Ventria's strategy for addressing infant diarrhea to others on the basis of safety, efficacy and cost-effectiveness.

POTENTIAL HUMAN HEALTH IMPACTS

Two of the pharmaceutical proteins in Ventria's rice are genetically engineered versions of the human milk proteins lactoferrin and lysozyme. According to infant nutrition experts, "...the commercial production of milk proteins using recombinant technologies [i.e. genetic engineering] may produce unintended and unexpected side effects," such as allergic reactions.⁹ In addition, infants are more vulnerable than adults to adverse nutritional and environmental influences, which can disrupt development of rapidly growing organs and systems. Adverse events in infancy can have long-term effects that are not immediately detectable, but can be irreversible. Infants and young children are 3-4 times more likely to have food allergies than adults,¹⁰ and sick infants are even more vulnerable than healthy ones. Specific potential effects that require more rigorous evaluation include:

Aggravated Infections: While human lactoferrin has antimicrobial properties, it paradoxically poses the potential hazard of exacerbating infections by certain pathogens capable of using it as a source of needed iron. Such pathogens include bacteria that cause gonorrhea and meningitis, as well as the *H. pylori* bacteria implicated in causing ulcers and certain forms of stomach cancer. According to Dr. Eugene Weinberg of Indiana University, human lactoferrin "might not be a successful therapeutic agent for *H. pylori* and, indeed, could intensify the infection."¹¹

Allergenicity: Ventria's rice-expressed lysozyme and lactoferrin have two characteristics of proteins that cause food allergies: resistance to digestion and to breakdown by heat. Its lactoferrin has two further allergenic characteristics, structural similarity to known food allergens (lactoferrin from cows and chick ovotransferrin) and plant-type glycosylation. These allergenic characteristics may explain why noted food allergist Steve Taylor stated that the FDA would not approve rice-grown lactoferrin."¹²

Autoimmune Disorders: Pharmaceutical proteins generated by inserting human genes into plants, bacteria or other mammals are usually different than their natural human counterparts. These differences may cause the body to perceive them as foreign, resulting in potentially dangerous immune system responses. Anti-lactoferrin antibodies are correlated with markers of disease activity in rheumatoid arthritis and systemic lupus erythematosus.¹³ Careful study is required to determine whether rice-expressed lactoferrin or lysozyme could cause such potentially dangerous reactions.

FDA REFUSES TO APPROVE VENTRIA'S PHARMACEUTICAL PROTEINS

Ventria has thus far evaded FDA's new drug review process by requesting the FDA to grant its proteins "generally recognized as safe" (GRAS) status as food additives (see Table 1). The FDA has properly taken no action on these requests. Significantly, Ventria's GRAS petition for lactoferrin languished at the FDA for two years before Ventria withdrew it in November 2006. FDA's letter announcing the withdrawal of the petition alludes to "complex scientific issues" regard-

ing Ventria's lactoferrin, suggesting that the agency has unanswered safety questions.¹⁴ Another company's recombinant human lactoferrin is currently undergoing FDA's new drug review process as a potent anti-cancer drug,¹⁵ underscoring the need for stringent review of Ventria's recombinant proteins as drugs.

VENTRIA'S PHARMACEUTICAL PROTEINS MAY BE USED IN UNREGULATED "MEDICAL FOODS"

Ventria intends to market its pharmaceutical proteins as additives to oral rehydration solution (ORS) to treat diarrhea. ORS falls into an unregulated category known as "medical foods" that have been associated with adverse health impacts, including deaths, as well as fraudulent health claims. Ventria must not be permitted to exploit this loophole in FDA regulation.

QUESTIONABLE EXPERIMENTATION ON PERUVIAN INFANTS

In 2002, a Ventria collaborator stated that Ventria's rice-derived lactoferrin (rhLf) would have to be tested on rats and infant rhesus monkeys before any human testing.¹⁶ However, a search on the comprehensive medical database PubMed reveals no such published studies of rice-derived rhLf on rats or monkeys.¹⁷ It is unclear whether such research has been conducted but remains unpublished, was published in some obscure journal, or whether Ventria and its collaborators chose to forego animal experiments and proceed directly to the trial on Peruvian infants.

At least one mother whose infant was enrolled in the Peruvian experiment and subsequently developed allergies reports that she was "deceived" in that she was not informed that the treatment was experimental and involved compounds from transgenic rice. The researchers observed the infants in the experiment for only 14 days,¹⁸ far too short a period to detect any adverse consequences of Ventria's compounds.

The authors of a paper on the Ventria-sponsored Peruvian experiment violate basic scientific protocol by not reporting the results for each of the three groups

tested. By improperly combining the results for two distinct treatment groups,¹⁹ the authors may have exaggerated the benefit (if any) of Ventria's compounds in treating diarrhea.

VENTRIA'S RICE A DIVERSION FROM PROVEN, COST-EFFECTIVE WAYS TO TREAT DIARRHEA

Diarrheal mortality in infants and young children has been reduced from about 4.6 million deaths in 1980 to 1.5 to 2.5 million deaths a year today, one of the greatest medical achievements of the 20th century.²⁰ This reduction in diarrheal deaths is due to a number of effective prevention measures—including improved sanitation facilities and drinking water supplies, improved hygienic practices, use of disinfectants, and more optimal breastfeeding²¹—as well as widespread introduction of oral rehydration therapy²²

Ventria's CEO Scott Deeter admits that foundation support would be necessary to make oral rehydration solutions (ORS) containing his company's proteins—which would likely be more expensive than existing ORS formulations—widely available.²³ Even if Ventria's proteins eventually do prove safe after proper testing, governmental or private foundation aid would be more cost-effectively spent on the proven measures mentioned above, which are not adequately funded.

In addition, Ventria intends to market its proteins as nutritional supplements in performance (sports) drinks, granola bars, yoghurt and similar products for wealthy consumers in developed countries.²⁴ This raises the question of whether premature experimentation on Peruvian children has been conducted primarily to obtain market approval for these more profitable applications.

CONCLUSION

Genetically engineered, pharmaceutical rice is not the answer to diarrhea. The bioactive proteins in Ventria's pharma rice may exacerbate infections or cause allergies or autoimmune disorders. They have apparently not been adequately tested on animals. A Ventria-

sponsored experiment on sick Peruvian infants was marked by lack of informed consent, incomplete presentation of data, and reports of adverse reactions in several study participants.

Ventria has failed to obtain FDA approval of its pharma rice or pharmaceutical proteins, despite four petitions to the FDA since 2003. Meanwhile, USDA is poised to allow Ventria to grow thousands of acres of pharmaceutical rice in Kansas, despite ample evidence of unapproved genetically engineered crops entering the food supply and extremely deficient USDA regulation.

Ventria's officers and promoters have likened the company's rice to the "Holy Grail,"²⁵ reminiscent of many past promises that pharma crops would deliver "miracle cures." Yet despite outdoor field tests dating back to 1991, not a single pharma crop-produced drug has received FDA approval or saved a single life.

Ventria's rice-grown drugs are not only unproven, they are not needed. Effective and inexpensive measures to prevent and treat diarrhea have already saved millions of lives, and could save millions more with adequate funding.

RECOMMENDATIONS

1. Reject field test permit

The U.S. Dept. of Agriculture is urged to reject all permit applications for cultivation of pharmaceutical-producing food crops, including Ventria's application for cultivation of pharmaceutical rice in Kansas and other states.

2. Reject GRAS additive review

The U.S. Food and Drug Administration is urged to reject applications to approve Ventria's rice-expressed, recombinant human milk and blood proteins in the context of the GRAS ("generally recognized as safe") food additive review process.

3. Moratorium on further human experimentation

Ventria and its collaborators are urged to refrain from further human experimentation with Ventria's recombinant human lactoferrin or lysozyme except in the context of the U.S. Food and Drug Administration's new drug review process.

4. Bar use of pharma rice compounds in "medical foods"

In view of the drug-like properties and potential hazards of Ventria's recombinant proteins, the FDA is urged to bar use of Ventria's recombinant human lactoferrin or lysozyme in unregulated products marketed as "medical foods."

5. Fund inexpensive and cost-effective to address diarrheal disease

Private and governmental public health funders are urged to invest in provision of safe water, sanitation facilities and other proven, cost-effective measures to reduce diarrheal morbidity.

ENDNOTES

- ¹ This executive summary contains citations mainly for quotations. See the body of the report for full references.
- ² Zavaleta, N. et al (2007). "Efficacy of rice-based oral rehydration solution containing recombinant human lactoferrin and lysozyme in Peruvian children with acute diarrhea," *Journal of Pediatric Gastroenterology and Nutrition* 44(2), January 2007, pp. 258-264.
- ³ Diaz, D. (2006). "Transgénicos: Niños ya sufren sus efectos," La Republica (Peru), July 14, 2006. http://archivo.larepublica.com.pe/index.php?option=com_content&task=view&id=116503&Itemid=38&fecha_edicion=2006-07-14.
- ⁴ Leighton, P. (2006). "Study on infants in Peru sparks ethics inquiry," Science and Development Network, July 18, 2006. <http://www.scidev.net/news/index.cfm?fuseaction=readnews&itemid=2992&language=1>.
- ⁵ See <http://www.cfsan.fda.gov/~lrd/biocon.html>. Note that BNF 82, the petition number for Ventria's lactoferrin-producing rice, is not present under FDA's "List of Completed Consultations on Bioengineered Foods." See also Appendix 1.
- ⁶ See <http://www.cfsan.fda.gov/~rdb/opa-g162.html>.
- ⁷ See <http://www.cfsan.fda.gov/~rdb/opa-g174.html>
- ⁸ See GRN No. 191 at <http://www.cfsan.fda.gov/~rdb/opa-gn06.html>
- ⁹ NAS (2004). "Infant Formula: Evaluating the Safety of New Ingredients," Committee on the Evaluation of the Addition of Ingredients New to Infant Formula, National Academy of Sciences, 2004, p. 120. <http://www.nap.edu/catalog/10935.html>
- ¹⁰ EPA SAP (2000a). "A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding: Assessment of Scientific Information Concerning StarLink Corn," FIFRA Scientific Advisory Panel, SAP Report No. 2000-06, December 1, 2000. <http://www.epa.gov/scipoly/sap/meetings/2000/november/one.pdf>
- ¹¹ Weinberg, E.D. (2001). "Human lactoferrin: a novel therapeutic with broad spectrum potential," *J. Pharmacy & Pharmacology* 53(10), pp. 1303-10. <http://munstermom.tripod.com/HumanLactoferrin2001.htm>
- ¹² As quoted in: Pearson, H. (2002). "Milk in rice could curdle," *Nature*, April 26, 2002. www.nature.com/nsu/020422/020422-19.html
- ¹³ Chikazawa, H. et al (2000). "Immunoglobulin isotypes of anti-myeloperoxidase and anti-lactoferrin antibodies in patients with collagen diseases," *J. Clin. Immunology* 20(4), pp. 279-86.
- ¹⁴ See <http://www.cfsan.fda.gov/~rdb/opa-g162.html>.
- ¹⁵ Agennix, based in Houston, Texas. See end of Section 5.2.
- ¹⁶ Lönnerdal, Bo (2002). "Expression of Human Milk Proteins in Plant," *Journal of the American College of Nutrition*, Vol. 21, No. 3, p. 218S-221S.
- ¹⁷ PubMed is the premier, comprehensive database of medical and biological science articles run by the U.S. National Institutes of Health and National Library of Medicine; it covers over 16 million biomedical and life science articles in 33,000 scientific and medical journals. Searches conducted on April 17, 2007 on the keywords "lactoferrin" AND "rice" yielded 13 hits; on "lysozyme" AND "rice" 25 hits. None of these studies involved feeding trials on rats or monkeys with rice-expressed rhLf or rhLys. One study involved a feeding trial with rhLys and rhLf on chicks designed to measure performance, not safety.
- ¹⁸ Bethell (2006), op. cit.
- ¹⁹ Zavaleta et al (2007), op. cit. The authors combine results for group 1 (glucose-based ORS) and group 2 (rice-based ORS without Ventria's compounds). Group 3 received rice-based ORS with Ventria's compounds added. Numerous studies show that rice-based ORS without Ventria's compounds is superior to glucose-based ORS in treatment of diarrhea. Given the widely-acknowledged superiority of rice-based ORS, the authors should have reported the results for the first two groups separately to show whether the addition of Ventria's compounds produced any benefit beyond rice-based ORS without them (group 2).
- ²⁰ Victora et al (2000). "Reducing deaths from diarrhoea through oral rehydration therapy," *Bulletin of the World Health Organization*, 2000, 78(10). [http://whqlibdoc.who.int/bulletin/2000/Number%2010/78\(10\)1246-1255.pdf](http://whqlibdoc.who.int/bulletin/2000/Number%2010/78(10)1246-1255.pdf); CDC (2003). "Managing acute gastroenteritis among children: oral rehydration, maintenance, and nutritional therapy," U.S. Centers for Disease Control, 2003. www.cdc.gov/mmwr/preview/mmwrhtml/rr5216a1.htm
- ²¹ Setty, V. (2006). "Better Breastfeeding. Healthier Lives," *Population Reports*, Series L, No. 14. Baltimore, Johns Hopkins Bloomberg School of Public Health, The INFO Project, March 2006. www.populationreports.org/114/.
- ²² Victora et al (2000), op. cit.
- ²³ USDA Advisory Committee on Biotechnology and 21st Century Agriculture (AC21), presentation of Ventria CEO Scott Deeter, transcript of 6/17/00 meeting, at http://www.usda.gov/agencies/biotech/ac21/meetings/mtg_june03/jun17AC21v1.txt
- ²⁴ USDA/APHIS Environmental Assessment, in response to permit application 04-309-01r from Ventria Bioscience for field-testing of rice genetically engineered to express human lysozyme, USDA APHIS BRS, p. 22. http://www.aphis.usda.gov/brs/aphisdocs/04_30901r_ea.pdf. See corresponding citation for lactoferrin at http://www.aphis.usda.gov/brs/aphisdocs/04_30201r_ea.pdf
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A GRAIN OF CAUTION: A CRITICAL ASSESSMENT OF PHARMACEUTICAL RICE

1. INTRODUCTION

Since 1997, California-based Ventria Bioscience or its predecessor Applied Phytologics has been conducting outdoor field trials of rice genetically engineered with modified human genes to produce experimental pharmaceuticals in several states. These pharmaceutical compounds include artificial versions of the human milk proteins lactoferrin and lysozyme. Once the rice is grown and harvested, the experimental pharmaceuticals are extracted from the grains of rice. Thus, the genetically engineered (GE) rice is regarded as a “bio-factory” for pharmaceutical production. Ventria has proposed a number of uses for its GE rice-grown pharmaceuticals. These include use in oral rehydration solutions to treat diarrhea, and as nutritional supplements in yogurt, performance drinks, granola bars and similar products. In 2004 and 2005, Ventria sponsored a clinical trial in which its rice-produced pharmaceuticals were administered to Peruvian infants suffering from diarrhea (Diaz 2006; Vecchio 2006).

So-called “pharma crops” such as Ventria’s rice represent an experimental, unproven and highly controversial application of biotechnology. There have been hundreds of outdoor field tests of crops engineered to produce experimental drugs since the first took place in 1991, but neither Ventria’s pharmaceutical proteins nor any other pharma crop-produced drug has been approved by the U.S. Food and Drug Administration (FDA). Ventria does not have any pharmaceutical products on the market.²⁶

Farmers, food companies and scientists have voiced strong concerns about the potential for pharma crops to contaminate food-grade crops and food products derived from them (Freese & Caplan 2006). In 2002, pharma corn was found contaminating 500,000 bushels of soybeans stored in an elevator in Nebraska, resulting in seizure and destruction of the contaminated soybeans, one step away from the food chain (Toner 2002). Opposition from rice growers blocked Ventria’s bid to grow 120 acres of its rice in California in 2004 (Silber 2004). Ventria’s attempt to grow over 200 acres of pharma rice in Missouri in 2005 was stopped by the states’s rice farmers and beer giant Anheuser-Busch, the nation’s largest rice purchaser (Bennett 2005). Ventria conducted field trials of its pharma rice in North Carolina in 2005 and 2006 over the objections of rice breeders at a government rice-breeding facility less than one mile away (UCS 2006a). Now, the company is seeking approval from the U.S. Department of Agriculture for cultivation of up to 3,200 acres of its pharma rice in Kansas this year (Weiss 2007).

Experimental genetically engineered (GE) crops have contaminated commercial food supplies on numerous occasions (CFS 2006). Commercial long-grain rice supplies have been found contaminated with two unapproved GE rice varieties over the past year, leading to export market rejection, substantial losses to rice farmers, and a scarcity of certified seed for planting this spring (Weiss 2006; Bennett 2007). These episodes have increased concerns that the U.S. Dept. of

²⁶ Ventria may sell small amounts of rice-produced lysozyme and lactoferrin *for research use only* (not medical use) through the chemical supply house Sigma-Aldrich (search on product numbers L1667 and L4040 at <http://www.sigmaaldrich.com>). It must be emphasized that none of Ventria’s products have undergone FDA’s stringent new drug review process; they cannot be sold or used as pharmaceuticals. For more, see Section 5.2 and Freese et al (2004), pp. 15-16.

Agriculture (USDA) is unable or unwilling to prevent experimental GE crops, including pharma crops, from entering the food supply. Criticism of USDA’s poor performance at regulating field trials of experimental GE crops has come from the National Academy of Sciences (NAS 2002), the Union of Concerned Scientists (UCS 2006), the USDA’s Inspector General (USDA IG 2005), and the food industry (Freese & Caplan 2006).

In light of this poor regulation and the inherent potential for unapproved drugs to enter the food supply, the Center for Food Safety supports a ban on the use of food crops for production of plant-made pharmaceuticals (PMPs), including Ventria’s pharmaceutical rice, a position shared by the Union of Concerned Scientists (UCS 2006b) and other groups.

Some have promised that pharma crops will bring “miracle cures” (Olson 1999). Ventria’s officers have claimed that the company’s rice will save children suffering from severe diarrhea (Bethell 2006); one biotech industry publicist even refers to it as “the Holy Grail in a grain of rice” (Miller 2006). Given the emotive rhetoric surrounding pharma crops, it is important to subject claims of “miracle cures” and the like to close scrutiny.

In this report, we assess the potential adverse consequences and regulatory status of Ventria’s recombinant proteins; critically examine the Ventria-sponsored Peruvian experiment and the claims made on its basis; and briefly review the status of diarrheal morbidity, treatment and prevention. Finally, we compare Ventria’s strategy for addressing infant diarrhea to others on the basis of safety, efficacy and cost-effectiveness. First, however, we present some basic facts on diarrhea.

2. OVERVIEW OF DIARRHEAL DISEASE

Diarrhea is the second-leading cause of mortality in children under 5 years of age, accounting for an estimated 1.5 to 2.5 million deaths each year, most attrib-

“In addition, we recognize the possibility of the inadvertent introduction of LFI64 [lactoferrin-producing rice] at low, adventitious levels into commercial rice varieties.”

Delia R. Bethell, Vice President, Ventria Bioscience, in letter to FDA’s Robert Martin, 11/24/03. See Appendix 1.

utable to severe dehydration (CDC 2003, Victora et al 2000). The overwhelming majority of these deaths occur in developing countries. For perspective, roughly 300 U.S. children die from diarrhea each year (CDC 2003). A baby borne in sub-Saharan Africa has over 500 times the chance of dying from diarrhea as a baby borne in Europe or the U.S. (WHO-UNICEF 2005, p. 12).

Most cases of diarrhea are due to gastrointestinal illnesses induced by a wide range of disease agents (viruses, bacteria and parasites). These mostly water- and feces-borne pathogens thrive under conditions of inadequate and unsafe water, poor sanitation, and unsafe hygienic practices—the major causes of diarrheal morbidity in children (UNICEF 2005).

Diarrheal deaths in infants and children under five years of age (the majority under one year of age) have declined by two- to three-fold since 1980, when diarrhea is estimated to have claimed 4.6 million young lives (Victora et al 2000). Factors responsible for this remarkable achievement include improved sanitation and drinking water supplies, better hygienic practices, a general increase in breast-feeding, and the widespread introduction of oral rehydration solutions. We assess these factors in more detail in Section 7.

3. INFANCY AS A PERIOD OF INCREASED VULNERABILITY

Infancy is a period of heightened vulnerability to illnesses, nutritional inputs, toxins and other environmental influences. Such influences can disrupt the development of rapidly growing organs and systems whose proper functioning is essential to the health and well-being of the future adult. Thus, adverse events in infancy can have effects that are not immediately detectable, but can be irreversible or amenable to only limited compensation in later life. Infants cannot communicate their experiences verbally, making it difficult to interpret or sometimes even detect any harms they

experience. For these and other reasons, any new substances proposed for administration to infants should undergo extremely rigorous testing that takes account of the infant's special vulnerabilities.

In this paper, we are considering new ingredients in oral rehydration formulas meant for occasional rather than the continuous use typical of infant formulas. However, the reduced exposure to new ingredients in oral rehydration solutions (ORS) versus infant formula must be weighed against the increased vulnerability of the infant due to the diarrhea-producing gastrointestinal infection. One such heightened vulnerability is the increased permeability of the sick infant's gut, which can raise the infant's risk of acquiring food allergies (NAS 2004, p. 37). In addition, it should be noted that Ventria is also seeking FDA approval to introduce at least one of its two lead recombinant rice proteins into infant formulas (see Table 1).

4. POTENTIAL ADVERSE IMPACTS OF RECOMBINANT HUMAN LACTOFERRIN AND LYSOZYME ON INFANT HEALTH

Besides these general concerns related to infants' special vulnerabilities, there is also abundant evidence to support a rigorous risk assessment specifically for Ventria's recombinant human lactoferrin and lysozyme (henceforth abbreviated as rhLf and rhLys, respectively).

First of all, lactoferrin and lysozyme must be counted among those human milk proteins that experts describe as having "drug-like effects" (NAS 2004, p. 60). Lysozyme is an enzyme that kills bacteria by breaking down their tough protective skin, causing them to burst under their own internal osmotic pres-

The World Health Organization (WHO) and UNICEF stress the importance of preventative measures in the fight against disease and death from diarrhea. The 2005 WHO/UNICEF report "Water for Life: Making it Happen" provides estimates of the efficacy of various preventative measures based on a comprehensive review of the available literature:

Improved water supply reduces diarrheal morbidity by 25% (if severe outcomes as from cholera are included)

Improved sanitation reduces diarrhea morbidity by 32%

Hygiene interventions such as hygiene education and promotion of handwashing reduce diarrheal disease by 45%

Improvements in drinking water quality (e.g. chlorination, adequate storage) reduce diarrheal disease by 39%

See WHO-UNICEF (2005), p. 13 and Section 7.

sure (Cummins 2004), and is classified as "bactericidal" (NAS 2004, p. 48). Extensive research on lactoferrin, however, suggests that it has numerous other effects, most of which are incompletely understood. These include the capacity to bind iron; antimicrobial, antifungal, antiviral and anti-inflammatory activity; toxin-binding properties; promotion of growth in some animal cells; platelet-binding activity; modulation of the immune system; wound-healing properties; and anti-cancer activity (OSU Bibliography). The NAS committee classifies it on the basis of its best-known antimicrobial properties as an "anti-infective" (NAS 2004, p. 48), though it is important to keep the less well-studied effects in mind.

While these drug-like properties evince promise for therapeutic application of lysozyme and lactoferrin, they also urge caution. Premature application of drug-like proteins, before their full repertoire of effects is understood, is no more advisable than administration of

any promising drug prior to a rigorous assessment process. Lactoferrin's multitudinous and still poorly understood properties create the potential for unintended and potentially hazardous effects; this hazard is exacerbated when researchers narrow their sights to focus on the one or two effects on human physiology that are relevant to their intended application.

Another concern with Ventria's proteins is their source in genetically engineered, or transgenic, rice. According to the NAS committee cited above: "The presence of the substance [i.e. new ingredient in infant formula] in human milk may not be a sufficient definition of a nutritional substance, and other factors, **such as whether the substance is produced from genetically modified sources**, need to be considered" (emphasis added, NAS 2004, p. 39). The committee

goes on to state that “the commercial production of milk proteins using recombinant technologies may produce unintended and unexpected side effects” such as increased allergenicity due to differences in structure vis-à-vis the native (i.e. natural human) proteins (NAS 2004, p. 120). The potential for such hazardous immune system responses is discussed further below (based in part on Freese et al 2004).

4.1 Rice-Derived, Recombinant Human Lactoferrin and Lysozyme Must be Treated as Novel Proteins

In public, Ventria and its supporters often refer to its recombinant proteins as if they were identical to the human milk proteins lactoferrin and lysozyme.²⁷ Such a characterization is incorrect. In general, recombinant proteins may differ from their native counterparts in two major ways:

- 1) Amino acid sequence, as encoded by the transgene; and
- 2) Post-translational processing, such as glycosylation, which is a function of the host organism.

Changes of either sort can alter the conformation (i.e. three-dimensional shape) of the protein, its stability, and its capacity to cause harm to human health, particularly with respect to the immune system.

As detailed below, several studies have confirmed that Ventria’s rhLf and natural human lactoferrin differ in their post-translational processing. In addition, Ventria has apparently *not* compared the full-length amino acid sequence of rhLf or rhLys to their natural human counterparts, so they may differ in this respect as well. ***For these reasons, rhLf and rhLys should undergo rigorous assessment as novel proteins to which humans have never been exposed, and must not be treated as if they were “natural human milk proteins.”***

“...the committee describes the period of infancy as so special and the consequences of any harm so great that the public should be unwilling to tolerate any harm due to the addition of an ingredient new to infant formulas, even if there are benefits to most who would be exposed to the new ingredient.”

National Academy of Sciences (2004), “Infant Formula: Evaluating the Safety of New Ingredients,” p. 36. See NAS (2004) in Bibliography.

4.2 Immune System Disorders

The mammalian immune system serves to protect the body from micro-organisms, viruses, and substances recognized as foreign and potentially harmful to the body. The immune system works by recognizing and responding to large molecules (usually proteins) called antigens. Any substance or organism that contains such antigens is recognized and attacked by the immune system. Proteins that the body recognizes as “self” (e.g. insulin) are normally not attacked. Immune system disorders occur when the immune response is excessive, inappropriate, or lacking. Allergies occur when the immune system overreacts to a

substance that, in the majority of people, the body perceives as harmless (such as a food protein). Autoimmune disorders occur when the immune system responds to certain of the body’s own proteins as if they were antigens, thus destroying or damaging normal body tissue. The studies discussed below raise questions about the potential for recombinant, rice-derived lactoferrin and lysozyme to lead to an allergic response and/or autoimmune disorder.

4.2.1 Allergenicity

Allergenicity has long been recognized as one of the key potential human health impacts associated with recombinant proteins in genetically modified crops (for instance, see Metcalfe et al 1996; Wal 1998; EC Scientific Steering Committee 2000). Many studies indicate that children are 3-4 times as likely to suffer from allergies as adults. Therefore, exposure to novel proteins such as rhLf and rhLys is of special concern in the case of children:

“Data from several studies estimate that 6-8% of children and 2-2.5% of adults have an immunologic-mediated reaction to foods... This suggests that

²⁷ See, for instance, Vecchio (2006), quoting potential Ventria collaborator William Greenough, who incorrectly describes Ventria’s recombinant proteins as “...purified, normal human breast milk proteins...”

children are the population at most risk for the introduction of novel food proteins.” (EPA SAP 2000a, pp. 11-12)

This is particularly true of children in the first two years of life. Speaking of this age group, leading U.S. food allergist Dr. Hugh Sampson states:

“...the younger child is definitely at higher risk for sensitization and likely...requires smaller amounts of a protein to cause a problem.” (EPA SAP Transcript 2000a, p. 447)

Dr. Sampson notes that 95% of a group of 5000 peanut-allergic patients were sensitized under the age of 2, “and so most of these allergies are initiated in the first few years of life” (Ibid, pp. 447-48).

In 2001, an international group of food allergy experts convened jointly by the United Nations’ Food and Agriculture Organization and World Health Organization established the first detailed testing scheme to assess the potential for novel proteins in genetically modified crops to cause allergies (FAO-WHO 2001). Though no allergenicity testing scheme is perfect, the FAO-WHO 2001 protocol represents the best thinking of international experts in the field.

Ventria’s rhLf possesses four characteristics of allergenic proteins—amino acid sequence similarity to known allergens, glycosylation, resistance to digestion and heat stability—while rhLys possesses the latter two characteristics. Below we evaluate rhLf and rhLys with reference to the FAO-WHO 2001 protocol and other authoritative experts. The following discussion is based in part on Consumers Union (2005), which includes discussion of contradictions in Ventria’s submissions to various U.S. government regulatory agencies.

Amino acid sequence similarity to known allergens

In a submission by Ventria to the U.S. Food and Drug Administration on November 24, 2003, Ventria’s Delia Bethell noted that human lactoferrin and chick ova-transferrin (conalbumin), a known human allergen,

“The presence of the substance [i.e. new ingredient in infant formula] in human milk may not be a sufficient definition of a nutritional substance, and other factors, such as whether the substance is produced from genetically modified sources, need to be considered.”

(underscore added) NAS (2004), p. 39.

have 52% amino acid sequence homology (Ventria BNF 2003). Human lactoferrin also bears 68% amino acid sequence homology to lactoferrin from cows (Groenink et al 1999), which according to leading French allergy expert Dr. Jean-Michel Wal is a significant allergen in cow’s milk (Wal 1998). The NAS committee on infant formulas also cites “allergic effects” as a possible adverse consequence of lactoferrin in infant formulas (NAS 2004, p. 23). Both the FAO-WHO experts and the international food safety authority Codex Alimentarius (Codex 2003) propose that any

global sequence homology between a transgenic protein and a known allergen that exceeds 35% should be considered significant and trigger further research.

In order to assess sequence similarity to known allergens, Ventria must be required to fully sequence rhLf and rhLys, which may differ from the natural human counterparts used for the studies noted above. In studies listed on Ventria’s website, and in its patent, company scientists did *not* do this (Nandi et al 2002; Ventria Patent 2004). Instead, they compared only the N-terminal sequences. In the case of lysozyme, a 130-amino acid protein, only 11 amino acids at the N-terminals of the native and recombinant versions were sequenced and compared; 10 of 11 of these amino acids were demonstrated to be identical (Huang et al 2002). The identity of the other 119 amino acids was apparently not determined, thus only 8% of the amino acids of the two proteins were demonstrated to be identical. A Scientific Advisory Panel to the U.S. Environmental Protection Agency (EPA) has recommended full-length amino acid sequencing of plant-produced recombinant proteins, as one or two point mutations can affect the protein’s allergenicity or other properties (EPA SAP 2000b, p. 14). For instance, it is noted in that report that two isoforms of beta-lactoglobulin, each with a single point mutation, display altered allergic properties. Ventria’s method of determining only the few amino acids at one end of the protein is considered “highly undesirable” by the EPA’s experts (Ibid, p. 14).

Thus, natural human lactoferrin possesses significant similarity to two allergenic proteins, chick ovo-transferrin and cow's milk lactoferrin. Until Ventria presents full sequencing information, it must be assumed that Ventria's rice-derived rhLf also possesses this similarity to known allergens.

Glycosylation

One form of post-translational processing is glycosylation, or attachment of carbohydrate groups to the surface of the protein. Glycosylation is considered to be a characteristic property of allergenic proteins. "The allergenic fraction of food is generally comprised of heat-stable, water-soluble glycoproteins..." (Sampson 1999). Animals and plants attach different types of carbohydrate groups to proteins. Plant-type glycosylation patterns have been implicated in the allergenicity of plant proteins that cause food allergies, especially carbohydrate groups with the -1,3 fucose and -1,2 xylose sugar residues (EPA SAP 2000b, p. 23). It has been demonstrated that rice-expressed rhLf is glycosylated differently than native human lactoferrin (Lönnerdal 2002), and that all eight types of carbohydrate groups found in rhLf have the 1,3-linked fucose and 1,2-linked xylose structures implicated in allergic reactions (Fujiyama et al 2004). Plant-type glycosylation of a "human" protein may also alter its stability (EPA SAP 2000b, p. 23). Any increase in stability would be a warning flag, as digestive stability is a characteristic of food allergens.

Digestive stability

Resistance to breakdown in the gastrointestinal tract is considered a key indicator of the potential allergenicity of a novel protein by the FAO-WHO's experts and many others (FAO-WHO 2001, Metcalfe et al 1996). Infants generally have less acidic guts than adults, meaning that proteins generally survive digestion longer in their immature digestive systems. Digestive stability is usually measured in a simple in vitro system consisting of a solution of digestive enzymes. A digestive stability test on rice-expressed rhLf and rhLys revealed that "the major part" of their activities

"...6-8% of children and 2-2.5% of adults have an immunologic-mediated reaction to foods... This suggests that children are the population at most risk for the introduction of novel food proteins."

Scientific advisors to the EPA, in a report on potential food allergies from exposure to genetically engineered corn. See EPA SAP (2000a), pp. 11-12.

remained after 30 minutes in a pepsin solution at low pH and 37° C, followed by adjustment of the pH to 7, addition of pancreatin (mixture of pancreatic enzymes), and incubation in this solution for 30 or 60 minutes (Lönnerdal 2002, p. 220S). This activity clearly indicates that rhLf and rhLys were largely undegraded under the conditions of the test, and hence possess digestive stability, another characteristic of proteins that cause allergies.

Heat stability

The stability of a protein to breakdown by heat is considered by

FAO-WHO experts to be a characteristic property of food allergens (FAO-WHO 2000; Helm 2001). Leading U.S. food allergist Dr. Hugh Sampson agrees: "The allergenic fraction of food is generally comprised of heat-stable, water-soluble glycoproteins..." (Sampson 1999). Taylor & Hefle (2001, p. 768) also assume that food allergens are stable to heat.

Dr. Bo Lönnerdal conducted heat stability tests on rice-expressed rhLf and rhLys as follows: "If recombinant human milk proteins are to be added to infant formula or baby foods, some degree of processing may be involved. We therefore exposed the recombinant proteins, both in pure form in solution and as added to infant formula, to various heat treatments, ranging from 78 – 100° C for 8 seconds up to 30 minutes. Except for the most severe treatment, 100° C for 5 minutes, which partially inactivated both recombinant and native human milk proteins, these proteins maintained activities similar to those of the native proteins" (Lönnerdal 2002, p. 220S).

In a background paper for the FAO-WHO 2001 meeting of international allergy experts, food allergy expert Dr. Ricki Helm recommends that a novel recombinant protein showing stability to heat treatment for 5 minutes at 90° C be considered stable to heat (Helm 2001, pp. 9-10). Both rhLf and rhLys, which retained some activity after 5 minutes at 100° C, would retain at least as much activity (and probably more) at the milder

90° C recommended by Helm, and so must be considered stable to heat.

It should be noted that in Lönnerdal's test system, digestive and heat stability are measured by the protein's retention of activity, not its resistance to breakdown. Allergists are interested in the extent to which a protein degrades into small fragments when exposed to heat or digestive solutions.

Proteins generally lose activity through denaturation (i.e. deformation) before they break up, and denatured proteins can still cause allergies. Therefore, Lönnerdal's results underestimate the stability of rhLf and rhLys for the purposes of allergenicity assessment.

Summary of allergenic properties

Rice-expressed, recombinant human lactoferrin possesses at least three, and probably four, characteristics of proteins that cause food allergies. These allergenic properties may explain why noted food allergist Steve Taylor of the University of Nebraska warned four years ago that the FDA would not approve Ventria's rhLf (Pearson 2002). Ventria's rhLys possesses at least two allergenic characteristics (heat and digestive stability). Ventria is marketing its rhLys for research use only (i.e. not for medical use of any sort) through Sigma Chemical Company. A material safety data sheet on rhLys from Sigma states that: "Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals" (Sigma MSDS). According to Steve Taylor, FDA regulations will have to be rethought before rice-grown lactoferrin, and other human proteins made by genetically modified organisms, can be approved for production (Pearson 2002).

This strong evidence of potential allergenicity, coupled with reports that two infants involved in the Peruvian experiment acquired allergies after their treatment (Diaz 2006), makes it essential to conduct full follow-up investigations into the health status of all Peruvian infants who received Ventria's compounds.

"... an important potential hazard of therapeutic use of hLf [human lactoferrin] in human patients is possible induction of an antibody response."

Dr. Eugene Weinberg, Indiana University. See Weinberg (2001)

4.2.2 Autoimmune Disorders

Two lines of evidence—one general and one specific to lactoferrin—suggest that Ventria's proteins may have the capacity to cause immune system dysfunction.

First, there is a growing body of evidence demonstrating puzzling, unexpected and in some cases dangerous immunologic responses to biopharmaceuticals produced in genetically engineered cell cultures

(FoE 2003). In these cell culture production systems, a human gene encoding a medically useful protein such as insulin is spliced into bacteria, yeast or mammalian cells, which then produce a recombinant version of the protein, known as a biopharmaceutical. While the immune system does not normally attack a bodily protein because it is recognized as "self," it may respond to the corresponding biopharmaceutical due to subtle differences that cause the body to recognize it as foreign. The precise nature of these differences has not been established in most cases and is a subject of intense research; they could involve differences in post-translational processing, tertiary structure, and/or primary amino acid sequence.

In some cases, the administered biopharmaceutical merely elicits an immune system response that reduces or eliminates the drug's potency. This phenomenon has been observed in some patients receiving recombinant blood clotting Factor VIII and the multiple sclerosis drug beta-interferon. In other cases, the immune system detects that the engineered drug is different (i.e. treats it as foreign), yet the antibodies produced against the engineered drug also target the natural counterpart, thereby leading to potentially disastrous consequences. For instance, a recombinant version of megakaryocyte growth and development factor (MGDF) produced by Amgen was discontinued in clinical trials because some patients receiving the drug mounted an immune attack on both Amgen's recombinant MGDF and their own natural version of MGDF, resulting in bleeding. A similar phenomenon might be responsible for up to 160 cases of red cell aplasia (virtual shutdown of red blood cell production) observed in patients treated with recombinant erythropoietin, a

hormone that stimulates red blood cell production. The important fact to keep in mind here is that these reactions to recombinant biopharmaceuticals have taken biotech companies and regulators alike *by surprise*. Dr. Burt Adelman, head of research & development at Biogen, found the immune reactions to MGDF “stunning.”

*“The conventional wisdom had been that this was a theoretical risk ... nobody saw it coming. If you’re in my business, it’s really unnerving.”
(as quoted in Aoki 2002)*

In other words, although the natural human protein and the corresponding engineered biopharmaceutical appear to be identical, the immune system is able to detect a difference that scientists, at present, cannot. The FDA has implicitly recognized this fact. At a meeting in 2002 about human plasma-derived drugs, the FDA’s Chris Joneckis noted that:

*“Despite best efforts to detect product differences and predict the impact of manufacturing changes, these surprises do continue to occur.”
(FDA CBER 2002)*

If tightly-controlled fermentation production of mammalian cell-produced “human” drugs is causing such stunning, unpredicted and in some cases hazardous immune reactions, what are we to think of plant-produced pharmaceuticals such as lactoferrin produced in plants subject to the “manufacturing changes” imposed by nature in the form of widely varying microclimates and microhabitats, insect infestation, etc.?

A second immunologic concern specific to rice-expressed lactoferrin is suggested by the unexplained presence of anti-lactoferrin antibodies in the blood stream of many patients suffering from a wide range of autoimmune disorders:

*“...anti-LF autoantibodies are found in several autoimmune conditions, including rheumatoid vasculitis, rheumatoid arthritis, systemic lupus erythematosus, ulcerative colitis, primary sclerosing cholangitis and Crohn’s disease.”
(Audrain et al 1996)*

While these anti-lactoferrin antibodies have not yet been demonstrated to have pathophysiological significance, they have been shown to be correlated with markers of disease activity in patients with rheumatoid arthritis and systemic lupus erythematosus (Chikazawa et al 2000). One report suggests that when anti-lactoferrin antibodies of the IgG class bind to lactoferrin in the synovial fluid of rheumatoid arthritis sufferers, they cause lactoferrin to release iron, which in its unbound state is implicated in arthritic inflammation and tissue damage (Guillen et al 1998). One team recommends that:

“Future research should address the pathophysiological role of anti-lactoferrin ANCA [antineutrophil cytoplasmic autoantibodies] and the influence of anti-lactoferrin ANCA binding on the functional properties of the lactoferrin molecule.” (Rozenendaal et al 1998)

In a review article on the potential uses of recombinant human lactoferrin in treating various medical conditions, Dr. Eugene Weinberg of Indiana University cautions that: “... an important potential hazard of therapeutic use of hLf [human lactoferrin] in human patients is possible induction of an antibody response” (Weinberg 2001). In short, there is great uncertainty concerning a possible pathophysiological role for anti-lactoferrin autoantibodies in autoimmune diseases. Could introduction to the diet of a rice-expressed “human” lactoferrin with subtle but clear differences to the native protein, and with demonstrated resistance to degradation in the gut, elicit potentially hazardous autoimmune reactions? We don’t know, but the appropriate research should be undertaken to answer such questions before any further experimentation on infants is undertaken.

4.3 Lactoferrin Inhibits But May Also Promote Certain Pathogens

Lactoferrin is found in bodily secretions, such as breast milk, tears, saliva, gastrointestinal and seminal fluid, as well as in the mucous membranes lining the nose, vagina and lungs. These are the body’s portals to the outside world, and hence the entry points for many pathogens. Lactoferrin is also an important component of infection-fighting white blood cells known as poly-

morphonuclear neutrophils, which circulate in the bloodstream. Accordingly, one of lactoferrin's better-understood roles is to fight microbial infection. The main weapon in lactoferrin's pathogen-fighting arsenal is thought to be its ability to bind free iron at infection sites. Iron is an essential nutrient, for microbes as for humans. Lactoferrin locks up iron, making it unavailable, and thus literally starves many microbial invaders (Weinberg 2001).

As so often in nature, however, closer examination reveals a more complex state of affairs. Several microbes have developed mechanisms to reclaim the iron they require for growth. Some compete with lactoferrin by secreting their own iron-binding compounds (called siderophores) that then provide them with the iron. Other pathogens have learned the trick of extracting iron directly from lactoferrin and its close relative transferrin—they actually feed on the weapon developed by the body to kill them. Pathogenic bacteria that can utilize lactoferrin and transferrin as an iron source include *Helicobacter pylori* (ulcers and stomach cancers), *Haemophilus influenza* (meningitis), *Bordetella pertussis* (whooping cough), *Legionella pneumophila* (legionnaires' disease), and two species of the genus *Neisseria* that cause gonorrhea and meningitis (Dhaenens et al 1997). *Trichomonas vaginalis*, a protozoan responsible for genital disease in both women and men, can also extract iron from lactoferrin.

According to Dr. Weinberg, therapeutic use of human lactoferrin could stimulate growth of such pathogens, resulting in an "adverse response." While Weinberg believes that human lactoferrin (Lf) has therapeutic potential, he argues that "[p]recaution is needed ... to avoid ... introduction of the protein [lactoferrin] to tissues that may be infected with specific protozoa or bacteria that utilize Lf in their acquisition of host iron" (Weinberg 2001). The potential for exacerbation of such infections deserves special consideration in the developing country context. Because it resides in the human gut, *H. pylori* may be of particular concern

"... [p]recaution is needed ... to avoid ... introduction of [lactoferrin, Lf] to tissues that may be infected with specific protozoa or bacteria that utilize Lf in their acquisition of host iron ... [human lactoferrin] might not be a successful therapeutic agent for H. pylori and, indeed, could intensify the infection."

Dr. Eugene Weinberg, Indiana University. See Weinberg (2001)

when rhLf is fed to infants suffering diarrhea. Weinberg notes that human lactoferrin "might not be a successful therapeutic agent for *H. pylori* and, indeed, could intensify the infection." *H. pylori* is implicated in causing ulcers, chronic gastritis and certain forms of stomach cancer (Weinberg 2001).

We find no evidence in publications by Ventria or its collaborators that this potential for exacerbation of certain infections has been evaluated, or even acknowledged.

4.4 Amyloidosis and Mutant Proteins

Hereditary systemic amyloidosis is a rare disease characterized by the deposition of insoluble protein fibers (called amyloid fibrils) in various organs and tissues. The amyloid fibrils result from mutant forms of certain cellular proteins. These mutations cause the cellular proteins to change their three-dimensional shape and become flatter (so-called beta-sheet structure), allowing them to stack up together like sheets of paper to form a fiber which becomes insoluble. Over time, the amyloid fibrils build up in various organs and tissues, making them stiff and reducing their ability to function. One rare form of the disease caused by mutant lysozyme usually presents in middle age and is marked primarily by "slowly progressive renal impairment that can take decades to reach end-stage" (Hawkins 2003). The three known mutant versions result from three point mutations in lysozyme: threonine for isoleucine at position 56, arginine for tryptophan at position 64, or histidine for aspartic acid at position 67. In each case, the mutant lysozyme auto-aggregates to form fibrils with a characteristic beta-sheet structure.

As noted above, Ventria reports sequencing only the 11 amino acids at the N-terminal of recombinant lysozyme; the identities of the amino acids at positions 56, 64 and 67 were not determined. Ventria scientists did demonstrate that their rice-expressed lysozyme has antimicrobial activity, which is presumably dependent on the protein molecule assuming its proper three-

dimensional conformation, which in turn argues against the conformation-changing point mutations discussed above. Yet circumstantial evidence is not adequate. Ventria should follow the advice of numerous expert bodies and fully sequence recombinant lysozyme to detect these or any other potentially hazardous mutations resulting from its production in rice.

A similar evaluation is needed for Ventria's recombinant lactoferrin, because a lactoferrin mutant with just a single amino acid alteration has been implicated as a cause of amyloidosis accompanied by trichiasis, a common condition of the eyelid that can impact vision (Cummins 2004).

It is perhaps unlikely that dietary exposure to human lysozyme and lactoferrin mutants could cause the amyloidosis conditions outlined above. However, the demonstrated resistance of rhLys and rhLf to breakdown by digestive fluids and heat at least raises the possibility that the mutant forms could be absorbed into the bloodstream, or aggregate in the gastrointestinal tract.

5. REGULATION OF VENTRIA'S RICE AND RECOMBINANT PROTEINS

5.1 Regulatory Options

In view of these potential human health impacts, one would hope that Ventria's transgenic rice and recombinant proteins would undergo a mandatory and rigorous assessment process, especially given their intended use in oral rehydration solutions for vulnerable infants and children. As of now, it is unclear how the U.S. Food and Drug Administration (FDA) will regulate Ventria's products.

Ventria's transgenic rice could undergo the FDA's "voluntary consultation" process for genetically engineered crops. This is the procedure that companies may use (if

The "Medical Foods Paradox"
"Medical foods intended for use by sick people are subject to much less scrutiny than virtually all other foods, which are intended for the healthy general population."

FDA, in explaining why it proposed to strengthen regulation of "medical foods" (FDA ANPR 1996). FDA never followed through on its proposal. Ventria proposes to introduce its pharmaceutical proteins in oral rehydration solutions as "medical foods."

they choose) to obtain FDA's sign-off before marketing their new GE crops for general food use (for more on this process, see Freese & Schubert 2004). This process, however, has never been employed for a GE *pharmaceutical* crop. Ventria's recombinant proteins (once extracted from rice) could be reviewed by the FDA as drugs or food additives, or regulated under the GRAS ("generally recognized as safe") notification system. Review as drugs would seem appropriate in view of the drug-like effects of these compounds and the claims made for them as agents to "treat" diarrhea, rather than, as in the case of

generic oral rehydration solutions, merely "manage" the condition. A second option would be to treat rhLf and rhLys as food additives. Food additives must undergo "an exhaustive, rigorous scientific evaluation of all appropriate safety studies" by the FDA (NAS 2004, p. 59). Neither drugs nor food additives can be marketed until the FDA has thoroughly reviewed all the relevant safety studies and approved the pertinent compounds as safe. An important difference between the two processes is that drug approval requires demonstration of both safety and benefit to the patient (i.e. efficacy), while food additives need not confer any benefit, but only be affirmed as safe.

The third option is GRAS notification, which has become by far the most frequent review process for introduction of new ingredients (especially proteinaceous ones) into infant formulas as well as the general food supply. In contrast to drugs and food additives, for which FDA "owns" the safety decision, GRAS notification places the burden for the safety of the new ingredient on the manufacturer (NAS 2004, p. 3). If the manufacturer concludes that the ingredient is safe for its intended use, possibly (but not necessarily) after convening a scientific panel of experts, the manufacturer notifies FDA. FDA reviews the notification, which includes a *summary* of the scientific evidence and historic use, if any, of the compound. If FDA has no questions, it issues a letter of no objection, which explicitly notes that the manufacturer bears responsi-

bility for the safety of the new ingredient. If FDA has concerns regarding the safety of the ingredient, it informs the manufacturer of the outstanding issues, which must be addressed to FDA's satisfaction. Since 1997, FDA has signed off on over 75% of GRAS notifications it has received (NAS 2004, p. 60). The major weakness of the GRAS process is the reliance on company-prepared "summaries" of studies they have conducted, which often present only results and lack crucial methodological details needed for a critical evaluation. Experience in the field of genetically engineered crops shows that company-prepared "data summaries" open up the possibility of selective presentation of data that can undermine the integrity of the FDA's review process (Freese & Schubert 2004). The NAS committee on new ingredients in infant formulas also argues that a more rigorous system than GRAS notification should be applied to new ingredients in infant formulas (NAS 2004, pp. 59-60).

Finally, the FDA has a problematic "medical foods" category intended for products used in the dietary management of certain diseases and conditions with "distinctive nutritional requirements," a category that commonly includes oral rehydration solutions (for following discussion, see FDA ANPR 1996). Ventria intends to market its recombinant proteins for use in oral rehydration solutions classified as medical foods (Ventria PR 2006), as well as in infant formulas and other foods and drinks.

It is interesting to note that prior to 1972, medical foods (e.g. infant formulas used to treat metabolic disorders²⁸) were regulated as drugs. In 1972, FDA removed such products from the stringent new drug review process for a number of reasons, including its desire to foster innovation by lowering regulatory costs for development of such products. Unfortunately, this led to the "medical foods paradox," described by the FDA as follows: "Medical foods intended for use by

"We believe that Ventria's recombinant human proteins should be subjected to the the new drug review process, which would require the FDA to undertake an exhaustive analysis to determine whether or not the proteins are in fact both safe and efficacious."

sick people are subject to much less scrutiny than virtually all other foods, which are intended for the healthy general population" (FDA ANPR 1996). In other words, medical foods should be subject to more stringent testing than general foods because the sick people who receive them are more vulnerable to any harm caused by such foods than healthy people would be. FDA issued an advance notice of proposed rulemaking (ANPR) in 1996 as a prelude to remedying this situation.

In particular, FDA intended to address safety problems (including deaths) that had occurred due to use of unsafe medical foods; and to stem the tide of fraudulent claims for health benefits, unbacked by scientific evidence, that proliferated with the increasing number of products marketed as medical foods.

Though FDA never implemented rules for medical foods due to "regulatory backlog" (FDA Withdrawal 2004), its 1996 notice provides valuable information about its thinking that is relevant to Ventria's proposed products. Most importantly, FDA acknowledged that the scientific standards used to evaluate the safety and claimed benefits of medical foods need to be more stringent than those applied to conventional foods, perhaps even as stringent as the standards used to evaluate the safety and efficacy of drugs. Its rationale, echoed by professional pediatric organizations, is that medical foods are meant for especially vulnerable patients (e.g. infants) who would often utilize the medical food as their sole source of nutrition, and that unfounded health claims could misguide even health professionals into prescribing ineffective, unsafe or unnecessarily expensive products.

The lack of regulatory requirements for medical foods means that Ventria need not provide any rigorous demonstration of safety or health benefits for oral rehydration solutions containing its recombinant human milk proteins, even though FDA believes such requirements are needed.

²⁸ The paradigmatic example of a medical food is the infant formula Lofenalac, which contains very low levels of the amino acid phenylalanine, for feeding to infants with a metabolic disorder (phenylketonuria, or PKU) that impairs their ability to metabolize it properly. When they eat normal foods, infants with PKU build up harmful end products of phenylalanine metabolism in their bodies, which cause severe mental retardation.

To summarize, the possible regulatory options in the case of Ventria are: 1) Review of Ventria's transgenic rice under FDA's voluntary consultation system for GE crops meant for general food use; 2) Review of the recombinant proteins derived from Ventria's transgenic rice as drugs, food additives or under the GRAS notification system for specific uses; 3) Though FDA believes that medical foods, such as an oral rehydration solution with Ventria's proteins, should meet standards for safety and efficacy similar to those for drugs, it has failed to implement regulations to this end. We believe that Ventria's recombinant human proteins should be subjected to the the new drug review process, which would require the FDA to undertake an exhaustive analysis to determine whether or not the proteins are in fact both safe and efficacious. Demonstration of efficacy is critical in light of Ventria's possible inflation of benefits in the Peruvian experiment (Section 6.4), and the potential adverse consequences of prescribing an ineffective product under the mistaken assumption that it is beneficial.

5.2 Current Regulatory Status of Ventria's Recombinant Proteins

Ventria has not received regulatory clearance from the FDA for any food or medical use of its recombinant proteins. Table 1 below presents the regulatory status of Ventria's transgenic rice/recombinant proteins in the United States. The first Ventria submission to the FDA that we have found dates to November 2003, over 3 years ago. In this limited request, Ventria did NOT ask the FDA to approve its pharmaceutical-producing rice (in this case, lactoferrin rice) for medical or any other use, but rather only to have FDA: 1) Agree that it was "generally recognized as safe" (GRAS) *in the event*

"FDA has not approved either of Ventria's two lead compounds for any human use whatsoever, and even refused to sign off on lactoferrin rice as a low-level contaminant in food, despite 4 requests to FDA to gain the agency's approval over the past three years."

*that it contaminated food-grade crops;*²⁹ and 2) Grant Ventria permission to put rice residues remaining after extraction of the lactoferrin into the food and feed supply (Ventria BNF 2003; Lee 2004; see also Appendix 1). The company's purpose was clearly to shield itself from liability in the event that its rice contaminates food-grade rice. Interestingly, Ventria's CEO Scott Deeter has repeatedly dismissed the possibility of such contamination in public statements made at this time and

subsequently. In any case, FDA did not respond even to this limited request.³⁰ Ventria's lactoferrin rice therefore not only remains unapproved for medical or food use, but could even be considered an adulterant if found contaminating the food supply at low levels. Rice or other food products adulterated with Ventria's lactoferrin rice could therefore be subject to seizure by FDA.

The three remaining requests were all GRAS notifications for Ventria's recombinant proteins, rather than for the transgenic rice containing them. In December 2004, Ventria submitted a request to have FDA sign off on its rhLf as GRAS for use in foods, beverages and medical foods.³¹ The request was withdrawn by Ventria nearly two years later, on November 20, 2006, suggesting that FDA has unanswered safety concerns.³²

In June 2005, Ventria submitted a GRAS notification to have FDA sign off on its rhLys for use as an antimicrobial agent or ingredient in various foods, but then withdrew this notification just three months later in September for unknown reasons.³³ Ventria submitted a new GRAS notice for rhLys in January 2006, this time for use of the compound in infant formulas or pediatric oral rehydration solutions.³⁴ This notification is still pending.

²⁹ In the words of Ventria vice-president Delia Bethell: "... we recognize the possibility of the inadvertent introduction of LF164 [lactoferrin rice] at low, adventitious levels into commercial rice varieties." (Ventria BNF 2003; see also Appendix 1)

³⁰ See <http://www.cfsan.fda.gov/~lrd/biocon.html>. Note that BNF 82, the notification number for Ventria's lactoferrin-producing rice, is not present under FDA's "List of Completed Consultations on Bioengineered Foods."

³¹ See GRN No. 162 at <http://www.cfsan.fda.gov/~rdb/opa-gn04.html>.

³² See <http://www.cfsan.fda.gov/~rdb/opa-g162.html>.

³³ See <http://www.cfsan.fda.gov/~rdb/opa-g174.html>.

³⁴ See GRN No. 191 at <http://www.cfsan.fda.gov/~rdb/opa-gn06.html>.

Table 1: Ventria’s Failed Attempts to Obtain FDA “Generally Recognized as Safe” (GRAS) Approval of its Rice-Derived Pharmaceutical Proteins

DATE OF PETITION	COMPOUND	INTENDED USE	STATUS AT FDA	COMMENTS
Nov. 2003	Lactoferrin (Lf) rice	None. Lf rice as contaminant; Lf rice residues after Lf extraction for human food & animal feed	BNF 082; no action (see Appendix 1)	Ventria sought approval of Lf rice as contaminant while publicly claiming Lf rice would not contaminate food
Dec. 2004	Lactoferrin	Ingredient in foods, beverages, medical foods	GRN. #162; withdrawn on Nov. 20, 2006	Ventria withdrew this petition because FDA indicated it would not approve lactoferrin as safe.
June 2005	Lysozyme	Antimicrobial agent; ingredient in various foods	GRN #174; withdrawn	Ventria withdrew this petition in Sept. 2005 for unknown reasons
Jan. 2006	Lysozyme	Ingredient in infant formulas & pediatric oral rehydration solutions	GRN #191; no action	A 2004 National Academy of Sciences panel recommends more stringent testing of new ingredients in infant formulas

Thus, FDA has not approved either of Ventria’s two lead compounds for any human use whatsoever, and even refused to sign off on lactoferrin rice as a low-level contaminant in food, despite 4 requests to FDA to gain the agency’s approval over the past three years.

While we cannot say for certain why the FDA has not granted GRAS status to Ventria’s compounds, a clue is provided in FDA’s November 20, 2006 response to Ventria, where the agency alludes to the “complex scientific issues” involved in Ventria’s proposed uses of rice-derived lactoferrin.³⁵ Because of the drug-like nature of these proteins, the FDA may be considering whether to regulate these substances as drugs or food additives rather than under GRAS notification. Another factor is the use of genetic engineering to produce the compounds, which as discussed above raises additional safety concerns that require rigorous evaluation. The heightened vulnerability of one target group for Ventria’s compounds—sick infants—may be influencing FDA to require more rigorous assessments than Ventria has thus far submitted.

Finally, a Houston, Texas-based company named Agennix is currently producing recombinant human lactoferrin in genetically modified fungus in a contained pharmaceutical manufacturing facility. Agennix has been developing rhLf as a pharmaceutical drug under FDA regulation since 1996. According to Agennix’s Peter Glynn:

“Recombinant human lactoferrin is a highly active pharmaceutical compound with promising activity in Phase 2 cancer trials. As with any new drug, caution must be exercised during the initial testing period when the record of human exposure is limited. Without conducting robust controlled clinical trials of sufficient size and duration, no claims as to the safety and efficacy of rhLf, from any source, can be supported. Further, since versions of rhLf from different sources and manufacturing processes are clearly distinct, safety or efficacy data with rhLf from one source or manufacturing process cannot prove the safety or efficacy of rhLf from a different source or manufacturing process.” (personal communication, 7/28/06)

³⁵ See <http://www.cfsan.fda.gov/~rdb/opa-g162.html>

6. ANALYSIS OF THE VENTRIA-SPONSORED EXPERIMENT ON PERUVIAN INFANTS

There are five aspects of the Peruvian experiment which may be considered highly suspect or unethical. First, Ventria's failure to obtain GRAS status for its proteins prior to their use in the experiment. Second, the apparent lack of peer-reviewed animal feeding studies prior to the trial on Peruvian infants. Third, reports that investigators did not fully inform the parents of trial participants about the experimental nature and potential risks of the trial before obtaining their consent, and the lack of follow-up. Fourth, the improper and partial reporting of results prior to peer review or publication, occurring moreover in the context of Ventria's public relations campaign to promote its experimental proteins. Finally, the possibility that Ventria's trial on Peruvian infants and the emotive hype surrounding it is being used for the ultimate end of selling its proteins as additives in products targeted to wealthy consumers in developed countries.

6.1 No FDA Approval

This point was addressed in the previous section. Here, we will only re-emphasize that Ventria's December 2004 notice to the FDA for GRAS approval of lactoferrin languished at the agency for nearly two years before being withdrawn by Ventria on November 20, 2006, strongly suggesting that the FDA has safety concerns that have not been adequately addressed.

6.2 No Record of Peer-Reviewed Rat or Monkey Studies

In 2002, Bo Lönnerdal of University of California, Davis, who has collaborated closely with Ventria in the past and was also involved in the Peruvian experiment, announced plans to test the antibiotic properties of rice-derived rhLf and rhLys on rats, followed by a trial on infant rhesus monkeys.

Further efficacy and safety trials in animals and humans will be needed next. Safety studies in rats will be necessary prior to human trials. For efficacy studies, we have chosen to first use a rat pup model, in which we can assess resistance against proteolytic activity as well as anti-infective properties (incubate with pathogens). We subsequently will use infant rhesus monkeys (Lönnerdal 2002, 220S).

Lönnerdal further noted that the studies on infant rhesus monkeys would involve infecting them with a diarrhea-causing pathogen to "evaluate the effect of the added protein on diarrhea prevalence, severity and duration. Such studies, of course, are not possible to do in human infants." (Ibid, p. 221S).

Despite these assurances, a search on PubMed reveals no such published studies of rice-derived rhLf or rhLys on rats or monkeys.³⁶ It is unclear whether such research has been conducted but remains unpublished, was published in some obscure journal, or whether Ventria and its collaborators chose to forego animal experiments and proceed directly to the trial on Peruvian infants. There is also no indication of rat or monkey trials with rice-derived rhLf or rhLys in any of the many studies (co-)authored by Lönnerdal on his UC Davis website,³⁷ or on Ventria's website.³⁸ Even if such experiments have been conducted, they should have been published in reputable, peer-reviewed journals prior to human experimentation. Peer review and publication are essential elements of the scientific method; without them, critical review and replication (or not) of an individual team's findings are not possible. We would also emphasize that studies on rats are not sufficient. For the testing of new ingredients in infant formula, experts recommend studies on at least two different test animals, "keeping in mind that the more animal models used, the better...." with infant non-human primates recommended as the closest analog to humans (NAS 2004, p. 78).

³⁶ PubMed is the premier, comprehensive database of medical and biological science articles run by the U.S. National Institutes of Health and National Library of Medicine; it covers over 16 million biomedical and life science articles in 33,000 scientific and medical journals. Searches conducted on April 17, 2007 on the keywords "lactoferrin" AND "rice" yielded 13 hits; on "lysozyme" AND "rice" 25 hits. None of these studies involved feeding trials on rats or monkeys with rice-expressed rhLf or rhLys. One study involved a feeding trial with rhLys and rhLf on chicks designed to measure performance, not safety.

³⁷ <http://nutrition.ucdavis.edu/faculty/lonnerdal.html>

³⁸ <http://www.ventria.com/collaborators/publications.asp>

The only clinical trial involving Ventria's rice-derived rhLf that we find on the comprehensive PubMed database involved a study of iron absorption from a meal supplemented with rhLf in young women. In this trial, the women were fed two meals, each supplemented with rhLf, at an interval of 4 weeks. The results indicated that iron was absorbed as well from rhLf as from ferrous sulfate (Lönnerdal & Bryant 2006). This limited study was intended only to evaluate absorption of iron from rhLf, was not intended to detect potential adverse effects it might have, involved only two exposures to rhLf at an interval of 4 weeks, and was carried out on healthy women. Therefore, it can have little bearing on the questions raised above concerning potential health impacts from administration of this compound to sick infants.

6.3 Lack of Informed Consent and Follow-Up

According to an account in Peru's *La Republica* (Diaz 2006), at least one mother whose infant was enrolled in the experiment was not informed that the treatment ("suero de arroz" or "rice serum") was experimental and involved compounds from transgenic rice. Diana Canessa Garay, who enrolled her son Fabrizio in the experiment on February 15, 2005, states that she was "deceived." According to Garay: "After they gave him the serum, my baby became sickly, delicate. Now he is allergic to everything..." A second mother, Johana Sanchez Turreate, says that her 3-year-old son Jordano also developed allergies after receiving the "serum." Peruvian Member of Parliament Mercedes Cabanillas has initiated an investigation of the experiment by the Public Defender's office. Wilfredo Ardito of the Peruvian Association of Human Rights doubts that the parents gave informed consent to their children's participation in the experiment (Leighton 2006).

In our view, informed consent should have involved a full explanation of the experimental nature of the treatment, including the lack of regulatory approval for Ventria's proteins anywhere in the world and their source in transgenic organisms. Furthermore, parents should have been advised (if they weren't) of exclusive

"After they gave him the serum, my baby became sickly, delicate. Now he is allergic to everything..."

Diana Canessa Garay, on the condition of her son Fabrizio after he participated in the Ventria-sponsored clinical trial to test Ventria's pharma rice compounds. (Diaz 2006).

breastfeeding as a possible alternative treatment option (see Section 7.2), given its universally acknowledged benefits (infants who were exclusively breastfed were excluded from the study (Bethell 2006)). Finally, the researchers reportedly followed-up on the infants for only 14 days (Bethell 2006), far too short a period to detect potential adverse consequences of the administration of Ventria's compounds.

6.4 Possible Inflation or Fabrication of Benefits

Scientists involved in the Peruvian experiment presented their results on April 30, 2006 at a conference of the Pediatric Academic Societies in San Francisco, CA. An abstract of this presentation is available at the conference website (Zavaleta et al 2006). A paper based on the experiment was published only eight months later (Zavaleta et al 2007).

The reporting of the trial results in both the abstract and the paper do not follow basic scientific protocol. The authors report that 140 Peruvian boys aged 3 – 36 months with acute diarrhea and dehydration were admitted to either Children's Hospital in Lima, Peru or the Pediatric Clinic of the Belen Hospital in Trujillo, Peru, and treated with either:

- 1) Group 1: Low osmolarity WHO oral rehydration solution (ORS);
- 2) Group 2: ORS based on conventional rice; or
- 3) Group 3: ORS based on conventional rice to which recombinant lactoferrin and lysozyme extracted from Ventria's rice had been added.

The serious flaw is that the results for each of the three treatment groups are not reported separately. Instead, the authors improperly combine the first two groups and report averaged figures for group 1+2 versus the results for group 3 infants, who were treated with ORS containing Ventria's compounds. The average duration of diarrhea was modestly reduced in the third group versus the combined group 1+2 (3.67 versus 5.21 days) and there were slightly more children in the third group who "achieved 48 hours with solid stool"

(85.1% versus 69.2% in combined group 1+2). There were no statistically significant differences between the combined group 1+2 and 3 in volume of diarrhea or in percent of children who had a new diarrhea episode (Zavaleta et al 2006), though a Ventria press release inexplicably claims that group 3 children “were less likely to relapse into another episode of diarrhea” (Ventria PR 2006), despite the fact that this finding was not statistically significant.

The obvious problem with omitting group 2 results is that it obscures differences between group 2 and groups 1 and 3. It is well-accepted in pediatric circles that infants suffering from diarrhea recover more quickly when simple glucose-based ORS (group 1 treatment), which contains electrolytes + sugar (glucose), is combined with early “nutritional support” (see discussion in CDC 1992). One popular form of early nutritional support is administration of cereal-based ORS (usually rice), which contains both electrolytes and carbohydrates from rice. A number of studies have shown that rice-based ORS reduces stool output and duration of diarrhea versus glucose-based ORS (Wall et al 1997; Sarker et al 2001; Dutta et al 2000; see also discussion in CDC 1992). For instance, in a meta-analysis covering 13 clinical studies, Gore et al (1992) found that rice-based ORS reduced the rate of stool output in children with cholera by 32% and for those with acute non-cholera diarrhea by 18% versus children treated with standard glucose-based (WHO) ORS. Thus, in the Peruvian experiment, one would expect that group 2 infants receiving rice-based ORS fared better than those in group 1, and thus that group 2 results would be better than the average for groups 1+2, and at the same time more nearly approximate group 3 results.

The failure of the authors to report group 2 results, combined with Ventria’s obvious interest in making its compounds appear as efficacious as possible, leads one

“a Ventria press release inexplicably claims that group 3 children [treated with Ventria’s rice compounds] ‘were less likely to relapse into another episode of diarrhea,’ despite the fact that this finding was not statistically significant.

to suspect that group 3 infants receiving Ventria’s compounds experienced minimal if any benefit over those in group 2 treated with rice-based ORS. If this reasoning is correct, the Ventria-sponsored researchers may have combined group 2 with group 1 in order to fabricate or at least inflate a “benefit” for Ventria’s compounds when in fact they contributed little if anything beyond rice-based ORS. Unfortunately, the published paper, like the abstract, fails to break out

results for group 1 versus group 2 (Zavaleta et al 2007). Even if Ventria’s compounds are eventually proven to deliver some marginal benefit, hyperbolic claims of “saving lives” and the “the Holy Grail in a grain of rice” are clearly inappropriate.

One final note. Dr. William Greenough, cofounder of Cera Products, Inc., has been extensively featured in Ventria’s promotional materials on the Peruvian experiment (for two examples, see Bethell 2006; Ventria PR 2006). Cera Products markets CeraLyte, a rice-based ORS, which the company promotes as offering superior performance to glucose ORS based on published studies.³⁹

“CeraLyte has been shown to be more effective than standard glucose Oral Rehydration Therapy; especially in severe cases of diarrhea from cholera. ... CeraLyte is especially effective in the most severe cases of diarrhea. Rice-based ORS has been shown to have a significant advantage (20 to 50% better) in conditions where purging is severe. ... CeraLyte has been shown to decrease the amount of fluid loss, shorten the length of the illness, and promote recovery.”⁴⁰

It is ironic that Dr. Greenough should agree to be featured in public relations material for the Ventria-sponsored Peruvian experiment, in which precisely the difference between the glucose ORS and rice-based ORS treatments (groups 1 and 2) went unreported.

³⁹ “Cera Products Rice-Based ORS Bibliography,” Cera Products, Inc. http://www.ceraproductsinc.com/research/RiceBased_ORs_Bib.pdf.

⁴⁰ “Why CeraLyte ORS?” Cera Products, Inc. fact sheet, <http://www.ceraproductsinc.com/research/WhyCeraLyte.pdf>.

One possible explanation is the reported interest of Cera Products in marketing a rice-based ORS containing Ventria's compounds:

“Greenough is also co-founder of Cera Products, a Maryland company that has been talking about adding Ventria’s lactoferrin and lysozyme to its product for treating diarrhea.” (Jacobs 2004).

Thus, Ventria and Cera Products may both find it advantageous to downplay the advantages of rice-based ORS without Ventria's compounds in order to pave the way for introduction of an ORS that contains them.⁴¹

6.5 The Holy Grail of Performance Beverages?

It is also important to understand that Ventria may view the oral rehydration solution market as merely a media-friendly venue in which to gain public acceptance and regulatory approval of its proteins. Ventria's CEO, Scott Deeter, was questioned about this at a presentation he gave to the USDA Advisory Committee on Biotechnology and 21st Century Agriculture in 2003. Committee member Dr. David C. Magnus, Assistant Professor at the University of Pennsylvania's Center for Bioethics in Philadelphia, commented as follows on Ventria's plans to use its proteins in oral rehydration solutions to treat children with diarrhea:

“So I think it’s, frankly, a specious argument to think that the real target of these kinds of technologies are likely to be the children in less developed countries where most of the problems you are identifying are a function of things that, actually, for a lot less money than that, could be fixed in terms of potable water and things like that” (USDA AC21 2003).

In response, Deeter candidly admits that his (prospective) investors find no value in oral rehydration solu-

“[T]he Ventria-sponsored researchers may have combined group 2 with group 1 in order to fabricate or at least inflate a ‘benefit’ for Ventria’s compounds when in fact they contributed little if anything beyond rice-based ORS.”

tions as an application for Ventria's proteins. Deeter also makes it clear that an ORS with his company's proteins would have to be subsidized by a charitable foundation (USDA AC21 2003), which of course begs Dr. Magnus's question as to the most cost-effective way to address diarrhea (see Sections 7 & 8). This need for subsidies also clashes with the oft-repeated claim of Ventria's officers and supporters that an ORS with Ventria's compounds would be “inexpensive” and “within reach of people who do not have resources” (Bethell 2006). Indeed, even existing ORS solutions are sometimes too expensive for those who need them (Meyers et al 1991).

A better answer to Dr. Magnus's question about the “real target” of Ventria's rice is perhaps provided in a submission by Ventria to the USDA, in which Ventria proposes to use its rice-derived, recombinant lactoferrin and lysozyme “as supplements in yoghurts, meal replacement and performance beverages, bars (for example, granola bars), and in nutritional supplement drinks.” (USDA EAs 2005).

Such products would clearly be targeted to wealthy consumers in the U.S. and other developed countries. Cera Products is a likely partner for Ventria in this market as well. Besides the rice-based ORS CeraLyte, Cera Products manufactures “CeraSport, a new generation hydration and performance drink [that] restores essential salts and fluids lost in sweat due to exercise, fever or heat stress...”⁴²

The upshot is that Ventria and Greenough may view the Peruvian experiment primarily as an initial step towards gaining public and regulatory acceptance of Ventria's recombinant proteins for use in Gatorade-type performance drinks and other high-end consumer products for sale to wealthy consumers in developed countries.

⁴¹ In Bethell (2006), Nelly Zavaleta, lead author of the abstract on the Peruvian experiment, states: “What current oral rehydration solutions don't have is a clear impact on cutting the duration or reducing the severity of acute diarrhea,” which appears to directly contradict Cera Products' contention (quoted above) that rice-based ORS does in fact reduce the severity and duration of diarrheal episodes versus glucose ORS.

⁴² <http://www.ceraproductsinc.com/productline/cerasport.html>.

7. ADDRESSING DIARRHEAL DISEASE AND MORBIDITY

Diarrhea is a condition whose fatal impacts are felt predominantly among the world's poor infants and children. Thus, cost-effectiveness becomes a primary factor when considering any prevention or treatment options. Expensive solutions, no matter how effective, will in most cases simply not be adopted due to lack of funds on the personal or national level. When expensive measures are nonetheless used, they may actually result in greater mortality relative to a foregone option that is more cost-effective. Below, we discuss the factors that appear to have contributed most to reducing diarrheal morbidity over the past 25 years, and consider the use of oral rehydration solutions containing Ventria's recombinant proteins in this broader context.

7.1 Improvements in Sanitation, Drinking Water Supplies and Hygienic Practices

According to a joint report by the World Health Organization and UNICEF, 2.6 billion people lacked even basic sanitation facilities and 1.1 billion were without access to improved drinking water sources in 2002 (WHO-UNICEF 2005, p. 4). As shocking as these statistics are, the situation has actually improved over the past years. According to UNICEF, over a billion people have gained access to improved sanitation facilities and drinking water over the past 15 years (UNICEF 2005). While it is unclear exactly how much these improvements have contributed to reducing diarrheal deaths among infants and young children from 4.6 million in 1980 to roughly 2 million in 2000, it is likely substantial. According to one comprehensive literature review, improved water supply and sanitation reduces diarrheal morbidity by 25% and 32%, respectively (Fewtrell et al 2005).

“So I think it’s, frankly, a specious argument to think that the real target of [Ventria’s rice compounds] are likely to be the children in less developed countries where most of the problems you are identifying are a function of things that, actually, for a lot less money than that, could be fixed in terms of potable water and things like that.

Dr. David C. Magnus, Assistant Professor at the University of Pennsylvania’s Center for Bioethics in Philadelphia

One hundred years ago, diarrhea was a leading cause of childhood deaths in the United States, with seasonal epidemics in the summer months (CDC 1992). As noted above, diarrhea now kills approximately 300 children in the U.S. (CDC 2003), a striking reduction that is likely attributable in large part to improved sanitation and drinking water, particularly when one considers that the most effective treatment for diarrhea (oral rehydration therapy) was not introduced on a widespread basis until 1979 (Victora et al 2000).

Despite the progress achieved thus far, the World Health Organization reports that achievement of the Millennium Development Goal to halve the number of people without access to sanitation facilities by

2015 will require substantially greater effort and investments by the international community, particularly in underserved regions such as sub-Saharan Africa (WHO-UNICEF 2005, p. 5). Of course, water and sanitation improvements have numerous other benefits, for instance, reducing the incidence of other water-borne diseases, and freeing up time spent fetching water from distant sources for productive uses.

Better hygienic practices such as handwashing with soap can reduce the risk of diarrheal diseases by a surprising 42-47% (Curtis & Cairncross 2003; Fewtrell et al 2005). Permanent rather than temporary improvements in hygienic practices, however, are “difficult to achieve and require significant resources, persistency and capacity” (WHO-UNICEF 2005, p. 30). Successful strategies include incorporation of hygienic practices in school curricula, practical demonstrations, pretesting instructional materials for efficacy, and extensive community participation, all of which require trained personnel.

Another effective preventive measure involves treatment of water with sodium hypochlorite or other disinfectants, as well as safe storage practices.

Contamination of water with pathogens can occur at the source (17% of the world's population lack access to improved water supplies), but also through contamination of water from "improved" sources from seepage of contaminated run-off water, or unhygienic handling during transport or in the home. Fewtrell et al (2005) estimate that water treatment and safe storage reduce the incidence of diarrhea episodes by 39%. Because these measures are so cheap, they can produce huge benefits at little cost, and can also have the added benefit of providing opportunities for local entrepreneurs to produce and supply the requisite disinfectant or safe storage vessels (WHO-UNICEF 2005, p. 28).

7.2 Breastfeeding to Prevent and Treat Diarrheal Disease

7.2.1 Breastfeeding for prevention of diarrhea

It is well known that breastfeeding substantially reduces the likelihood of diarrheal disease (Setty 2006, p. 12), with exclusively breast-fed infants benefiting more than partially breast-fed infants. In one case-controlled study in Brazil, infants that were not breast-fed at all were 14 times more likely to die of diarrhea than those who received only breast milk; partially breast-fed infants were 4 times more prone to death from diarrhea than the exclusively breast-fed (Victora et al 1987). Breastfeeding is also known to protect against acute respiratory infections, such as pneumonia, and middle-ear infections; and is thought to reduce the risk of diabetes, asthma and childhood cancers (Setty 2006, p. 12). It is estimated that each year, improved breast-feeding practices⁴³ could save the lives of 1.3 million children (UNICEF 2006a).

“Factors that hinder higher rates of exclusive breastfeeding include the continued unethical marketing practices of some infant formula manufacturers, the decline of governmental and donor support for breastfeeding initiatives, the provision of infant formulas to countries in crisis situations, and the rise of AIDS and the risk of transmission of HIV through breast milk.”

Based on: Setty (2006), pp. 4, 10

Breastfeeding protects against diarrhea in numerous ways (Setty 2006, p. 12; Onnela 1997):

1) Reduced exposure to pathogens: By reducing the baby's exposure to contaminated drinking water, which is the leading source of diarrheal disease in many countries;

2) Protective compounds: By the presence of numerous compounds in breast milk that protect against diarrhea-causing pathogens:

a) Anti-infective substances like antibodies (e.g. secretory IgA), macrophages, lactoferrin and lysozyme that inhibit or destroy pathogens;

b) Anti-inflammatory factors, which reduce the mucosa's

response to infection and thus limit damage;

c) Growth factors (e.g. epidermal growth factor) that promote proliferation of beneficial bacteria that prevent the growth of gastrointestinal pathogens and foster the mucosa's renewal and healing after infection;

d) Immunomodulating agents that influence the growth and functioning of the infant's own immune system;

3) Optimal nutrition: By providing all the nutrients and water needed for the majority of infants for about the first 6 months of life, contributing to optimal nutrition and growth, which some consider perhaps the most important diarrheal-prevention effect of breastfeeding; and

4) Optimal pH: By helping maintain a low pH in the gut, which is favorable for the growth of bifidobacteria, which may play a role both in prevention of infection, and in modulating the clinical course of diarrhea.

⁴³ Defined as increasing the percentage of infants who exclusively breastfeed for the first 6 months of life to 90% levels.

It is important to emphasize here that breast milk provides numerous compounds beyond lactoferrin and lysozyme that protect against diarrhea or mitigate its severity. Thus, even if Ventria's recombinant versions of these natural substances do eventually prove to be effective and safe, any oral rehydration solution containing them would remain far inferior to breast milk. With all these benefits, it is not surprising that experts recommend continued breastfeeding during diarrheal episodes: "Breastfeeding should be continued at all times, even during the initial rehydration phases" (CDC 2003).

7.2.2 Breastfeeding for treatment of diarrhea:

Breastfeeding has even been recommended as a proactive treatment for diarrhea-suffering infants of mothers who do not breastfeed, or who do so only sporadically:

"The re-establishment of exclusive breastfeeding in partially breastfed infants below about 6 months of age is a new and promising approach in the treatment of diarrhoea." (Onnela 1997, p. 13)

Even mothers who have stopped breastfeeding can "re-lactate":

If an infant has stopped breastfeeding completely, it is usually possible for his mother to start breastfeeding again, that is to relactate ... Relactation is well recognized as a feasible intervention and is already practiced in many places. It is easier with younger babies, who are willing to suckle, and when the time since the mother stopped breastfeeding is shorter. However, it is possible at any age, and at any time. Infants who are unwilling to suckle can be encouraged to do so by using a breastfeeding supplementer or syringe to provide them with milk while they suckle at the breast. Mothers require skilled support and frequent encouragement. It usually takes from 1-3 weeks to restart the production of breast milk (Onnela 1997, pp. 13-14).

"Breastfeeding substantially reduces the likelihood of diarrheal disease, with exclusively breast-fed infants benefiting more than partially breast-fed infants."

Based on: Curtis & Cairncross (2003); Fewtrell et al (2005)

Breastfeeding has been promoted for many years, especially since the World Health Organization's and UNICEF's Innocenti Declaration in 1990. Based on data from 37 developing countries, the percentage of babies in the developing world who were exclusively breast-fed in the first six months of life rose from 34% in 1990 to 41% in 2004, a 21% rise (UNICEF 2006b). Clearly, there is much room for further improvement.

Factors that hinder higher rates of exclusive breastfeeding include the continued unethical marketing practices of some infant formula manufacturers, the decline of governmental and donor support for breastfeeding initiatives, the provision of infant formulas to countries in crisis situations, and the rise of AIDS and the risk of transmission of HIV through breast milk (Setty 2006, pp. 4, 10).

7.3 Oral Rehydration Solution (ORS)

Oral rehydration therapy is the administration of oral rehydration solution (ORS) consisting of essential electrolytes and carbohydrates in water to replace fluids and thus treat dehydration in infants suffering from diarrhea. Introduced in 1979, oral rehydration therapy has been vigorously promoted in developing countries over the past quarter-century and is widely credited with saving many lives. However, even proponents of ORS recognize that it is one of many factors that has helped reduce the number of diarrheal infant deaths from two- to three-fold since 1980 (Victora et al 2000).

For many years, the standard ORS solution was a formulation recommended in 1975 by the World Health Organization (WHO) and UNICEF. In 2002, WHO recommended an improved ORS with reduced osmolarity associated with less vomiting, reduced stool output and a reduced need for unscheduled intravenous infusions compared to the WHO's 1975 formulation (CDC 2003). ORS formulations based on cereals, in particular rice, tend to have lower osmolarity (CDC 2003, Table 3), and a number of studies have demonstrated that rice-based ORS reduces stool output and

duration of diarrhea relative to glucose-based (WHO) formulations (see Section 6.4).

Barriers to increased use of ORS for diarrhea include the cost of commercially available ORS (Meyers et al 1991), cultural practices, lack of parental knowledge, and medical professionals untrained in its use (CDC 2003).

8. COMPARISON OF MEASURES TO PREVENT OR TREAT DIARRHEA

Infant and early childhood deaths from diarrhea have been reduced by more than 2 million per year since 1980. This remarkable achievement is due to many factors, including improved sanitation facilities, safe drinking water supplies, training in improved hygienic practices, safe storage vessels for drinking water, use of disinfectants, improved breastfeeding, immunization against measles and widespread introduction of oral rehydration solutions. Nevertheless, an estimated 1.5 to 2.5 million children still die each year from diarrhea.

Given resource limitations in poor developing countries and aid institutions, both governmental and private, it is essential to direct funding to the most cost-effective options to further reduce diarrheal mortality. In general, of course, prevention is more cost-effective and superior in terms of averting human suffering than treatment (WHO-UNICEF 2005, p. 31). Of the measures discussed above, all but oral rehydration solutions fall into the prevention category. As we have seen, considerably more investment is needed to meet Millennium Development Goals for reducing the number of people without improved sanitation facilities by 2015 (WHO-UNICEF 2005, p. 5). Training in proper hygiene is personnel-intensive, but if pursued in a vigorous and sustained manner, with follow-up community support measures, it yields enormous benefits lasting far beyond the immediate diarrheal episode. Progress in improved breastfeeding is

“Barriers to increased use of oral rehydration solution (ORS) for diarrhea include the cost of commercially available ORS, cultural practices, lack of parental knowledge, and medical professionals untrained in its use. ... In this broader context, experimental modifications to existing oral rehydration solutions appear to be of lesser importance in reducing diarrheal morbidity.”

also essential for prevention (and in some cases, treatment) of diarrhea, yet governmental and donor support in this area has fallen off in recent years (Setty 2006, p. 4). Safe storage vessels and disinfectants are low-cost preventive measures with huge benefits, and can also spur development of local, small-scale industry.

Of course, more widespread introduction of existing oral rehydration solutions and education in their proper use is also important, especially in the shorter-term, since infrastructure improvements in sanitation facilities and drinking water supplies, and lasting behavior

change, take time. Increasing the use of ORS is mainly a matter of education and training to overcome inappropriate cultural practices and to inform parents and medical professionals about its advantages (CDC 2003).

In this broader context, experimental modifications to existing oral rehydration solutions appear to be of lesser importance in reducing diarrheal morbidity. Such modifications—which include supplementing ORS with zinc, amino acids, amylase-resistant starch from corn, partially hydrolyzed guar gum, and probiotics—were addressed in a few short paragraphs in the U.S. Centers for Disease Control’s comprehensive report on managing diarrhea in children (CDC 2003). CDC viewed all of these additives as experimental, requiring further validation, and devoted the majority of its report to practical measures on the proper and expanded use of existing oral rehydration solutions.

Given the acknowledgement of Ventría CEO Scott Deeter that foundation support would be needed to make their rHLf and rHLys-supplemented oral rehydration solutions available in poor countries, one should ask whether this would divert already inadequate funding from more cost-effective solutions, even assuming the compounds are eventually demonstrated to be safe and efficacious.

9. CONCLUSION

Ventria's recombinant human lactoferrin and lysozyme as additives to oral rehydration solutions must be evaluated from a number of perspectives. First, Ventria's proteins pose potential risks to infants that have not been adequately investigated, which may explain why the U.S. FDA has not signed off on their safety. It is also troubling that animal feeding studies with Ventria's proteins, promised in 2002, have either not been completed, or at least not published in PubMed-available publications, prior to the experiment on Peruvian infants. As we have seen, two mothers whose infants were involved in the Ventria-sponsored experiment report that their infants developed allergies after their treatment. Provision of appropriate medical care to these two young children, and comprehensive follow-up on all who were treated with Ventria's compounds, is crucial to detect and ameliorate any harm these infants may have suffered. Second, Ventria's improper reporting of the results of the Peruvian

experiment raise serious questions about whether the company's proteins offer any real benefits over existing rice-based ORS. Third, Ventria is clearly interested in selling the company's proteins for use in performance drinks and other food products for wealthy consumers in developed countries, raising the question of how committed the company is to alleviating diarrhea at all. Fourth, it is likely that any ORS with Ventria's recombinant proteins would be substantially more expensive than existing, already effective ORS. As we have seen, commercially available ORS is already beyond the means of some poor families whose infants suffer from diarrhea (Meyers et al 1991). Finally, a strong argument can be made that the best and most cost-effective way to decrease suffering and death from diarrhea is to redouble flagging efforts to prevent and treat diarrhea that have already been proven enormously successful.

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APPENDIX 1

*Copy of Letter from Ventria Notifying FDA That Pharma Rice (LF164)
May Contaminate the Food Supply, and Seeking FDA Approval of Contamination*⁴³

November 24, 2003

Dr. Robert Martin
Office of Food Additive Safety
Division of Biotech and GRAS Notice Review
HFS-255
Center for Food Safety and Applied Nutrition
Harvey W. Wiley Federal Building
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: BNF 082

Dear Dr. Martin:

Please find enclosed one (1) original, one (1) paper copy and one (1) electronic copy on CD of a Premarket Biotechnology Notice. This notice on “Safety, Compositional, and Nutritional Aspects of LF164 Transformed Rice” is submitted by Ventria Bioscience for evaluation by the Food and Drug Administration (FDA), Center for Food Safety and Nutrition (CFSAN) and the Center for Veterinary Medicine (CVM). This document is submitted under 21 CFR Part 192.25 and was prepared according to the FDA’s proposed rule for Premarket Notice Concerning Bioengineered Foods, Federal Register: January 18, 2001, Volume 66, Number 12 (66FR4706).

As Vice President of Clinical Development for Ventria Bioscience, I attest to the following:

Although Ventria Bioscience does not intend to commercialize LF164 rice for use in human food or animal feed, we have initiated the consultation process to demonstrate the safety of the plant material remaining following the extraction and removal of the new protein transferred into the plant. **In addition, we recognize the possibility of the inadvertent introduction of LF164 at low, adventitious levels into commercial rice varieties.** We are providing data and information to address the safety of the residual materials or adventitious levels of LF164 for inclusion in human food and animal feed.

⁴³ This letter and the accompanying submission to FDA was obtained by the Center for Food Safety in a Freedom of Information Act request (see Ventria BNF (2003) in Bibliography). LF164 = lactoferrin rice. **FDA did NOT grant Ventria’s request.** See Section 5.2 and Table 1 for fuller discussion. Bolded, underlined sentence not highlighted in the original.

The intended use of the bioengineered LFI64 rice is as a source of human recombinant lactoferrin. The lactoferrin protein will be used for the development of products that will be submitted through the appropriate regulatory pathways in compliance with all aspects of the Federal Food, Drug and Cosmetic Act.

Ventria Bioscience has collected and reviewed data and information to address the areas of concern in the proposed rule. Our data and information establish that the materials remaining following extraction of lactoferrin from LFI64 rice are not materially different in composition, safety or relevant parameters from the same materials following extraction of the parental rice. In addition, we have provided data on the safety of LF164 rice should it inadvertently be introduced into commercial rice at low, adventitious levels.

Ventria Bioscience agrees to make available to the FDA any relevant data or information not included in this submission during the process of evaluation of the PBN or for cause. The additional data will be made available to the FDA for review at Ventria Bioscience, 4110 N. Freeway Blvd, Sacramento, California 95834, during regular business hours or by paper or electronic copy to the FDA.

It is the view of Ventria Bioscience that the information in this PBN is appropriate for disclosure under the FOIA.

To the best of my knowledge, the information included in this notice is representative and a balanced presentation of all information available about LF164, both favorable and unfavorable. All data that are pertinent to the evaluation of the safety, nutritional or other issues associated with the use of the by-products or the adventitious presence of this bioengineered food have been included in this submission.

Sincerely,

Delia R. Bethell, Ph.D.
Vice President
Clinical Development

Enclosure

Cc: File