Pharmaceutical Rice in California

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Potential Risks to Consumers, the Environment and the California Rice Industry

SUBMITTED TO:

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Background

- Ventria Bioscience or its predecessor Applied Phytologics has been conducting outdoor field trials of genetically engineered, pharmaceutical-producing rice in California's Central Valley since 1997.
- Field trial acreage has increased from 6 acres in 1999 to 93 acres in 2003.
- Ventria's bid to begin commercial production on 120 acres in Southern California in 2004 was temporarily blocked by California and federal authorities, though the company may re-apply. Meanwhile, Ventria has received permission to grow still another test plot in the Central Valley.

Why California Authorities Need to Take Action

- The National Academy of Sciences, the food industry, and even the editors of a leading biotechnology journal all acknowledge that it is virtually inevitable that plant-made pharmaceuticals will contaminate the food supply when drug-bearing food crops such as Ventria's rice are grown out-of-doors.
- Federal regulators properly maintain a "zero tolerance" standard for drugs in food, yet they continue to condone outdoor cultivation of pharm crops rather than ban this hazardous practice.
- Ventria's rice-grown pharmaceutical proteins pose potential health threats to all consumers and environmental risks to the California environment (see Executive Summary).
- Discovery of pharmaceuticals in California's rice could have devastating consequences for the state's farmers. Quality-conscious export markets like Japan and South Korea would likely shun California rice, much as they shunned U.S. corn after the StarLink corn contamination debacle.
- Federal regulation of genetically engineered (GE) crops is seriously deficient. Field trials of GE pharmaceutical crops are not monitored to detect potential contamination of neighboring fields. These experimental pharm crops are not subject to mandatory health or environmental assessments, and no consideration is given to the likely economic impacts of contamination.

Experimental and Unproven

- Pharmaceutical crops such as Ventria's rice represent an *experimental* and *unproven* application of biotechnology. Not a single "plant-made pharmaceutical" (PMP) has been approved by the U.S. Food and Drug Administration (FDA), despite numerous clinical trials, industry promises, and field trials dating back to 1991.
- Meanwhile, over 100 biotech pharmaceutical proteins produced in contained and controlled fermentation facilities have been approved by the FDA and are already helping people in need.

Recommendations

We call on the California Department of Food and Agriculture (CDFA), the California Department of Health Services (CDHS), and the California Environmental Protection Agency (Cal-EPA) to conduct a thorough review of Ventria's pharmaceutical-producing rice. CDFA should examine the likely economic impacts of contamination on rice farmers. CDHS should subject Ventria's rice to a thorough health assessment, while Cal-EPA should review Ventria's rice for potential environmental impacts. However, because of the potential risks and the great scientific uncertainty surrounding this unproven application of biotechnology, we believe a prudent approach is called for to protect the interests of California consumers and farmers. Thus, we further urge California authorities to consider a moratorium on the cultivation of Ventria's pharmaceutical rice and other pharm crops.

EXECUTIVE SUMMARY

Background

Since 1997, Ventria Bioscience or its predecessor Applied Phytologics has been conducting outdoor field trials of rice varieties genetically engineered to produce pharmaceuticals in California's rice-growing Central Valley. These pharmaceutical compounds include artificial versions of the human milk proteins lactoferrin, lysozyme and alpha-1-antitrypsin. Proposed uses for the whole rice and/or extracted pharmaceuticals include poultry feed, treatment of diarrhea, infant food and topical wound treatment. Field trial acreage has increased from 6 acres in 1999 to 93 acres in 2003. Ventria's bid to begin commercial production on 120 acres in Southern California in 2004 was temporarily blocked by California and federal authorities, though the company may reapply next year. Meanwhile, Ventria has received permission to grow still another test plot in the Central Valley.

Experimental and Unproven

Pharmaceutical crops such as Ventria's rice represent an *experimental* and *unproven* application of biotechnology. Not a single "plant-made pharmaceutical" (PMP) has been approved by the U.S. Food and Drug Administration (FDA), despite numerous clinical trials, industry promises, and field trials dating back to 1991. While pharm crops have failed to provide useful drugs, over 100 biotech pharmaceutical proteins produced in contained and controlled fermentation facilities have been approved by the FDA and are already helping people in need.

Contamination is Inevitable

The federal government has a "zero tolerance" standard for PMPs in food. Yet scientists and agronomists agree that it is virtually impossible to keep PMPs from entering the food and feed supply when food crops are engineered to produce these compounds. The National Academy of Sciences warned of this risk in two recent reports. The editors of a leading journal in the field, *Nature Biotechnology*, recently compared growing drugs in food crops to a pharmaceutical manufacturer "packaging its pills in candy wrappers or flour bags or storing its compounds or production batches untended outside the perimeter fence." These concerns are validated by numerous episodes in which conventional crops and *certified seed stocks* have become contaminated with transgenic traits. In two incidents in 2002, pharmaceutical corn adulterated 500,000 bushels of soybeans in Nebraska and 155 acres of corn in Iowa; the adulterated soy was seized and destroyed, the corn burned, costing millions of dollars. Continued cultivation of Ventria's rice could have a similar outcome.

Deficiencies in Ventria's Cultivation Protocol

Ventria's pharmaceuticals could contaminate food-grade rice through transport of seeds in the guts of birds, flooding, "volunteer" pharm rice sprouting from unharvested seed, pollen dispersal by bees or in high winds, or human error in transport and processing. Ventria reportedly has not adequately explained how it will prevent birds from spreading its rice, what constitutes proper disposal of rice plants, or whether nearby growers will be notified.

Potential Human Health Impacts

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, human lactoferrin "might not be a successful therapeutic agent for *H. pylori* and, indeed, could intensify the infection." The possibility of aggravated infections is a potential risk from the contamination of food rice by lactoferrin that argues against growing this rice outdoors.

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 a third characteristic, structural similarity to a known food allergen, lactoferrin from cows. These allergenic properties may explain why noted food allergist Steve Taylor stated that FDA regulations "will have to be rethought before rice-grown lactoferrin ... can be approved for production."

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 immune system responses. These immune reactions can deactivate the pharmaceutical, and in some cases also deactivate the body's natural version of the protein, resulting in autoimmune disorders. Careful study is required to determine whether rice-expressed lactoferrin or lysozyme could cause such potentially dangerous reactions.

Amyloidosis and Mutant Proteins

Certain mutations to human lysozyme have been associated with a condition known as heredity amyloidosis. Although it is unknown whether consumption of these mutant proteins could cause amyloidosis, the available evidence suggests that Ventria has not adequately examined its rice-expressed lysozyme to rule out these or other mutations.

Potential Environmental Impacts

Ventria's rice-produced pharmaceuticals have antibacterial and antifungal properties. If these traits are passed to related weed species such as wild and annual red rice, they could lend these weeds a fitness boost, promoting their spread. These weed species, as well as contaminated food-grade rice that sprouts in subsequent years from unharvested seed, could harbor these pharmaceutical traits and thus serve as a "genetic bridge" to pass the traits back to food-grade rice in the future.

Economic Impacts of Adulteration

Any adulteration of food rice with Ventria's pharmaceuticals would likely lead to a decline or even an end to rice exports to Japan and other quality-conscious export markets. Since the U.S. has a zero tolerance standard for PMPs in food, adulterated rice, like StarLink corn, would be excluded from the domestic market as well.

State Action Needed Due to Loopholes in Federal Regulation

Federal regulators have a fundamentally contradictory policy. While they properly ban even trace amounts of plant-made pharmaceuticals in food or feed, they allow open-air cultivation of Ventria's crops, virtually ensuring contamination of rice meant for food and feed use.

- *FDA:* The FDA does not regulate Ventria's pharm rice in the field, and does not consider the potential human health impacts of exposure to these pharmaceuticals as contaminants in food.
- *EPA:* The EPA has not assessed Ventria's pharm rice despite evidence that its pharmaceutical proteins have pesticidal properties and could disrupt soil ecology.
- *USDA:* Though USDA has authority over Ventria's pharm rice in the field, it has not done a single environmental assessment to determine whether Ventria's pharm traits are spreading to food-grade rice or related weed species, nor has it examined the potential for a noxious weed risk from the spread of Ventria's traits.

Introduction

Since 1997, Ventria Bioscience or its predecessor Applied Phytologics has been conducting outdoor field trials of rice varieties genetically engineered to produce pharmaceutical proteins in California's rice-growing Central Valley. These proteins, which are generated in and extracted from grains of rice, include artificial versions of human lactoferrin, lysozyme and alpha-1-antitrypsin. In 2003, Ventria was authorized by the U.S. Department of Agriculture (USDA) to grow 93 acres of pharmaceutical rice in the Central Valley. In 2004, the company's bid to grow 120 acres of pharm rice in Southern California was rejected; on May 13th, 2004, Ventria was granted permission to grow one acre in the Central Valley under permit # 03-365-01R.[1]

While over 100 biotech pharmaceutical proteins produced in contained and controlled fermentation facilities have been approved by the FDA and are already helping people in need, pharmaceutical crops such as Ventria's rice represent an ***experimental*** and ***unproven*** application of biotechnology. Not a single "plant-made pharmaceutical" (PMP) has been approved by the U.S. Food and Drug Administration (FDA), despite several clinical trials, numerous industry promises, and field trials dating back to 1991. Federal regulators frankly admit that they are treading new ground. The comment of the FDA's Michael Brennan at a conference on PMPs four years ago is still applicable today:

> *"And I think to be honest, the FDA is used to applying regulations to manufacturing plants, but not to plants used for manufacturing. So a lot of this is new to us as well, and that's why I won't be able to answer any questions at the end!"* [2]

As we discuss below, contamination of conventional rice by Ventria's pharm rice appears inevitable. This report details a number of serious concerns and unanswered questions regarding the potential human health, environmental and economic impacts of Ventria's pharmaceutical rice. These concerns have not been adequately addressed by the U.S. Dept. of Agriculture (USDA), the Environmental Protection Agency (EPA), or the FDA. Therefore, we call on the California Department of Food and Agriculture (CDFA), the California Department of Health Services (CDHS), and the California Environmental Protection Agency (Cal-EPA) to conduct a thorough review of Ventria's pharmaceutical-producing rice to address these concerns. CDFA should examine the likely economic effects on California rice growers should Ventria's traits be discovered in food-grade rice. CDHS's Division of Environmental and Occupational Disease Control and Division of Food, Drug and Radiation Safety could address the potential human health impacts of Ventria's pharmaceutical traits as contaminants in food rice, while Cal-EPA should carefully review the possible environmental impacts of these pharm crops.

Because of the potential risks and the great scientific uncertainty surrounding this unproven application of biotechnology, we believe a prudent approach is called for to protect the interests of California consumers and farmers. Thus, we further urge California authorities to consider a moratorium on the cultivation of Ventria's pharmaceutical rice and other pharm crops.

Contamination is Inevitable

There is a fundamental contradiction in the federal government's policy on pharmaceutical-producing food crops. While the government properly maintains a zero-tolerance standard for contamination of food with plant-made pharmaceuticals, it nevertheless permits them to be grown outdoors in the direct vicinity of food-grade crops of the same species, posing a high risk of contamination.

The zero tolerance standard currently in force for pharm crop residues in food or feed is unlikely to be changed because zero tolerance is strongly supported by the powerful food industry. The National Food Processors Association demands "no use of food or feed crops for plant-made pharmaceutical production without a '100% guarantee' against any contamination."[3] The Grocery Manufacturers of America also demand zero tolerance: "Anything less than 100% containment also will subject all participants in the drug development efforts—from farmers to pharmaceutical companies—to potential liability for bodily injury to consumers..."[4]

But is 100% containment of food crops engineered to produce drugs likely or even possible? Numerous authorities have made it perfectly clear that it is not. Two committees of the National Academy of Sciences have warned of the high risk that pharmaceuticals from pharm crops will contaminate the food supply.[5] Leading agronomists such as Dirk Maier of Purdue University have made the same point.[6] A leading journal in the field, *Nature Biotechnology*, has published two editorials on this theme,[7] asking whether pharm crops are "...really so different from a conventional pharmaceutical or biopharmaceutical manufacturer packaging its pills in candy wrappers or flour bags or storing its compounds or production batches untended outside the perimeter fence?"

Contamination of human foods with plant-made pharmaceuticals can occur through dispersal of seed or pollen. Wildlife, especially waterfowl, can transport seed for long distances, as can extreme weather events such as floods or tornadoes. Harvesting equipment can carry seed residues to conventional fields, seeds can be spilled from trucks, or unharvested seeds can sprout as volunteers amid the following year's crop. Cross-pollination occurs at considerable distances in high winds or by insect, even with self-pollinating crops such as rice.

That these are more than theoretical concerns is abundantly demonstrated by a growing list of transgene contamination incidents in other crops. In early 2000, at a USDA/FDA-sponsored meeting on pharm crops, Chris Webster of Pfizer stated that: "We've seen it on the vaccine side where modified live seeds have wandered off and have appeared in other products."[8] In the same year, StarLink corn, approved only for use as animal feed, was found contaminating the entire food chain, from processed foods to grain to seed stocks.[9] Despite massive efforts to eliminate it, residues of StarLink continue to be found in the corn supply even today, over 3 years later.[10] In 2001, a variety of GE canola unapproved for sale to Canada's major export markets was found in commercial canola, leading to recalls of thousands of bags of seed and the incineration of some 10,000 acres of the unapproved GE canola variety. In 2002, ProdiGene, Inc. allowed its pharmaceutical corn to contaminate half a million bushels of soybeans in Nebraska and 155 acres of corn in Iowa.[11] The adulterated soybeans and corn had to be destroyed. In 2003, wheat grown in the U.S. was found to be contaminated with biotech crops. Supposedly conventional tomato seeds were unwittingly sent around the globe for seven years until transgene contamination was detected in late 2003, ironically, by UC Davis researcher Nicholas Ewing, who was conducting

research on pharmaceutical-producing tomatoes.[12] The Union of Concerned Scientists recently demonstrated widespread low-level contamination of conventional corn, soy and canola *certified seed* stocks with commercialized transgenic traits.[13] These findings were anticipated by authorities like Walter Fehr, Director of the Office of Biotechnology at Iowa State University, who was quoted as saying that transgenic contamination of even breeder seed stocks of corn and soy "happens routinely."[14] When certified and even *breeder* seeds, whose cultivation is subject to extraordinary gene confinement measures, become contaminated, it becomes impossible to believe in 100% containment of pharm genes, no matter how stringent the gene confinement measures that are applied (including geographic isolation).[15]

Ventria's Rice Protocol Will Not Prevent Contamination

Nothing in Ventria's draft protocol for cultivation suggests that it will achieve 100% containment of its pharmaceutical rice. According to the *Sacramento Bee*, Ventria's protocol:

> *"...is light on some details, including how Ventria will prevent birds from spreading its rice; what constitutes "proper" disposal of rice plants; and whether the company will notify nearby growers."* [16]

The lack of detailed plans to prevent birds from spreading the pharm rice is particularly disturbing. California's Central Valley is one of the most important wintering areas for waterfowl in North America. Viable seed are known to pass through the gut of many waterfowl species, making waterfowl effective dispersal agents for many wetland plant species, including rice.[17] The same study also found that mallard ducks could transport viable seeds for up to 1400 kilometers, about 870 miles.

Ventria's draft protocol also does not deal with the possibility of seed dispersal through flooding. Ventria has grown its rice in the Central Valley, for instance in Sutter County (2001)[18] and Butte County (1997).[19] Historical records show that floods of various magnitude occur not infrequently in the Sacramento Valley.[20] Such flooding would carry pharm rice an indeterminate distance from its original field.

Ventria's recent switch from a two-year to one-year fallow period following cultivation of its pharm rice[21] means a greater likelihood of pharm rice volunteers contaminating a commercial rice crop grown subsequently on the same field.

The 100-foot isolation distance from food-grade rice stipulated in the permit conditions for cultivation of Ventria's rice may not be adequate to prevent cross-pollination. Rice pollen may be able to move up to 360 feet from its source in 22 mph winds, and it has been shown to travel at least 126 feet in 5.6 mph winds. Wind speeds in the Sacramento Valley often exceed 22 mph, and could result in even greater pollen dispersal.[22] Bees can also cross-pollinate rice plants, and rice breeders have observed that the out-crossing rate increases in the presence of honeybees.[23]

Finally, one press report suggests that Ventria may have violated its 2003 USDA permit by growing its pharm rice "within 100 feet of rice intended for human and animal food."[24] USDA established this mandatory isolation distance for plantings of pharmaceutical rice in 2002,[25] and has

confirmed that it applies to Ventria's 2004 pharm rice trial.[26] If this report is true, it casts further doubt on Ventria's ability to keep its pharm rice from contaminating food-grade rice.

In light of the expert testimony and history of contamination cited above, and given the deficiencies in Ventria's draft protocol, we should assume that contamination of food-grade rice with Ventria's pharmaceutical rice, either through cross-pollination, inadvertent seed movement, or human error, is inevitable.

Potential Human Health Impacts

If contamination is inevitable, then the potential human health impacts of exposure to Ventria's pharmaceutical proteins becomes a serious question. While some might argue that consumers' exposure to these proteins from contaminated rice would be at levels too low to be of concern, there is little basis for such assertions. First, the federal government does not monitor for contamination, so any statements about the level of actual or potential contamination of food are speculative, not science-based. Secondly, Ventria is generating extremely high levels of its pharmaceutical proteins in rice, up to 1% by weight of the rice grain, equivalent to 40-50% of the grain's soluble protein.[27] Food rice contaminated with Ventria's pharmaceutical genes could generate equally high levels. Finally, there has not been adequate study to determine what levels of these proteins might cause human health impacts, though we do know that allergies and immune system disorders (see below) in general can be triggered by extremely low levels of immunogenic compounds.

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 polymorphonuclear neutrophils, which circulate in the bloodstream. Accordingly, one of lactoferrin's chief roles is to fight microbial infection. The main weapon in lactoferrin's pathogen-fighting arsenal is 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.[28]

Several of Ventria's proposed uses for rice-derived lactoferrin are based on this antimicrobial property, including treatments for bacterial-induced diarrhea and topical infections, as well as (partial) replacement of antibiotics in poultry feed.

As so often in nature, however, closer examination reveals a more complex state of affairs. Microbes have developed several 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 in this latter class include *Helicobacter pyloris* (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.[29] *Trichomonas vaginalis*, a protozoan responsible for genital disease in both women and men, can also extract iron from lactoferrin.

According to Dr. Eugene Weinberg, therapeutic use of human lactoferrin could stimulate growth of such pathogens, resulting in an "adverse response." Weinberg notes that human lactoferrin "might not be a successful therapeutic agent for *H. pylori* and, indeed, could intensify the infection." The gut bacterium *H. pylori* is implicated in causing ulcers, chronic gastritis and certain forms of stomach cancer. Thus, if food rice were to be contaminated with Ventria's lactoferrin, consumers of this rice who happened to have *H. pylori* infections in their guts could find those infections, and their associated conditions, exacerbated. This potential risk deserves careful evaluation. 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."[30]

Rice-derived recombinant human lactoferrin and lysozyme are not identical to native human lactoferrin and lysozyme

Ventria and its collaborators often refer to its recombinant proteins as if they were the human milk proteins lactoferrin and lysozyme. 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; 2) Post-translational processing, a function of the host organism. One form of post-translational processing is glycosylation, or attachment of carbohydrate groups to the surface of the protein. Animals and plants attach different types of carbohydrate groups to proteins. It has been demonstrated that recombinant, rice-expressed human lactoferrin and alpha-1-antitrypsin are glycosylated differently than their native counterparts in humans.[31] The author notes that the latter difference may affect the recombinant proteins' stability. Any increase in stability would be a warning flag, as digestive stability is a characteristic of food allergens (see "Allergenicity" below).

The recombinant versions might also have different primary amino acid sequences than the native human proteins because genetic engineering sometimes results in integration of a fragmented or otherwise disrupted transgene into the plant's genome (i.e. total genetic material).[32] In studies listed on Ventria's website, company scientists compared only the N-terminal sequences of the human and recombinant proteins rather than the full sequences.[33] 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. 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. Overall, the equivalence testing conducted by Ventria scientists and its collaborators in studies listed on Ventria's website and in its comprehensive patent[34] does not meet standards established by this EPA Scientific Advisory Panel in a similar context.[35] There is no mention of tertiary structure comparisons, so the recombinant and natural versions could differ in conformation as well.

Thus, researchers have found clear differences in glycosylation between human lactoferrin and its rice-expressed counterpart that could cause the latter to differ from the former in stability or allergenic properties. Rice-expressed lysozyme and lactoferrin may differ in amino acid sequence from their native human counterparts. The extent of the differences, as well as the potential human health implications, should be examined.

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.

Allergenicity

Any novel transgenic protein bears close scrutiny as a potential allergen. According to Bo Lönnerdal, a scientist at the University of California, Davis, recombinant, rice-expressed lactoferrin and lysozyme are stable to digestion and heat,[36] two properties widely regarded as characteristic of food allergens.[37] Digestive stability is particularly pronounced in infants, whose guts secrete less pepsin and are less acidic (pH 4-5) than adult guts (pH 1-3). A material safety data sheet on rice-expressed human lysozyme states that: "Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals."[38] Ventria's lactoferrin has a third characteristic of food allergens: significant amino acid sequence homology to a known human allergen, bovine lactoferrin, an allergen found in cow's milk.[39] These allergenic properties may explain why noted food allergist Steve Taylor stated that 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..."[40]

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.[41] In these cell culture production systems, a human gene encoding a medically useful protein such as insulin is spliced into bacteria 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 sub-

ject 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."*[42]

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."*[43]

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, admittedly more speculative, 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."*[44]

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.[45] 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.[46] 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."*[47]

Lactoferrin expert Dr. Eugene Weinberg agrees: "... an important potential hazard of therapeutic use of hLf [human lactoferrin] in human patients is possible induction of an antibody response."[48] 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.

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 then 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."[49] 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 autoaggregates 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 presumably is 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.

FDA Fails to Consider Unintended Exposure

The inevitable contamination of food-grade rice with Ventria's recombinant proteins raises the question of unintended exposure, which is not even considered by our federal regulators. The

FDA plays virtually no role in pharm crop regulation unless a company, often after 5 to 10 years of outdoor field trials, reaches the clinical trial stage. To the limited extent that FDA may exercise authority in the field, its oversight will be focused on preventing contamination of the pharm crop, not on preventing pharm crop contamination of the food supply.[50] Because the Central Valley is a major rice-growing region, widespread contamination of food-grade rice and exposure of some people to at least low levels of the experimental pharmaceuticals is entirely conceivable, especially given the gene containment lapses described above. Of course, allergy-prone infants and young children as well as adults could be exposed unintentionally through food contamination. One would think that the issue of unintended exposure of the population to untested, potentially hazardous novel proteins would have been dealt with by now, over three years after StarLink corn massively contaminated the food supply and potentially caused food allergies in the exposed population, but such is not the case.[51]

Potential Environmental Impacts

Ventria's pharmaceutical rice varieties could also have negative environmental impacts, such as creation of hardier weeds, damage to non-target insects, and/or disruption of soil ecology. Experimental cultivation of Ventria's pharmaceutical rice in the Central Valley since 1997 has provided the opportunity for recurrent gene flow to two related weed species as well as to food-grade rice. Wild rice *(Oryza rufipogon)* is a federally listed noxious weed that has been found in California in the past, and gene flow between cultivated rice (*Oryza sativa*) and wild rice is well known.[52] Annual red rice (also *O. sativa*) was recently identified in the northern Central Valley's Glenn County, by some accounts in a field grown from certified seed, and is considered a "serious risk to the California rice industry."[53] The increasing scale of Ventria's field trials— from 6 acres in 1999 (the first year for which acreage is reported) to 93 acres in 2003—increases the risk of gene flow and effectively reduces the level of governmental oversight. We are not aware of any testing to determine whether gene flow has occurred into weed species, but believe such monitoring should be required, with the results made publicly available.

Most importantly, Ventria's pharmaceutical traits may confer a fitness boost on contaminated cultivars or weeds, creating or exacerbating a noxious weed risk. Three of these substances—lysozyme, lactoferrin and alpha-1-antitrypsin—have antibacterial, antifungal and/or insecticidal properties. Recombinant human lysozyme expressed in transgenic tobacco has been shown to confer enhanced resistance to the fungus *Erysiphe cichoracearum* and the phytopathogenic bacterium *Pseudomonas syringae pv. tabaci*.[54] Carrots transformed to express recombinant human lysozyme exhibit enhanced resistance to the carrot pathogens *Erysiphe heraclei*, a fungus causing powdery mildew, and Alternaria dauci, a pathogen causing leaf blight.[55] Alpha-1-antitrypsin is a serine protease inhibitor, a class of compounds being tested in many plants as plant insecticides, and some members of which also cause pancreatic damage in animals upon medium-term oral exposure.[56] It is possible that these same proteins will lend rice resistance to similar rice pathogens or insect pests. If these traits are transferred to red rice or wild rice, they may confer a fitness boost to these weedy species, enhancing their survival and making these already noxious weeds still more difficult to control. Crossing with cultivated rice would likely create hardier volunteers, which could also become more difficult to control. These hardier weeds or volunteers could then serve as a genetic bridge or reservoir to transfer the traits back to cultivated rice.[57]

Transgenic proteins can also "leak" from plant roots in a process called rhizosecretion, even when the plant has not been engineered for this purpose. The Bt protein found in most Bt corn varieties has been shown to rhizosecrete into soil and survive in active form adhering to soil particles for at least 180 days.[58] Such rhizosecreted proteins may have significant impacts on soil microbiota. Lysozyme, which as noted above has been experimentally engineered into carrots and tobacco, has also been introduced into potatoes. Lysozyme-containing root exudates of potatoes engineered with the T4 lysozyme gene have been shown to kill 1.5 to 3.5 times as many bacteria (*B. subtilis* as indicator species) as the root exudates of a control line.[59] Rhizosecretion may not be an issue with Ventria's pharmaceutical rice, because the seed-preferred promoters used by the company direct most or all of the transgenic protein to the rice endosperm rather than the roots or other tissues.[60] Still, it would be advisable to analyze the rhizosphere (root-associated soil) of pharm rice to rule out the presence of Ventria's transgenic proteins as well as to detect any adverse impacts on soil microbiota due to unintended effects of the transformation process.

Despite these potential environmental risks, the EPA is not involved in the regulation of Ventria's pharm crops. This might seem surprising given that the EPA is the federal agency responsible for genetically engineered "plant-incorporated protectants," a category that includes antifungal, antibacterial and antiviral agents as well as insecticidal compounds, and considering that Ventria's pharmaceutical proteins possess one or more of these properties. There are two reasons that Ventria can bypass EPA regulation: 1) GE plants are regulated according to the *intended use* rather than the *intrinsic properties* of their transgenic proteins, and Ventria has not indicated that it intends its rice to be pesticidal; and 2) Even when the intended use *is* pesticidal, EPA regulation is triggered only by field trials of over 10 acres. The USDA has also failed to conduct any environmental assessment of Ventria's lactoferrin- and lysozyme-producing rice varieties. Thus, Ventria's two major pharm crops have not been subjected to any review for environmental impacts in the seven years of their cultivation in California.

Deficiencies in the Federal Regulatory System

The discussion above makes it clear that the State of California cannot rely on the federal government to ensure that the State's consumers, farmers and environment are protected from potential harm by Ventria's experimental plantings of pharm rice in California. Loopholes in federal regulation, many of which were pointed out by a National Academy of Sciences' committee two years ago,[61] can be summarized as follows:

1) Despite a proper zero tolerance standard for Ventria's plant-made pharmaceuticals in food and feed, USDA and FDA allow open-air cultivation of these crops in a rice-growing region, which will almost inevitably result in pharmaceuticals adulterating the food and feed supply.

2) The FDA does not regulate Ventria's pharm rice at the field trial stage, and will not regulate it at any stage if the intended use of the rice is production of a research chemical, a medical food,[62] or for export. Although FDA may ultimately review lactoferrin and/or lysozyme produced from Ventria's pharm rice, it will not consider the potential human health impacts of exposure to these pharmaceuticals as contaminants in the food supply.

3) The EPA has not reviewed Ventria's pharmaceutical rice despite evidence that its pharmaceutical proteins possess pesticidal properties and could harm beneficial organisms, create more aggressive weeds, or disrupt soil ecology, because the intended uses of these proteins are not pesticidal.

4) The USDA has not done a single environmental assessment of lysozyme- or lactoferrin-producing rice field trials, despite the potential for a noxious weed risk from transfer of these traits to related cultivars or weed species.

Regulatory Confusion: Pharmaceutical, Food Additive or Food?

We have referred to Ventria's rice as "pharmaceutical rice" for several reasons: 1) USDA field trial permits for this rice granted to Ventria's predecessor, Applied Phytologics, in the period from 1997 to 2001 listed the "pharmaceutical protein produced" phenotype; 2) The permit conditions stipulated by USDA for Ventria's field trials (e.g. 100-foot isolation distance) are those for rice engineered to produce pharmaceuticals and industrial chemicals, not those for field trials of other GE rice varieties; 3) Lactoferrin and lysozyme possess antimicrobial properties; and 4) Several of Ventria's proposed uses for its recombinant proteins—for instance, as additives to oral rehydration formula for treatment of severe diarrhea,[63] or as "topical treatment for bacterial infections"[64]—are explicitly medical in nature.

However, it now appears that Ventria's pharmaceutical proteins may have been reclassified as something other than pharmaceuticals. Consider the following facts. First, for the 2003 and 2004 field trials, USDA changed its designation of Ventria's products from "pharmaceutical proteins produced" to "value added protein for human consumption."[65] Secondly, Ventria reportedly initiated a voluntary consultation on its rice with the FDA in November 2003.[66] The voluntary consultation process is used for GE crops intended for general food use, and it falls far short of FDA's mandatory pharmaceutical review process. Finally, Ventria representatives have told the California Rice Commission that the company is seeking GRAS (Generally Recognized As Safe) status for its recombinant human lactoferrin and lysozyme from FDA.[67] GRAS status *exempts* a food additive from the food additive review process, which is similar in stringency to the FDA's pharmaceutical review process. When contacted, FDA officials refused to comment on how Ventria's rice and recombinant proteins are being regulated—as pharmaceuticals, food additives, GRAS food additives, GE food or otherwise.

This apparent attempt to reclassify Ventria's products from pharmaceuticals to "value-added proteins" is troubling. Ventria's recombinant proteins have pharmaceutical properties, proposed pharmaceutical uses, and they were once classified accordingly by USDA. As detailed above, they pose a number of potential health risks that have not been adequately investigated. In the interests of public health, they should be stringently regulated as pharmaceuticals. Anything less is unacceptable.

Finally, there is evidence that Ventria has already commercialized its rice-expressed lysozyme as a research chemical. The chemical supply house Sigma-Aldrich Inc. currently offers for sale "Lysozyme from human milk, recombinant, expressed in Rice min. 100,000 units/mg protein," as product number L1667 (see www.sigmaaldrich.com for details). Sigma-Aldrich does not state

the source of this product, but it is likely to be Ventria, given that Sigma-Aldrich is cited as a collaborator on Ventria's website (see www.ventriabio.com/collaborators/).

Sigma-Aldrich sells this lysozyme "[f]or R&D use only. Not for drug, household or other use."[68] Nevertheless, commercialization of an experimental GE plant-produced compound with pharmaceutical properties is troubling. According to the National Academy of Sciences, such commercialization provides additional incentive for large-scale plantings that increase the likelihood of gene containment lapses,[69] and hence food contamination. Another concern is conflict of interest. USDA oversight of GE crop field trials depends to a great extent on company reports filed with the USDA at the end of the trial, or annually for multi-year permits. Such reports are to include any adverse impacts of the experimental crop. Because self-reporting of adverse impacts to the USDA could entail revocation or non-renewal of the permit, and thus loss of profits, the company's duty to report such adverse effects is clearly in conflict with its financial interest.

Potential Economic Impacts

California authorities should also give serious consideration to the impact on both domestic and export markets should Ventria's pharmaceutical proteins be discovered in rice intended for food or feed. Within the U.S., the FDA's zero tolerance standard for plant-made pharmaceuticals in food would condemn such rice as adulterated, and hence unsaleable. The impact on exports would be even more serious. Approximately one-third of the rice produced in California is exported, primarily to countries that have restrictions on GE foods. In 2002, almost 65% of California's rice exports went to Japan, Taiwan and Korea, all of which require labeling and certification of GE foods. Another 15% of California's rice was exported to Turkey, which is poised to join the European Union and so will have to comply with the EU's strict laws governing GE food importation.[70] None of these important export markets has approved GE rice, much less pharmaceutical rice, for importation. Thus, pharmaceutical contamination of California rice could put at least 80% of the state's rice exports at risk.

The likely response of these GE-sensitive export markets to even low levels of pharmaceuticals in rice can be gauged by their actions in response to contamination of the U.S. corn supply with unapproved StarLink GE corn in 2000. According to the USDA, outstanding sales of U.S. corn to Japan at the end of 2000 were down about 21% from the previous year, and the gap had widened to 44% by mid-April 2001.[71] Japan turned to Brazil, Argentina, China and South Africa to make up the difference.[72] Corn exports to South Korea also experienced a decline.[73]

Japanese consumers have already voiced their concerns. A letter from Consumers Union Japan to the California Rice Commission dated March 27, 2004 stated: "We wish to inform you that if you approve Ventria's request, California's rice market in Japan will be seriously threatened."

Conclusion

As demonstrated above, there are many serious deficiencies in federal regulation of Ventria's pharmaceutical crops. These deficiencies expose California's consumers, farmers, and rice industry to potential human health, environmental and economic risks. It should also be kept in mind that the manner in which Ventria's products are regulated could well set a precedent for the regulation of future pharmaceutical crops in California and elsewhere. If stringent standards are not established now, it may well prove more difficult to give future trials of pharm crops the degree of regulatory scrutiny they merit. Therefore, we urge the California Department of Food and Agriculture, the California Department of Health Services and the California Environmental Protection Agency to conduct their own independent review of the human health, environmental and economic concerns posed by Ventria's rice, including those raised in this letter. In the interests of prudence, we further encourage California authorities to establish a moratorium on the open-air cultivation of pharmaceutical crops, especially food crops.

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