October 18, 2011

Ms. Martha E. Marrapese
Keller and Heckman LLP
1001 G Street, N.W.
Suite 500 West
Washington, D.C. 20001

Re: GRAS Notice No. AGRN 000-001

Dear Ms. Marrapese:

The Food and Drug Administration (FDA) is responding to the notice, dated November 11, 2010, that you submitted, on behalf of Resonant Biosciences, under FDA's Center for Veterinary Medicine (CVM) Pilot Program for substances generally recognized as safe (GRAS) added to food for animals (See 75 FR 31800; June 4, 2010). FDA's Center for Veterinary Medicine received the notice on November 11, 2010, filed it on December 22, 2010, and designated it as GRAS Notice No. AGRN 000-001.

The subject of your notice is chlorine dioxide. The notice informs FDA of the view of Resonant Biosciences, Inc., that chlorine dioxide is GRAS, through scientific procedures, for use as an ingredient in animal feed for food producing animals as a processing aid in the production of non-food grade and food grade ethanol with a maximum application rate of 55 ppm per batch.

FDA has evaluated the information that Resonant Biosciences, Inc. discusses in its GRAS notice as well as other data and information that are available to the agency. As discussed more fully below, the notice does not provide a sufficient basis for a determination that chlorine dioxide is GRAS under the conditions of its intended use in animal food.

Data and information that Resonant Biosciences, Inc. presents to support its GRAS determination

Resonant Biosciences describes the common name of the ingredient, conditions of use, specifications and product analyses, analysis of lots, physical description, method of manufacture, calculated residue levels in dried distillers grains (DDGs), and self-limiting levels of use. Resonant Biosciences provided an unpublished internal report to demonstrate that the technology could replace the current antibiotic addition for control of bacterial contamination with no negative effect on the ethanol fermentation process.

Resonant Biosciences stated that "chlorine dioxide is an oxidizing agent and broadspectrum antimicrobial agent. It is intended to control bacterial contamination that can grow under fermentation conditions and compete with the growth of the intended yeast, affecting the production of ethanol. The PureMash® chlorine dioxide technology used for generating the chlorine dioxide is the same as used for generating chlorine dioxide cleared under 21 C.F.R.

§§ 173.300(a)(l)(ii) and 173.300(a)(2) where an aqueous solution of sodium chlorate is treated with hydrogen peroxide in the presence of sulfuric acid, and the generator effluent contains at least 90% by weight of chlorine dioxide with respect to all chlorine species."

Resonant Biosciences stated that chlorine dioxide is the subject of numerous GRAS determinations by FDA. Resonant Biosciences noted that FDA review of Food Additive Petitions (FAPs) 4A4415, 0A4716, 4A4751, and Food Contact Notification (FCNs) 391, 445, 644 and 645 on chlorine dioxide (incorporated by reference) concluded that it rapidly degrades during use, and due to its degradation, chlorine dioxide per se does not raise any toxicology concern for the purpose of a GRAS determination.

Resonant Biosciences presented analytical results of chlorite and chlorate levels from the water effluent from its chlorine dioxide generating system, PureMash®; estimated the levels of chlorate, chlorite, sulfate and sodium salt residues in distiller grains through calculations; and projected the maximal exposure levels for some animal species to chlorite and chlorate also through calculation. To further support its target animal safety determination, Resonant Biosciences provided published acute-phase metabolism, distribution, elimination, or residue data of chlorate generated from beef cattle, growing pigs, broilers, and rats. Resonant Biosciences also provided data in which residual chlorate concentrations and other residual ions were analyzed in DDGs.

Resonant Biosciences presented microbial food safety information including a brief summary of the notifier's reasoning for why this use of chlorine dioxide does not pose a concern.

With regard to the toxicology aspect of human food safety, Resonant Biosciences discussed published toxicology studies as well as decisions by FDA, US Environmental Protection Agency (EPA), and the WHO Joint FAO/WHO Expert Committee on Food Additives (JECFA) pertaining to the safety evaluation of chlorine dioxide and anticipated residues in the DDGs from the use of chlorine dioxide.

Resonant Biosciences provided an evaluation of the GRAS status of chlorine dioxide based on these anticipated residues in the DDGs. The human food safety of residual chlorine dioxide and its various chlorinated species were discussed with the main focus on chlorite and chlorate. Resonant Biosciences provided a toxicological evaluation of chlorate. Among the published studies evaluated by Resonant Biosciences were absorption, distribution and metabolism studies in animals (cattle, rats, swine and broilers), acute toxicity studies in humans and animals, short-term and subchronic toxicity studies in animals (rats, dogs and monkeys) and humans, chronic toxicity/carcinogenicity studies in rats, developmental studies in rats and rabbits, and genotoxicity studies. A summary of these toxicity studies as well as some of the cited literature were provided.

Resonant Biosciences discussed the safety of chlorite based on decisions made by FDA and EPA. Resonant Biosciences stated that FDA has evaluated the safety data on chlorite in conjunction with the Agency's review of previous notifications and petitions relating to chlorine dioxide; the EPA Integrated Risk Information System (IRIS) reviewed the available literature on the toxicity of chlorite and established an acceptable daily intake (ADI) of 30 µg/kg bw/day. In addition, Resonant Biosciences stated that published literature has reported

that any chlorite that is consumed by animals, such as cattle, swine, and poultry, is not detected in the animal after consumption and is believed to be metabolized to chloride ion; consequently no dietary exposure to chlorite for human is expected as a result of the intended use of the notified substance.

Resonant Biosciences discussed the safety of sodium ions based on decisions made by FDA, JECFA and AAFCO. Resonant Biosciences indicated that sodium ions are GRAS based on GRAS listings for numerous sodium salts as direct food ingredients (sodium acetate 21 CFR 184.1721, sodium benzoate 21 CFR 184.1733, and sodium chloride in 21 CFR 182.1(a)). Sodium chloride is GRAS on a prior sanction basis as well under 21 U.S.C. 321(s). With respect to sodium sulfate, JECFA found no toxicity associated with this sodium sulfate to justify establishing an ADI for this substance. Sodium sulfate has an AAFCO listing as a mineral source under 57.109. Sodium sulfate as sulfuric acid is GRAS affirmed for direct addition to food under 21 CFR 184.1095; sodium sulfate is the sodium salt of sulfuric acid which, if ingested, will result in exposure to sulfate ions and sodium ions.

FDA's evaluation of the data and information in Resonant Biosciences' notice

FDA has the following comments regarding manufacturing chemistry:

1. Your notice does not adequately describe how the PureMash® generation method ensures that only 55 ppm of chlorine dioxide is the maximum amount used in the fermentor since chlorine dioxide can come into the system from other points such as the piping system.

2. Your notice does not describe how the UV spectrophotometer continuously monitors the fermentation system.

3. The October 24, 2008 internal report used to demonstrate the intended use of controlling bacterial contamination and the level of use of chlorine dioxide was not published and generally available to experts. The notice should include generally available information to support the intended conditions of use of the chlorine dioxide.

FDA has the following comments regarding target animal safety:

4. Resonant Biosciences did not specify in a clear and consistent manner the intended animal species/life stages that would consume the DDGs. For example, Resonant Biosciences refers to "food producing livestock" and "food producing animal;" however, the specific target animal species/life stages were not provided.

5. Resonant Biosciences failed to provide sufficient peer-reviewed and published safety, exposure, and absorption, distribution, metabolism, excretion (ADME) information directly supporting the safe use of the chlorine dioxide in the target animal species/life stages. A large part of the critical data relied upon by Resonant Biosciences was from a private source.

6. Resonant Biosciences did not address the presence/absence of a consensus of qualified experts of safety for the intended use in the target species/life stages. The following is a list of example publications that reported observations of *in vitro* or *in vivo* toxicity of chlorine dioxide, its metabolites, or reaction products in food-producing animals or established bioassay test systems, that were noted absent in Resonant Biosciences' comprehensive safety discussion. On page 31802-3 of the June 4, 2010 Federal Register notice, a GRAS notice should include a comprehensive discussion of any reports of investigations or other information that may appear to be inconsistent with the GRAS determination, and the basis for concluding, in light of the data and information submitted, that there is a consensus among experts that there is a reasonable certainty that the substance is not harmful under the conditions of use.

Elia AC, Anastasi V, Dorr AJ. Hepatic antioxidant enzymes and total glutathione of Cyprinus carpio exposed to three disinfectants, chlorine dioxide, sodium hypochlorite and peracetic acid, for superficial water potabilization. Chemosphere. 64(10):1633-1641, 2006.

Ferett D, Zerbini I, Ceretti E, Villarini M, Zani C, Moretti M, Fatigoni C, Orizio G, Donato F, Monarcas S. Evaluation of chlorite and chlorate genotoxicity using plant bioassays and in vitro DNA damage tests. Water Research 42(15):4075-4082, 2008.

Harrington RM, Shertzer HG, Bercz JP. Effects of chlorine dioxide on thyroid function in the African Green monkey and the rat. J Toxicol Environ Health 19(2):235-242, 1986.

Hayashi M, Kishi M, Sofuni T, Ishidate M Jr. Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. Food Chem Toxicol 26(6):487-500, 1988.

Ishidate M Jr, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M, Matsuoka A. Primary mutagenicity screening of food additives currently used in Japan. Food Chem Toxicol 22(8):623-636, 1984.

Orme J, Taylor DH, Laurie RD, Bull RJ. Effects of chlorine dioxide on thyroid function in neonatal rats. J Toxicol Environ Health 15(2):315-322, 1985.

Steffen C, Wetzel E. Chlorate poisoning: mechanism of toxicity. Toxicology 84(1-3):217-231, 1993.

Svecevicius G, Syvokiene J, Stasiunaite P, Mickeniene L. Acute and chronic toxicity of chlorine dioxide (ClO_2) and chlorite (ClO_2^-) to rainbow trout (Oncorhynchus mykiss). Environ Sci Pollut Res Int. 12(5):302-305, 2005.

VanWijk DJ, Hutchinson TH. The ecotoxicity of chlorate to aquatic organisms: A critical review. Ecotoxicol Environ Safety 32(3):244-253, 1995.

Yonkos LT, Fisher DJ, Wright DA, Kane AS. Pathology of fathead minnows (*Pimephales promelas*) exposed to chlorine dioxide and chlorite. Marine Environ Res. 50(1-5):267-271, 2000.

FDA has the following comments regarding human food safety:

FDA has evaluated the information in AGRN 000-001 as well as other available information pertaining to toxicology, microbial food safety, and residue chemistry. The notice does not provide a sufficient basis for a determination that chlorine dioxide is GRAS under the conditions of its intended use. This conclusion is based on questions regarding the impact on human intestinal flora and cancer risk.

7. Resonant Biosciences did not adequately assess the microbial food safety impact of any residues or metabolites of chlorine dioxide present in edible tissues of animals fed DG on the intestinal flora of human consumers of animal-derived food products. Specifically, Resonant Biosciences did not adequately answer the question whether the residues of chlorine dioxide or its metabolites would be microbiologically active against representative human intestinal flora for the following two reasons. Resonant Biosciences presented data from the study conducted by R.C. Anderson et al., to justify that chlorine dioxide and its metabolites would not be microbiologically active against human intestinal flora. However, the Anderson paper clearly states that bacterial levels of *E. coli* are reduced, and that levels of similar bacteria are similarly reduced, contradicting the claim that chlorine dioxide and its metabolites are not microbiologically active. Further the paper states that anaerobes and other "beneficial" gut microbes are not affected; however, the levels of other bacteria are not measured. Therefore, we have questions regarding the microbiological effect of other human intestinal flora, such as species of *Enterococcus, Lactobacillus*, or *Clostridium*, etc.

8. The GRAS notice is deficient with respect to the carcinogenic risk. The deficiencies include, but are not limited to: a) the carcinogenic risk assessment of human exposure to chlorate from the consumption of edible tissues is not published and generally available to experts and b) the carcinogenic risk assessment is not generally recognized by experts.

We have the following administrative recommendations regarding the notice:

9. The notice should include consecutive page numbers throughout the entire notice. A bibliography list at the end of the notice (instead of under each text page) should facilitate review of the submission.

10. The notice should clearly and consistently describe the intended conditions of use of chlorine dioxide. For example, the notice inconsistently refers to the use of chlorine dioxide to treat highly fouled fermentation water, fermentation apparatus and piping, and any process equipment in contact with the fermentation water such as heat exchangers, piping, and transfer lines.

Conclusions

FDA has evaluated the data and information in AGRN 000-001 as well as other available information. The notice does not provide a sufficient basis for a determination that chlorine dioxide is GRAS under the conditions of its intended use in animal food.

In accordance with the Federal Register notice announcing the CVM Pilot Program, a copy of the text of this letter responding to AGRN 000-001, and a copy of the information in this notice that conforms to the information described in your GRAS exemption claim is available for public review and copying via the FDA home page at http://www.fda.gov. To view or obtain an electronic copy of this information, follow the hyperlinks from the "Animal & Veterinary" topic to the "Products" section to the "Animal Food & Feeds" to the "Generally Recognized as Safe (GRAS) Notifications" page where the Animal Food GRAS Inventory is listed.

If you have any questions about this letter, please contact Dr. M. Thomas Hendricks at 240-453-6869 or by email at thomas.hendricks@fda.hhs.gov. Please reference AGRN 000-001 in any future correspondence regarding this submission. If Resonant Biosciences wishes to have FDA consider any new information that Resonant Biosciences submits regarding chlorine dioxide, the appropriate mechanism would be for the notifier to submit, in accordance with proposed 21 CFR 570.36, a complete GRAS notice. FDA would assign a new file number to a new notice regarding chlorine dioxide.

Sincerely,

Sharon A. Benz, Ph.D., PAS
Director
Division of Animal Feeds
Center for Veterinary Medicine