TEXAS A&M
HEALTH SCIENCE CENTER
COLLEGE OF MEDICINE

August 9, 2010

Dr. Robbin Weyant
Director, Division of Select Agents and Toxins
Centers for Disease Control and Prevention
1600 Clifton Road
MS A-46
Atlanta, GA 30333

**RE: Comments on the changes to the list of select agents and toxins**

Dear Dr. Weyant:

I am writing in response to the call for public comment on the current HHS list of select agents and toxins as part of the biennial review and re-publication of the list as was announced in the *Federal Register* Volume 75, Number 139 (July 21, 2010).

My name is Vernon L. Tesh. I am a Professor in the Department of Microbial and Molecular Pathogenesis at the College of Medicine, Texas A&M – Health Science Center in College Station, Texas. The institution is currently registered with the CDC DSAT Program and my laboratory is approved for the possession and use of a select agent. I am, therefore, aware of the HHS list of select agents and toxins, as well as with rules and regulations pertaining to the possession, use and transport of select agents and toxins.

I am writing to recommend that Shiga toxins be removed from the HHS list of select agents and toxins. When using the term Shiga toxins, I am referring to a genetically and functionally related family of bacterial proteins expressed by *Shigella dysenteriae* serotype 1 and certain serotypes of *Escherichia coli.* Shiga toxins may alternatively be referred to as "Shiga-like toxins", "Shiga-like ribosome-inactivating proteins", "verotoxins", or "verocytotoxins".

According to the information provided in the advance notice of proposed rulemaking and request for comments published in the *Federal Register*, it states: "The HHS Secretary considers the affect on human health upon exposure to the agent or toxin; the degree of contagiousness of the agent; the methods by which the agent or toxin is transferred to humans; the availability and effectiveness of pharmacotherapies and immunizations to treat and prevent illnesses resulting from the agent or toxin; the potential for the agent or toxin to be used as a biological weapon; and the needs of children and other vulnerable populations. With your

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indulgence, I would like to briefly comment on each of these factors used in the decision-making process.

*The affect on human health upon exposure to the agent or toxin*: The deleterious impact of ingestion of Shiga toxin-producing bacteria on human health is incontrovertible, as evidenced by multi-state outbreaks of bloody diarrhea in the United States caused by the ingestion of contaminated ground beef products, unpasteurized fruit juices, and spinach. Thus, "wildtype" or clinical isolates of toxin-producing *S. dysenteriae* or *E. coli* are excellent toxin delivery systems, colonizing the large intestine, even after ingestion of doses as low as 10 to 100 bacteria. Once the organisms have colonized the gastrointestinal tract, they produce Shiga toxins. Mechanisms regulating toxin gene expression, toxin assembly and secretion, and toxin transport across the human intestinal epithelial barrier are poorly understood. Colonization and toxin production lead to bloody diarrhea. The transport of the toxins across the gut may lead to the hematogenous spread of the toxins and the development of systemic complications, such as acute renal failure and central nervous system abnormalities (seizures and paralysis). The diarrheal disease may be incapacitating, but is usually self-limited. Unfortunately, the extra-intestinal complications are life-threatening.

*The degree of contagiousness of the agent*: As outlined above, "wildtype" or clinical isolates of Shiga toxin-producing *S. dysenteriae* and *E. coli* are contagious, and person-to-person spread does occur. As discussed below, recombinant *E. coli* strains expressing Shiga toxin genes for the purpose of toxin purification are non-pathogenic. Shiga toxins are not contagious.

*The methods by which the agent or toxin is transferred to humans*: Naturally occurring Shiga toxin-producing *S. dysenteriae* and *E. coli* are excellent delivery systems for the fecal/oral transmission of the toxin-producing agents. Therefore, clinical isolates, (*i.e.,* organisms that have acquired the constellation of genes conferring: i) survival of passage through the acidic environment of the stomach; ii) the ability to invade or tightly adhere to the gut wall; and iii) production of Shiga toxins), should be considered potential bioterrorism threats and subjected to select agents and toxins rules and regulations. I am not aware of published studies examining the inhalational hazard of Shiga toxin-producing bacteria, nor am I aware of studies on the pathogenicity of the bacteria following injection into the bloodstream. However, I would note that Shiga toxin-producing bacteria do not cause bacteremia, *i.e.*, extra-intestinal disease is associated with the presence of toxin in the bloodstream rather than the presence of the bacteria.

In the late 1980's, a number of laboratories in the U.S. and elsewhere, reported the cloning and sequencing of genes encoding Shiga toxins from *S. dysenteriae* and *E. coli* clinical isolates. This led to the construction of recombinant *E. coli* strains designed to maximize the

production of Shiga toxins for the purpose of toxin purification. This development was essential in acquiring sufficient quantities of the purified toxins to perform experiments exploring the mechanisms by which the toxins damage cells *in vitro*, or cause disease in animals. The recombinant toxin-producing organisms were generally constructed in *E. coli* K12 strains. These strains are considered non-pathogenic. They are "deep rough mutants" which means they lack the genetic material necessary to encode a complete outer membrane. Furthermore, it was shown in experiments using human volunteers, that these strains did not stably colonize the human intestinal tract and did not cause disease. As an additional safety means, the toxin genes were placed under control of promoters which mediated optimal toxin expression only under certain conditions (for example, temperatures greater than human body temperature of $37^{O}$C). While I am unaware of published studies involving the ingestion of Shiga toxin-producing *E. coli* K12 strains by human volunteers, it is unlikely that these strains would cause disease. I am not aware of studies on the pathogenicity of these recombinant strains following inhalation or injection.

Shiga toxins are composed of two protein subunits. In order to be toxic, Shiga toxins must form a macromolecular structure composed of a ring of 5 individual B-subunits (referred to as the B-pentamer) in non-covalent association with a single A-subunit protein. This multi-protein structure is called the holotoxin. The B-pentamer is responsible for binding to a membrane glycolipid expressed on human cells. Following binding, the holotoxin is internalized, and the A-subunit mediates the toxic activity resulting in protein synthesis inhibition and cell death. In the absence of achlorhydria or pharmacological treatment to reduce stomach acid, oral ingestion of purified Shiga toxins will probably not lead to intoxication. The normal acidic environment of the stomach may cause denaturation of the holotoxin, so that functional Shiga toxins are not delivered to the intestinal tract. I am not aware of published studies examining the inhalational hazard of purified Shiga toxins. However, I note that a group of Japanese scientists did report that Shiga toxins are cytotoxic for pulmonary epithelial cells. The intrapulmonary administration of ricin, a plant toxin possessing the identical enzymatic activity of Shiga toxin A-subunits, was shown to cause massive pulmonary inflammation, renal damage and death in mice (Wong *et al., Am. J. Pathol.* 170:1497-1510 [2007]; Lindauer *et al., J. Immunol.* 183:1419-1426 [2009]). Given these data, it is prudent to assume that Shiga toxins may represent an inhalational hazard in the laboratory. I am not aware of studies on the means to deliver Shiga toxins *via* aerosols to large target populations. By far the most dangerous route of administration of purified Shiga toxins is by injection into the bloodstream. This is not a realistic means of widespread delivery.

*The availability and effectiveness of pharmacotherapies and immunizations to treat and prevent illnesses resulting from the agent or toxin:* There are no vaccines or effective therapeutic regimens to prevent or treat diarrheal and systemic disease caused by Shiga toxin-producing bacteria.

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*The potential for the agent or toxin to be used as a biological weapon*: In my opinion, clinical isolates represent a serious risk for dissemination *via* ingestion. The organisms can be cultured in large volumes using straightforward microbiological techniques and apparati. Small quantities of organisms in water or food supplies could cause outbreaks of bloody diarrhea which could be incapacitating for troops in the field, and may lead to death from extra-intestinal complications in 10% - 15% of cases. Risks from inhalational means are poorly characterized. Recombinant *E. coli* K12 strains expressing toxin genes used in toxin purification are not readily available and frequently require specific growth conditions or media to maximize toxin expression. As these strains are highly attenuated, they would be a poor choice for use as a bioterrorism agent. Purified toxins probably do not represent a major risk when ingested. The risk of harm following aerosolization is largely unknown, but the limited amount of published data available suggests it is a concern if effective delivery methods have been developed. Even using recombinant high-expression strains, the purification protocols for Shiga toxins require a working knowledge of biochemical techniques and the purification of large volumes would require large-volume fermentation and purification apparati. The greatest risk of working with purified Shiga toxins is accidental inoculation *via* needle stick or sharps.

*The needs of children and other vulnerable populations*: Shiga toxin-producing bacteria are capable of causing bloody diarrhea in healthy people. Epidemiologic studies suggest that children, the elderly, and immunocompromised individuals are at increased risk for developing the life-threatening sequelae that may follow bloody diarrhea.

In summary, "wildtype" or naturally occurring strains of Shiga toxin-producing bacteria are excellent delivery vectors for a family of toxins that cause an incapacitating illness and may cause death in some cases. The inclusion of these strains as select agents seems prudent. Recombinant strains producing the toxins are possessed by only a handful of laboratories and are attenuated in virulence, making them poor bioweapons candidates. The complex molecular architecture of Shiga toxins limits the effectiveness of the purified toxins as bioweapons, and the toxins should, in my opinion, be removed from the list of select agents and toxins.

Thank you for the opportunity to comment of the biennial review and re-publication of the select agents and toxins list. Please feel free to contact me at the letterhead address or by e-mail at tesh@medicine.tamhsc.edu should you have additional questions or concerns.

Respectfully yours,

Vernon L. Tesh, Ph.D.
Professor, Microbial and Molecular Pathogenesis