

NADC Waste Water Disposal Evaluation

Report of the Scientific Review Panel, November 21, 2006

I. Preamble

The National Animal Disease Center (NADC) of the Agricultural Research Service (ARS), United States Department of Agriculture (USDA) and the City of Ames, Iowa, convened a scientific review panel to assess practices used at NADC to treat liquid waste generated from facilities where prion disease research is being conducted and review scientific information about decontamination of the agent causing prion diseases. (See Appendix 1) The Panel was convened after allegations were made by two NADC employees that wastes from NADC research facilities were not properly treated prior to discharge to the City of Ames waste water treatment plant, raising concerns about possible environmental contamination and prion disease spread. The Panel was tasked to identify acceptable prion decontamination methods, assess concerns raised about NADC's methods for prion decontamination, determine the risk posed to humans from possible environmental prion contamination, and provide scientifically sound approaches for corrective action that may be needed. The Panel was asked to prepare a written report to document its findings and present the report to the City of Ames, USDA, and the general public.

To carry out their responsibilities, the scientific review Panel members toured NADC's facilities and the adjoining USDA Animal and Plant Health Inspection Services (APHIS) National Veterinary Services Laboratories (NVSL), attended briefings by key NADC leadership staff, interviewed NADC employees, reviewed several relevant NADC records, and toured the City of Ames Water Pollution Control (WPC) plant. The Panel also held public deliberations on several occasions. This report summarizes the Panel's review findings and recommendations.

This Panel recognizes the unique research and facilities at NADC and strongly supports and endorses the research efforts at these laboratories. The findings of this Panel are intended to serve as recommendations for NADC to improve its waste handling procedures and to address potential concerns raised by the general public regarding waste disposal practices.

II. Introduction

A. Background

There are three USDA laboratories in Ames, Iowa, which are maintained as separate facilities. The ARS NADC is responsible for conducting basic research on animal diseases including the transmissible spongiform encephalopathies (TSEs). These TSEs include diseases such as scrapie (in sheep) and chronic wasting disease (CWD, in deer and elk), and transmissible mink encephalopathy (TME); there is a plan to initiate research on bovine spongiform encephalopathy (BSE, in cattle). NADC also engages in

work on host ranges of these TSEs. The USDA APHIS NVSL laboratories are focused on the diagnosis and tracking of diseases in the domestic livestock herd, including three prion diseases (scrapie, CWD and BSE). The USDA APHIS Center for Veterinary Biologics (CVB) is co-located with NVSL and is responsible for regulatory oversight for veterinary biologics. Collectively these three laboratories are referred to as the National Centers for Animal Health (NCAH).

In the spring of 2006, it came to the attention of animal caretakers at the NADC that the methods for managing potentially prion disease contaminated or exposed wastes differed from those used at the NVSL facility. The employees were concerned about the significance of these differences, and they felt that they were unable to obtain satisfactory explanations from NADC personnel. On May 4, 2006, they took the initiative to discuss their concerns with personnel from the City of Ames, IA. The concern was that wastes from animals challenged with prions and prion-associated materials were not properly treated prior to discharge to the City of Ames sanitary sewer system and the WPC plant.

On May 8, 2006, representatives from the City of Ames and USDA ARS met to discuss the concerns and to develop plans to address the issues. The USDA and the City of Ames agreed to convene an expert panel to review scientific information to evaluate these concerns.

The Panel includes individuals selected and approved by both USDA and the City of Ames. They are listed alphabetically:

Alwynelle (Nell) Ahl, PhD, DVM, Risk Analyst, Adjunct Professor, Michigan State University, East Lansing; Ermias Belay, MD, Medical Epidemiologist, Centers for Disease Control and Prevention, Atlanta; James Colbert, PhD, Environmentalist, Associate Professor, Iowa State University, Ames; Linda Detwiler, DVM, prion livestock disease specialist, Adjunct Professor, University of Maryland, College Park; Robert Ellis, PhD, Professor and University Biosafety Officer, Colorado State University, Ft. Collins; Fran Kremer, PhD, Senior Science Advisor, U.S. Environmental Protection Agency, Cincinnati; Katherine McMahon, PhD, Environmental Engineer, Assistant Professor, University of Wisconsin, Madison; Robert Rohwer, PhD, Director, Molecular Neurovirology Laboratory, Veterans Affairs Medical Center (a prion laboratory) and Associate Professor, University of Maryland, Baltimore.

B. The charge to the expert Panel

The City of Ames and the USDA ARS agreed to convene a Panel to evaluate four issues related to the handling and disposal of potentially prion-contaminated materials in waste water from the NADC. The issues to be considered are (1) the identification of scientifically accepted methods for effectively destroying prions in waste water; (2) assessment of the concerns raised regarding NADC's current and past methods for the destruction of prions in waste water; (3) determination of the risk posed to humans and the environment from the current as well as previous methods for the destruction of prions in waste water utilized at NADC; (4) recommendations for remediation, if needed,

to provide scientifically sound approaches for corrective action(s) that may be taken. (The official charge to the Panel is included as Appendix 1)

Because this report is to be released to the public, efforts were made to ensure that it is scientifically sound, and written and presented in a way that promotes public understanding of the facts. Every attempt has been made to provide this clarity and transparency.

C. Prion diseases

Transmissible spongiform encephalopathies (TSEs) also known as prion diseases are a family of invariably fatal central nervous system diseases in humans and animals. Naturally occurring prion diseases that occur in humans include kuru, Creutzfeldt-Jakob disease (CJD), variant CJD, Gerstmann-Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI). Prion diseases that affect animals include scrapie in sheep, goats and moufflon, bovine spongiform encephalopathy (BSE) in cattle, feline spongiform encephalopathy in domestic and zoo cats, ungulate spongiform encephalopathy in exotic zoo ruminants, chronic wasting disease (CWD) in deer, elk, and moose, and transmissible mink encephalopathy (TME) in mink. The nature of the causal agent(s) is still uncertain and controversial and highly debated. There are three primary theories of which the leading theory is that the agent is composed of an abnormal form of a host protein called a prion. This abnormal form of the prion protein is highly resistant to treatments (e.g., moderate heat, protein digesting enzymes, radiation, and formalin) that would inactivate typical proteins, as well as viruses or bacteria.

Prion diseases can be transmitted among the same species and in some instances across species. The best example of cross-species transmission of prion diseases is the transmission of bovine spongiform encephalopathy to humans causing variant Creutzfeldt-Jakob (CJD) disease. Since its first description in 1996, over 195 variant CJD cases have been reported worldwide with over 80% of the cases reported from the United Kingdom where the largest number of BSE cases was identified. Laboratory studies have demonstrated the existence of a species barrier reducing the efficiency of cross-species transmission of prion diseases. This species barrier may have helped to reduce the number of variant CJD cases resulting from BSE and may have protected humans from acquiring CWD from deer and elk and scrapie from sheep.

The BSE outbreak, which is known to have affected many European countries, North America, Japan, and Israel, is presumed to have spread via consumption of feed containing meat and bone meal from rendered BSE-infected carcasses. There is evidence that BSE infectivity survives the rendering process and could thus contaminate animal feed. There is strong evidence indicating that scrapie and CWD can be transmitted among their respective hosts upon exposure of the animals to contaminated environments. Hence, environmental contamination may play an important role in local maintenance of scrapie and CWD outbreaks. However, little is known about the persistence of TSE infectivity in contaminated waste water generated in abattoirs or prion disease research laboratories.

D. Scientifically accepted methods for destroying prions

TSE infectivity is notoriously difficult to inactivate. Established TSE disinfection methods are highly destructive to macromolecules or macromolecular function. Accepted physical methods are steam sterilization and incineration. Accepted chemical disinfectants are 1N (normal) or stronger sodium hydroxide (NaOH) and 5% or greater sodium hypochlorite (bleach). A commercial phenolic disinfectant, Environ LpH, has also proven highly effective as a TSE inactivant. Even these powerful inactivants can be defeated if challenged by whole tissue fragments, undiluted tissue macerates or tissue that has been fixed either by drying, or with aldehyde crosslinkers and perhaps other fixatives as well. Crosslinkers and fixatives stabilize proteins and other macromolecules against denaturation. The kinetics of inactivation for all four methods of disinfection are biphasic with a rapid destruction of the vast majority of the infectivity upon contact with the inactivant leaving a residual fraction that is resistant to further inactivation, or is inactivated at a much slower rate. The concentration of this resistant fraction is often at the limit of detection of the bioassay where only a few animals inoculated with the undiluted sample actually develop disease. Depending upon the starting concentration of the infectivity in the sample this amounts to a survival at the level of parts per million or less of the starting infectivity. There is growing indirect evidence that this refractory material derives its resistance, not from any intrinsically greater resistance to the inactivant by the surviving infectivity but rather because it has, at the level of parts per million or less, ended up in some physical microenvironment that has sheltered it from the inactivant. For example, if infectivity were embedded in an anhydrous (not in contact with water) particle (lipid micelle, fat globule, etc) it might be isolated from the surrounding environment of aqueous NaOH or hypochlorite. If this particle were dry and oxidized onto the hot surface of an autoclave, it might never see the hydrolytic effects of the steam and instead experience the autoclave as dry heat. These could be very rare structures and very rare events and still account for the very small fraction of the infectivity that escapes the inactivant. It is also important to realize that there is very little direct experimental evidence to support this mechanistic explanation for the surviving sub-fraction of the infectivity. However, there is direct evidence that well-dispersed aqueous suspensions of the infectivity give more complete disinfection than tissue macerates, and fixation by drying or crosslinking stabilizes the infectivity against otherwise effective inactivation procedures.

As a general principle, the application of multiple methods of inactivation using different mechanisms such as, physical and chemical methods, is generally more effective than the application of only one modality. This has been true for TSE agents where a combination of hydrolytic steam and alkali (alkaline hydrolysis) has been shown to sterilize TSE infectivity even in crude tissue. However, even with that process, care must be taken to make sure that the entire sample is contacted and digested by the process.

NADC operates a waste water pretreatment plant (WWPTP) that steam processes all waters directed to it prior to their discharge to the Ames sanitary sewer system (see section III.D.). For the task at hand, it would be reasonable to presume that if there is any residual survival of TSE infectivity from the WWPTP at the NADC it would likely be

associated with particles in the waste water system or be localized to material dried onto the head space in the waste water autoclaves.

III. Waste Water Flow and Treatment

A. Composition of waste

NADC research projects utilize several of the TSE agents (prions) for development of diagnostic tests as well as transmission and pathogenesis studies. In many of the experiments the agents are transmitted to a wide number of species and may be found in an array of tissues including blood as well as secretions and excretions.

Waste water is generated at several sites. In addition to the water itself, the discharge may contain extraneous materials from different NADC facilities as listed below.

Barns

The waste from the barns consists of water, urine, feces, secretions and excretions as well as feed which mostly would consist of pellets and occasionally hay. Straw is used occasionally and measures are taken in an attempt to prevent the majority of the bedding material from going down the drain.

Necropsy Building

The waste from the Necropsy Building consists of water, urine, feces, secretions, excretions, blood and small amounts of tissue. Although efforts are made to collect as much central nervous system tissue as possible, if some of it goes down the drain, it may contribute the greatest level of infectivity to the waste water. Recently, a procedure which includes pre-charging the drains with bleach has been initiated.

Laboratories

The waste from laboratories primarily consists of water but may contain aqueous based buffers and solutions and solids.

Procedures for handling most of the laboratory wastes are described in NADC TSE Standard Operating Procedures (SOP) 001, section 4 dated, 10/20/05. (See Appendix #2) Aqueous waste such as buffers which are considered non-hazardous chemicals are collected and treated with bleach (>20,000 ppm) or 2 N NaOH for at least one hour. In some cases, it is treated overnight. After treatment, the liquid is poured down the drain with excess water to dilute the chemicals and is discharged to the waste water pretreatment plant (WWPTP). Solvents are collected in hazardous waste containers labeled as containing TSE wastes. The final disposition of the chemically hazardous solvents is incineration by a contractor specializing in handling such wastes. Other solid wastes, such as gels, are incinerated on site.

B. Current source and flow of waste water

Waste water at NADC comes from research laboratories and barns, a necropsy facility as well as from the administrative offices, engineering shops, and buildings which house normal animals. The cloth laundry of personnel working with TSE infectivity is autoclaved at 132° C for 30 minutes before washing. Personnel performing necropsies wear disposable clothing which is then incinerated after use. The waste water may be routed directly to the Ames sanitary sewer system or to the WWPTP (see section III.D.) before going into the sanitary sewer system. A map which illustrates the flow from each of the buildings is attached. (See Appendix #3) The blue lines show waste water which flows directly into the Ames sanitary sewer system and the red lines show waste water going to the WWPTP. The bullet points listed below provide a listing of the primary sites and their route of discharge:

- NADC Building 1, which is at the front of Building 2, is comprised of administrative offices, conference rooms, computer server rooms, a cafeteria, and visual services offices. The waste water from this building goes to the Ames sanitary sewer connection.
- Buildings 10 and 11 at the north end of the campus are engineering shops and buildings 191-197 and 200 are used to house normal animals or equipment. The waste water from these buildings goes into the Ames sanitary sewer system.
- Building 154 is the Utility facility that goes directly into the Ames sanitary sewer. The waste water generated is from the sinks, toilets and other plant operations.
- Building 155 is the WWPTP.
- Within the primary facility (Bldg. 2) there are 36 laboratories of which the majority are BSL-2*¹, although a few are BSL-3. The waste water from the laboratories goes to the WWPTP facility. Waste from non laboratory drains within Building 2 (eg. toilets and sinks in the engineering sections) go directly to the city sewer.
- Buildings 3 and 4 (animal containment buildings), 5 (necropsy building), 6 (housing TSE-infected mice), 174 (TSE/BSE large animal building), 157 (truck decontamination building), 149 (animal holding facility) and animal buildings 108-137 (animal containment buildings) are connected only to the WWPTP.
- Buildings 230 and 231, which are used to house sheep infected with US strains of scrapie, are not connected to the sanitary sewer system or the WWPTP. Water in these drains goes to a tile that leads to a drainage ditch near the animal buildings. This is the same as the water that would run off of the concrete.

(Note: The Special Site Visit Report of Drs. Rohwer and Ellis were used to provide the specifics for part of this discussion; this visit was in addition to the one made by the entire Panel. A copy of that report can be found in Appendix 4.)

¹ Laboratories at NADC are designated as BSL-1, BSL-2 or BSL-3 depending on the level of hazard associated with the research. Work done in BSL-1 laboratories is of lowest safety concern. BSL-3 laboratories deal with the most hazardous materials; work with BSE infected tissues is restricted to BSL-3 facilities. All other TSE work can be performed in BSL-2 facilities.

C. Comparison of the present and future route of wastewater at NADC

- BSL-2 and BSL-3 animal areas-waste water is currently heat-treated and will be heat-treated in the new facility.
- BSL-3 laboratories- waste water is currently heat treated and will be heat treated in the new facility.
- BSL-2 laboratory waste water at NADC is heat treated at the point of generation. Liquid wastes with infectious agents are steam sterilized in autoclaves within the laboratories and are then discharged to the WWPTP.
- In the new facility, inactivated liquid wastes from BSL-2 laboratories other than those handling TSE agents will be discharged to the Ames sanitary sewer.

Currently, the TSE BSL-2 laboratory waste water is collected and chemically treated with bleach (>20,000 ppm) or 2 N NaOH for at least one hour prior to discharge to the WWPTP. Solvents from processes such as immunohistochemistry are collected as hazardous waste and are incinerated by a contractor specializing in handling the wastes. In the new facility chemical pretreatment will continue with heat treatment in the laboratory or at the WWPTP before discharge to the Ames sanitary sewer system. The decision has not yet been made as to where the heat treatment will take place.

D. Existing NADC WWPTP operation

Waste water other than that which is discharged directly to the city sewer as specified above is directed from the barns or laboratories where it was generated, into holding tanks at the WWPTP (see red lines on Appendix 3). It is then moved into smaller tanks for treatment.

Figure 1 depicts a schematic of the existing waste water pretreatment process at NADC. In 2005 and 2006, total waste water discharges to the Ames sanitary sewer ranged from 63,000 to 159,000 gallons per day, the majority of which originates from the WWPTP. Daily loads have reached as high as 252,000 gallons per day. (Information provided by the City of Ames.)

Wastewater entering the WWPTP is collected in a series of three 50,000 gallon storage tanks. Movement of the solids is facilitated by ¼ inch per foot slope to the tank and an air powered agitation system; water is drawn off the bottom of the tank. As long as the solids remain in the tank they present no risk. Large fragments that break away could present an increased resistance to inactivation due to a physical state that may protect the agent.

Stored waste water is next transferred to one of four heat treatment cook tanks (6,500 gallons each) that uses direct steam injection, at 250°F, at one atmosphere of pressure

with a batch retention time of 30 minutes. Each tank has one temperature probe located at the bottom in the front of the tank. Waste water is mixed in the tank by steam injection. (See Figure 1 for a cut-away diagram of the tank) Timing of the process is not begun until the tank has reached the required temperature. Computerized system controls help ensure the needed retention time at the required temperature.

Effluent from the heat treatment is passed through heat exchangers and diluted with tap water to cool the waste water to 150°F prior to discharge, to comply with the temperature requirements for the pretreatment permit.

E. Future NADC WWPTP operations

NADC has constructed a new waste water pre-treatment plant that will be operational tentatively March 2007 (Figure 2). This has been designed with increased automation for operational control, monitoring, and data logging. This facility will provide five 30,000-gallon storage tanks with a total capacity of 150,000 gallons. Both the wastewater storage tanks and cook tanks are vertically oriented, virtually eliminating any solids accumulation. There will be four cook tanks, 10,000 gallons each. This new WWPTP will have the operational flexibility to provide heat treatment up to 277°F, with a residence time of up to 60 minutes. The thermal treatment unit will be able to treat up to 150,000 gallons in 16 hours when using the current processing cycle of 250°F for 30 minutes. The operational plans are to run the new plant at 250°F for 30 minutes, as is currently done. There are four probes in each tank, one at the top, one at the bottom and two spaced in the middle.

F. Treatment at the Ames Water Pollution Control (WPC) Plant

Heat treated water from NADC is pumped into the sanitary sewer system of the City of Ames. It is further treated along with municipal sewage at the City's WPC Plant which is located at 56797- 280th Street, Ames, Iowa. The treatment process yields water and biosolids (sludge).

At the WPC Plant, digested (treated) biosolids (sludge) are stored in a lagoon for approximately four to six months prior to land application. Sludge is generally applied to land during the fall, though last year some was applied in the winter while the digesters were cleaned. Field application rates are limited by crop nutrient uptake rates. During calendar year 2005, application rates varied from 2.0 to 6.5 dry tons per acre. This was higher than in average years because of digester and lagoon maintenance activities. During average years, around 40% of the total solids entering the facility are ultimately land applied. This includes the screened "grit" (inorganic silt, fine sands that will not break down); this is mixed with lime and land applied on the surface. The majority of land applied solids are digested biosolids which are land applied by injection or surface applications. Approximately 3% of the solids are discharged in treated effluent and the rest are destroyed by microbial degradation. Around half of the land applied solids are not "volatile" meaning that they do not combust at 550°C, and are comprised mainly of

biologically stable compounds such as minerals. After treatment the water is discharged into the South Skunk River. Schematics of the Ames WPC Plant are shown in Figure 3.

IV. Hazard Assessment and Recommendations

A. Concerns regarding NADC's current/past methods for destruction of TSE infectivity

The concerns regarding the NADC's current and past methods for destruction of TSE infectivity are: 1) exposure of personnel to prion-containing material, and 2) contamination of the environment with prion-containing material which could expose humans, domestic animals or wildlife.

B. Risks to humans and the environment from the current and previous methods for destruction of TSE infectivity at NADC

The fate of TSE infectivity during municipal waste water treatment is currently unknown. The efficiency of transmission of TSE infectivity between species is uncertain. The Panel believes that the current state of knowledge is insufficient to conduct a meaningful quantitative risk assessment. Instead, the Panel chose to conduct a qualitative evaluation of risk to humans and the environment from TSE infectivity potentially discharged by NADC. The Panel's evaluation of the potential exposure of humans, domestic animals and wildlife is based on the best available information consistent with our charge, expertise and resources.

In concept, the NADC waste water pretreatment facility as designed and operated should be an effective process to inactivate TSE infectivity using heat as previously described. Provided that the pretreatment process is operated according to NADC current standard operating procedures, it should effectively inactivate infective material in the liquid stream to below what can currently be detected. The largest risk of downstream exposure is associated with particles in the waste, since prions adhered to the particles may not be as effectively inactivated by the steam heat.

In March 2007, the new pretreatment facility will provide improved automated control, monitoring, and data logging and minimize particle accumulation (vertical discharge).

In the unlikely event that a small amount of infectivity remains following the waste water pretreatment process, it would travel through the sanitary sewer to the City of Ames WPC plant. Of the total flow entering the WPC plant, between 63,000 - 159,000 gallons per day originate at NADC. This value includes all waste water leaving NADC in the sanitary sewer and constitutes about an average of 1.7% of the total sewage treated at the City of Ames WPC plant. Particle concentrations in water leaving NADC are around 200-250 mg/L, which is also typical for domestic municipal sewage. Particles originating from NADC are estimated to constitute less than 2% of the total particles entering the WPC plant, from the entire service area. The WPC plant consistently achieves greater than the regulatory standard of 85% removal of particles, with an average removal that is actually much higher (97%). Thus, particles originating from NADC are likely to be

removed from the water during the municipal wastewater treatment process. Particles entering the municipal waste water treatment process are either broken down by microbial activity or removed from the water by sedimentation. Sedimented particles are treated further by anaerobic digestion or lime stabilization.

A proportion of solid material (i.e. particles) entering the City of Ames WPC plant eventually comprises the biosolids that are land applied. If this material contained TSE infectivity, the extent of reduction of prion infectivity during waste water treatment is unknown. Because of their physical and chemical characteristics, TSE infectivity surviving the treatment processes would likely be present in the biosolids and not the treated effluent water. Thus, it is expected that any TSE infectivity remaining in the WPC plant effluent would be extremely low, representing a negligible risk of exposure to humans, domestic animals and wildlife.

Solid material separated from the water during treatment at the WPC is land applied. Grit (sand and rock) is treated with lime for four hours to pH 10.5-11 and is applied on the surface of the soil. Biosolids are treated using anaerobic digestion are stored for four to six months in a lagoon prior to being land applied, 90% by injection at 3-6 inches below the surface of soil; 10% of biosolids are surface applied (due to frozen ground.) Given the properties of the prions, the anaerobic digestion process is the most likely of the treatment process components to contribute to prion degradation.

The current and past methods of treating the waste water at the NADC sufficiently inactivate the potentially prion-contaminated waste from the research laboratories and animal holding facilities to ensure that the exposure of personnel and animals is extremely unlikely. Even if there were residual infectivity remaining in the waste water and that material were to enter the sanitary sewer down stream of the waste water pretreatment plant, the risk of exposure resulting in prion-associated disease would be extremely minimal.

The procedures in place, when followed, for the personnel conducting animal care and other research-associated activities also make the risk of exposure to prion-associated material, and subsequent human or animal disease extremely unlikely. The personal protection requirements (dedicated clothing, boots, gloves and masks) plus showering out of the prion animal research areas are sufficient to ensure that exposure of personnel would not be likely to occur.

The cumulative effect of all steps in the waste handling process creates multiple barriers that are expected to reduce potential residual contamination. These barriers include, 1) minimizing infective material going into NADC drains; 2) treatment of materials at the building and laboratory levels to reduce infectivity; 3) Heat treatment in the NADC WWPTP 4) downstream WPC processing. The premise established above is that any residual infectivity should be associated with the biosolids not the effluent. It must be emphasized that the level of any residual infectivity in the biosolids is expected to be extremely low.

C. Recommendations

The Panel recognizes and acknowledges that NADC is a highly unique facility in the types of research it conducts. The recommendations that follow reflect the need to apply additional measures given the unknowns associated with cross species TSE transmission experiments.

There are two possible paths by which TSE infectivity might enter the sanitary sewer system of Ames, Iowa. Waste exposed to TSE infectivity could be discharged directly to the sanitary sewer system without prior treatment. The panel found no evidence of direct discharge to the sanitary sewer system, thereby eliminating this as a current pathway.

The second path by which TSE infectivity might enter the sanitary sewer system of Ames, Iowa would be via inadequately treated waste water from the WWPTP. The WWPTP is an impressive facility that processes thousands of gallons of water daily at 1 atmosphere and 250°F for 30 minutes before discharge to the Ames sanitary sewer and ultimately the Ames WPC plant. The feed to the WWPTP autoclaves is very dilute and thereby well dispersed. The water in the autoclave tanks is agitated during treatment to ensure uniform exposure to the sterilizing conditions and the conditions are adequate to reduce well dispersed TSE infectivity at least ten million fold. The main vulnerabilities of the process are the presence of insoluble particulates in the water. These theoretically could provide sanctuaries for very low level survival of some infectivity. The other vulnerability is the head space in the autoclave tanks where infectivity could dry and be fixed in such a way that it becomes much more resistant to inactivation. Retention of very small amounts of infectivity immobilized in the head space of the tank would not expose the Ames sanitary sewer system. It is highly likely that this infectivity would also be inactivated in time but at a slower rate than for well-dispersed infectivity. This possibility should be considered when servicing these tanks however.

Currently the particulates from the NADC WWPTP are discharged along with the water to the Ames sanitary sewer where they are presumably recovered in the sludges (biosolids) removed by the Ames WPC. It must be emphasized that the level of any residual infectivity in these solids would be expected to be very low, and it is highly unlikely for animals and humans to be exposed in such a way to cause transmission and disease. Nevertheless, it would be advisable for NADC to limit where possible the level of particulate contamination containing infectivity that is discharged to the drains to maximize the margin of safety. This is especially important where the waste water has been contaminated by tissues, for example at necropsy sites. The Panel recommends that NADC:

- Collect and pretreat all liquid and particulate wastes from high exposure sites before delivery to the pretreatment plant, e.g. Building 5, necropsy facility. (The Panel does not consider precharging the drain as meeting the intent of this recommendation.)
- Minimize discharges of solids into the waste water system from the source of generation, e.g. pen wastes, manure.

- If it is found that solids cannot be controlled at the source, an additional measure to consider would be removal of the solids after the pretreatment of the wastewater at NADC before discharge to the sanitary sewer.

A key element in successful implementation of an effective prion management program is clear, consistent and accessible standard operating procedures along with well-trained and knowledgeable personnel at all levels. Therefore, the Panel recommends that:

- National Centers for Animal Health (NCAH) should harmonize prion disease infection control procedures as much as possible.
- NADC should ensure that personnel working with prion disease infected animals and specimens are trained in prion disease infection control issues and safety procedures.
- It would be wise for NADC to eliminate potential sources of concern with their operations in the interests of maintaining a positive relationship with the Ames community. It would also be advisable for a formal reporting and audit system to be developed between Ames and NADC whereby NADC would certify the integrity of the isolation of the NADC discharges of water from TSE exposed sites from direct discharge to the Ames sanitary sewer (except via connection with the NADC WWPTP plant).

V. Summary

- a) The Scientific Review Panel was tasked to identify acceptable prion decontamination methods, assess concerns raised about NADC's methods for prion decontamination, determine the risk posed to humans from possible environmental prion contamination, and provide scientifically sound approaches for corrective action that may be needed. The Panel was asked to prepare a written report to document the Panel's findings and present the report to the City of Ames, USDA, and the general public. This report will be available on the City of Ames website after final editing.
- b) The Panel recognizes that NADC at Ames constitutes a unique and essential National resource and strongly supports and endorses the research conducted at these laboratories. The findings of this Panel are intended to serve as recommendations for NADC to improve its waste handling procedures and to address potential concerns raised by the general public regarding waste disposal practices.
- c) The cumulative effect of all steps in the waste handling process creates multiple barriers that are expected to reduce potential residual contamination. These barriers include, 1) minimizing infective material going into NADC drains; 2) treatment of materials at the building and laboratory levels to reduce infectivity; 3) Heat treatment in the NADC WWPTP; and 4) downstream WPC processing. The properties of the agent suggest that residual infectivity should be associated

- with the biosolids not the effluent. It must be emphasized that the level of any residual infectivity in the biosolids is expected to be extremely low.
- d) It must be emphasized that the level of any residual infectivity in the biosolids would be expected to be very low, and it is highly unlikely for animals and humans to be exposed in such a way to cause transmission and disease. Nevertheless, it would be advisable for NADC to limit where possible the level of particulate contamination containing infectivity that is discharged to the drains to maximize the margin of safety. This is especially important where the wastewater has been contaminated by tissues, for example at necropsy sites. The Panel recommends that NADC:
 - i) Collect and pretreat all liquid and particulate wastes from high exposure sites before delivery to the pretreatment plant, e.g. Building 5, necropsy facility. (The Panel does not consider precharging a drain as meeting the intent of this recommendation.)
 - ii) Minimize discharges of solids into the wastewater system from the source of generation, e.g. pen wastes, manure.
 - iii) If it is found that solids cannot be controlled at the source, an additional measure to consider would be removal of the solids after the pretreatment of the wastewater at NADC before discharge to the sanitary sewer.
 - e) A key element in successful implementation of an effective prion management program is clear, consistent and accessible standard operating procedures along with well trained and knowledgeable personnel at all levels. Therefore, the Panel recommends that:
 - i) National Centers for Animal Health (NCAH) should harmonize prion disease infection control procedures as much as possible.
 - ii) NADC should ensure that personnel working with prion disease infected animals and specimens are trained in prion disease infection control issues and safety procedures.
 - f) It would be wise for NADC to eliminate potential sources of concern with their operations in the interests of maintaining a positive relationship with the Ames community. It would also be advisable for a formal reporting and audit system to be developed between Ames and NADC whereby NADC would certify the integrity of the isolation of the NADC discharges of water from TSE exposed sites from direct discharge to the Ames sanitary sewer (except via connection with the NADC WWPTP plant).

VI. References

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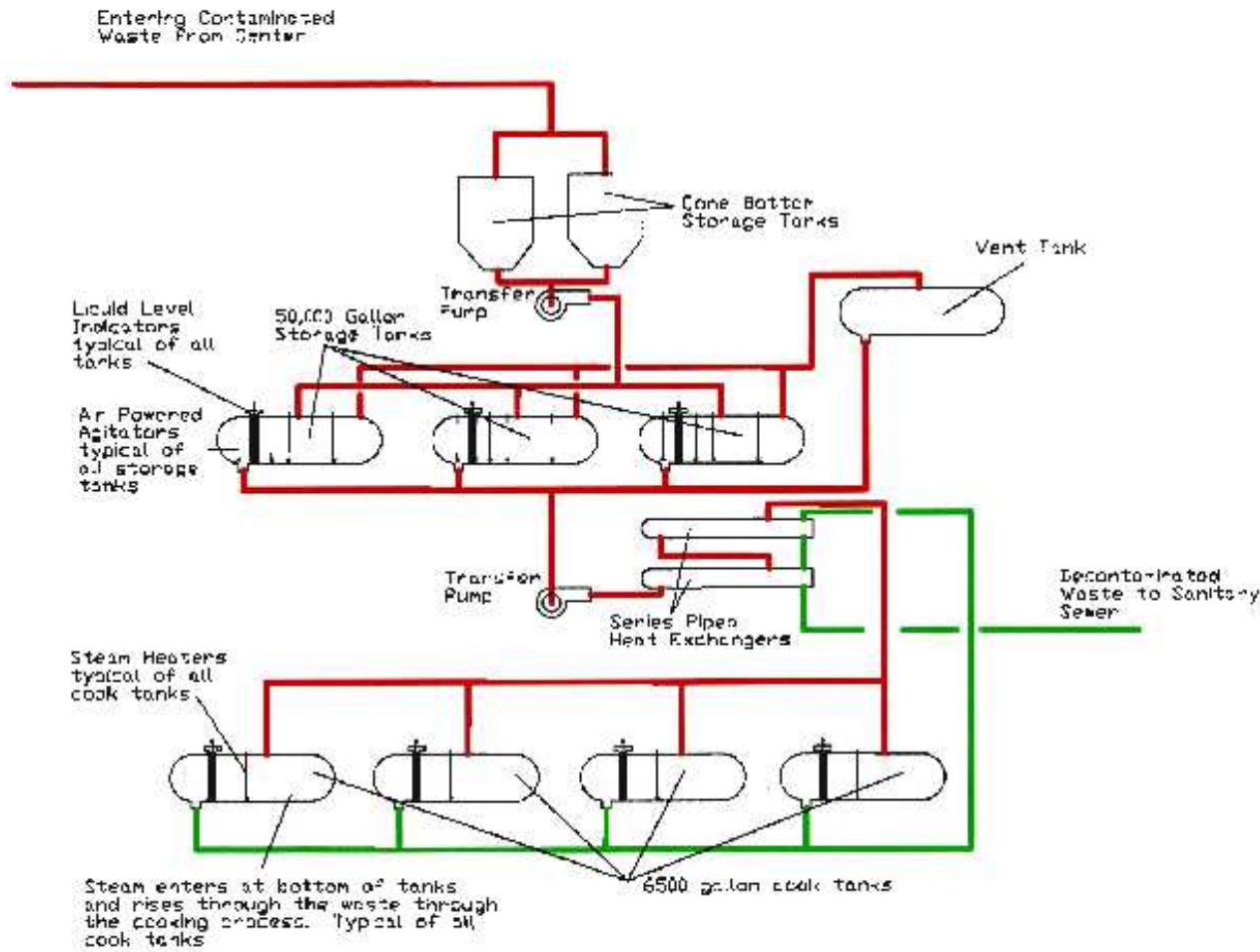
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FIGURE 1

Existing Bldg 155 Waste Treatment System



NEW WASTEWATER PRETREATMENT PROCESS

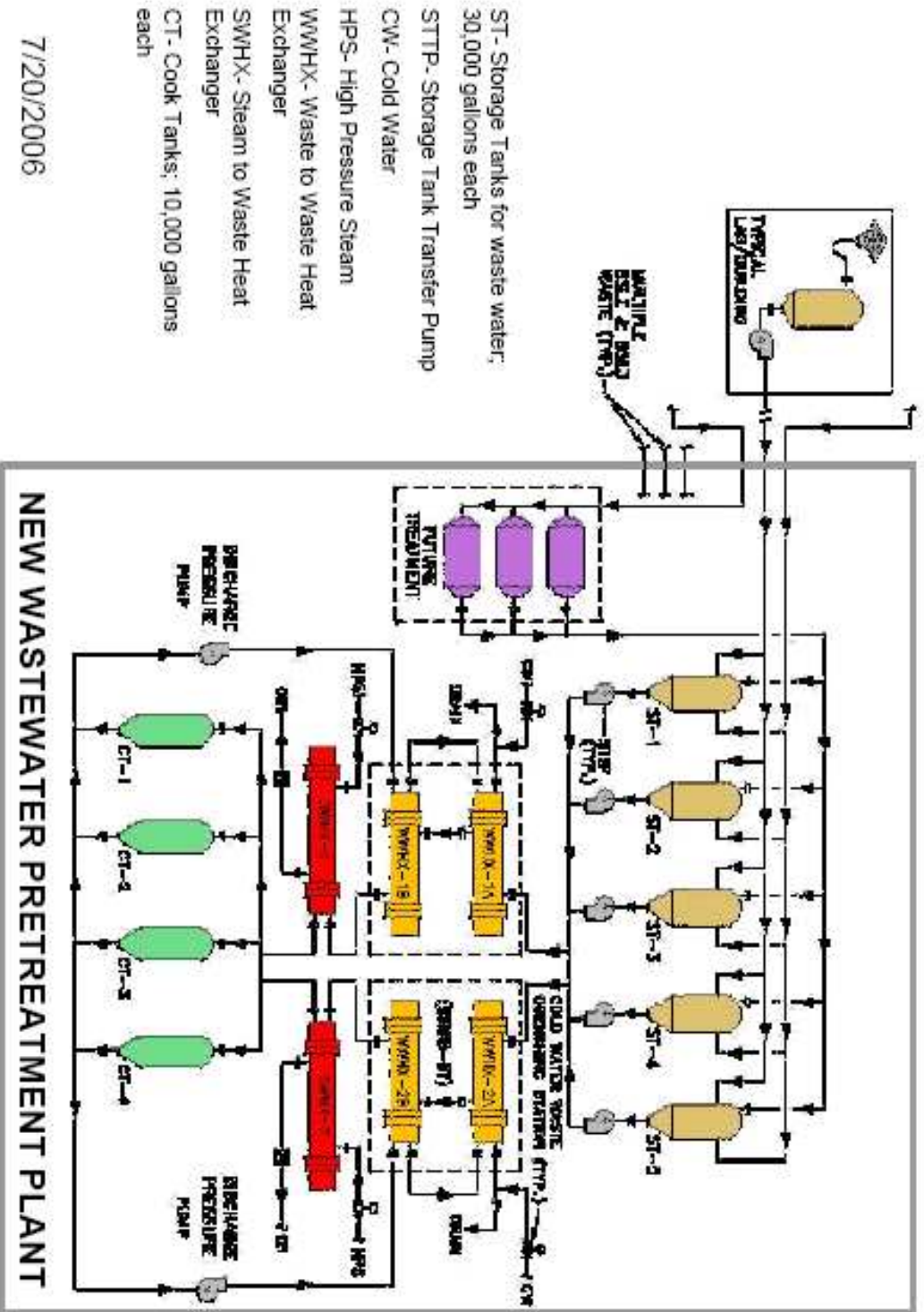
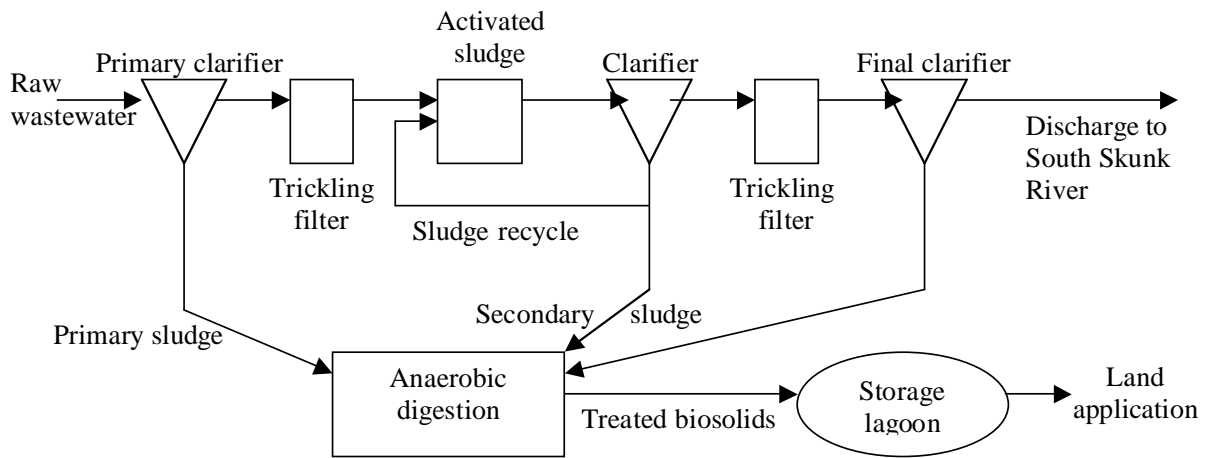


FIGURE 2

7/20/2006

FIGURE 3. Schematic of the Ames Water Pollution Control (WPC) Plant



Appendix 1

NADC Waste Disposal Evaluation

Scientific Review Panel Charter

(August 23, 2006)

Background:

On May 4, 2006, the City of Ames received allegations that wastes from areas at NADC with animals challenged with prions were not properly treated prior to discharge to the City wastewater plant. On May 8, 2006, representatives from the City of Ames and USDA met to discuss the concerns and develop plans to address the issues. USDA and the City of Ames agreed to convene an expert panel to review scientific information about deactivation of prions and assess practices used at NADC to treat liquid wastes from areas where animals with prions are housed and handled that enter the Ames wastewater treatment system.

Project:

To evaluate four main issues related to the handling and disposal of potentially prion-contaminated materials in wastewater from the National Animal Disease Center. Prions for the purposes of this document are defined as specific proteins that are abnormally shaped and can cause transmissible diseases associated with the allegations.

1. Identify scientifically accepted methods for effectively destroying prions.
2. Assess the concerns raised regarding NADC's current and past methods for the destruction of prions.
3. Determine the risk posed to humans and the environment from the current, as well as previous, methods for the destruction of prions utilized at NADC.
4. If remediation is needed, provide scientifically sound approaches for corrective action(s) that may be taken.

Deliverables:

Prepare a written report that documents the panel's findings for the four main issues being evaluated. Participate in presentations of the report to the City of Ames, USDA, and the general public.

Boundaries and Parameters:

The following parameters should be observed by the panel.

1. The purpose of this panel is to evaluate potential prion discharges into wastewater. Other issues not related to prion discharges are outside the scope of this panel's activities. If other issues are identified by the panel during its evaluation, this information should be provided to the co-coordinators of this evaluation (see Panel Process below). Except as it relates directly to the issue of prion discharges, the panel should not spend time evaluating NADC's internal administrative or personnel issues.
2. Similarly, this panel is not charged with trying to determine if any laws or regulations were violated. This panel's work is to focus on the technical issues surrounding potentially prion-contaminated waste disposal practices.
3. While it is desirable to have the final panel report express a unanimous opinion, for some issues there may be dissenting opinions. Where appropriate, the final report may include discussions about differing opinions.
4. Minutes, meeting summaries and the final report will be public information and available upon request.
5. Conclusions should be based on peer-reviewed science whenever available. Considering the nature of this area of research, peer-reviewed work may not be available for every issue. In these cases, panel members may be asked to provide information that provides a benchmark for what other research institutions are doing. In some instances, panel members may be asked to simply offer their best professional judgment. Professional opinions should be identified as such and the basis for the opinion should be expressed.
6. Panel members are acting as experts in their fields and are not speaking as agents of their agencies or universities.

Starting and Ending Date:

The panel's activities begin with an initial on-site panel meeting to be held as soon as possible. The panel's final report should be completed within 90 days.

Information and Knowledge:

Panel members will be given access to all available information sources related to prion waste disposal at NADC. This includes interviews with NADC administration, researchers, animal care staff, and environmental health and biosafety staff. The City Manager of Ames and USDA-ARS's Midwest Area Director have pledged their support to provide access to City and NADC personnel, information, facilities, and documentation applicable to this evaluation to panel members. Panel members may also use public records available through relevant federal, state and local agencies.

Panel Process

The entire process of this evaluation will be under the co-coordination of Mr. Tom Neumann of the City of Ames and Dr. Steven Shafer of USDA-ARS. Mr. Neumann and Dr. Shafer will approve and facilitate the panel's access to personnel, records, facilities, and information needed by the panel. The panel will communicate with the City and USDA exclusively through the co-coordinators. Mr. Neumann and Dr. Shafer may observe any of the panel's activities and may clarify the panel's charge if needed; however, Mr. Neumann and Dr. Shafer are not members of the panel and will have no role in the panel's deliberations or development of its conclusions.

All written communications to Mr. Neumann must be copied to Dr. Shafer, and vice-versa. Verbal communications should involve both co-coordinators whenever possible.

Panel members will develop their own methods for conducting the evaluation. All panel members should be present for all meetings; participation via conference call will be acceptable if circumstances prohibit travel.

The City and USDA strongly recommend that the panel select a chairperson early in the process. The panel may define the responsibilities of the chair, but one recommended role for the chair would be to serve as the focal point for communication between the panel and the co-coordinators.

The panel's meetings will be announced in advance and open to the public. Mr. Neumann and Dr. Shafer will arrange for public notices. We anticipate a minimum of two meetings. During the first meeting, the panel will be instructed on its charge by Mr. Neumann and Dr. Shafer; select a chairperson (suggested); and establish its methods. The last meeting should be held no more than 90 days later, during which the panel will develop its conclusions based on information it has gathered.

A period of time (e.g., 30 minutes) may be provided at the end of each public meeting for public comment. Panel members will not be required to respond to public comments.

IMPORTANT: Federal regulations for this panel require that the panel or any subset of its members may not engage in deliberations outside a publicly accessible meeting. "Deliberations" are verbal or written discussions intended to arrive at consensus or conclusions.

In addition to these publicly accessible meetings, the panel may perform preparatory work during which information will be gathered through interviews, site visits, document review, etc.. Preparatory work will be coordinated through Mr. Neumann and Dr. Shafer and conducted by the full panel. Preparatory work will not be open to the public. Per federal regulations, preparatory work may not include deliberations as defined above; they are restricted to information-gathering.

Any questions that arise outside meetings or preparatory work must be forwarded in writing (email preferable) to *both* co-coordinators (Mr. Neumann and Dr. Shafer). If such questions need to be

addressed by an employee of the City or NADC, the appropriate co-coordinator will facilitate the communication.

Appropriate secretarial support will be provided to the panel for each meeting and for preparation of the final report.

Individual Roles

Co-Coordinators (roles described above; present at all meetings and as needed for consultation): Tom Neumann (City of Ames; alternate John Dunn); Steve Shafer (USDA)

Technical Resources for Facilities (routine information on facilities; available for all meetings and as needed for consultation): John Dunn (City of Ames wastewater treatment plant); Diana Whipple (NADC).

Panel Members (primary charge); Alwynelle Ahl (Michigan State University), Ermias Belay (Centers for Disease Control and Prevention), Jim Colbert (Iowa State University), Linda Detwiler (VA-MD Regional College of Veterinary Medicine), Robert Ellis (Colorado State University), Fran Kremer (US Environmental Protection Agency), Katherine McMahon (University of Wisconsin), Robert Rohwer (Veterans' Administration Medical Center). The quorum required for a panel meeting is any five members present in person or via conference call.

Observer: Byron Rippke (USDA-APHIS).

Reporting Process:

The City of Ames has established an Internet web page for the dissemination of information related to the panel's work. Panel agendas and meeting summaries will be posted on this site. This web page is hosted by the City of Ames, and is located at <http://www.city.ames.ia.us/waterweb/nadc/nadc.htm>. In addition, printed copies will be available to the public through the City Manager's Office and the Water and Pollution Control Department's administrative office.

Any dissemination of information from the panel to the press during the evaluation process will be through the co-coordinators. Panel members will defer all media contacts to these individuals until the conclusion of the panel's evaluation and submission of the final report.

After the final meeting, the panel will make a concluding presentation that will be open to the public. At the conclusion of the presentation, the panel will be dissolved.

Panel Expenses

All panel expenses are being covered through the USDA. Expenses for additional resources must be approved in advance by the USDA.

Appendix 2. NADC TSE Standard Operating Procedures (SOP), 001, section 4, dated 10/20/05

4. Procedure / Method

4.1 Use of the TSE Laboratory

4.1.1 Put on all protective clothing indicated in section 2.2 before working with any TSE agent. Disposable laboratory coats must be worn when homogenizing any TSE tissue. When working with the BSE agent wear additional personal protective equipment; exception: staining histology slides, where basic protective clothing is adequate. Standard chemicals for neutralizing TSEs are Sodium Hypochlorite (bleach) at concentrations $\geq 20,000$ PPM for 1 hour contact time and 2N NaOH for 1 hour contact time. After appropriate contact time, bleach and NaOH containing wastes can be disposed of in the customary manner.

4.1.2 On completion of BSE work, remove disposable lab coat, gloves, shoe covers, bouffant cap and place in designated, lined BSE waste container, which will be incinerated. Face shield will be soaked in Sodium Hypochlorite (bleach) at concentrations $\geq 20,000$ PPM for 1 hour and then rinsed with water.

4.1.3 When work with other TSEs is completed, disposable items are placed in the lined TSE waste container and will be incinerated. Clothing will be placed in the laundry bags in the locker rooms and sent for washing. Lab shoes are kept in the locker room.

4.1.4 Staff may retain the same disposable laboratory coat for up to one week before disposal, if there has been no visible TSE material spilled on it.

4.1.5 All personnel must wash hands before leaving the laboratory.

4.1.6 To prevent the need to decontaminate the benches and protect from minor spills coming in contact with the lab bench, disposable pads or plastic backed laboratory paper, etc. will be used whenever possible

4.1.7 If spillages occur on a bench or floor, contain or absorb the spill as per EHS guidelines and decontaminate with Sodium Hypochlorite (bleach) at concentrations $\geq 20,000$ PPM or 2N NaOH. Bleach and 2N NaOH should be left on the surface for one hour, blotted dry and then rinsed with water and the waste must be disposed of in the customary manner. Contact EHS for assistance with clean up of spills which are beyond the immediate capabilities of laboratory staff.

4.1.8 If fixatives, such as phosphate buffered formalin are spilled, the proper absorbent material (spill kit) should be applied to the spill immediately to contain and absorb any liquid. The surface should then be decontaminated as above and all waste should be collected for incineration. If unsure how to deal with a spilled substance, consult the Principal Investigator or the Biosafety Officer.

4.2 Waste / Garbage Disposal

4.2.1 All solid, TSE contaminated waste must be double bagged in extra heavy duty plastic garbage bags (ordered from NADC warehouse). The top of the bag will be tied off with duct tape after which the plastic garbage bag is placed inside of a burlap bag, which is sealed with duct tape and a red biohazard label tied to the bag. The red label must have the laboratory and building number, type of waste, agent, and the first and last name of the person preparing it. After TSE garbage is sealed and labeled, it is placed in the outer airlock of the laboratory module. Laboratory personnel should call the NADC Facilities Engineering and Management Unit Maintenance Trouble Phone (NADC Help Desk) @ ext 7328 for pick-up and incineration.

4.2.2 Liquid, non-hazardous, TSE waste (other than organic solvents) is inactivated with either 2N NaOH or Sodium Hypochlorite (bleach) at concentrations $\geq 20,000$ PPM for a minimum of 1 hour. After inactivation, liquid waste can be poured down the drain. In addition to inactivation of TSEs as described above, NADC waste water is heat treated at 121°C for 30 minute before release to city sewer.

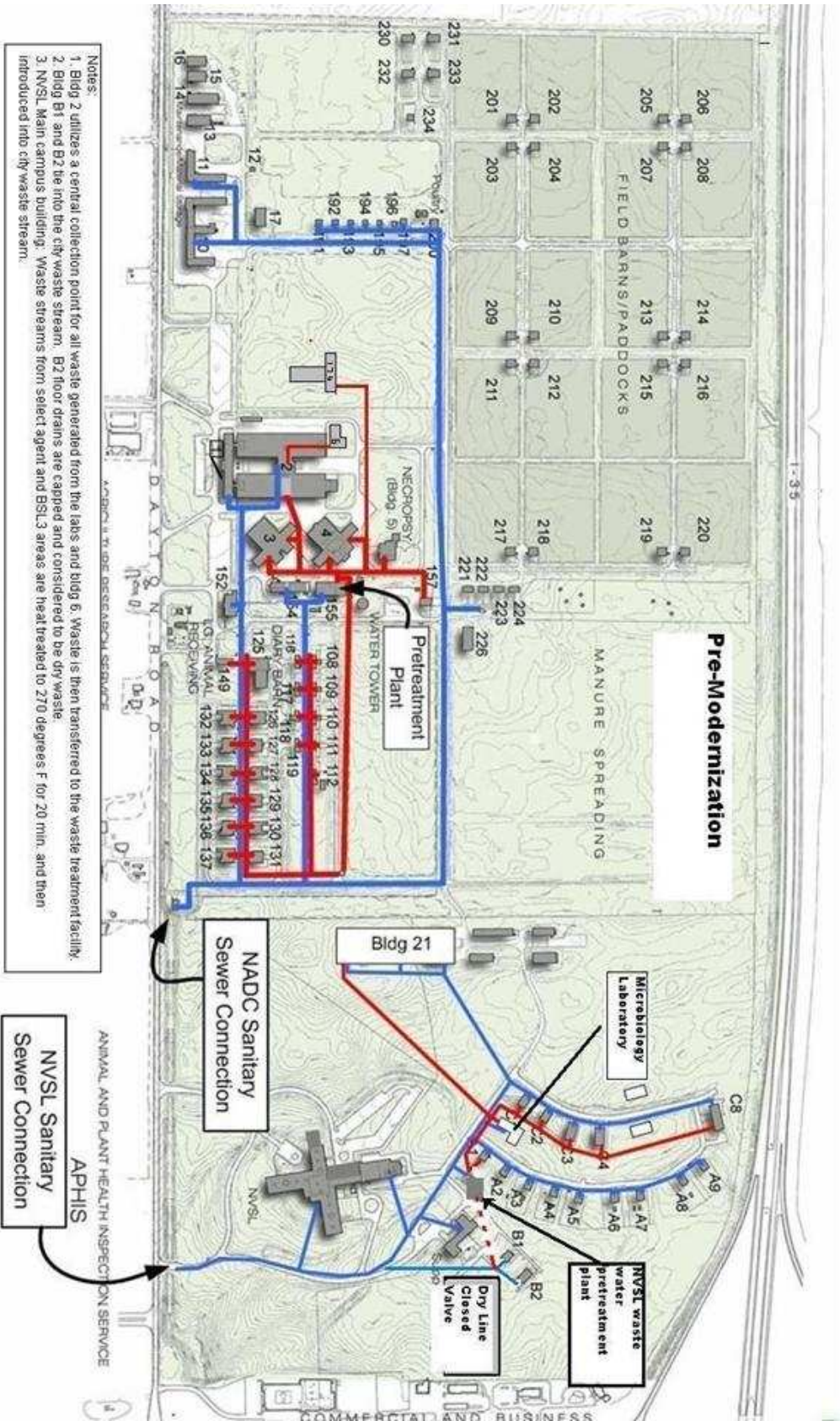
4.2.3 Contact EHS for the disposal of solvent waste and other hazardous liquid waste so that it can be disposed of according to EHS guidelines.

4.2.4 All infectious, BSE waste must be controlled, so a chain of custody form must be filled out. Call the EHS office for BSE waste pick-up. Waste should be bagged (as described in 4.2.1).

4.2.5 Liquid, non-hazardous, BSE waste will be treated with either Sodium Hypochlorite (bleach) at concentrations $\geq 20,000$ PPM.

4.2.6 Non-TSE contaminated waste should be placed in autoclave bags as regular lab waste. Non-TSE lab waste will be autoclaved and then placed in the hallway where it will be picked up. Laboratory garbage cans will be clearly designated as TSE, BSE and Non-TSE Laboratory Waste.

Appendix 3. Map of NADC Buildings and waste water flow



APPENDIX 4

Unavailable for security reasons