An Analysis of Risks Associated with the Disposal of Deer from Wisconsin in Municipal Solid Waste Landfills

1. Background

The purpose of this document is to evaluate risks associated with the landfilling of CWD-infected deer carcasses. It uses the current scientific information available on this subject and, as such, should be considered an interim document that will be updated as new information is available.

1.1 The Challenge

In February 2002 the first cases of chronic wasting disease (CWD) in free-ranging white-tailed deer east of the Mississippi River were reported in southwest Wisconsin. Further surveillance revealed a 3% rate of infection in an 11-mile radius around the initial cases. Based on these findings and the input of wildlife disease control and CWD experts, a disease eradication program was developed. This program calls for the harvest of all of the deer within a 360 square mile area and for population reductions in the surrounding areas.

The zone targeted for complete depopulation has been designated the *eradication zone* (EZ) and the surrounding area has been termed the *management zone*. It is estimated that, as of fall of 2002, there are approximately 25,000 deer in the EZ alone. Citizen-hunters are expected to be the primary means of removing deer from the EZ. It is expected that the majority of these deer will not be used as a source of venison. Therefore, the carcasses will need to be disposed of in a manner that does not jeopardize animal or human health or environmental quality.

Any disposal method must also have the following attributes: the capacity to handle a large number of carcasses; the ability to conform to local, state and national laws and regulations; and to be in place by October 2002. A final consideration is the cost of disposal. Although the latter is not the primary consideration, it is likely that disposal costs will be one of the largest expenses of Wisconsin's CWD control program. The four primary options currently under consideration for the disposal of deer are landfilling, rendering, incineration and chemical digestion.

Deer carcasses and tissues are often sent to municipal solid waste landfills. This material is incorporated in with other waste at the landfill. Landfills generate a certain amount of liquid, termed *leachate*, which is collected and processed. This leachate results primarily from precipitation falling on the landfill surface. Composite landfill liners prevent leachate from entering groundwater. The leachate is collected at the base of the waste just above the liner. In most instances the leachate is transferred to a wastewater treatment plant (WWTP) for treatment. Less commonly, some landfills may recirculate a portion of the leachate. At the WWTP, the leachate is processed along other wastewater. Solids are separated from the water portion. This material, termed "sludge" or biosolids, is commonly applied on farm fields or landfilled.

Based on the above, the primary pathway of potential risk identified for the CWD prion following landfill disposal of infected deer can be described as:

carcass→landfill→ leachate→wastewater treatment plant→sludge →

farm field→ingestion by humans or deer.

1.2 The Disease

CWD is a member of the transmissible spongiform encephalopathies (TSEs) a group of diseases that includes scrapie of sheep, bovine spongiform encephalopathy (BSE) of cattle and Creutzfeldt-Jakob Disease of humans. BSE is the only animal TSE for which there is experimental and epidemiological evidence of transmission to humans (Bruce *et al.* 1997, Hill *et al.* 1997 and Scott *et al.* 1999). All of the diseases in this group are characterized by a prolonged incubation, insidious onset of neurological signs, typically slow progression and eventual death. As a group, the TSEs are infectious, but not highly contagious. The specific transmission routes (i.e. portal(s) of agent exit and entry) of CWD between infected and susceptible animals have not been established. There is evidence that CWD can be transmitted by direct and indirect means; that is by animal-to-animal contact or by animal contact with contaminated items or the environment.

1.3 Biochemical and Physical Properties of the TSEs

Biochemically, the TSEs are characterized by a resistant form of a normal protein that is found in all mammalian and avian species examined to date. This protein is termed *prion protein* (PrP). The abnormal form, termed *PrP-res*, is associated with TSE infectivity and pathogenicity. The "res" refers to the fact that the abnormal prion protein is partially resistant to proteinase K digestion. Unlike the normal host prion protein, PrP-res forms ordered oligomeric structures; which are units composed of more than one protein chain. PrP-res has both hydrophilic and hydrophobic regions (Meyer *et al.* 1986). The hydrophobic region will be an important determinant of the behavior of the CWD agent in the landfill environment. Other distinctive properties of PrP-res include resistance to many of the commonly used disinfectants and inactivation procedures that are typically used to destroy infectious agents. Finally, there are multiple strains of TSE agents that have been identified, even within a particular disease group. Research has shown that some strains are more resistant to inactivation than others. With respect to CWD, it is unknown whether there are multiple strains and what relative degree of resistance to inactivation CWD has with respect to some of the better characterized TSE agents such as scrapie and BSE.

2. Behavior of the Prion Protein (PrP-res) in the Environment

2.1 Soil and Solid Waste

Due to the hydrophobic regions of the CWD PrP-res molecule, infectious prions in the environment can be expected to adsorb to organic material and soil. Initially the infectious agent is likely to adhere to the protein and carbohydrate components of the animal carcass. As the carcass decomposes, the undegraded PrP-res will adhere to adjacent soil or waste material in a landfill. The ability of scrapie prions to bind to metals and plastics has been reported (Flechsig et al, 2001; Weissman et al., 2002). All municipal solid waste landfills in Wisconsin employ a thick plastic membrane (generally polyethylene) as a component of the liner. This liner acts as a protective barrier and should prevent the movement of 'free' prions to subsurface soils or to groundwater.

In the only experiment to examine the fate of PrP-res in an outdoor environment, Brown and Gajdusek (1991) buried perforated petri dishes containing hamster scrapie in a residential garden for three years. They found that approximately 1% of the original infectivity in the original location

survived this term. Examining surrounding soil layers, no infectivity was found above the original location, a small amount of infectivity was found in the 4 cm soil layer that was directly beneath the perforated dish containing the original inoculum and no infectivity was found at 4-8 cm below the dish. The authors conclude that the hamster scrapie agent used in this experiment can persist in contaminated soil for three years under natural environmental conditions, but that there is little leaching to surrounding soil layers. To date there has been no further work that specifically examines the fate of TSE agents in the soil/solid waste environment.

Land application of municipal sludge that potentially contains CWD PrP-res may result in the presence of CWD PrP-res in surface soils. The application rates of municipal sludge are dependent on the chemical characteristics of the sludge and therefore will vary. The mechanism and time course of PrP-res degradation in soil/solid waste environment is unknown. Normal biodegredation processes are expected to inactivate the CWD prion over time.

2.2 Water

The hydrophobic nature of PrP-res (Bennett 1992) indicates that leaching of the CWD agent into an aqueous environment is unlikely to occur in the landfill or in soil. This assumption is echoed in a 2000 BSE risk assessment produced for the British Ministry of Agriculture, Fisheries and Food (now Department for Environment, Food & Rural Affairs [DEFRA]). In section 3.1 titled "Fundamental Assumptions" it states that "BSE agent is stuck to particulate matter and, hence, is removed with the particulate matter from the effluent." Gale *et al.* (1998) in examining the risk from BSE in the aquatic environment state, "With the possible exception of flows in the vicinity of extraction wells, the rate of flow through landfilled wastes is generally slow and non-turbulent, with the result that particulate material is unlikely to be taken up in suspension."

Should any PrP-res exit the landfill as part of the leachate, it will, due to its hydrophobic nature, be attached to particulates (colloids) suspended in the leachate. Once that leachate reaches the wastewater treatment plant the suspended solids will be separated from the effluent. Those suspended solids will then be termed "sludge" or biosolids. Again, due to its hydrophobic nature, the PrP-res is expected to selectively partition with the solids into the sludge portion, and is not expected to be present in wastewater discharged to surface water. Gale and Stanfield (2001) discuss this expectation in their risk assessment for BSE in sewage sludge.

2.3 Air

Air transport is not considered in this document because there is currently no evidence that PrP-res can be released into the air or volatilized in any way under natural conditions. In addition, there is no evidence of airborne transmission between animals or people.

3. Human vs. Animal Exposure to the CWD Agent

To date, no human illness has been associated with exposure to the CWD agent. However, systematic surveillance has only recently begun. Given that humans have likely been exposed to the CWD agent for decades from animals, in laboratories and from the environment, this is a significant observation. Surveillance of prion-related diseases in humans is in its infancy. The primary routes of exposure in the future are likely to be through hunting and the consumption of CWD-contaminated venison and elk. To date, test-tube experiments in which normal human prion protein is exposed to

PrP-res from white-tailed deer have shown a limited degree of infectivity, but at a less efficient rate than that for BSE or scrapie (Raymond et al., 2000).

In contrast to interspecies transmission of CWD from deer to humans, there is good evidence that deer and elk can contract CWD by animal-to-animal contact as well as by contact of a susceptible animal with a contaminated environment.

Domestic cattle have failed to develop disease when housed with CWD-infected deer (Williams & Miller 2002). Under experimental conditions, however, 3 of 13 cattle inoculated (~ 5 years ago) intracerebrally with CWD did succumb to a TSE illness (Hamir et al., 2001). The remaining cattle are still alive and will remain under observation for another 5 years. Animals orally inoculated have, to date, not succumbed to the disease (Williams, 2002). Based on the above observations, the following pathway is not considered further in this document:

deer→landfill→leachate→ wastewater treatment plant→sludge→ farm field→ animal fodder (surface contamination)→domestic livestock→commercial meat.

4. Impact Assessment

4.1 Minimum Level of Exposure Known to Cause Disease

As described above, it is likely that over the past several decades, thousands of hunters, taxidermists, meat processors, and research staff have ingested the CWD prion, as well as been exposed via eye splashes and through cuts and wounds. Exposure from these routes is likely to have been significantly greater than any that would be expected to result from contact with leachate from a well-managed landfill.

The issue of how much infected material an individual (human or animal) must consume or be exposed to in order to become infected with CWD or any other TSE is not known. Neither is it known if repeated small doses can result in infection. In an experimental setting, low-dose inoculation studies have revealed a decreased probability of infection and prolonged incubation periods. In some animal experiments, the incubation period extended beyond the natural life span of the animal; that is, at the time of death due to "natural causes", the animal was infected with the TSE agent, but was not symptomatic (Dickinson, 1977; Thackray et al., 2002).

4.2 Transmission of TSEs to Humans and Among Animals of the Same Species

As stated in section 2., the only animal TSE that is known to have been transmitted to humans is BSE. Transmission of TSEs from one animal to another is likely to depend on a number of factors. These include the specific TSE, the strain of the TSE, the dose, the route of exposure, the human PrP genotype, and likely other unidentified factors.

The dose for any TSE is typically expressed as a "infectious/lethal dose 50" or an "I/LD_{50}" per gram of tissue. It represents the dose of material at which 50% of the recipients become infected and will die. An I/LD_{50} is always species and route specific. The I/LD_{50} is determined by serial dilutions of the original material and subsequent inoculations into groups of animals to determine the endpoint at which 50% of the animals succumb or are diagnosed as infected (The term "LD_{50}" is often replaced by "ID_{50}" or "infectious dose 50" to indicate that experimental animals are not allowed to progress

fully through clinical disease to death). For the TSEs, a lower dose can decrease the likelihood that an exposed animal will become infected.

The route of exposure is also an important factor. The intracerebral route of inoculation is the most efficient. However, it is not a natural route of exposure. The following additional routes of infection are listed in descending order of efficiency (generally): intravenous, intraperitoneal and oral. The oral infectious dose of CWD has not been determined for deer or elk (E. Williams, personal communication, 2002).

4.3 Movement of Prions to Landfill Leachate

While the assumptions in this analysis are based on limited data, they serve to provide an approximation of the range of conditions likely to be encountered in the environment under the scenario described in this document.

4.3.1 Permeability

Permeability is defined as the time needed for liquids to percolate through the waste mass at a landfill. There are limited data available regarding the saturated hydraulic conductivity of municipal waste. The EPA Hydrologic Evaluation of Landfill Performance (HELP) model for predicting the movement of liquids through landfill caps and liners uses a default value of 1×10^{-3} cm/sec for the saturated hydraulic conductivity of municipal waste. This value is based on work by Oweis et al (1990). More recent data (Shaw and Carey, 1996, Bleiker et al,1993;Townsend et al,1995) indicates a broader range of permeabilities from 10^{-3} to 10^{-6} cm/sec. Permeability, however, can vary based on waste composition, age (degree of decomposition) and depth of fill.

4.3.2 Distance to the Leachate Collection System

Typical municipal waste landfills in Wisconsin range in final height from 100 to 300 feet above the leachate collection system. The landfills are typically constructed in a series of phases over time, so that there are disposal areas available in the upper portions of the previous phase, while filling is occurring at the base of a new phase. DNR recommended in a June 6, 2002 letter to landfill operators that the deer carcass burial area should be "strategically sited high in the landfill such that any liquids will have to pass through many feet of waste material before reaching the leachate collection system"(WDNR, 2002).

4.3.3 Summary

Considering these factors in tandem with the observations in section 2.1 about the hydrophobicity of prions and their tendency to degrade in soil, it is expected that if prions were to move into landfill leachate, their movement would be slow enough that their concentration would be significantly reduced by degradation and retention in the remaining waste mass.

4.4 Ingestion of potentially CWD-contaminated soil by humans and deer after sludge application

As described in section 2 of this document, it is expected that any prions present in leachate will adhere to sludge during the wastewater treatment process. Furthermore, the incorporation of sludge

into the 9-inch plow layer, which is standard for land application practices, would provide significant dilution within the soil. This combination of concentration reduction factors at the landfill, the sewerage treatment plant, and in the soil as well as the natural degradation processes is expected to greatly reduce the potential for infectious CWD prions to be present in sludge-amended soil.

5. Discussion

A quantitative or semi-quantitative assessment of the risk is not possible because the amount of infectivity present in a carcass is unknown. In addition, the dose needed to infect a human or deer is also unknown. Nonetheless, existing information suggests that landfilling large numbers of deer from an area with a low incidence of CWD is unlikely to pose a significant risk to humans or to wildlife.

This document provides support for the following conclusions:

1. The disease specific agent is hydrophobic and is expected to adhere to organic materials present in a landfill.
2. It is likely to take the CWD agent several months to move through a landfill. During that time the agent will be subject to biodegredation and is likely to lose a significant amount of its infectivity. Based on the findings of Brown and Gajdusek (1991), up to 98% loss of infectivity can be anticipated within a 3-yr period.
3. Any infectivity that exits the landfill will be captured in the effluent and transferred to a wastewater treatment plant or recirculated in the landfill.
4. CWD prions present in wastewater are expected to partition with the sludge fraction.
5. Land-applied sludge will be greatly diluted by surface soils and incorporated with soil at a depth of 9 inches.

5.1 Likelihood of Human Exposure

Two factors strongly influence human risk:

1. The presence of a species barrier
2. The route and dose of the exposure

The precise nature of any species barrier for CWD transmission between white-tailed deer and humans has, as yet, not been described. Given the fact that controlled experiments cannot be conducted in human beings, the existence of a species barrier cannot be directly tested. However, there is limited experimental evidence of a species barrier (Raymond et al. 2000). Further supporting the notion that such a species barrier exists is the observation that humans have been handling and consuming tissues from infected deer for decades with no evidence to date of any correlation with any human illness. The U. S. Centers for Disease Control (CDC) has investigated Creutzfeldt-Jakob disease (CJD) among three individuals thirty years of age or younger who had some association with hunting or consumption of venison (not related to the ongoing CDC investigation in Wisconsin) and has concluded that there was no evidence for a causal link with the consumption of venison (Belay et al., 2001).

The route of exposure to TSEs is also an important determinant of the efficiency of transmission. Oral (i.e. ingestion) exposure is among the least efficient means of transmitting any TSEs. In many

circumstances, TSEs that can be transmitted by the artificial route of intracranial (IC) inoculation directly into the brain cannot be transmitted by the oral route. Under experimental conditions when the dose can be controlled, it generally requires a far greater dose (typically 1,000- to 100,000-fold more) to transmit a given TSE at the same rate by the oral route than by the IC route.

The collection of leachate from a large landfill, the co-mingling of the solids from the leachate with other solids from the sewerage system and its mixing with 9 inches of topsoil provides an extremely large dilution factor. In addition, any prions that enter the environment will degrade with time.

In summary, it is reasonable to conclude that while absolute numbers relating to human health risk cannot be generated, the available knowledge about CWD and other TSEs suggests that landfilling of CWD-infected deer does not pose a significant risk to human health.

5.2 Likelihood of Transmission to Deer

The major factors that influence the risk to deer from the landfilling of a population of deer some of which are infected with CWD are:

1. The absence of a species barrier
2. The route of exposure
3. The concentration reduction factors inherent in sludge production and application
4. The consumption of soil by deer.

For any TSE that is transmitted within the same species there is assumed to be no species barrier. The consequence of this is that deer are the most susceptible species to any exposure to viable CWD agent that enters the environment. For deer there may, however, be genetic influences on susceptibility and incubation period. For CWD this information is not known. Therefore, the approach in this document has been to assume that all white-tailed deer are equally susceptible to CWD infection.

The primary route of exposure to the CWD prion for deer from sludge amended soil is by ingestion. Typically for the TSEs the oral route is among the least efficient means of transmission. However, the oral infectious dose of CWD for deer has not been determined.

The collection of leachate from a large landfill, the co-mingling of the solids from the leachate with all the other solids from the sewerage system and then its mixing with 9 inches of topsoil provides an extremely large concentration reduction factor. Should any viable CWD prions make it out into the environment it is likely that they will degrade with time and will be diluted due to the mixing to a depth of 9 inches.

In conclusion, it is deemed likely that the risk of spreading CWD among Wisconsin's deer population by landfill disposal of infected carcasses is quite small.

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