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Transport of the Pathogenic Prion Protein through Landfill

Materials

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Abstract

Transmissible spongiform encephalopathies (TSEs, prion diseases) are a class of fatal neurodegenerative diseases affecting a variety of mammalian species including humans. A misfolded form of the prion protein (PrP^{TSE}) is the major, if not sole, component of the infectious agent. Recent TSE outbreaks in domesticated and wild animal populations has created the need for safe and effective disposal of large quantities of potentially infected materials. Here, we report the results of a study to evaluate the potential for transport of PrP^{TSE} derived from carcasses and associated wastes in a municipal solid waste (MSW) landfill. Column experiments were conducted to evaluate PrP^{TSE} transport in quartz sand, two fine-textured burial soils currently used in landfill practice, a green waste residual material (a potential burial material), and fresh and aged MSW. PrP^{TSE} was retained by quartz sand and the fine-textured burial soils, with no detectable PrP^{TSE} eluted over more than 40 pore volumes. In contrast, PrP^{TSE} was more mobile in MSW and green waste residual. Transport parameters were estimated from the experimental data and used to model PrP^{TSE} migration in a MSW landfill. To the extent that the PrP^{TSE} used mimics that released from decomposing carcasses, burial of CWD-infected materials at MSW landfills could provide secure containment of PrP^{TSE} provided reasonable burial strategies (e.g., encasement in soil) are used.

Introduction

Transmissible spongiform encephalopathies (TSEs, prion diseases) are fatal neurodegenerative diseases affecting a variety of mammalian species and include bovine spongiform encephalopathy (BSE or “mad cow” disease); chronic wasting disease (CWD) of cervids (deer, elk, and moose); scrapie of sheep and goats; and Creutzfeldt-Jakob disease (CJD), fatal familial insomnia, and Gerstmann-Sträussler-Scheinker disease in humans. The significance of TSEs in North America has become increasingly apparent. Sixteen cases of BSE have been reported in North America in recent years (www.oie.int/eng/info/en_esbmonde.htm), and the known geographic range of CWD has expanded dramatically in the past 8 years.

The infectious agent in these diseases is the prion, a pathogen apparently lacking nucleic acids and composed primarily, if not solely, of a misfolded isoform of the normal cellular prion protein (designated PrP^{TSE}) (1). Spongiform degeneration of the brain occurs as abnormal

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prion proteins accumulate, resulting in personality and memory changes, loss of coordination, and inevitably death (1). No cure exists for these diseases. Interspecies transmission of some prion diseases has been documented (e.g., ² and ³), including humans contracting new variant CJD from consumption of BSE-infected beef (4). Thus, the presence of TSEs in wild or farmed animal populations represents a potential risk to both human and domestic animal health, and governments are acting to safeguard human and livestock populations.

Countermeasures to minimize the risk of TSE transmission often involve depopulation of infected herds and extensive post-mortem screening. For example, hundreds of cattle were slaughtered, and 2000 Mg (metric tons) of potentially infected beef products were discarded when the first US BSE case was discovered in 2003 (5). In Wisconsin and other states, CWD management efforts include depopulation of infected herds to limit intra- and potential interspecies transmission. Large volumes of infected waste are generated by these responses to TSE threats, and a significant need exists for safe and effective disposal of infected carcasses and other materials. Because of the large volume of material, underground burial and landfilling are attractive disposal options. However, the risks associated with these options remains unknown. Prions are highly resistant to degradation (e.g., refs. ^{6,7,8}), and therefore may persist in environments that may lead to human and animal exposure. In addition, virtually nothing is known about the transport and fate of prions in porous media (e.g., soils, landfills, subsurface environments) (9).

The objective of this study was to assess the potential for PrP^{TSE} transport through materials present in conventional municipal solid waste (MSW) landfills. A series of laboratory-scale column experiments were conducted to quantify migration of PrP^{TSE} through landfill burial materials and solid waste, and mathematical modeling was used to estimate the concentration of PrP^{TSE} in landfill leachate for a typical disposal scenario. All experiments were conducted using the pathogenic form of the prion protein (PrP^{TSE}) as a biomarker for infectivity.

Materials and Methods

Porous Media

Six porous media were selected for use in prion transport experiments: quartz sand, two natural soils, green waste residual material (GWR), and fresh and aged MSW. Quartz sand was selected as a material with low potential for binding of PrP^{TSE} (10,¹¹), whereas the natural soils, GWR, and MSW were intended to represent more typical burial materials that might be encountered in landfill practice.

The quartz sand (Iota 6, Unimen, New Canaan, CT) was uniformly graded, consisting of particles with a nominal size between 0.18 and 0.25 mm. Metal and organic contaminants were removed from the fractionated sand by 24-h soaking in 12 N HCl, rinsing with distilled deionized water (ddH₂O; 18 MΩ-cm resistivity), and baking overnight at 800°C (12). Prior to use, the sand was re-hydrated by 1-h boiling in ddH₂O water. Attachment of PrP^{TSE} to this quartz sand was described previously (11). Characteristics of the sand are summarized in Table 1.

Two soils used as burial materials at MSW landfills were selected for study: Boardman silt and Bluestem clay soils. These soils represent a range of PrP^{TSE} binding potentials that might be encountered in practice (silt: lower binding potential, clay: higher binding potential). Sorption of PrP^{TSE} to these soils is described by Johnson et al. (10). The soils were dried for 24-h at 110 °C and then moistened to gravimetric water contents typical of field conditions prior to being compacted in the columns (15% Boardman, 11% Bluestem, ref 13). Characteristics of these soils are presented in Table 1.

Fresh MSW was obtained from the Wake County Transfer Station in Wake County, North Carolina and was composed of 70.7% (w/w) paper, 14.6% plastic, 3.9% metal, 3.5% glass, 0.6% wood, and 6.7% miscellaneous materials (e.g., fabric, food debris, cigarette butts, rubber, excrement). Aged MSW (~10 y old) was obtained from a bioreactor landfill in Kentucky and was composed of 50.3% (w/w) soil, 24.0% wood, 10.3% paper, 4.1% plastic, 2.2% glass, 0.5% metal, and 8.5% miscellaneous materials. Both types of MSW were shredded to a maximum particle size of 10 mm prior to packing into the columns. GWR was obtained from a composting operation at a MSW landfill.

Leachate

Landfill leachate was obtained from a MSW landfill in southern Wisconsin operating with leachate recirculation. Leachate was collected from a lift station with polytetrafluoroethylene (PTFE) bailers and stored at 4°C under inert gas until used. Major cations and anions, pH, redox potential, and alkalinity of the leachate are described in Table S2 in the Supporting Information (SI).

Prion Source

Prion-enriched fractions were prepared from brains of hamsters clinically affected with the HY agent using a modification of the Bolton et al. (14) procedure described by McKenzie et al. (15). Such prion enrichments contain $\sim 10^9$ infectious units (IU₅₀)·mL⁻¹ (11). Hydrodynamic radii of PrP^{TSE} aggregates present in these prion enrichments range from 250 to 500 nm under the pH and ionic strength conditions relevant for the present study (11).

PrP^{TSE} Transport Experiments

Column tests to evaluate PrP^{TSE} transport were conducted with quartz sand, Boardman silt, Bluestem clay, GWR, and MSW. All column tests were conducted in custom-fabricated columns (10-mm ID, 24-mm height for quartz sand, soil, and GWR experiments; 50-mm ID, 50-mm height for MSW experiments). Different sized columns were employed to allow even packing of each material and to minimize the amount of PrP^{TSE} used. Poly(tetrafluoroethylene) (PTFE) was used for all column parts based on preliminary experiments that indicated minimal PrP^{TSE} binding to PTFE relative to other potential column materials (viz. glass, polyvinyl chloride, polymethyl methacrylate). A 1-mm thick perforated PTFE frit was placed at the bottom of each column to retain the porous medium. Ferrules were PTFE or ethylene-tetrafluoroethylene, fittings were made of fluorinated ethylene polypropylene (Teflon[®] FEP), and seals were PTFE-coated Viton[®].

For quartz sand experiments, sand was placed by sedimentation in ddH₂O while gently tapping the column. The Boardman silt and Bluestem clay were compacted to dry densities of 1.62 and 1.65 Mg·m⁻³, the GWR was compacted to a dry density of 1.09 Mg·m⁻³, and the fresh and aged MSW were compacted to dry densities of 0.49 and 0.77 Mg·m⁻³. These densities represent typical field conditions (16).

End plates were attached to the columns, and the enclosed medium was saturated by pumping ddH₂O (quartz sand) or 5 mM CaCl₂ (soils, GWR, MSW) through the column for at least 15 pore volumes (PV). A pore volume was defined as the total volume of pore space in the specimen as defined by weight-volume computations. This gross pore volume may neglect micropores within particles. Flow was oriented downward to be consistent with the landfill setting. The effluent discharge point was maintained above the upper surface of the column to ensure saturation.

To determine the hydraulic properties of the porous media, a tracer test was conducted by pumping 1.2 mM KBr through each column. The effluent Br⁻ concentration was determined

using a microplate-based colorimetric method (17). The effective porosity and dispersivity (Table 1) were determined by fitting the advection-dispersion-reaction equation (ADRE) to the Br^- breakthrough curve using the method in Lee and Benson (18). Tailing was absent in the Br^- effluent data and good agreement was obtained between the data and the fit of the ADRE, indicating that classical advection-dispersion theory represented the transport processes in the experiments. However, in some waste materials with significant secondary porosity within the organic fraction, other transport processes could be important (e.g., micropore diffusion).

Columns were flushed for ≥ 15 PV with the chosen eluent (10 mM Tris pH 7.0 + 10 mM NaCl for quartz sand to allow comparison with previous research (11); leachate for soils, MSW, and GWR to represent conditions prevailing in landfill environments) after the tracer test. PrP^{TSE} -enriched preparations were then applied directly to the top of the column (10 μL , or ~ 50 ng for quartz sand, soil and GWR; 300 μL or ~ 1500 ng for MSW). Forty PV of eluent were then pumped downward through each column at a seepage velocity of $\sim 0.2 \text{ m}\cdot\text{d}^{-1}$. For the quartz sand, soil and GWR columns, four samples were collected per PV in Protein Lo-Bind microcentrifuge tubes (Eppendorf AG, Hamburg, Germany). After elution, samples were snap-frozen in liquid nitrogen and stored at -80°C until analysis. For the MSW column, two or four samples were collected per PV in 50-mL PTFE centrifuge tubes (Nalg Nunc, Rochester, NY) and stored at -80°C prior to analysis. All column experiments were conducted at least twice.

Prior to analysis, PrP^{TSE} was precipitated from solution by addition of ice-cold methanol ($4\times$ sample volume), overnight storage at -20°C , and 30-min centrifugation at 0°C (24,400g). Supernatants were aspirated and discarded. The resulting pellets were dried by vacuum centrifugation (Speed Vac SC110, Thermo Savant, Waltham, MA), and re-suspended in a 1:1 mixture of water and $5\times$ sample buffer (100 mM Tris, 7.5 mM EDTA, 100 mM DTT, 350 mM SDS pH 8.0) used for SDS-PAGE. All samples were analyzed by immunoblotting (*vide infra*).

After eluting 40 PV, the columns were frozen for 1 h at -80°C and sectioned using a razor blade (quartz sand, soil and GWR columns) or overnight at -20°C and sectioned with an Oster 2803 reciprocating electric knife (MSW columns). PrP^{TSE} was extracted from a portion of each section with $5\times$ SDS-PAGE sample buffer at 100°C and analyzed by immunoblotting (10).

Immunoblot Analysis

Samples containing PrP^{TSE} were fractionated by SDS-PAGE and analyzed by immunoblotting following the method in Johnson et al. (10). The PrP -specific monoclonal antibody 3F4 (Signet, Dedham, MA) was used at a 1:40,000 dilution. Detection was achieved with HRP-conjugated goat anti-mouse immunoglobulin G (Bio-Rad, Hercules, CA) and West Pico peroxidase detection substrate (Pierce, Rockford, IL). Control experiments with leachate and soil extracts in the absence of PrP^{TSE} demonstrated no non-specific binding of the primary antibody and limited non-specific binding of the immunoglobulin G to constituents in the leachate. This non-specific binding gave signals near 50 kD and 37 kD and did not interfere with detection of PrP . Spiking PrP^{TSE} into leachate or column eluent did not significantly diminish immunoreactivity of the PrP^{TSE} .

Estimation of Transport Parameters

When PrP^{TSE} was observed in the effluent (i.e., present above the detection limit), transport parameters were obtained by fitting the breakthrough curve with the one-dimensional ADRE with linear and instantaneous sorption and first-order attachment and detachment (19):

$$\frac{\partial \theta C}{\partial t} = \frac{\partial}{\partial z} \left(\theta D \frac{\partial C}{\partial z} \right) - \frac{\partial q C}{\partial z} - \frac{\rho_d K_d}{\theta} \frac{\partial \theta C}{\partial t} - \theta k_{att} C + \frac{\rho_d k_{det} S}{\theta} \quad (1)$$

where θ is volumetric water content, C is the PrP^{TSE} concentration in the aqueous phase, t is time, z is distance in vertical direction, v is seepage velocity, D is the dispersion coefficient, q is the Darcy velocity, ρ_d is the dry density of media in the column, K_d is the equilibrium distribution coefficient describing linear, rapid and reversible sorption, k_{att} is a first-order attachment coefficient, k_{det} is a first-order detachment coefficient, and S is the solid-phase (attached) PrP^{TSE} concentration. Various forms of Eq. 1 have been used successfully for modeling the transport of colloids in porous media (e.g., ^{19–21}).

Eq. 1 was fitted to the data using the inverse algorithm in the program HYDRUS (v1.02; ²²), which uses the finite-element method to solve Eq. 1 and employs a Marquardt-Levenberg optimization algorithm for inversion. The seepage velocity was set at q/n_e , where n_e is the effective porosity, and the dispersion coefficient was set as the product of the dispersivity and seepage velocity using parameters from the tracer tests (Table 1). Molecular diffusion was ignored because advection dominated transport in the columns (Peclet number $\gg 10$). The reaction parameters (K_d , k_{att} , and k_{det}) were independent variables fitted via the inversion algorithm.

When no PrP^{TSE} was observed in the column effluent (i.e., below detection limit), the governing equation was simplified by neglecting K_d and k_{det} :

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial z^2} - v \frac{\partial C}{\partial z} - k_{att} C \quad (2)$$

The solution to Eq. 2 for instantaneous slug input of mass M at $z = 0$ is (23):

$$C(z, t) = \left\{ \frac{M/A}{\sqrt{4\pi Dt}} \exp \left[-\frac{(z - vt)^2}{4Dt} \right] \right\} e^{-k_{att}t} \quad (3)$$

where A is the cross sectional area of the column. This approach was based on a parametric evaluation of the potential impacts of K_d , k_{att} , and k_{det} on PrP^{TSE} transport, which showed that the absence of PrP^{TSE} in the effluent over at least 40 PV was possible only when K_d and k_{det} were nil for the column testing conditions that were employed (see SI). Eq. 3 was solved for k_{att} assuming that the PrP^{TSE} concentration in the effluent was 80 ng·L⁻¹ (based on detection limit for the immunoblot analysis of the sand and soil column effluent samples) at one PV. The seepage velocity and dispersion coefficient were set as previously described. Use of Eq. 3 in this manner provides a lower bound estimate of k_{att} .

Results and Discussion

Transport of PrP^{TSE} through Quartz Sand and Soils

Effluent from the quartz sand and soil columns contained no PrP^{TSE} detectable by immunoblotting (detection limit ~25pg PrP^{TSE}, equivalent to 0.05% of the protein loaded onto columns). Dissection and analysis of sections from the quartz sand, Boardman silt, and Bluestem clay columns revealed intense PrP^{TSE} signals from the top-most section (upper 3 mm) and strongly attenuated signals from the subsequent two or three sections (Figure 1).

Mass balances for the quartz sand and soil columns could not be closed with the analytical methods used (i.e., not all of the PrP^{TSE} loaded onto the columns could be recovered). More than 95% of the mass of protein applied was extracted from the sand column, while ~55% and ~38% were recovered from the Boardman silt and Bluestem clay columns. The inability to account for all of the PrP^{TSE} may have been due to time-dependent decline in the extractability of the protein from soil as previously reported (24,25), and as observed in soil persistence experiments conducted in our laboratory. Degradation of PrP^{TSE} within the columns cannot be ruled out. However, past studies have demonstrated that PrP^{TSE} is remarkably stable (7,8), and our experiments indicate that PrP^{TSE} does not degrade in landfill leachate in the time frame studied. Thus, degradation within the columns is considered unlikely.

Our finding of negligible PrP^{TSE} transport in soil is consistent with previous observations of low migration of infectivity (6) and prion protein (25) in soil persistence experiments. Limited mobility in soil was also noted for a recombinant, non-infectious form of the prion protein that is folded in a considerably different manner (26). The aggregate state in which prions are released from decomposing carcasses is not presently known. The procedure we used to isolate PrP^{TSE} study produces rod-shaped prion aggregates that are larger than those present in infected tissues. This may have promoted retention in these porous media.

Transport of PrP^{TSE} through MSW

PrP^{TSE} was detected in effluent samples from both the fresh and aged MSW columns (Figure 2). Relative to the aged MSW, the breakthrough observed for the fresh MSW included a larger percentage of the loaded protein (28% versus 0.3%) and was spread over a larger number of pore volumes (8 vs. 3 PV) (Figures 2a and 2d). For both materials, no PrP^{TSE} was detected beyond 8 PV. When sectioned (Figures 2b and 2e), both columns show a PrP^{TSE} distribution similar to those of the soil and quartz columns, with all detectable protein residing in the topmost sections. These results suggest that PrP^{TSE} may migrate through MSW. Migration of PrP^{TSE} in MSW, but not in soils, may be due to the larger pores in MSW, and the higher porosity of MSW. Similarly, more migration may have occurred in fresh MSW relative to aged MSW because of the higher porosity and larger pores of the former.

The MSW used in the transport experiments was shredded to a maximum particle size of 10 mm to permit compaction in the columns. The pore spaces of actual (i.e., unshredded) MSW are expected to be considerably larger, potentially permitting migration of larger amounts of PrP^{TSE} than observed in these experiments.

Transport of PrP^{TSE} through Green Waste Residual

PrP^{TSE} eluted in the first two pore volumes from the GWR column (Figure 3a). The first PV sample produced a very strong signal, while the second PV sample yielded a substantially weaker signal (Fig. 3a). Signal was not detected in later samples. The total amount of PrP^{TSE} detected in the first two pore volumes accounted for ~2% of the PrP^{TSE} loaded onto the column. Analysis of the column sections (Figure 3b) showed detectable signal in only the topmost section, suggesting that the majority of the PrP^{TSE} did not migrate through the column. These results indicate that, as with MSW, a fraction of PrP^{TSE} can migrate through GWR. At present, the reason for larger migration through the GWR is not clear. However, the large pore size and higher organic matter content (relative to the soils used) of the GWR may have contributed to transport of PrP^{TSE}.

Estimated PrP^{TSE} Transport Parameters

Transport parameters for PrP^{TSE} in the MSW and GWR (Table 2) were obtained by inversion of Eq. 1 using HYDRUS. Since no PrP^{TSE} was observed in the effluent of the column tests conducted with quartz sand, Boardman silt, or Bluestem clay, Eq. 3 was used to calculate

k_{att} for these media. These k_{att} for quartz sand, Boardman silt, or Bluestem clay represent lower-bound estimates; the actual k_{att} are larger. These estimated k_{att} are an order of magnitude higher than calculated environmental k_{att} for the most comparable colloidal contaminants, viruses ($0.17 - 0.3 \text{ h}^{-1}$, refs. ²⁷ and ²⁸).

The k_{det} for MSW and GWR are small, and the uncertainty in these values is as large as, or larger than, the mean estimates, suggesting that detachment for these materials is negligible (Table 2). Estimated k_{det} are several orders of magnitude larger than values reported for virus particles ($2.2 \times 10^{-5} - 1.4 \times 10^{-4} \text{ h}^{-1}$, ref. ²⁸). Similarly, k_{att} for the GWR does not differ from zero. Additionally, K_d is nil or near zero for all three materials for which the effluent data could be analyzed with HYDRUS. This finding, along with the parametric analyses reported in the SI, suggest that attachment is the dominant mechanism affecting binding of PrP^{TSE} to the solid phase in porous media, and that distribution coefficients reported in previous batch studies (e.g., ref. ¹¹) likely reflect attachment rather than sorption.

Implications for Landfill Disposal of Prion-contaminated Waste

To evaluate the implications of our experimental results on the disposal infected carcasses in a MSW landfill, we simulated PrP^{TSE} transport in a profile characteristic of a landfill setting and estimated PrP^{TSE} concentrations in the leachate collection system. The disposal scenario simulated was consistent with management and disposal practices anticipated in Wisconsin, a state with a significant need for safe and economical disposal of CWD-infected materials. Disposal of infected carcasses was assumed to occur annually in a single pit excavated within MSW at an operating landfill. The annual carcass volume requiring disposal was estimated to be 450 m^3 based on the mass of carcasses collected each year in Wisconsin (130 Mg) and recommendations for mass livestock burial (29). The disposal pit was assumed to be $15\text{-m} \times 15\text{-m} \times 2\text{-m}$ (Figure S2) and underlain by a 0.5-m layer of burial soil. The burial soil was separated from the leachate collection system by 2m of MSW. This geometry is consistent with operations in practice, and disposal requirements under consideration in Wisconsin stipulate even larger separation using MSW ($>6\text{m}$). Leachate was assumed to contact carcasses directly from MSW placed over the pit. This assumption overestimates contact between leachate and the carcasses, because disposal practices require that carcasses be encased in burial soils to reduce the likelihood for contact by leachate and to provide containment of PrP^{TSE} should flows occur laterally within and through the sides of the disposal pit.

Transport Model—One-dimensional transport of PrP^{TSE} in the assumed landfill profile (Figure S2) was predicted using Eq. 3 and with HYDRUS. The PrP^{TSE} mass initially assigned to each disposal pit was $1.5 \text{ g PrP}^{\text{TSE}}$. This value corresponds to the amount of PrP^{TSE} estimated to be disposed of annually in Wisconsin (see the SI for detailed discussion). All PrP^{TSE} was assumed to be instantaneously released from the disposed CWD-contaminated material. This assumption overestimates PrP^{TSE} release, because the agent would be mobilized slowly in a landfill as carcasses degrade, resulting in gradual release into underlying materials. The remainder of the domain was assigned zero initial concentration.

For analyses including burial soils (quartz sand, Boardman silt, or Bluestem clay), the PrP^{TSE} concentration at the bottom of burial soil was calculated using Eq. 3. This approach was used because the large k_{att} of these materials (Table 2) resulted in numerical instability in HYDRUS. When the burial soil was GWR, transport through the GWR and MSW was analyzed directly using HYDRUS. Transport parameters obtained from tracer tests (Table 1) and the experiments conducted with PrP^{TSE} (Table 2) were assigned as input. A 46-y time period was used for modeling (6 y as an open cell, 40 y of post-closure care as stipulated by Wisconsin regulations).

Flow in the profile was defined by Richards' equation:

$$\frac{\partial \theta}{\partial t} = \frac{\partial}{\partial z} \left(K(h) \frac{\partial h}{\partial z} \right) - \frac{\partial K(h)}{\partial z} \quad (5)$$

where h = pressure head and K = hydraulic conductivity. Eq. 5 was solved by HYDRUS using a constant flux boundary at the surface and a unit gradient boundary at the base of the MSW layer. When the cell was open, the surface flux was assumed to be $0.3 \text{ m} \cdot \text{y}^{-1}$, an upper bound leachate generation rate in the eastern United States (30). For closed conditions, the surface flux was assumed to be $0.003 \text{ m} \cdot \text{y}^{-1}$, characteristic of percolation rates from landfill final covers with composite barriers in the eastern United States (13).

For all simulations, van Genuchten's equation was used to describe the relationship between θ and pressure head:

$$\theta = \theta_r + (\theta_s - \theta_r) \frac{1}{[1 + (\alpha h)^n]^{1-1/n}} \quad (6)$$

where θ_r is the residual volumetric water content, θ_s is the saturated water content, and α and n are the van Genuchten parameters describing the water retention function. Parameters assigned to Eq. 6 for GWR and MSW in HYDRUS are summarized in Table 1.

Estimated Concentration of PrP^{TSE} in the Leachate Collection System—For the quartz sand, Boardman silt, and Bluestem clay, PrP^{TSE} concentrations at the bottom of burial soil were at the computational limit of the computer used for the simulations and, therefore, $\sim 0 \text{ ng} \cdot \text{L}^{-1}$. Consequently the concentration in the leachate was $\sim 0 \text{ ng} \cdot \text{L}^{-1}$. Penetration into the burial material over the 46-y simulation period was $< 20 \text{ mm}$. Simulations were also conducted for GWR as the burial material and for disposal directly on the existing MSW (no burial soil or GWR). For these cases, the concentration of PrP^{TSE} at the bottom of the profile (i.e., at the interface of the MSW and leachate collection system) was also zero. These findings indicate that concentrations of PrP^{TSE} in a leachate collection system should be effectively zero for the conditions that were simulated, regardless of whether the CWD waste is encased in burial soil. These simulations were based on column experiments that used PrP^{TSE} enriched from brain tissue using a method that causes the protein to aggregate to a larger extent than appears to occur *in vivo*. Future experiments should employ partially disaggregated (31) and N-terminally truncated (32) PrP^{TSE} and track the migration of prion infectivity.

As noted above, however, the transport parameters for MSW were derived for experiments conducted with shredded MSW. The pore spaces of actual, unshredded MSW are expected to be considerably larger, potentially permitting migration of larger amounts of PrP^{TSE} than observed in this experiment. Thus, while this analysis may suggest that PrP^{TSE} may not migrate even if the CWD waste is disposed directly on MSW, encasement in burial soil is still recommended until data from larger-scale experiments are available. As a further safeguard, PrP^{TSE} migration may be further limited by the use of reactive burial materials (33).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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(a) Quartz

(b) Boardman

(c) Bluestem

FIGURE 1.

PrP^{TSE} extracted from (a) quartz sand, (b) Boardman and (c) Bluestem soil columns. Each section represented ~3 mm, 12.5% of the column height. Section 1 is the topmost section of the column. The PrP^{TSE} preparation (10 μ L) was loaded onto the top of the column. Positive controls are given as a percentage of the total PrP^{TSE} preparation initially applied to the column. Protein molecular mass is indicated at the left. The quartz sand column experiment was repeated three times; the Boardman and Bluestem soil columns were repeated twice.

FIGURE 2.

PrP^{TSE} in fresh and aged MSW column eluents and extracted from MSW. (a) Immunoblot of the initial pore volumes eluted from the fresh MSW column (one pore volume = 62.8 mL). No further PrP^{TSE} was detected in subsequent pore volumes. (b) Comparison of observed PrP concentrations with those predicted from the transport parameters in the fresh MSW column. Observed protein concentrations were determined by immunoblotting diluted samples and comparison with samples of known concentrations (not shown). (c) Immunoblot of the distribution of PrP extracted from the fresh MSW column. Each section represented ~10 mm, 19% of the column height. (d) Immunoblot of the breakthrough of PrP from the aged MSW column (one pore volume = 51.1 mL). No further PrP^{TSE} was detected in subsequent pore volumes. (e) Comparison of observed PrP concentrations and concentrations predicted by the transport parameters in the aged MSW column. (f) Immunoblot of the distribution of PrP extracted from the aged MSW column. In the immunoblots, positive controls are given as a percentage of the total PrP^{TSE} preparation initially applied to the top of the column (300 µL). Bands at ~50kD and ~37kD were due to non-specific binding of the secondary antibody to constituents of the leachate used. Left-hand labels indicate protein molecular mass as determined using molecular mass standards. In all immunoblots, positive controls (PrP^{TSE} spiked into leachate) are given as percentages of the volume of PrP^{TSE} enriched preparation initially applied to the top of the columns (300 µL).

FIGURE 3.

(a) Immunoblot of the first seven pore volumes eluted from the green waste residual (GWR) column (one pore volume = 0.85 mL). No further PrP^{TSE} was detected in subsequent pore volumes. MeOH Precip = methanol-precipitated. (b) PrP^{TSE} extracted from the GWR solids. Each section represented ~3 mm, 12.5% of the column height. In both immunoblots, positive controls (PrP^{TSE} spiked into leachate) are given as percentages of the volume of PrP^{TSE} enriched preparation initially applied to the top of the column (10 μ L). Left-hand labels indicate protein molecular mass as determined using molecular mass standards.

TABLE 1

Characteristics of packing materials used in column experiments^a

parameter	quartz sand	Bluestem clay	Boardman silt	GWR ^b	fresh MSW	aged MSW
specific surface area ^c (m ² ·g ⁻¹)	0.2	10.3	13	11	ND	ND
f_{oc} ^d	0	0.0042	0.0047	0.022	0.42	0.18
dry density (Mg·m ⁻³)	1.38	1.62	1.65	1.09	0.49	0.77
effective porosity	0.38	0.27	0.34	0.45	0.64	0.52
dispersivity (mm)	1.03	2.68	0.60	0.60	32	51
residual volumetric water content, θ_r	0.45	0.068	0.034	0.095	0.11	0.11
saturated water content, θ_s	0.43	0.38	0.46	0.45	0.53	0.53
content, θ_s	0.43	0.38	0.46	0.45	0.53	0.53
α (m ⁻¹)	14.5	0.8	1.6	20	26	26
n	2.68	1.09	1.37	1.31	2.22	2.22
K (m·d ⁻¹)	7.128	0.048	0.06	0.062	0.086	0.086
particle size distributions						
% sand	100	50	37	26	ND	ND
% silt	0	23	55	56	ND	ND
% clay	0	27	8	18	ND	ND

^a Abbreviations: f_{oc} , mass fraction of organic carbon; GWR, green waste residual; MSW, municipal solid waste; α and n, van Genuchten parameters for the water retention function; K, saturated hydraulic conductivity ND, not determined.

^b GWR was obtained from the composting operation of a landfill in southern Wisconsin where disposal of infected carcasses has been proposed.

^c Specific surface area measured by N₂ adsorption (BET method [1]).

^d Mass fraction of organic carbon determined by the organic carbon dry combustion method using a Leco CNS-2000 (± 0.0001) (St. Joseph, MI).

^e Calculated from loss on ignition data using the relationship $f_{oc} = 0.476 (f_{lo} - 0.0133)$ (Mort Barlaz, personal communication).

TABLE 2

PrP^{TSE} transport parameters from column tests.

porous Medium	amount of loaded PrP ^{TSE} eluted (%)	analysis method	K_d (L·kg ⁻¹)	k_{att} (h ⁻¹)	K_{det} (h ⁻¹)	MSE ^b
quartz sand	0	Eq. 3 ^a	-	>2.9	-	-
Boardman silt	0	Eq. 3	-	>2.6	-	-
Bluestem clay	0	Eq. 3	-	>3.3	-	-
fresh MSW	28	Eq. 1	0.15 ± 0.05	0.55 ± 0.04	0.003 ± 0.003	4.0×10 ⁻²⁰
aged MSW	0.3	Eq. 1	4.5 ± 0.2	6.6 ± 0.3	0.001 ± 0.002	2.9×10 ⁻²³
green waste residual	2	Eq. 1	0.0 ± 1.2	1.9 ± 1.8	0.01 ± 0.38	2.3×10 ⁻¹⁸

^a Analysis with Eq. 3 permits lower-bound estimate of k_{att} only.

^b Mean square error of effluent concentration (g·mL⁻¹) vs. time (h) function