Risk Assessment of Bovine Spongiform Encephalopathy Transmission Through Bone Graft Material Derived From Bovine Bone Used for Dental Applications*

A. Sogal and A.J. Tofe

Background: Several commercial products are currently available for clinical application as bone graft substitutes. These products can be broadly classified into two categories: synthetic and natural. Bovine bone is a popular source for several of the natural bone substitutes. The availability of bovine-derived xenogenic bone substitutes has made it possible to avoid traumatic and expensive secondary surgery to obtain autogenous bone once thought essential for effective bone replacement. While autogenous bone still remains the undisputed “gold standard” in bone grafting, the realization that bone requirement in several clinical applications is as effectively met by xenografts has lead to their widespread use. But the convenience of using xenografts is tempered by the possibility of disease transmission from cattle to humans. The recent incidents of bovine spongiform encephalopathies (BSE) in humans have underscored this likelihood. In this paper, we report a risk analysis performed to assess the possibility of such disease transmission from a commercially available bone graft substitute (BGS) that is popularly used in clinical dentistry.

Methods: An extensive review of current literature on the status of risk assessment of BSE transmission was conducted, and two risk assessment models were identified as applicable to the present study. Risk assessment models developed by the German Federal Ministry of Health and by the Pharmaceutical Research and Manufacturers Association of America were applied to BGS.

Results: Results from the analyses conducted using both models showed that the risk of disease (BSE) transmission from BGS was negligible and could be attributed to the stringent protocols followed in sourcing and processing of the raw bovine bone used in the commercial product.

Conclusions: Based on the risk analysis, it is evident that the risk of BSE infection from BGS is several orders of magnitude less than that posed by the risk of death related to, lightning, tornadoes, or similar remote events. However, this low risk can only be maintained as long as an effective and active risk management program is implemented in operations that involve processing xenogenic tissue for human use. J Periodontol 1999;70:1053-1063.

KEY WORDS
Bone substitutes; encephalopathy, bovine spongiform; risk factors; models, biological.

* CeraMed Dental, LLC, Lakewood, CO.
BSE is a form of transmissible spongiform encephalopathy specific in origin to the bovine species. Although the motivation for neutralizing all immunogenic components in xenografts precedes this discovery, the event has heightened awareness of disease transmission across species. A particularly concerning aspect of this discovery has been the realization that the safety previously thought to be afforded by the “species barrier” can no longer be taken for granted. Agents that cause/transmit BSE have been identified as prions. Prions show remarkable resistance to physical and chemical treatments that would inactivate other conventional microorganisms by destroying their nucleic acid. But the cause of BSE is not completely understood; neither is infection by this pathogen immediately obvious. Its effect can manifest after an extended incubation time, yet almost always results in fatality.† These factors, and because so much of this pathogen and its consequences are unknown, provide a strong motivation for the assessment of risk of disease transmission from bovine related products. To the best of our knowledge, the present paper is the first published risk analysis on any commercially available product for bone regeneration.

**MATERIALS AND METHODS**

**Preparation of the Bone Graft Material**

The common factor for most bovine-derived bone substitute products is their origin: raw bovine bone. However, a variety of manufacturing methods are used to extract the final product from the raw bone. The article reports on a commercially available product‡ (BGS), manufactured using a high temperature (HT) extraction process (Fig. 1). BGS is manufactured from raw bone obtained from cattle born, reared, and slaughtered in the United States. The raw bone is cleansed of fat and other non-skeletal attachments through a non-chemical process that involves essentially water, steam, and pressure. The cleansed raw bone is then deorganified by high temperature treatment for extended periods of time. The time periods at temperatures in excess of 600°C exceed 6 hours, and the peak temperature is in excess of 1000°C. The crystal structure and chemical phase are characterized/confirmed by x-ray diffraction (XRD) and Fourier transform infrared (FTIR) analyses. The deorganification process is confirmed through Kjehdahl nitrogen and carbon analyses.⁶,⁷

**Risk Analysis**

The real and perceived risks of transmitting BSE through bovine-derived bone substitutes can be managed through formal risk analysis. Risk is a measure of the probability of occurrence of an undesirable event. The principal objective of a formal risk analysis is to highlight hazards, and structure a strategy to select alternatives to reduce the resultant risk. Mathematically, risk is a function of uncertainty and associated injury, or:

\[
\text{Risk} = f(\text{uncertainty, injury})
\]

As uncertainty increases, so does the potential for injury, and the resultant risk increases. On the other hand, because risk analysis identifies hazards and suggests avenues for remedies, it can also be represented as:

\[
\text{Risk} = f(\text{hazards, remedies})
\]

In this case, as remedies increase, the hazards decrease and so risk decreases. Therefore, a formal analysis of risk associated with clinical utilization of bovine-derived bone substitutes must identify uncertainties, possible injuries, hazards, and possible remedies. Because risk analysis involves inclusion of several “unknowns,” at least part of such a study is expected to be qualitative.

In the particular context of bone substitutes derived from bovine materials, the specific hazard is the chance that a commercial product is manufactured from raw bone infected with BSE. In the unlikely event that the high temperature production process fails to completely deorganify the raw bone, the pathogen could be transmitted to humans through the infected bovine-derived graft material. Because of the unusual resistance of the BSE prion to inactivation, process methods play a critical role in determining the potential risk. Methods that ensure complete inactivation result in products of less risk. Empirical data have shown that a titer of infective agent from conta-

‡ OsteoGraf/N, CeraMed Dental, LLC, Lakewood, CO.
minated tissue can be as high as $10^9$ ID$_{50}$ units per 1 mg of infected tissue. Therefore, an effective manufacturing method will ensure that this initial risk is mitigated through proper choice of processing methods and risk management techniques.

The source of the raw bone, that is the country of origin, has a significant impact on the final risk associated with a product. Both the World Health Organization (WHO) and the Office International des Epizooties, Paris (OIE) Animal Health Code (May 1997) have focused on sourcing as the best way and the most critical control point to secure maximum safety of active substances, excipients and reagents used in the manufacture of medicinal products. Therefore, proper sourcing of raw materials appears to be the critical point for BSE risk management.

In the specific instance of bovine-derived bone substitutes, this concern can be circumvented by using raw bone only from cattle which are born, reared, and slaughtered in countries like the United States, where no cases of BSE have been discovered. Obviously, the lowest risk of BSE is from countries which have not reported indigenous cases of BSE, which have compulsory notification, with compulsory clinical and laboratory verification of suspected cases and a surveillance program. BSE has not been found in the U.S. and industry regulations meet and exceed all of the criteria of the WHO and OIE for sourcing.

Additional steps to reduce the risk of contamination include screening type of tissue, designing effective processing methods, control of tissue collection techniques, amount of material administered clinically, and route of administration. Transmission of BSE is considered most potent from organs such as the brain, spinal cord, spinal marrow, and the eyes. Bones, without the marrow, are considered potentially low risk. Since the marrow content of bone is organic, minimum risk in this category would imply complete deorganification. Products derived from some organs are of greater concern than others are. Table 1 lists the organs of concern based on the WHO recommendation for classification of tissue for potential BSE infectivity.

Another factor that has to be considered in a risk analysis is the choice of a model or framework for analysis. Because expression of BSE has been relatively recent, to date, there is no single model that has been accepted by the scientific community and society at large. Thus, the risk assessment presented in this article contains all the essential elements as described above, and reflects the latest thinking on managing risk of BSE transmission from bovine-derived products to humans based on reports from the 2nd International Conference on Transmissible Spongiform Encephalopathies held in Washington, DC, November 1997. Of all the effort directed toward developing a concise framework for risk assessment, two models stand out due to the advanced stage of their development: in Europe, the risk model developed by the German Ministry of Health, and in the United States, the model proposed by the Pharmaceutical Research and Manufacturers of America (PhRMA).

### Risk Assessment Based on the German Federal Ministry of Health Method

In February 1994, the German Federal Ministry of Health issued guidelines on “safety measures in connection with medicinal products containing body materials obtained from cattle, sheep or goats for minimizing the risk of transmission of BSE and scrapie.” These guidelines classify xenogenic products according to perceived risk for 6 different parameters, and are identified with 3-letter abbreviations as indicated in Table 2. Each of the 6 parameters are rated on a base 10 logarithmic scale, with increasing powers directed toward

---

### Table 1. WHO Recommendations for Tissue Classification Based on Potential BSE Infectivity

<table>
<thead>
<tr>
<th>Category</th>
<th>Infection Potential</th>
<th>Tissue Source</th>
<th>Max Estimated Infectivity Bovine Units/Gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>High</td>
<td>Brain, spinal, (eye)</td>
<td>10,000,000</td>
</tr>
<tr>
<td>2</td>
<td>Medium</td>
<td>ileum, lymph nodes, proximal colon, spleen, tonsil, dura cord mater, pineal gland, placenta, cerebrospinal fluid, pituitary, adrenal</td>
<td>25,000</td>
</tr>
<tr>
<td>3</td>
<td>Low</td>
<td>Distal colon, nasal mucosa, peripheral nerves, bone marrow, liver, lungs, pancreas, thymus</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>Not detectable</td>
<td>Blood clot, faeces, heart, kidney, mammary gland, milk, ovary, salivary gland, seminal vesicle, serum, skeletal muscle, testis, thyroid, uterus, fetal tissue, bile, bone, cartilaginous tissue, connective tissue, hair, skin, urine.</td>
<td>0.1</td>
</tr>
</tbody>
</table>

---

increased safety, i.e., decreased risk. A power or score of 0 indicates the greatest possible, yet realistic, risk contributed by a specific parameter. Scores higher than the baseline of 0 indicate the increased safety of a product relative to the baseline. It is important to note that while attributing the greatest possible risk to a parameter, it must be realistic to the product application. For example, the country of origin, ORG cannot be 0, as tissue known to be infected with BSE cannot be used in clinical practice. A brief explanation and scoring range for each parameter is presented in Tables 3 through 8.

**PhRMA BSE Risk Assessment**

The PhRMA model builds on the model presented by the German Ministry of Health, and includes other techniques. It offers a comprehensive set of guidelines for analyzing risk from bovine-derived products. Unlike the German model, rather than a single value of acceptable risk, the PhRMA model makes provision for a benefit-to-risk analysis applicable to a particular situation. Risk is computed from a set of 6 equations, and several values used in the equations are derived from scientific literature or guidelines and demographic data compiled by international authorities, such as WHO. In principle, the protocol for this model involves identifying factors that influence risk, magnitude of the factors, choices among factors, and, finally, methods for reducing risk.

**Equation 1.** The potential risk \( R_1 \) of an animal sourced in the United States being infected with BSE is determined from the regional information compiled by the WHO, and is based on reports of outbreak of the disease in various regions of the world.

\[
R_2 = R_1 \times n_a
\]

\( n_a = \text{number of animals used per batch of bone substitute manufactured} \)

**Table 2. Classification of the German Federal Ministry of Health**

<table>
<thead>
<tr>
<th>Classification</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Country of origin and animal environment (ORG)</td>
<td>(Cattle are rated separately from sheep)</td>
</tr>
<tr>
<td>2. Raw materials; tissue (MAT)</td>
<td></td>
</tr>
<tr>
<td>3. Methods used to inactivate or remove TSE pathogens (RED)</td>
<td></td>
</tr>
<tr>
<td>4. Quantities of animal material required to provide one daily dose of the product (QTY)</td>
<td></td>
</tr>
<tr>
<td>5. Number of daily doses (NDD)</td>
<td></td>
</tr>
<tr>
<td>6. Route of administration (APL)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Explanation of Scores for Country of Origin and Handling of Cattle (ORG) in the German Model**

| ORG 0 | This category does not exist as use of tissue from infected animals is prohibited. |
| ORG 1 | Country of origin of cattle is known. |
| ORG 4 | Country of origin known. Prevalence of BSE-infected cattle is between one in every \( 10^4 \) to \( 10^5 \) (e.g., Switzerland, UK). |
| ORG 5 | Country of origin is known. Prevalence of BSE-infected cattle exists but is certainly less than one in \( 10^5 \). However, at least one case of BSE has been discovered in an animal that did not originate from the UK. The cattle is from a country without known cases of BSE, but where there is no obligation to report BSE prior to early 1992. |
| ORG 6 | Cattle from countries which have a legal obligation to report BSE through veterinary surveillance procedures, but where either no case of BSE has been reported or only one case involving cattle that can be traced to the UK. These countries have a ban on import of cattle from the UK since 1990. |
| ORG 7 | Country of origin like ORG 6, with import restrictions on animal feed and ingredients from UK or a ban on feeding cattle animal feed, or ban on sale of animal feed that was not processed at least \( 133^\circ \)C for 20 minutes in pressurized steam. |
| ORG 8 | Cattle from this category are from selected herds in specific countries, except the UK, where there are no reported cases of BSE and no cattle or animal feed has been imported from countries with known BSE, and no cattle have been fed with animal feed since 1983. |
### Table 4.

**Explanation of Scores for Raw Material (MAT) in the German Model**

<table>
<thead>
<tr>
<th>MAT 0</th>
<th>Brain, spinal, (eye)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT 3</td>
<td>Ileum, lymph nodes, proximal colon, spleen, tonsil, dura cord mater, pineal gland, placenta, cerebrospinal fluid, pituitary gland, adrenal gland</td>
</tr>
<tr>
<td>MAT 5</td>
<td>Distal colon, nasal mucosa, peripheral nerves, bone marrow, liver, lungs, pancreas, thymus, and all organs listed under MAT 0 from animals under 6 months old</td>
</tr>
<tr>
<td>MAT 7</td>
<td>All materials listed under MAT 3 and MAT 5 from animals under 6 months old and all materials from fetuses</td>
</tr>
<tr>
<td>MAT 8</td>
<td>Blood clot, feces, heart, kidney, mammary gland, milk, ovary, saliva, salivary gland, seminal vesicle, serum, skeletal muscle, testis, thyroid, uterus, fetal tissue, bile, bone, cartilaginous tissue, connective tissue, hair, skin, urine</td>
</tr>
</tbody>
</table>

### Equation 3. **Assuming that infected bone is used, the risk of human infectivity per infected raw bone is calculated as:**

\[
R_3 = i \times w \times s \times c \\
i = \text{estimated infectivity of tissue for tissue category of animal} \\
w = \text{weight of infected tissue used per treatment} \\
s = \text{correction for species barrier} \\
c = \text{correction of LD}_{50} \\
LD_{50} \text{ is the median Lethal Dose; the dose that is lethal in 50% of the implanted population.} 
\]

### Equation 4. **Assuming that a batch of bovine-derived bone substitute is produced from contaminated bone, the potential number of human infections per batch is calculated as:**

\[
R_4 = R_3 \times p \times e \\
p = \text{infectivity reduction by process} \\
e = \text{transmission efficiency of route of administration} 
\]

### Equation 5. **The potential risk of a patient becoming infected per dose of bone substitute manufactured using the HT process is calculated as:**

\[
R_5 = R_4 \times R_2/nd \\
nd = \text{number of doses of product manufactured per production batch} 
\]

### Equation 6. **The potential risk of a patient becoming infected per treatment of bone substitute manufactured using the HT process from raw bone obtained from a BSE-free source is calculated as:**

\[
R_6 = R_5 \times n_t \\
n_t = \text{number of doses per treatment regimen} 
\]

### RESULTS

**Preparation of the Bone Graft Material**

The crystal structure and chemical composition of BGS is contrasted with that of pure (synthetic) hydroxyapatite in Figures 2 and 3. The proper combination of water and temperature results in the conversion of raw bovine bone to a highly crystalline calcium phosphate.
phase that corresponds to hydroxyapatite, the major mineral component of bone. Two spectrometric techniques that are extensively used in identification and characterization of chemical compounds are X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). The XRD pattern is a representation of the atomic structure of compounds, while the FTIR spectrophotograph serves to identify the various chemical groups that make up a compound. The XRD pattern (Fig. 2) of BGS is characteristic of pure well crystallized hydroxyapatite. The FTIR spectra (Fig. 3) indicate that BGS and pure hydroxyapatite have very similar compositions. However, the BGS FTIR spectrum shows the presence of

Table 7.

<table>
<thead>
<tr>
<th>NDD</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Long term or continuous administration; 100 to 365 daily doses per year</td>
</tr>
<tr>
<td>1</td>
<td>Continuous or long-term use with several-day intervals</td>
</tr>
<tr>
<td>2</td>
<td>Short term or single administration</td>
</tr>
</tbody>
</table>

Table 8.

<table>
<thead>
<tr>
<th>APL</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Products applied directly into the central nervous system.</td>
</tr>
<tr>
<td>1</td>
<td>Products administered directly into blood vessels.</td>
</tr>
<tr>
<td>2</td>
<td>Parenterally administered products used in open wounds.</td>
</tr>
<tr>
<td>3</td>
<td>Subcutaneous or intracutaneous applications. Products applied directly on mucous membranes and products remaining in the mouth.</td>
</tr>
<tr>
<td>4</td>
<td>Parenterally administered products used in open wounds.</td>
</tr>
<tr>
<td>5</td>
<td>Products administered orally.</td>
</tr>
<tr>
<td>6</td>
<td>Products applied to undamaged external skin.</td>
</tr>
<tr>
<td>7</td>
<td>Same as APL 6, but with a warning note prohibiting application on damaged skin or mucous membrane.</td>
</tr>
</tbody>
</table>

Figure 2.

XRD patterns of pure HA (top) and BGS (bottom).
CO$_3$ (at approximately 1412 and 1450 cm$^{-1}$) characteristic of biological calcium phosphate apatites.$^{14}$

Kjehdahl analysis showed the product had 14 ± 6 ppm residual nitrogen ($n = 42$), and carbon analyses showed less than 0.05% carbon ($n = 20$). This confirms that the HT process results in a product completely devoid of the organic material contained in raw bone. The final product conforms to accepted standards for composition of anorganic bone for surgical implants.$^{15}$

**Risk Assessment**

Risk assessment based on the German Federal Ministry of Health method. Application of the German model to the bovine-derived bone substitute is presented in Table 9. A total of 30 points were accrued, which exceeds the minimum of 20 required for a product to be classified as acceptable for human use. The design of this system implies that the risk from each of the six categories is multiplied, and the total risk reflects the “degree of safety” of a product, relative to a product with known infection. Thus in establishing a minimum of 20 points, the model requires that a product be 20 orders of magnitude safer than a product carrying the highest potential risk factor. A total score of 30 points indicates that if the raw bone is acquired from a BSE free source and manufactured using the high temperature process, the resultant bone substitute product is $10^{10}$ times safer than a product of acceptable safety.

**PhRMA BSE Risk Assessment**

Results of risk calculations are shown below:

**Equation 1.** As compiled by the WHO based on reports of outbreak of the disease in various regions of the world, the potential risk ($R_1$) of an animal sourced in the United States being infected with BSE is:

$$R_1 = 1 \times 10^{-6}$$

**Equation 2.** Assuming occurrence of the disease, the potential risk of BSE contaminating a batch of bovine bone used in manufacturing of bone substitute material is:

$$R_2 = 1 \times 10^{-6} \times 10,000$$

$$R_2 = 1 \times 10^{-2}$$

The vendor of raw bovine bone used for the manufacturing of BGS estimates that a maximum of 10,000 animals are rendered in a single batch. Although the amount of raw bone used in the manufacturing of a batch is a small fraction (1000 pounds) of a batch of cattle rendered by the vendor, an accurate estimate of
the number of animals contained in this amount cannot be calculated. Therefore, a worst case scenario of 10,000 animals is attributed per batch.

**Equation 3.** Assuming that infected bone is used, the risk of human infectivity per infected raw bone is:

\[
R_3 = 0.1 \times 10 \times 0.001 \times 0.5
\]

\[
R_3 = 5 \times 10^{-4}
\]

The estimated infectivity of 0.1 units per gram was obtained from the WHO/OIE guidelines for Category 4 tissue (Table 1). In most dental applications such as extraction sites, furcations, intrabony defects, etc., bone substitute graft material is applied in 0.25 to 1 gram doses. However, a maximum dose of 10 grams is assumed for this calculation. The species barrier represents the reduction in infectivity probability due to the higher threshold that exists across species. A mouse has approximately 1,000 fold species barrier, a human is assumed to be equal to a mouse. Correction for LD50 is made as by definition only 50% of patients get disease with an LD50 dose.

**Equation 4.** Assuming that a batch of bovine-derived bone substitute is produced from contaminated bone, the potential number of human infections per batch is:

\[
R_4 = 5 \times 10^{-4} \times 10^{-10} \times 0.9
\]

\[
R_4 = 4.5 \times 10^{-14}
\]

The 1010 factor reduction by process is extrapolated from literature, where a 200°C increase in temperature, from 160° to 360°C has been reported to result in a reduction of infectivity by two orders of magnitude. Since the HT process involves temperatures in excess of 1000°C, a 1010 factor of reduction is attributed. Transmission efficiency is considered highest if intracerebral route of administration is considered. Based on empirical data, the estimate for an intravenous application is 0.9.

**Equation 5.** The potential risk of a patient becoming infected per dose of bone substitute manufactured using the HT process is:

\[
R_5 = (4.5 \times 10^{-14} \times 1 \times 10^{-2})/6,000
\]

\[
R_5 = 7.5 \times 10^{-20}
\]

A maximum of 6,000 1 gram doses is manufactured in one production batch.

**Equation 6.** The potential risk of a patient becoming infected per treatment of bone substitute manufactured using the HT process from raw bone obtained from a BSE-free source is:

\[
R_6 = 7.5 \times 10^{-20} \times 10
\]

\[
R_6 = 7.5 \times 10^{-19}
\]

BGS is normally applied in less than 1 gram doses per surgical site and the clinical procedure is seldom repeated more than once. But for the purposes of this analysis, a worst case scenario of 10 repetitions, or application in 10 different surgical sites in a single patient is assumed.

Based on the above reasoning, the risk analysis demonstrates that we can expect 7.5 infections for every 1019 1 gram doses or one case of infection for
every $1.3 \times 10^{18}$ doses (1 gram each) of bone substitute graft material processed by the high temperature process using raw bone from BSE-free sources.

DISCUSSION

There were several assumptions that were made during application of the PhRMA model to commercially available BGS. The number of animals used per batch of bone substitute graft material was an approximate number obtained from the vendor of the raw bone. Although this number illustrates a worst case scenario, it was used due to lack of a more accurate estimate of the number of animals that constitute a batch. The factor for species barrier has been included in the calculations because the model is intended to assess the transfection of the BSE agent from a bovine source to a human. It may be argued that the discovery of CJD in humans precludes this factor from calculations. However, this would only be true if the risk of CJD transfection from one human to another were being calculated. In this instance, while the species barrier is not assumed to provide immunity from the disease, it is expected to provide a higher threshold for infection and thus decrease the odds of transmission. Finally, none of the proposed routes of administration described in the model are directly applicable to the clinical utility of the bone substitute graft material. The intravenous route was chosen based on the closest resemblance.

Several techniques have been used to inactivate prion infectivity of biological tissue. These range from physical treatments such as short-term exposure to dry heat and steam autoclaving, to chemical inactivation procedures like exposure to alcohol, bases, acids, organic solvents such as phenols, SDS (sodium dodecyl sulphate), hexylene glycol, formaldehyde, and various combinations of the above. Some of these procedures have proven to be ineffective, while most result in significant reductions in infectivity.19-27 There have also been instances where exposure to chemical solvents increased the resistance of the pathogens to inactivation. In some cases, authors that previously reported success in inactivation using low temperature now report new findings that dispute their earlier results.28,29 Obviously, a consensus as to the best/most effective treatment procedure does not exist. Nonetheless, a detailed scrutiny of current literature reveals that short-term exposure to low temperature, such as standard autoclaving procedures, does not unequivocally demonstrate complete inactivation. But there is clear evidence suggesting that the degree of inactivation is directly related to temperature and time. Exposure to high temperatures (in excess of 1000°C) for extended periods of time (in excess of 6 hours) have been most effective in inactivation of the BSE agents, whether or not combined with chemical solvents.19,30,31

While using dry heat, a titer of surviving infectivity is known to be reduced progressively as temperature is increased. No infectivity was recovered in a study conducted under dry heat at 200°C for 1 hour.2

Studies conducted at much lower temperatures have shown complete inactivation by autoclaving at 132°C for 1.5 hours, while autoclaving at 132°C for 1 hour was ineffective.30 Other studies have shown that exposure of BSE infected tissue to 160°C for 1 hour resulted in a $10^{5.3}$ factor reduction in infectivity and exposure to 360°C for 1 hour showed a $10^{7.3}$ factor reduction.1 Thus, a 200°C increase in temperature, from 160°C to 360°C, alone resulted in a two order of magnitude reduction in infectivity—from $10^{5.3}$ to $10^{7.3}$. Relative to these parameters, bovine-derived bone substitutes manufactured by the HT process using raw bone from BSE free sources are exposed to extremely high temperatures for prolonged time periods. Therefore, the reduction factor in theoretically infected high temperature processed bovine bone will be much higher than $10^{7.3}$. Thus a reduction factor of $10^{10}$ chosen in this assessment appears conservative.

A particularly significant study conducted to examine the effectiveness of high temperature as a means of BSE inactivation was commissioned by the United Kingdom Environmental Agency. This extensive study was focused on the risk of infection to people through environmental pathways, not via implantation in the body. Nonetheless, it is worthwhile to note that the conclusions from the report state that incineration is an effective means of BSE inactivation and the consequent risks of infection to people is negligible.32

CONCLUSIONS

From a technical perspective, the current risk assessment and consequent risk associated with clinical application of this product should be applicable to any bovine-derived bone substitute that is manufactured by the HT process as described in Figure 1. Conversely, the risk associated with using products that are not extracted by the above method will be different. As depicted in Figure 1, transformation of raw bone to the final product involves 3 distinct stages: procurement of raw bone from an acceptable source; processing the raw material using techniques that minimize potential disease transmission; and final packaging. As described in this analysis, each of these stages has a significant impact on the computation of the final risk parameter. Thus a change in a single factor can result in higher risk by several orders of magnitude. That does not mean to say that the hazard of undesirable procurement cannot be countered with other remedies to reduce the final risk, as described in the mathematics of the risk function. In this instance, unequivocal evidence of absence of organic material in the final product is of utmost importance. Failing this, the
uncertainty associated with the final product and the consequent harm will result in a product that poses a considerable risk of disease transmission. For instance, let us apply the German Model to an anorganic bovine matrix based bone substitute manufactured from raw bone that originated in a country with known BSE (category ORG - 4). Further, let us assume the manufacturing process did not ensure complete removal of organic matter. Thus, the reduction of infectivity by process would only be a factor of 3 (RED - 3). The total score, assuming all other conditions are similar to that exhibited in Table 9, would be 19. This product would then be considered unsafe for clinical application.

The conclusions from the German model match those arrived at using the PhRMA model for risk assessment—that the risk of BSE transmission through bovine-derived bone substitutes manufactured from the HT process using raw bone from BSE free sources is clearly negligible. In a continuing operation, this negligible risk will remain true only as long as the conditions under which the present assessment has been made continues to be maintained. Management of risk includes maintaining the present conditions of restricting sourcing of raw materials from disease-free sources, and processing the raw material at high temperature for extended periods of time. The key to a successful risk assessment is continual updating. Therefore, it is essential that manufacturers of bovine-derived products maintain a close relationship with their raw materials vendor(s) in order to ensure communication of discovery of BSE or any form of disease.

In reality, a zero risk does not exist. However, an insignificant risk has been generally accepted as that which affects less than one person in any given country’s population is affected per year. If earth’s population is estimated at 6 billion, and there were a billion planets like earth, and every person on these billion planets was treated with 1 gram of BGS, then we would expect to find approximately one infection among these billion populated planets. A more reasonable perspective is that the risk of BSE infection from BGS is several orders of magnitude less than that posed by the risk of death related to, lightning, tornadoes, or similar remote events.11

ACKNOWLEDGMENTS

The product designated BGS is manufactured by CeraMed Dental, LLC, Lakewood, Colorado, as OsteoGraf/N. The authors are employed by CeraMed Dental in the following capacities: A. Sogal, Chief Engineer, Research and Development and A. Tofe, President and Chief Executive Officer.

REFERENCES

8. Guidelines on safety measures in connection with medicinal products containing body materials obtained from cattle, sheep, or goats for minimizing the risk of transmission of BSE and scrapie. 2-16-1994; Federal Ministry of Health (Germany). Federal Bulletin No. 40.


Send reprint requests to: A. Sogal, CeraMed Dental, LLC, 12860 W. Cedar Drive, Lakewood, CO 80228. Fax: 303/989-5669.

Accepted for publication November 20, 1998.