ウシ等由来原料を使用した医薬品、医療用具等に対する現行措置の評価と今後の対応方針（案）

１．現行措置の評価等

（１）平成 12 年 12 月、ウシ等由来原料の原産国にかかわらず、医薬品、医療用具、医薬部外品及び化粧品（以下、「医薬品、医療用具等」という。）の原料として、BSE の伝播のリスクが高い部位（脳、脊髄、眼等）の使用を禁止したことにより、狂牛病の発生が確認された現時点においても、医薬品、医療用具等として通常使用される範囲では、公衆衛生上のリスクは回避されていると評価している（現時点で厚生労働省が有する科学的知見に基づく評価（別紙１参照））。

（２）なお、平成 13 年９月 19 日、念のため、（１）の措置の徹底のほか、
① 狂牛病サーベイランスで BSE 陽性と診断されたウシ等由来原料の使用を禁止
② 原料となる国内産のウシの飼育過程で動物性飼料（肉骨粉）の使用を禁止
等を指導したところ。

２．今後の対応方針（案）

わが国が BSE 発生国となったことを踏まえ、現時点における更なる安全性確保策として、ウシ等由来原料によるリスクの可能性をできるかぎり排除し、今後の一層の安全性に対して万全を期すため、製造業者等に対し、以下の措置を講じる。

（１）念のため、平成 12 年 12 月の措置に対応していない医薬品、医療用具等が医療機関等で使用されていないことを確認するための現状調査を行うとともに、仮に当該医薬品、医療用具等の存在が確認された場合にあっては、当該医薬品、医療用具等の回収を指導する。

（２）さらに、将来的なプリオン感染のリスクを最小限とするため、製造業者等に対し、以下の措置を講じる（別紙２参照）。
① 医薬品、医療用具等の原料として、BSE の伝播のリスクが高い部位（脳、脊髄、眼等）の使用を禁止する（平成 12 年 12 月の措置の継続）。
② 日本を含む BSE 発生国及び BSE 発生リスクの高い国に加え、リスクの評価がなされていない国（リスク不明国）を原産国とするウシ等由来原料について、医薬品、医療用具等に使用することを原則禁止する(*)。

（*）リスク不明国を原則使用禁止とする措置は、現段階では他の先進諸国でも行われていない厳しい規制である。

なお、やむを得ずこれらの国（ただし、高発生国である英国、ポルトガルを除く。）を原産国とするウシ等由来原料を使用する場合は、次の事項等によって BSE に汚染していないことを科学的に確実に証明できなければならない。

イ　原料のウシ等に BSE の疑いがないこと

ロ　原産国の BSE 防疫体制が組まれていること

ハ　原料のウシ等の飼育管理での動物性飼料が使用されていないこと

③　②に該当する製品については、今後、製造又は輸入を禁止することとし、これに伴い、製造業者等においては、②に対応する製品への切り替えを直ちに行うとともに、製造承認書等の一部変更承認申請を要する場合は、可及的速やかに（遅くとも平成 13 年 12 月 28 日までの約３ヶ月以内に）当該申請手続きを行うこととする。

（３）なお、ワクチン類の取扱いについては、他の製品とは異なり以下の特性を有していることに鑑み、製造業者等において、（２）の措置にかかわらず、製造工程で用いるウシ由来原料を可及的速やかに（遅くとも平成14年３月29日までの約６ヶ月以内に）より安全な原料に切り替えること（別紙３）。

イ　ワクチン類の製造工程において用いられるウシ等由来原料は、血清、肝臓、胆汁等感染リスクが低い部位を使用していること

ロ　最終製品となるまでの製造工程中で、ウシ等由来原料は概ね 10 の６乗以上に希釈されていること

ハ　通常、ワクチン類の製造に要する期間が概ね数ヶ月ないしそれ以上であることから、ウシ等由来原料の即時切り替えが困難であること

（別紙１）

現状のリスクの評価の考え方

１．疫学的に、ＢＳＥ高発生国／ｖＣＪＤ発生国の英国においても、ｖＣＪＤの原因として医薬品は指摘されていない。

２．臓器分類毎の感染リスク（感染ヒツジの臓器のマウスへの脳内注射感染実験による。）を基に、現在使用を禁止しているウシ等の部位はカテゴリーＩ及びＩＩであり、使用が認められているＩＩＩ及びＩＶは、感染動物を仮に使用していたとしても、通常の医薬品としての使用において感染リスクは考えにくい。

カテゴリー	リスク	臓器の例	脳内投与時の ID_{50}/g（注１）	リスク(注２)
Ｉ	高リスク	脳、神経組織等	10^7	１
ＩＩ	中リスク	脾臓、リンパ節、腸、胎盤等	$< 2.5 \times 10^4$	400分の１以下
ＩＩＩ	低リスク	膵臓、肝臓、肺等	< 100	10万分の１以下
ＩＶ	リスクなし	骨格筋、骨、心臓、血液、乳等	< 0.1	１億分の１以下

出典：　欧州医薬品庁評価及びＦＤＡレポート「Estimating Risks for vCJD in Vaccines Using Bovine-Derived Materials」

（注１）ID_{50}/g：　臓器１グラムあたりの感染単位数（number of infectious units per gram of tissue)。

（注２）カテゴリーＩに属する臓器（脳、神経組織等）のリスクを１とした場合の他のカテゴリーに属する臓器のリスクを示す。

なお、脳内投与に比して、筋肉注射や静脈注射等通常の注射を行った場合は、さらに200分の１程度リスクが減じられると言われている。

（別紙２）

１．ＢＳＥ発生国又は発生リスクの高い国

	国　名
BSE 発生国	英国＊、ポルトガル＊、スイス、フランス、チェコ、アイルランド、オマーン、オランダ、ベルギー、デンマーク、ルクセンブルグ、ドイツ、ギリシャ、イタリア、スペイン、リヒテンシュタイン、日本
BSE 発生リスクの高い国	アンドラ、アルバニア、オーストリア、ボスニア・ヘルチェゴビナ、ブルガリア、ノルウェー、クロアチア、ユーゴスラビア、フィンランド、ハンガリー、マケドニア、モナコ、ポーランド、ルーマニア、スロバキア、スウェーデン、サンマリノ、キプロス、エストニア、リトアニア、スロベニヤ

（注１）米国連邦規則第９巻第一章第９８条第１８項（米国農務省告示）（9CFR Ch.I § 94.18）をもとに、新たに米国で輸入制限国となった国、欧州委員会の地理的 BSE リスク評価結果（GBR）クラス III（高発生国以外の国及びリスクの高い国）となった国を追加。（下線部）

（注２）＊はＢＳＥ高発生国

２．ＢＳＥ発生リスクの低い国

	国　名
BSE のリスクの低い国	アルゼンチン、オーストラリア、ボツワナ、ブラジル、チリ、コスタリカ、エルサルバドル、ナミビア、ニカラグア、ニュージーランド、パナマ、パラグアイ、シンガポール、スワジランド、ウルグアイ、カナダ、コロンビア、インド、ケニア、モーリシャス、ナイジェリア、パキスタン、米国

（注３）欧州委員会の地理的 BSE リスク評価結果（GBR）クラスⅠ及び II

３．ＢＳＥ伝播のリスクの高いウシ等の部位

脳、脊髄、眼、腸、扁桃、リンパ節、脾臓、松果体、硬膜、胎盤、脳脊髄液、下垂体、胸腺又は副腎

（別紙３）

ワクチンのＢＳＥリスクについて
（平成１２年７月のＦＤＡの判断を参考）

ワクチンの製造工程において、ウシ等由来原料はマスターシードの製造、菌、ウイルス等の培養に用いられる。そこで用いられるウシ等由来原料は、もともと、血清、肝臓、胆汁等の感染リスクの低い部位であること、最終製品となるまでに製造工程中で、これら原料が概ね１０の６乗以上に希釈がされていることからも、現時点で厚生労働省が有する知見に基づけば、安全性において懸念はないと思われる。

Recommendations for the Use of Vaccines Manufactured with Bovine-Derived Materials

Bovine-derived materials have traditionally been used in the manufacture of many biological products, including vaccines. Bovine spongiform encephalopathy (BSE), so-called "mad-cow disease," was first recognized in the United Kingdom (UK) in the 1980s[(1)]. The Center for Biologics Evaluation and Research (CBER) of the U.S. Food and Drug Administration (FDA) has been concerned about eliminating any potential for contamination of biological products with the BSE agent. This concern was heightened by the appearance of the human transmissible spongiform encephalopathy known as variant Creutzfeldt-Jakob Disease (vCJD, also referred to as new-variant CJD) in the UK in 1996; vCJD has been attributed, among other possibilities, to eating beef products from cattle infected with the agent of BSE [(2)]. To date, there are no reports of BSE contamination of pharmaceutical or biological products. To minimize the possibility of contamination in such products, the FDA, in 1993 (published in the Federal Register on August 29, 1994, 59 FR 44591), and again in 1996, recommended that manufacturers not use materials derived from cattle that were born, raised, or slaughtered in countries where BSE is known to exist; the FDA referred manufacturers to the listing of such countries that is maintained by the U.S. Department of Agriculture (USDA)[(3)].

In 1991 the USDA list included only countries and other regions in which BSE was known to exist, such as France, Great Britain, Northern Ireland, the Republic of Ireland, Oman, and Switzerland. In 1998, the USDA expanded the list to include countries and other regions in which BSE had not been documented but in which import requirements were less restrictive than requirements that would be acceptable for import into the United States or in which surveillance was inadequate. Thus, all European countries, even those that have had no reported BSE cases, are currently on the USDA list, which is published in the Code of Federal Regulations, title 9, part 94 (9 C.F.R. part 94).

In 2000, CBER learned that its recommendations regarding the sourcing of bovine materials for the manufacture of vaccines had not been followed in at least one instance. As a result of this finding, CBER requested all vaccine manufacturers to review the source for all bovine-derived materials used in the manufacture of their vaccines. This review identified additional vaccines manufactured with bovine-derived materials that had been obtained from European countries on the USDA list.

No evidence exists that any case of vCJD has resulted from the administration of a vaccine product[(4)], and no cases of vCJD have been reported in the United States. To evaluate the risk of disease that might result from a vaccine manufactured with a process that utilizes bovine materials potentially contaminated with the BSE agent, CBER conducted risk assessments and convened a special joint meeting of the Transmissible Spongiform Encephalopathy Advisory Committee and the Vaccines and Related Biological Products Advisory Committee on July 27, 2000. In assessing the potential risk of vaccines, CBER and the joint Committees considered: (1) the likelihood that any cattle that were used might be infected (i.e., the time period and country of origin) and animal husbandry procedures; (2) the amount of bovine material that might be present in the final vaccine; and (3) the inherent infectivity of the various types of bovine materials that were used. The joint Committees concluded that the risk of vCJD posed by vaccines in the scenarios that were presented was theoretical and remote. They also noted that the benefits of vaccination far outweigh any remote risks of vCJD. The joint Committees made several recommendations.

- Bovine-derived materials used in the routine production of vaccines that are sourced from countries on the USDA list should be replaced with bovine-derived materials from countries not on the USDA list.
- Working bacterial and viral seed banks and working cell banks that were established using bovine-derived materials sourced from countries on the USDA list should be re-derived with bovine-derived materials from countries not on the USDA list. However, master bacterial and viral seed banks established in a similar manner do not need to be re-derived; the potential risk presented by the master seed banks is even more remote than that presented by the working seed banks and is outweighed by the risk of altering the bacterial or viral vaccine through re-derivation.
- These issues are of public interest and, therefore, the public should be informed about the safety of vaccines that used materials sourced from countries on the USDA list, and the assessment of the nature of any risk of vCJD from such vaccines.

As noted above, there is no evidence that any case of vCJD has been caused by or is related to vaccines manufactured with bovine-derived materials obtained from countries in which BSE or a significant risk of BSE exists (i.e., countries on the USDA list), and thus the risk of vCJD is theoretical. The joint Committees recommendation to replace such bovine-derived materials with bovine-derived materials from countries no on the USDA list is a precautionary measure intended to minimize even the remote risk of vCJD from vaccines.

The vaccines that use bovine-derived materials from countries on the USDA list include: Aventis Pasteur, S.A.'s *Haemophilus influenzae* type b conjugate vaccine, ActHIB® (ActHIB® is also marketed as OmniHIB™ by SmithKline Beecham Pharmaceuticals); BioPort's anthrax vaccine; North American Vaccine Inc.'s diphtheria and tetanus toxoids and acellular pertussis (DTaP) vaccine, Certiva™ (the tetanus toxoid manufactured by Statens Seruminstitut for use in Certiva™ is the only component of the vaccine manufactured with bovine-derived materials from a country on the USDA list); SmithKline Beecham Biological's DTaP vaccine, Infanrix®(the diphtheria toxoid manufactured by Chiron Behring GmbH & Co. fo use in Infanrix® is the only component of the vaccine manufactured with bovine-derived materials from a country on the USDA list),hepatitis A vaccine, Havrix®, and the hepatitis A inactivated and hepatitis B (recombinant) vaccine, TWINRIX® .

In some other cases, the source of the bovine-derived materials is unknown, in part because manufacturer have not always maintained or had access to records of the source of such materials, particularly in the 1980s and early 1990s, before the connection between BSE and vCJD was first suggested. Vaccines that use bovine-derived material of unknown origin obtained in 1980 or thereafter (the current best estimate is that BSE first emerged in 1980) include: Aventis Pasteur, S.A.'s inactivated polio vaccine, IPOL® and Lederle Laboratories' pneumococcal polysaccharide vaccine, PNU-IMUNE® 23.

Vaccines using bovine-derived materials from a country on the USDA list or from an unknown source to manufacture only the master seed are not listed above; the joint Advisory Committees indicated that maste seeds need not be re-derived. Additional information on such vaccines can be obtained upon request.

The FDA has requested that manufacturers of vaccines using bovine-derived materials obtained from countries on the USDA list or from an unknown source replace these materials with materials from countries not on the USDA list, consistent with the recommendations of the joint Advisory Committees. The manufacturers have agreed to fully implement these changes. Indeed, several manufacturers initiated a number of these changes before the July 27, 2000, joint Advisory Committee meeting. FDA anticipates tha the majority of these changes will be completed within one year. The FDA will revise the list of vaccines using bovine-derived materials from countries on the USDA list or from an unknown source as the requested changes are implemented and the vaccines come to market (see section VIII for the current listing).

The Public Health Service (PHS) recommends that all children and adults continue to be immunized according to current immunization schedules[(5)]. At the present time, the PHS has no preference for using one licensed vaccine product over another based on the source of bovine-derived materials used in vaccin production. The recommendations of the FDA Advisory Committees and the actions of the FDA are, as described, precautionary and have been taken to reduce even the remote potential of a risk of vCJD and to maintain public confidence in the safety of vaccines. Failure to obtain the recommended vaccinations with licensed vaccines poses a real risk of serious disease.

References

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Bovine Spongiform Encephalopathy (BSE)

Estimating Risks for vCJD in Vaccines Using Bovine-Derived Materials

The risk of vCJD from bovine-derived materials

The risk of developing an illness such as vCJD from the use of bovine-derived material in the manufacture of vaccines is a function of a number of factors, including the nature and the amount of the bovine tissue that is used in manufacture, as well as the date and country of origin of the cows [(1)]. Other factors, such as how the cows were fed, are also important. In this regard, the CDC estimates that the risk, if any, for vCJD from eating a beef meal in Europe is less than approximately 1 in 10 billion [http://www.cdc.gov/travel/madcow.htm].

CBER's survey of vaccine manufacturers revealed a number of vaccines that utilized bovine materials that were obtained from countries where BSE or a significant risk for BSE exists. An estimate of the risk that the use of these materials might pose is presented in the following sections. Two examples have been chosen for presentation here, namely, the risk from the use of fetal calf serum sourced from the United Kingdom (UK) in the derivation of a viral working seed that is subsequently used in vaccine manufacture and the use of European-sourced (excluding the UK) beef broth in the production of a bacterial toxoid. Based on CBER's survey of the use of bovine-derived materials sourced from countries on the USDA BSE-list, the potential risk that would be associated with other uses of bovine-derived materials in vaccine production would be less than might be associated with these two situations.

The infectivity of most bovine-derived materials has not been determined experimentally. More is known about the infectivity of various ovine-derived (from sheep) materials. The knowledge of the infectivities of different ovine tissues relative to each other can be used to estimate the relative infectivities of bovine tissues. For example, if we know that, on a gram for gram basis, sheep brain is 100 million times more infective than sheep muscle, we can assume that bovine brain is also 100 million times more infective than bovine muscle. Thus, if the infectivity of bovine brain has been measured, and contains 10 million infective doses per gram, then we can estimate that bovine muscle is 100 million times less infective and contains 0.1 infective dose per gram.

Not surprisingly, since BSE and scrapie (the corresponding disease in sheep) are neural diseases, the greatest infectivity is found in neural tissue. Based on experimental studies, infected bovine brain contains approximately ten million infectious units per gram when administered to other cattle [(2,3)]. In other tissues, such as serum or skeletal muscle, no infectivity has been detected. This does not mean that there is no infectivity associated with these materials; only that, if they are infectious, then the infectivity is at a level that is too low to be measured by current tests.

Table I presents the estimated infectivity of different bovine-derived tissues as determined by The Agency for the Evaluation of Medicinal Products (EMEA) The actual infectivity of skeletal muscle or serum, for example, may be well below the values shown; we will, nevertheless, use these values in our risk estimates. It should be noted that these values are based on experiments in which animals were infected intra-cerebral injection with affected tissue; this is the most effective means of infecting experimental animals. When another route of administration, namely intramuscular injection, is used, infection rates are estimated to be approximately 200 fold lower [(4)].

The risk assessments follow.

Fetal calf serum used to derive viral seed and cell banks

Fetal calf serum from the United Kingdom was used in the production of certain viral seeds and cell banks. The calf serum that was used was produced in the mid-1980s, when the BSE epidemic was just getting underway in the UK [(5)]. The U.S. Department of Agriculture estimated the incidence of BSE in adult cattle a about 1 in 200 at that time[(6)]. [Although many fewer cattle were observed to suffer from mad cow disease a that time, the long incubation time for the disease means that more cattle were infected than appeared diseased.] Since fetal calf serum was used in the production of the cell and viral seed banks, it is necessar to address the question of maternal-fetal transmission. Whether there is mother to fetus transmission of BSE is still unknown. One study may be interpreted as indicating that maternal-fetal transmission occurs a a rate of approximately 10%; i.e., that the calves of one of ten infected mothers may become infected with the BSE agent [(7)]. However, other data indicate that maternal-fetal transmission does not occur or, if it doe occur, it is below this 10% rate [(8)]. As noted above, the U.S. Department of Agriculture estimates that, during the mid 1980s, approximately 1 in 200 cows in the United Kingdom was infected with BSE. Assuming that the rate of transmission from mother to fetus is 10% we would then estimate that 1 in 2000 fetal calves would have been infected.

When fetal calf serum is manufactured, the sera from approximately 1500 calves are pooled together. If 1 i 2000 calves is infected, it is likely that any given serum pool is infected. As mentioned above, although no infectivity has been observed with serum, there are limits to detectability. These experiments only rule out an infectivity that is greater than 1 infectious unit per milliliter (mL) of blood [(3,9,10)]. Although serum is listed as category IV, we are using the highest estimate consistent with infectivity experiments. In the following risk estimate, we assume that the serum of an infected fetal calf can contain up to 1 infectious unit per mL.

In our risk calculation, we assume that the number of infectious BSE units that enters the vaccine production process is equal to the number of infectious units that remain in the vaccine at the end; that is, that the risk for vCJD is the input number of infectious units divided by the number of doses of vaccine that is in the batch. Thus, the risk estimate does not account for any purification step that might be present in the viral vaccine manufacturing process; although there are steps that probably remove infectivity, these are not considered in our risk estimate since none of the manufacturing steps have been demonstrated to remove BSE infectivity. We have also assumed that the BSE agent does not replicate during the manufacturing process; this is a reasonable assumption, bolstered by the many failed attempts to propagate the BSE agent in cell culture [(11)]. The BSE infectivities that are estimated in Table I are derived from data using direct intra-cerebral inoculation (direct injection of the material into the brain). Vaccines are given intramuscularly, a less efficient route of transmitting the disease. In our risk estimate, we have allowed a factor of 200 for reduced transmission by the intramuscular route.

In general, there is a species barrier for the transmissible spongiform encephalopathies; that is, it is easier to infect the same species of animal than another species (for example, bovine material is more infectious for cows than it is for other animals, such as mice) [(3,4)]. The species barrier from cows to humans is not known; in our calculations, we will therefore assume that there is none.

Given these assumptions, we can estimate the risk for vCJD from fetal calf serum (FCS) being used to prepare a viral working seed as the product of four separate risk factors. The level of BSE agent in the serum of an infected calf is estimated at 1 infectious unit per mL. Approximately 1 infected calf is present ir each pool, deriving from approximately 1500 calves, of fetal calf serum. The infectivity of the pooled FCS is thus diluted to 1/1500 infectious units per mL (ca. 6.7 x 10-4 infectious units/mL). The amount of FCS that was used to produce a vial of a working viral seed is approximately 4 mL, and the number of doses of vaccine coming from that batch is approximately 500,000. The risk for acquiring vCJD is therefore:

The number of infected calves in each pool	1/1500
Multiplied by	
The number of infectious units per mL of serum	1
Multiplied by	
The number of mLs of serum used	4

Divided by	
The number of doses of vaccine	500,000
Divided by	
The reduction in infectivity related to the route of	200

This yields a final risk estimate for vCJD of approximately 2.5 per 100 billion or 1 in 40 billion doses of vaccine [(1/1500) x 1 x 4 x (1/500,000) x (1/200)]. This level of risk would correspond to one case of vCJD arising every 5,000 years (assuming two doses per child) when vaccinating the entire birth cohort of the Unites States (four million children). Because of the assumptions that were used, this is an overestimate of the risk, and the true risk is likely to be significantly less. The risk that would be calculated for the use of a master seed that was prepared with fetal calf serum is again considerably less, due to an additional dilution that attends the preparation of the working seed from the master seed.

Beef broth used to manufacture a bacterial vaccine: a bacterial toxoid as an example

The potential risk of vCJD from a bacterial vaccine that used bovine-derived material in the nutrient broth to grow the bacterial strain during vaccine production is as follows. In the example that we are using, tissue derived from a single cow is used to prepare the fermentation broth. For this estimate, the incidence of BSE in European cows is taken to be 1 in 10,000. This value was derived by multiplying the average BSE rate in this region over the last five years by a factor of ten [(1)] to account for any uncertainty in the actual rates. The nutrient medium that is used to grow the bacteria for the vaccine contains approximately 750 grams of skeletal muscle (a Category IV material) and 200 grams of a pancreatic extract (a Category III material); see Table I. Because the broth is autoclaved (heated at high temperature), some of its potential infectivity is lost; a reduction factor of 20 is assigned to the autoclaving process(2).

The risk, per dose of vaccine, for vCJD from a vaccine using a beef/pancreatic extract can be calculated as the product of the risk of using an infected cow (1 in 10,000) times the inherent risk of the bovine material after correction for the autoclaving process (approximately 1000 units; [200 grams of Category III material is estimated to contain no more than 20,000 infectious units and the 750 grams of Category IV material no more than 75 infectious units (20, 075 units total); the autoclaving process reduces this infectivity to approximately 1000 units]), divided by the number of doses that are in a batch of vaccine (approximately 1 million), corrected for the route of administration (a reduction factor of 200).

Risk of an infected cow	1/10,000
Multiplied by	
Amount of infectious material	1000
Divided by	
The number of vaccine doses	1,000,000
Divided by	
The reduction in infectivity related to the route of	200

This yields a risk estimate for vCJD of 1 case in 2 billion doses of vaccine [(1/10,000) x 1,000 x (1/1,000,000) x (1/200)]. A second scenario can also be considered, namely one in which a small amount of neural tissue inadvertently might contaminate the beef broth. We consider a 0.01% contamination with neural tissue. This would increase the amount of infectious material from 1000 units to 50,000 units, raising the total risk to 1 in 40 million. Because of the overestimates that were used in the risk calculation, the true risk is likely to be significantly less.

Potential sources of error

In estimating the risk of BSE contamination, it is important to note that each risk factor carries its own uncertainty. The overall risk, which is the product of these factors, compounds these uncertainties. For example, we have assumed no species barrier and no purification effect. The actual risk could be 10 to 1,000 fold lower, but probably no greater. On the other hand, we have assumed a 200-fold reduction due to an intramuscular route of administration. In fact, this risk could be 10-fold greater or 10-fold lower. Finally, in the case of viral vaccines, and based on experiments with analogous cell lines, we have assumed that BSE cannot replicate in cell cultures that were used. These uncertainties must be considered in order to

interpret the risk of BSE in viral vaccines. These calculations are not a formal risk assessment, but an attempt to estimate risk based on information currently available.

It should be noted that for both the viral and bacterial vaccine examples used, the exposure to this risk is temporary. Manufacturing changes have already been implemented which eliminate exposure during vaccine manufacture to bovine materials from countries at risk of BSE contamination. Vaccines made by these procedures are expected to be available in 2001.

Table 1

Estimated infectivity of bovine tissue by category

Category	Tissue	ID_{50}/gram*
I	Nervous tissue	10^7
II	Spleen, lymph nodes, colon	$<2.5 \times 10^4$
III	Pancreas, liver, lung	<100
IV	Muscle, bone, heart	<0.1

Adapted from: Bader et. al, 1998 BioPharm *ID_{50}/gram = number of infectious units per gram of

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Bovine Spongiform Encephalopathy (BSE)

Overview of Vaccine Manufacturing

The common childhood vaccines (www.cdc.gov/nip/recs/child-schedule.pdf) and many other vaccines, derive from bacteria, yeast, or viruses that are grown in culture. Bacteria are free-living organisms that are able to grow and reproduce when placed in a medium containing various nutrients (the culture medium) such as sugars, salts, or amino acids. Growing bacteria in culture simply refers to placing a small quantity of bacterial cells into a nutrient medium, and then allowing the cells to multiply.

Viruses, on the other hand, are not free-living organisms, and can reproduce (multiply) only in cells (they require many of the cellular enzymes and machinery for reproduction). Human- and animal-derived cell cultures are commonly used for growing vaccine viruses. The cells that are used as substrates for viral growth can, like bacterial cells, maintain themselves and grow only in a medium that contains their needed nutrients. Growing viruses in culture, therefore, involves adding a small quantity of virus to an existing culture of cells and letting the virus replicate in these cells. Commonly, the virus will kill the cell in which it is grown.

In order to manufacture vaccines consistently, it is essential to begin the manufacturing process (the growt of the virus or bacteria) with the exact same virus or bacterium; moreover, it is essential to start the proces with a virus or bacterium that is pure. This is accomplished through the development and maintenance of “seed banks.” A seed is the general term that is used to describe the small amount of bacteria or virus that is added to growth media or cell cultures to initiate further expansion of the bacterial cells or viruses.

Consider, for example, the manufacture of a bacterial vaccine; see Figure 1. During the period when a new bacterial vaccine is being developed, a small amount of a pure culture of that vaccine bacterium is grown and frozen away; this material is termed the “master seed bank.” A small portion of this master seed bank i then expanded (grown in culture) and frozen away in many small portions (for example, in a thousand different vials); these small portions are collectively referred to as the “working seed bank.” The “master” and “working” cell banks are purified, characterized and shown to be free of known contaminants prior to using them in production. Each time a batch of the vaccine is manufactured, the process begins with one o the vials from the working seed bank. Through the use of “working seed banks” the manufacturing process always begins with the same material. If the vials of working seed are exhausted, another small portion of the master bacterial cell bank can be used to produce a new working seed bank, for example, another thousand vials. Since the number of batches of vaccine that are produced each year is limited, this banking system provides a sufficient amount of material to last, in a practical sense, indefinitely.

The viruses that are used in vaccine manufacturing also use a seed lot system. “Master” and “working” vira seed banks are produced and stored away in a similar fashion, the difference being that the viruses are propagated in cells as opposed to a simple nutrient medium. In order to ensure the consistency of the viral vaccines, “master” and “working” cell banks are also kept in those instances for which the virus is propagated in cell lines (as opposed to primary animal cell cultures). When a cell line is developed, a substantial amount is frozen away as a “master cell bank;” a small portion of the master cell bank is grown and stored away in many small vials, thus forming the "working cell bank."

The nutrient media that are used to support the growth of bacteria and the cell cultures in which viruses are grown often contain animal-derived components and, commonly, bovine-derived (from cows) components. As examples, the cells that are used to propagate viruses generally require calf serum for their

maintenance and growth and the nutrient broths that are commonly used to grow bacteria contain beef extracts (e.g., a beef broth).

A typical viral vaccine production scheme is outlined in Figure 2. The seed banking system has been described above. At the start of vaccine production, the manufacturer thaws a vial of the working cell bank and grows it to large amounts, whereupon it is infected with a vial of the working seed virus, which will ther grow to large numbers. One vial of the working cell bank and working seed virus may result in a half millior or more doses of vaccine.

One of the major difficulties in the manufacture of vaccines arises from the possibility of introducing adventitious agents into the process. Obviously, if the seed virus or cells contain a viral agent other than th vaccine virus, it too may grow. Similarly, and the topic of current concern, if the BSE agent enters into the vaccine manufacturing process at any point, that agent may be carried through into the final vaccine formulation. Variant Creutzfeldt-Jakob disease, as mentioned in other sections of this Web Site, has been attributed to, among other possibilities, the consumption of products from BSE-infected cattle.

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Figure 1. Manufacture of a bacterial vaccine

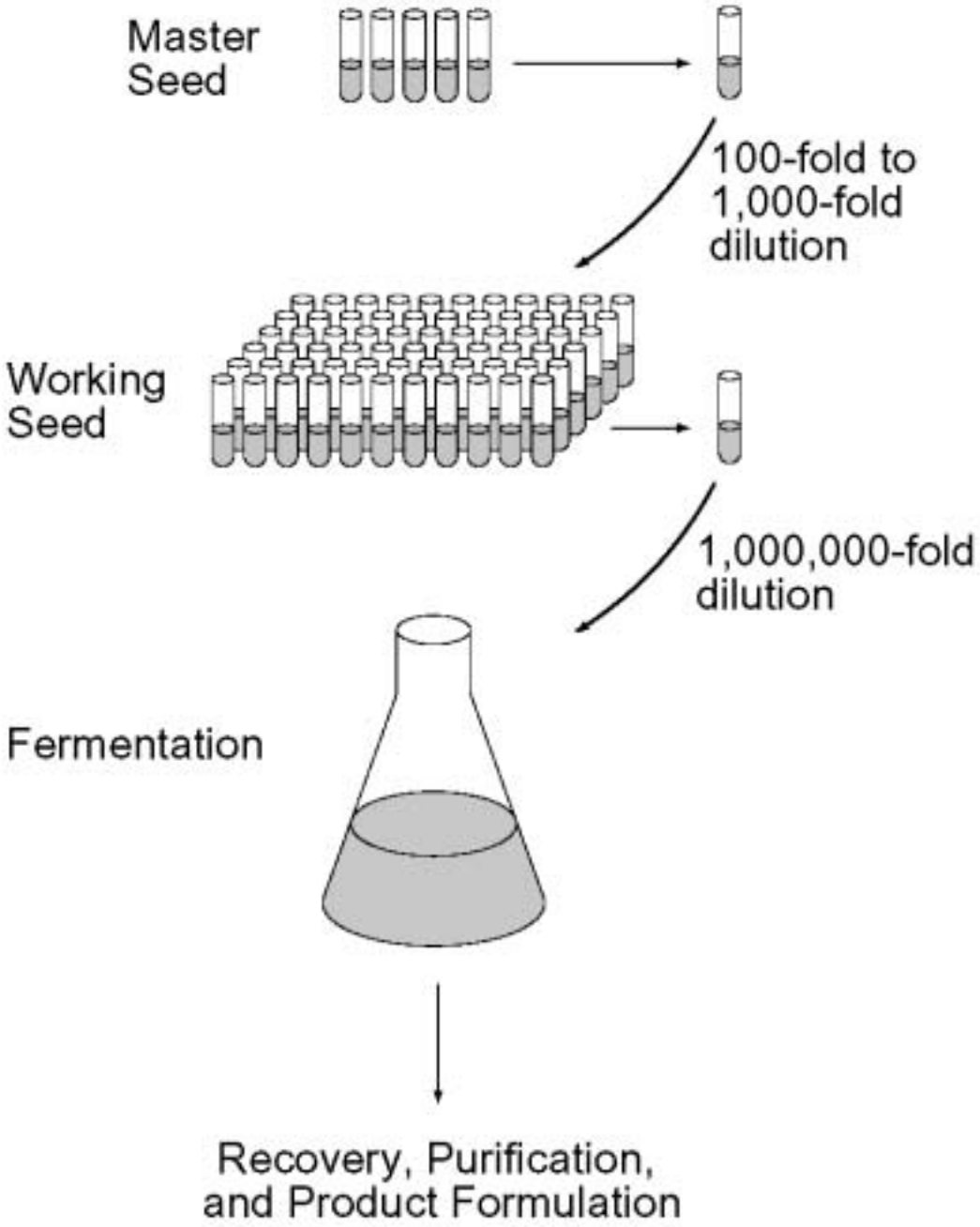

Figure 2: Viral Vaccine Production