

Table 1: Tentative summary of preliminary estimations on classification of tissues of cattle according to infectivity after experimental oral or natural exposure to the agent of BSE.

Infectivity titres**:

A = high $10^{3.0} - 10^{5.0}$ in mouse; $10^{5.7} - 10^{7.7}$ in cattle ***
 B = medium $10^{1.5} - 10^{3.0}$ in mouse; $10^{3.3} - 10^{5.6}$ in cattle ***
 C = low $\leq 10^{1.5}$ in mouse; $\leq 10^{3.2}$ in cattle ***

	EXPERIMENTAL			NATURAL
			clinical	clinical
months after exposure	6-26	32	36-40	-
Brain	-	B / C	C	A
Spinal cord	-	C	C	A
Dorsal root ganglia	-	C	C	C
Trigeminal ganglion	-	-	C	

· Refer to the report for further detail

** The classification used is preliminary and arbitrary because of a skewed range of infectivity in cattle with BSE compared to sheep with scrapie. It does not correspond to the Groups or Categories used in previous similar estimates of scrapie infectivity in sheep tissues. Ranges of values are given as: Log₁₀ mouse intracerebral/intraperitoneal LD/50 per g tissue, or Log₁₀ cattle intracerebral LD/50 per g tissue.

*** Categories in bold in the table are based on bioassays in cattle and the remainder on bioassays in mice.

-: Negative

- b. In cattle after experimental oral exposure to the agent of BSE⁵ detection of infectivity in brain and spinal cord (by mouse bioassay) was relatively late (80-90%) in relation to the minimum incubation period recorded in that specific experiment). This however, does not provide information on the first occurrence of infectivity in CNS tissues, relative to incubation in field cases of BSE. It must be borne in mind that in this study tissue bioassays in mice were carried out on the pooled single tissue from 2-3 cattle killed at each sequential time point. As cattle for each kill group were unselected, animals within a group would have different incubation periods, dependant on the range of incubations for the given exposure dose. Dose response data of cattle infected orally with a dose of BSE infectivity closely similar to that administered to induce disease in the Pathogenesis Study (G. A. H. Wells, unpublished data) suggests a mean incubation of almost 45 months (range 33-55 months). At the time of clinical onset of disease in the exposed cattle in the Pathogenesis Study only 8 animals remained in the study and their

⁵ Wells, G.A.H., Hawkins, S.A.C., Green, R.B., Austin, A.R., Dexter, I., Spencer, Y.I., Chaplin, M.J., Stack, M.J. & Dawson, M., 1998. Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update. *Veterinary Record* 142, 103-106..

clinical statuses relative to the times after exposure at which they were killed was as follows (Wells *et al*, 1998):

Months after exposure:	Clinical status:
36	+/-, +/-, -
38	+, +/-, +/- *
40	+, +/-*

- + : Definite Clinical Signs of BSE
- +/- : Probable/early Clinical Signs of BSE
- : No consistent clinical signs of BSE
- * : Individual cattle in which no pathology (vacuolation or PrP) was detected in the CNS

It is clear from this that most of the animals killed after the earliest clinical onset, at 35 months after exposure, were at an equivocal or early clinical stage. Two animals, one at 38 months and another at 40 months, did not show CNS lesions and may not have contributed to the CNS infectivity detected for each of their respective groups. If this were so, then in these animals, we can assume for the purpose of the present argument, that infectivity had not reached the CNS. This assumption places the interpretation of observed clinical signs in these animals into question and leaves the possibilities that they either remained preclinical cases or had not been infected. If it is then assumed that the incubation periods of all of the animals in the Pathogenesis Study would fall somewhere in the range determined by the dose response data of cattle infected orally (33-55 months) then the possible range of the interval between onset of detectable CNS infectivity (by mouse bioassay) and incubation period can be suggested from the combined data from these studies. Since infectivity was not detected at 26 months after exposure, but was present at 32 months, the interval between earliest possible CNS infection and minimum incubation period might be $33-27=6$ months, whereas the interval between the earliest possible CNS infection and maximum incubation period could be $55-27=28$ months. In attempting to relate this to natural disease the differences in the mean incubation period for the experimental study (45 months) and that estimated from age specific incidence in the epidemic (60 months, UK data), must be considered and is addresses further at c) below.

In its opinion of 12 January 2001 (EC, 2001) the Scientific Steering Committee concluded that, as a reasonable worst case scenario, based on available experimental results, it could be assumed that infectivity in the CNS can become detectable as from approximately half the incubation period. The justification for this assumption were:

- Animal numbers per experimental group in the Pathogenesis Study were small and so the above percentage (i.e 80-90%, in relation to the minimum incubation period recorded in *that specific* experiment) could well be revised downwards by studies still in progress.

- The time at which, during incubation, infectivity can first be detected in the CNS of animals with TSEs varies with the specific natural disease and, in experimental models, with host and agent variables, particularly those of PrP genotype, agent strain and route of exposure. In certain mouse models of scrapie using non-neural peripheral inoculation routes (including intragastric) detection of infectivity in brain occurs at 40-50% of the incubation period. In 263K hamster scrapie in hamsters the equivalent value is 25%. In certain models this has been shown to be preceded by infectivity demonstrable in the spinal cord.

As BSE has been found in animals below 24 months, a logical conclusion was to lower the threshold age for considering the vertebral column and dorsal root ganglia as risk materials to, for example, to 12 months.

- c. However, an alternative argument has sometimes been advanced that infectivity would reach the CNS in the greater proportion of BSE cases at a much later age, because with a mean estimated age of onset of clinical signs of 60 months in field cases and assuming calfhood exposure, half of the incubation period is at approximately 30 months of age.

The doses of infected brain used in the Pathogenesis Study (100g) was relatively high and it is known that the incubation period shortens with increasing dose (EC, 2002). As all animals received the same dose, (and in cattle no genetic factors have been shown to affect susceptibility to BSE) it might also be expected that the incubation period distribution in the animals in this experiment would fall within the range of the incubation periods determined from the dose-response data of cattle infected orally with a dose of BSE infectivity closely similar to that administered to induce disease in the Pathogenesis Study (G. A. H. Wells, unpublished data) As discussed above a preliminary estimate from that study suggests a mean incubation of almost 45 months (range 33-55 months). This mean is clearly less than that indicated from epidemiological observations, for the majority of cases, but the range is, nevertheless, within that estimated for incubation periods for field cases. Thus, following this argument if one allows for a safety margin of some months, for the presence of infectivity at as yet undetectable levels, and given that infectivity in dorsal root ganglia may well be secondary to established CNS infection, it may be concluded that residual risk of vertebral column after removal of the spinal cord would be negligible in the vast majority of infected cattle aged below 24 months.

The TSE/BSE *ad hoc* Group however considers that the BSE in cattle pathogenesis study (Wells, 1998) cannot be exploited to express the time of detectable infectivity in the Central Nervous System tissues as a fraction of the total incubation period and that the limited number of animals used in this study do not allow to conclude that infectivity is absent in the spinal cord until a few months before clinical signs are manifested.

From other experiments with other animal species and for which more data are available (e.g., mice, hamster, primates, sheep, ...) it may be concluded

that the assumption made by the SSC on 12 January 2001, i.e., that in general, as a reasonable worst case assumption, the dorsal root ganglia and the spinal cord are considered to pose a higher risk as from the second half of the incubation period, remains valid.

- d. A possible increase of the current age limit of 12 months for treating vertebral column as SRM in bovine animals, depends upon the age below which the probability of infectivity being present [at levels that pose a risk for humans] in the spinal cord and dorsal root ganglia would be remote. This in turn requires a [cost/benefit?] decision of what can be considered as an "acceptable" BSE incidence in terms of human exposure risk.

The human exposure risk on its turn depend upon the he cattle – human species barrier, which is not known: it could be non-existent (barrier = 1) or even very high (e.g., 10.000). According to the SSC (EC, 2000c), values greater then one are likely to be more realistic.

IV. ANALYSIS OF THE AGE DISTRIBUTION OF RAPID-TEST BSE-POSITIVES IN EUROPEAN UNION IN SECOND SEMESTER OF 2001.

According to the EC (2002) draft report on BSE testing in 2001 a total of 8.501.457 bovine animals were tested in 2001 in the framework of the monitoring programme, 2150 of which turned out positive. 8.441.360 bovine animals were tested by active monitoring (rapid tests on risk animals and animals slaughtered for human consumption) while 3.634 bovine animals were tested in the passive surveillance (animals reported as BSE suspects by the farmer or the veterinary practitioner and subject to laboratory examination). In addition, 56.463 animals were tested in the framework of BSE eradication. 49 % of positive cases were detected by the Active Monitoring and 51 % were detected by Passive Surveillance. Positive cases were found in all Member States except Luxembourg and Sweden.

Tables 2 to 9 hereafter are extracted from that report and provide a summary of the information which is relevant in the context of the current opinion.

Annexes 1 - 6 provide the details of the Age Distribution of Positive Cases in 2001, for the following categories of animals: fallen stock, (Healthy slaughtered animals), Risk Animals, Suspects and risk animals.

Table 2: Age limits used in sampling

	Age Limit				
	Emergency Slaughter	Clinical Signs at ante mortem	Fallen Stock	Healthy Animals	BSE Suspects
Belgique/België	>24months	No age limit	>24 months.	>30 months.	No age limit
Danmark	>24 months	>24 months	>24 months	>30 months	No age limit
Deutschland	Compulsory testing of animals > 24 months. Voluntary testing of animals <24 months				No age limit
Ellas	>24/30 months ¹	>24/30 months ¹	>24/30 months ¹	>30 months	No age limit
España	>24/30 months ¹	>24/30 months ¹	>24/30 months ¹	>30 months	No age limit
France	-	-	>24 months	>24/30 months ¹	No age limit
Ireland	-	>24/30 months ¹	>24/30 months ¹	>30 months	No age limit
Italia	>24/30 months ¹	>24/30 months ¹	>24/30 months ¹	>24/30 months ²	No age limit
Luxembourg	>24/30 months ¹	>24/30 months ¹	>24/30 months ¹	>30 months	No age limit
Nederland	>24/30 months ¹	>24/30 months ¹	>24/30 months ¹	>30 months	No age limit
Österreich	> 24 months ⁵				No age limit
Portugal	>24/30 months ¹	>24/30 months ¹	>24/30 months ¹	>30 months	No age limit
Suomi-Finland	>24/30 months ¹	>24/30 months ¹	>24/30 months ¹	>30 months	No age limit
Sverige	>24/30 months ¹	>24/30 months ¹	>24/30 months ¹	>30 months	No age limit
United Kingdom	> 30 months	No age limit	>24/30 months ³	>30 months ⁴	No age limit

¹From January to June: >30 months

From July to December: >24 months

²From January to September: >30 months

From October to December: >24 months

³Northern Ireland: >24 months⁴Northern Ireland: No age limit⁵In Austria, since January 2001, all bovines older than 20 months, which show unspecific central nervous symptoms or are emergency slaughtered are tested on BSE. Since October 2001 also all fallen stock from 20 months onwards is tested on BSE.**Table 3: Total testing**

	Nr of tests performed						Total
	Emergency Slaughter	Clinical signs at ante mortem	Fallen Stock	Healthy slaughtered	BSE Suspects	BSE eradication	
Belgique/België	1.513	137	13.060	359.435	242	3.522	377.909
Danmark	1.796	99	20.297	250.414	73	4.286	276.965
Deutschland	7.972	185	268.776	2.565.341	214	13.849	2.856.337
Ellas	224	2	1.429	15.360	3	95	17.113
España	3.353	195	50.033	328.517	464	3.700	386.262
France	171	0	133.718	2.382.225	469	11.117	2.527.700
Ireland	0	893	24.614	636.930	482	12.196	675.115
Italia	8.282	14.648	47.214	388.494	10	5.098	463.746
Luxembourg	30	35	1.330	19.475	14	2	20.886
Nederland	13.279	2	31.056	454.649	97	2.558	501.641
Österreich	2.490	0	7.023	216.045	2	28	225.588
Portugal	1.468	5.403	1.162	28.384	326	2.012	38.755
Suomi-Finland	8.140	5.940	3.880	9.882	3	31	27.876
Sverige	1.393	2	22.248	4.433	25	0	28.101
United Kingdom	46.189	13	27.228	21.033	1.211	407	96.081
Total	96.300	27.554	653.068	7.680.617	3.635	58.901	8.520.075

Table 4: Monitoring in relation to the total population

	Adult cattle ¹ (in million)	Risk Animals ²		Healthy Animals	
		Nr Tests	% Tests/Adult cattle	Nr Tests	% Tests/Adult cattle
Belgique/België	1,5	14.710	0,98%	359.435	24,0%
Danmark	0,9	22.192	2,47%	250.414	27,8%
Deutschland	6,5	276.933	4,26%	2.565.341	39,5%
Ellas	0,3	1.655	0,55%	15.360	5,1%
España	3,4	53.581	1,58%	328.517	9,7%
France	11,2	133.889	1,20%	2.382.225	21,3%
Ireland	3,4	25.507	0,75%	636.930	18,7%
Italia	3,4	70.144	2,06%	388.494	11,4%
Luxembourg	0,1	1.395	1,40%	19.475	19,5%
Nederland	1,8	44.337	2,46%	454.649	25,3%
Österreich	1,0	9.513	0,95%	216.045	21,6%
Portugal	0,8	8.033	1,00%	28.384	3,5%
Suomi-Finland	0,4	17.960	4,49%	9.882	2,5%
Sverige	0,7	23.643	3,38%	4.433	0,6%
United Kingdom	5,0	73.430	1,47%	21.033	0,4%
Total	40,4	776.922	1,92%	7.680.617	19,0%
		Total Tests		8.457.539	

¹ Source: Eurostat² Fallen stock, emergency slaughtered animals, animals found sick at ante mortem inspection

Table 5: Age distribution of tested animals. Extrapolated number of tested cattle in each age group
(This information was not available from the Netherlands)

Age Months	UK	DE	ES	IRL	IT	FR	BE	DA	AU	FI	PT	EL	LU	SV	Total 14
<24	0	726.275	0	3.991	0	0	1.014	0	0	0	0	0	0	0	731.280
24-30	14.653	405.652	21.987	11.974	71.035	76.721	2.660	2.778	14.084	1.960	2.370	2.505	1.400	660	630.440
31-36	799	262.903	23.521	269.707	53.190	341.142	35.503	31.613	23.306	2.152	1.975	821	3.351	2.550	1.052.533
37-42	6.099	189.047	14.986	86.026	38.395	327.014	39.562	31.978	16.185	2.701	6.045	923	2.612	2.600	761152*
43-48	6.777	162.757	23.564	39.317	36.540	216.898	45.113	34.912	14.374	2.589		1.017	1.879	2.350	591110*
49-54	5.424	148.009	17.424	23.693	29.449	171.754	40.193	32.329	12.725	3.092	4.973	1.187	1.661	2.692	492119*
55-60	12.088	137.014	27.029	28.872	27.751	153.662	37.501	28.881	13.153	2.690		1.185	1.321	2.360	475993*
61-66	13.922	123.784	14.825	17.663	27.138	142.343	31.277	25.124	12.518	2.788	4.746	1.283	1.295	2.675	419007*
67-72	11.464	112.811	26.584	17.238	23.742	130.258	28.469	21.312	13.107	2.186		1.185	1.113	1.914	393755*
73-78	4.109	99.955	14.134	10.446	20.660	118.989	23.660	16.748	11.647	1.940	4.157	1.168	1.017	1.736	328289*
79-84	4.637	87.903	24.026	18.342	20.660	107.674	20.622	13.059	11.815	1.492		892	839	1.090	315129*
85-90	4.654	74.818	12.507	8.832	16.037	95.989	15.575	10.022	10.277	1.191	3.253	866	834	443	253672*
91-96	3.997	64.624	20.413	20.211	15.110	87.466	13.293	7.633	10.668	884		610	620	1.553	248708*
>96	7.457	260.785	145.260	118.802	84.039	557.790	43.467	20.577	61.729	2.212	4.157	3.471	2.946	5.478	1.318.170
all	96.081	2.856.337	386.262	675.115	463.746	2.527.700	377.909	276.965	225.588	27.876	31.675	17.113	20.886	28.101	8.011.354

*: since the age distribution of cattle in Portugal was only available per year, the number of Portuguese cattle was equally distribute over both 6 months periods

Table 6: Incidence of BSE in different age categories. Incidence of BSE (positive cases per 10.000 animals) in cattle of different age

Age	UK	DE	ES	IRL	IT	FR	BE	DA	PT	AU	FI	EL	LU	SV	EU 14 - UK - PT		
															Samples	Positive	Ratio
24-30	0,00	0,05	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	613.173	2	0,03
31-36	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1.048.366	0	0,00
37-42	0,00	0,00	0,00	0,00	0,00	0,03	0,00	0,31	0,00	0,00	0,00	0,00	0,00	0,00	750.643	2	0,03
43-48	0,00	0,06	2,55	0,00	0,27	0,05	0,00	0,29		0,00	0,00	0,00	0,00	0,00	580.420	10	0,17
49-54	11,77	0,68	1,72	0,00	2,04	0,12	0,00	0,00	22,12	0,00	0,00	0,00	0,00	0,00	483.514	21	0,43
55-60	4,53	2,34	4,81	1,39	2,52	0,26	1,60	0,00	48,46	0,00	0,00	8,45	0,00	0,00	460.812	67	1,45
61-66	21,62	2,67	8,09	9,06	4,42	2,61	3,20	1,19		0,00	0,00	0,00	0,00	0,00	402.153	112	2,79
67-72	86,73	2,66	3,76	27,84	3,79	3,93	3,51	0,00	69,77	0,76	0,00	0,00	0,00	0,00	379.420	138	3,64
73-78	372,98	0,60	6,37	31,59	2,90	5,83	3,80	0,00		0,00	0,00	0,00	0,00	0,00	321.644	143	4,45
79-84	405,22	0,57	3,33	32,17	1,45	4,95	1,94	0,00	92,22	0,00	6,70	0,00	0,00	0,00	308.010	101	3,28
85-90	278,33	0,27	5,60	21,51	1,25	3,77	1,28	1,00		0,00	0,00	0,00	0,00	0,00	247.030	107	4,33
91-96	292,15	0,15	2,94	14,84	0,00	1,49	2,26	0,00	15,13	0,00	0,00	0,00	0,00	0,00	242.766	43	1,77
>96	472,19	0,12	0,55	2,78	0,36	0,18	0,46	0,00		0,00	0,00	0,00	0,00	0,00	1.304.474	61	0,47

Table 7: Incidence of BSE (positive cases per 10.000 animals) in healthy slaughtered cattle of different age

Age years (months)	DE	ES	IRL	IT	FR	BE	DA	LU	AU	EL	10 Member states		
											Nb of Samples	Positive	Incidence
2 (24-35)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1.498.411	0	0,00
3 (36-47)	0,00	0,31	0,00	0,00	0,02	0,00	0,17	0,00	0,00	0,00	1.312.008	3	0,02
4 (48-59)	0,39	1,87	0,00	1,64	0,06	0,27	0,00	0,00	0,00	4,66	860.809	30	0,35
5 (60-71)	0,84	1,13	3,34	2,74	0,85	2,63	0,48	0,00	0,41	0,00	709.975	84	1,18
6 (72-83)	0,29	1,84	2,56	1,41	1,91	1,43	0,00	0,00	0,00	0,00	574.493	70	1,22
7 (84-95)	0,00	3,55	2,86	0,36	0,87	1,10	0,00	0,00	0,00	0,00	449.300	37	0,82
>8 (>96)	0,13	0,40	0,92	0,13	0,04	0,49	0,00	0,00	0,00	0,00	1.184.085	22	0,19

It should be noted, however, that in the first half of 2001, testing programmes were not yet fully harmonised across all Member States: target groups and their definition may have been different. In the second half, the programmes had become more in line with each other and the testing results for the second half of 2001 therefore provide a better tool for analysis. **Table 8** provides the BSE incidence in cattle (all target groups confounded) of different age during the second semester of 2001.

Table 8: Incidence of BSE (positive cases per 10.000 animals) in cattle of different age during the 2° semester

Age (months)	UK	DE	ES	IRL	IT	FR	BE	DA	PT	AU	FI	EL	LU	SV	EU 14 - UK - PT		
															Samples	Positive	Ratio
24-30	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	374.169	0	0,00
31-36	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	687.356	0	0,00
37-42	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,59	0,00	0,00	0,00	0,00	0,00	0,00	401.186	1	0,02
43-48	0,00	0,00	2,59	0,00	0,00	0,07	0,00	0,00		0,00	0,00	0,00	0,00	0,00	367.276	5	0,14
49-54	7,38	0,40	1,59	0,00	2,60	0,11	0,00	0,00	18,82	0,00	0,00	0,00	0,00	0,00	264.710	11	0,42
55-60	4,45	0,26	4,02	0,00	3,12	0,31	1,27	0,00		0,00	0,00	12,95	0,00	0,00	289.562	22	0,76
61-66	4,89	2,73	1,98	0,00	5,90	1,15	2,49	2,17	46,21	0,00	0,00	0,00	0,00	0,00	223.005	45	2,02
67-72	48,59	2,78	2,39	7,66	2,95	4,02	5,48	0,00		1,16	0,00	0,00	0,00	0,00	238.538	82	3,44
73-78	213,16	0,61	4,27	20,71	1,44	6,13	5,54	0,00	43,04	0,00	0,00	0,00	0,00	0,00	176.574	89	5,04
79-84	279,36	0,00	1,95	31,71	1,44	6,39	2,18	0,00		0,00	11,15	0,00	0,00	0,00	197.792	74	3,74
85-90	205,41	0,00	4,73	32,01	1,08	4,59	1,17	0,00	66,07	0,00	0,00	0,00	0,00	0,00	132.822	83	6,25
91-96	205,57	0,26	1,54	27,18	0,00	1,38	3,33	0,00		0,00	0,00	0,00	0,00	0,00	159.838	29	1,81
>96	376,83	0,07	0,43	14,15	0,60	0,29	0,00	0,00	19,44	0,00	0,00	0,00	0,00	0,00	810.970	43	0,53