This is provisional English translation of an excerpt from the original full report.

Risk Assessment Report Enterohaemorrhagic *Escherichia coli* and *Salmonella* Species in Beef for Raw Consumption

Food Safety Commission of Japan (FSCJ)
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Executive summary

The Food Safety Commission Japan (FSCJ) assessed risk reduction effects to be generated by introduction of the proposed draft safety standards which includes processing standards upon enterohaemorrhagic *Escherichia coli* (EHEC) and *Salmonella* species in beef eaten raw, based on documents submitted by the Ministry of Health, Labour and Welfare(MHLW), as well as other relevant scientific data available elsewhere.

Based on current knowledge, it was indicated that a Food Safety Objective (FSO) for EHEC and *Salmonella* species must be set at <0.04 cfu/g, in an assumption that the minimum number of *E.coli* O157 (i.e. 2cfu per 50g of meat per patient, equivalent to 0.04 cfu/g of causal food) found in a food poisoning case in Japan could be considered to be a baseline to lead an appropriate level of health protection (ALOP). It was also pointed out that inter-individual differences in the susceptibility and characteristics of the causative bacteria must be taken into account when setting an FSO. Given the view above, it was suggested that the proposed FSO of 0.014 cfu/g from MHLW was three times more conservative than the FSO set at 0.04 cfu/g,

FSCJ also concluded that setting Performance Objective (PO) at one tenth of the proposed FSO would be appropriate while taking into account the rations of the pathogens from utenciles to meat at cross contaminations and potential growth rate of the pathogens, under the assumption that adequate hygienic control measures were implemented.

Although the proposed processing standards only have a certain risk reduction effect, such effect is not always necessarily achive the ALOP, therefore the processing standards must be accompanied by microbiological tests, in order to make sure that the PO for raw meat is met.

Degree of risk reduction cannot be confirmed unless the number of samples units for microbiological sampling plan is indicated, even though microbiological criteria of meat are established. Given the microbiological characteristics of a batch/lot that complies with the PO (a log normal distribution of concentrations and a standard deviation of 1.2 log cfu/g as well as a mean concentration of -0.85 log cfu/g) and *m* = absence in 25g, probability of rejection =95%, and to detect such a lot with more than 97.7% probability, 25 samples would need to be taken.When setting food safetycontrol system including process criteria for heat treatment (e.g. time and temperature), validation is necessary to confirm the time and temperature combinations are compliance with the process criteria.

Assessment of the risk reduction effects of foods on health (extracted from Part IV of the original risk assessment report)

1. Hazard identification
2. Hazard characterization
3. Exposure assessment

4. **Risk characterization**

Objective

Risk reduction effects by application of the proposed safety standards by the Ministry of Health, Labour and Welfare (MHLW) was estimated by assessment of the following three points:

(1) Proposed Food Safety Objective (FSO[1]) at 0.014 cfu[2]/g;
(2) Performance Objective (PO[3]) set at 0.0014 cfu/g based on the proposed FSO: and
(3) Feasibility of the PO set at 0.0014 cfu/g by application of draft standards (microbiological standards and processing standards)

(1) Assessment of the proposed FSO of 0.014 cfu/g

(i) Approach based on incidence and mortality data

a. Hazard-based risk analysis

Among the reported food poisoning cases caused by enterohaemorrhagic *E. coli* (EHEC), the minimum number of ingested bacteria was 2 cfu per person, which is equivalent to 0.04 cfu/g in the causative food (raw beef liver: below 50 g). Therefore, FSO must be below 0.04 cfu/g.

There is no data on EHEC contamination in retailed beef in Japan. Carney et al. reported that the number of *E.coli* O157 on the chopped beef in Ireland ranged 5.0-40.7 cfu/g using the direct plate count method, which is the only available data to our knowledge on EHEC in raw beef [1].

Assuming that the bacterial count in contaminated beef meat in Ireland is nearly equal to that in Japan, contamination level must be reduced from 40.7 cfu/g to below 0.04 cfu/g, or at least by 1/1,018.

The lowest bacterial count among the food poisoning cases by *Salmonella* species, on the other hand, was 4.3 MPN/100 g (0.04 MPN/g) found in chocolate. Thus we concluded that there was no much difference in hazard characteristics between EHEC and *Salmonella* species. In addition, according to International Commission on Microbiological Specifications for Foods (ICMSF), risk of *Salmonella* species is regarded to be lower than that of O157 [2].

b. Estimation of incidence of food poisoning caused by raw beef consumption at home and restaurants

Since we were not able to find information about the proportionshared by cases due to raw beef consumption in the total food poisoning cases in the Food Poisoning Statistics (MHLW) and published papers, we used various methods and existing survey data to estimate the number.

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[1] FSO: The maximum frequency and/or concentration of a microbial hazard in a food considered tolerable for consumer protection at the time of consumption that keeps health impact caused by consumption of the food below ALOP (Appropriate Level of Protection)

[2] cfu: colony forming unit

[3] PO: The maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable.

Consumption pattern was divided into eight according to 1) the place of consumption; 2) the type/part of meat consumed (dressed carcasse or offal); and 3) the preparation method (row or cooked). Annual number of cases per person for each of the 8 consumption patterns can be calculated by multiplying annual number of consumption by risk of illness per serving. The number of EHEC cases for each consumption pattern is obtained by further multiplying the annual number of case per person by the total population of Japan. Based on the results, the proportion of EHEC poisoning related to raw beef consumption was estimated at 11.2%.

It should be noted that the annual number of patients due to beef consumption as estimated in the mentioned studies includes diseased persons not presented at medical institutions, and therefore cannot be straightly compared to those in the Statistics of Food Poisoning and other data.

According to the National Epidemiological Survey of Infectious Diseases (NESID) conducted as stipulated by the Infectious Disease Law, reported number of EHEC cases ranged from 2,999 to 4,167 each year between 2001 and 2009 (3,837 cases on average per year), and about 65% of reported cases were symptomatic. About 68% of O157 cases are estimated to be food-borne [6]; therefore, the average annual number of food-related cases among reported cases to the NESID is estimated to be about 1,700.

Assuming that all these food-borne cases are caused by beef consumption, and applying 11.2% as the proportion of cases due to raw beef consumption, about 190 out of 1,700 cases are supposedly reported on the basis of the Infectious Disease Law.

c. Risk analysis based on the number of cases

Dose-response relationship is known to be linear in the range of a small number of bacteria [7]; thus reduction rate of contamination level of beef can correspond to reduction rate of incidence. Based on the estimation of the previous section, contamination level of beef must be reduced at least to 1/190 in order to keep the incidence below one case per year.

However, the above reasoning is based on average values for 2001~2010, and one should take into account yearly fluctuations, interindividual susceptibility difference, and other factors.**d. Risk analysis based on mortality**

According to the Food Poisoning Statistics by MHLW, thelargest number of fatal cases in a year reported to date is 9. Assuming that the proportion shared by fatal cases due to raw beef consumption is also 11.2%, the fatalities from raw beef consumption at home or restaurant can be estimated to be one person per year at maximum.

Thusthe number of deaths will be kept below 1 person per year with some kind of risk reduction measures.

e. Analysis based on literature review

ICMSF has set an FSO in the risk assessment and sampling plan for O157 in ground beef patty in the US. This FSO for O157 in the ground beef patty should be given necessary conservativeness, reflecting uncertainty degree, infection at relatively small bacterial count and severity of the disease. Assuming that there is no more than one O157 cell in two portions of ground beef patty (one portion: 125 g) produced and distributed commercially in the US, the FSO is established as 1 cfu/250 g = 0.004 cfu/g = -2.4 log cfu/g [8].

Conclusion 1

FSO must be set at below 0.04 cfu/g. Interindividual variability in host susceptibility and bacterial characteristics must be taken into consideration.

(ii) Verification of disease probability calculated using dose-response functions

When calculating the disease probability, we adopted α = 0.157 and β = 9.17 for Beta-Poisson model suggested by FSIS (2001) [9], and r = 5.1×10^{-3} for Exponential model suggested by Nauta et al. (2001) [10].

Beta-Poisson formula: $P = 1-(1+D/\beta)^{-\alpha}$

Exponential formula: $P = 1- e^{-rD}$

Assuming one serving of yukhoe is 50 g, the number of ingested bacteria per serving is 0.7 cfu/50 g if FSO is 0.014 cfu/g as proposed, and 2.0 cfu/50 g if FSO is 0.04 cfu/g. According to the data presented by Carney et al. (2006) [1], contamination level of EHEC O157 in the chopped beef is about 40 cfu/g at the maximum; hence the maximum bacterial count in one yukhoe serving is calculated to be about 2,000 cfu.

Table 1 shows incidence rates estimated from calculated bacterial counts.

Table 1

Bacterial count	Beta-Poisson model	Exponential model
FSO (0.014 cfu/g): 0.7 cfu/ 50 g	0.011483	0.003564
FSO (0.04 cfu/g): 2 cfu/50 g	0.0305	0.010148
Upper limit of contamination: 2,000 cfu/50 g	0.57094	0.999963

Reduction from the current contamination limit to FSO (0.04 cfu/g) would result in risk reduction per yukhoe serving by 1/18.7 by Beta-Poisson model and 1/98.5 byExponential model. Similarly, when FSO was reduced to 0.014 cfu/g, risk reduction rates would be 1/49.7 and 1/280.6, respectively.

Conclusion 2

By setting FSO at 0.014 cfu/g, risk reduction was expected to be nearly three times more effective compared with FSO of 0.04 cfu/g.

(2) Assessment of the proposed PO of 0.0014 cfu/g set from the proposed FSO

There is no data on the probability of cross-contamination and inappropriate temperature control, and temperature rise during slicing and other food processing operations at restaurants.

According to the model developed with Pathogen Modeling Program (ver.7.0), in case of O157 Broth Culture[4] under the condition of 10°C and pH 6.5, the lag phase[5] is estimated 2.25 days, the generation time[6] is estimated 0.22 days, and it takes 3 days for one log growth. Supposing that it is at a room temperature of 20°C, the lag phase is 6.6 hours, the generation time is 1.1 hours, and it takes 10 hours for one log growth.

Growth of O157 in beef estimated using the data of USDA-ARS Eastern Regional Research Center and Microbial Response Viewer developed by Koseki was 14 to 18 hours for one log at 10°C. On the other hand, at 20°C, the growth of 3.11-3.71 log cfu, 3.2-3.72 log cfu, and 3.14-3.69 log cfu in 7 hours is reported [11].

[4] Broth culture: cultivation of bacteria in a liquid medium containing meat extract.

[5] Lag phase: a preparatory period during which bacteria adapt to a new environment prior to fission (also called induction phase).

[6] Generation time: bacteria grow by binary fission, and the period between fissions is called generation time.

Mathematical modeling of cross-contamination of O157 on the surface of ready-to-eat meat productssuggested that the number of surface transfer of O157 from contaminated ham (10^3 cfu) via slicer blade was $10^{1.3}$ (20 cfu), 2% of the total number [12] .

Therefore, it is highly unlikely that the number of bacteria increases more than 10-fold in meat for which POs are met, as far as appropriate sanitary control measures are taken.

Conclusion 3

Setting PO at one tenth of the FSO ensure a considerablly high level of safety when adequate sanitary control is provided.

(3) Assessment of feasibility of PO set at 0.0014 cfu/g by introducing the draft standards

(i) The processing standards and microbiological standards for beef for raw consumption

The meat products to be assessd here are raw parts of beef not to be heat-treated directly. Surface heating process prescribed in the processing standards aims to control surface contamination through stirilization in order to ensure reduction of microbial level in consumable parts, because microbial contamination in appropriately slaughtered beef carcass is mainly surface contamination. Higher efficiency can be expected when heat-treatment is applied on the carcass during dressing in the first stage of the meat processing. The processing standards stipulated that the beef for raw consumption to be heat treated to the depth of 10 mm from the surface at 60°C for 2 minutes or longer, as a practicable means to improve safety; however, such processing alone cannot guarantee reduction of microbial level in parts intended for raw consumption, and it should be accompanied by appropriate microbiological examination.

According to the documents of MHLW onbacterial infiltration [13], , a small amount of bacteria less than one MPN/cm^3 were detected at 10 to 15 mm below the surface when 10^6 O157 were inoculated to beef in the second week after carcass dressing. Most infiltrated bacteria were localized within 5 mm depth but some bacteria were detected at the position of about 10 mm from the surface in the case of higher level of contamination. Thus, storage period and conditions after slaughtering are the points to be considered.

Bacterial count at the depth of 10 mm and deeper was reduced to $1/10^3$ to $1/10^4$ as compared to the surface, and highly likely to be reduced to below $1/10^4$ when heated at 60°C for 2 minutes. Therefore, assuming that the surface contamination level is below 40 cfu/g, the surface heating under the mentioned conditions would highly likely to reduce bacterial count to below 0.004 cfu/g in meat parts intended for raw consumption.

However, unlike suggested under the experimental condition, it is envisaged that massive bacterial infiltration would occur deep in the meat block depending on the shape of the meat.Moreover, the same level of bacterial reduction effect may not be achievable depending on fat content and freshness of meat. Therefore, it is necessary to employ appropriate combination of other processing standards and microbiological standards to ensure sufficient pasteurization effect.

There are no internationally validated testing methods exhaustively applicable for EHEC, and a vast number of samples would be required for direct inspection of pathogenic bacteria in order to confirm compliance with POs. Therefore, *Enterobacteriaceae* [1] are considered as internationally approved indicator bacteria that can be used to detect and estimate the level of fecal contamination as well as *Salmonella* species and EHEC.

According to the documents of MHLW [14], when *Enterobacteriaceae* are regarded as indicator bacteria for microbiological standards, PO by testing for *Enterobacteriaceae* is -0.85 log cfu/g, given the abundance ratio of 1:100 EHEC/*Enterobacteriaceae*.

As regards the relative abundance of EHEC and *Enterobacteriaceae* (1:100), MHLW refers to the fact that maximum concentrations of EHEC and *Enterobacteriaceae* in the cattle head meats are 10 cfu/g and 1,000 cfu/g, respectively, according to the report of Carney et al. (2006) (ref.1). Besides, according to a study on EHEC and *Enterobacteriaceae* in 43 samples of the minced beef products in Ireland by Crowley et al. (2005) (ref. 15), the bacterial count of EHEC occasionally closed to that of *Enterobacteriaceae* but the average counts are 0.88 log cfu/g and 3.44 log cfu/g, respectively, which gives the difference of about 2.55 log cfu/g (1:355).

(ii) Assessment of achievement of POs

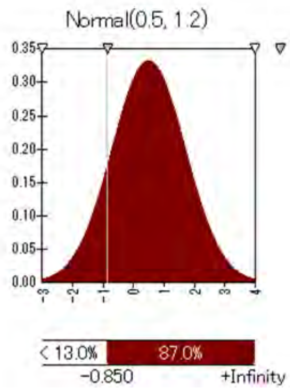
The draft safety standards (1) stipulate that "a 25 g sample of meat for raw consumption is negative for *Enterobacteriaceae*", yet the number of samples to be collected is not specified.

Case 1. Number of samples is not specified

Microbiological correlation between products in question and POs cannot be determined quantitatively unless a sampling plan, including the number of samples, is specified.

Conclusion 4

Unless the number of samples is specified, degree of risk reduction cannot be estimated, and achievement of POs by introduction of microbiological standards cannot be confirmed.



Case 2. Assessment with one sample

According to the documents of MHLW [16], in case that the sampling plan stipulates that one 25 g sample is taken and proven negative for *Enterobacteriaceae*, the average contamination level of *Enterobacteriaceae* per lot (95% rejection ratio) is 0.5 log cfu/g, that is, 3 cfu/g.

As indicated in the previous data of microbiological examinations, microorganisms in food products follow a log-normal distribution; the proposed standard deviation is 0.4 log cfu/g for homogenous food products, 0.8 log cfu/g for somewhat non-homogenous products, and higher (e.g., 1.2 log cfu/g) for heterogenous products [17-18]. Considering differences in contamination levels, homogeneity and processing facilities with different hygiene conditions, a standard deviation of 1.2 log cfu/g is used in calculations below.

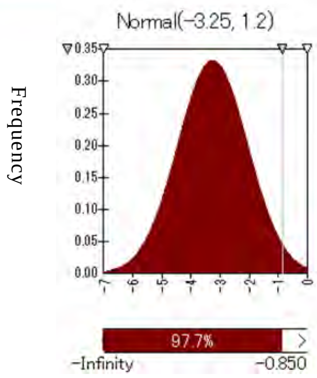
Assuming PO for O157 is 0.0014 cfu/g, which is 0.14 cfu/g (= -0.85 log cfu/g) for *Enterobacteriaceae*; as shown in Fig. 1, 87% of this lot exceeds the PO. In this case, the mean concentration is 3 cfu/g; however, concentrations of 1,000 cfu/g in *Enterobacteriaceae*, or 10 cfu/g in EHEC and *Salmonella* species is possible, though at a low probability; hence cases beyond 2-9 cfu per person are envisaged, which can cause a disease.

Fig. 1. A log normal distribution of contamination level in meat products to be rejected with 95% probability

(one sample; m=absence in 25 g)

Conclusion 5

PO cannot be met if the number of samples to be tested is one.



Case 3. Achievement of PO with 97.7% probability (a standard deviation of 1.2 log cfu/g) with 95% confidence

In case that PO is set at 0.0014 cfu/g in O157, with a standard deviation of 1.2 log cfu/g, the probability of compliance with the PO of 0.14 cfu/g = -0,85 log cfu/g in *Enterobacteriaceae* is 97.7%, when the number of samples is 25 as shown in Fig. 2. However, the probability does not reach 97.7% when 24 samples are tested (97.6%) [19].

Fig. 2. A log normal distribution of contamination level in meat products rejected with 95% probability

(25 samples; m=absence in 25 g)

Conclusion 6

If 25 or more samples are taken and proven negative, then achievement of the proposed PO at 97.7% probability (a standard deviation of 1.2 log cfu/g) can be confirmed with 95% confidence.

(iii) Evaluation of achievement of the PO due to the draft processing standards

The requirement "Meat blocks used for processing are not frozen, and shall be cut from the carcass in a sanitary manner" in the draft processing standards (6) is distinct in that meat for raw consumption is cut and separated from other meat parts as soon as possible when meat is shipped from slaughterhouses complying with sanitary regulations stipulated in the Slaughterhouse Act. We concluded that such handling manner of meat for raw consumption reflects the concept that sanitary control must be applied as early as possible in the food chain.

As regards the requirement "Meat blocks processed according to (6) shall be promptly packed into air-tight sanitary containers and sealed, and pasteurization shall be performed by heating to the depth of 1 cm or more from the surface at 60°C for 2 minutes or longer, or by another method offering equivalent effect " in the draft processing standards (7), no bacteria infiltration to meat parts intended for raw consumption was confirmed in the experiments conducted by MHLW using meat blocks obtainedon 4th day after carcass breaking.

However, one cannot expect for pasteurization effect in meat parts for raw consumption. Though the processing standards have a certain risk reduction effect, such effect is not necessarily always achieved as mentioned above in (3) (i); therefore, complete achievement of PO is impossible by only means of the processing standards, and the microbiological examination mentioned in Conclusion 6 must be applied in combination. In addition, when setting up a processing system including heat treatment, it should be taken into account that validation is a necessary step to confirm whether food sanitation control is performed appropriately in the processing system of each establishment.

Conclusion 7

Though the processing standards have a certain risk reduction effect, such effect is not necessarily always achieved; therefore, complete achievement of PO is impossible by means of the processing standards only, and the microbiological examination mentioned in Conclusion 6 must be applied in combination. In addition, when setting up a processing system including heat treatment, it should be taken into account that validation is necessary to confirm whether food sanitation control is performed appropriately in the processing system.

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