Food Allergen Labeling Regulation and its implementation in Japan

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In 1998, Sapporo, Hokkaido

The child (6th grade) having unease eating buckwheat, accidentally ate buckwheat by the lunch provided by school.

- Then he had rash around his mouse. The teacher called on the parents, and consequently sent him back to the house.
- On the way back, he got the anaphylaxis, and passed away by suffocation.
- The teacher and the Sapporo educational committee (belonged to the Ministry of Education) were sentenced for the violation of safety duty of care.

※ In 2004, Ministry of Education surveyed Food allergy prevalence in all public school (36,830 schools, 12,770,000 pupils).

According to the survey, pupils having food allergy were 329,423 (2.6%) and the one experienced the anaphylaxis shock were 18,323 (0.14%).
Government action

The Special Subcommittee for Labeling under government Food Sanitation Investigation Council concluded that “Because there were a large number of health hazards caused by food containing allergens, mandatory labeling should be required for allergens in foods (Study Report on Food Labeling: fiscal 1998).

Accordingly, Ministry of Health Labor and Welfare (MHLW) made a decision to amend the Food Sanitation Act, and foods containing allergens be labeled to the effect that they contain such substances was necessary from the view point of preventing health hazards to consumers.
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<table>
<thead>
<tr>
<th>Food ingredient</th>
<th>Allergy incidence</th>
<th>Anaphylactic shock</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rank</td>
<td>Cases</td>
</tr>
<tr>
<td>Eggs</td>
<td>1</td>
<td>1486</td>
</tr>
<tr>
<td>Milk and Dairy products</td>
<td>2</td>
<td>616</td>
</tr>
<tr>
<td>Wheat</td>
<td>3</td>
<td>311</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>4</td>
<td>179</td>
</tr>
<tr>
<td>Shrimp/Prawn</td>
<td>5</td>
<td>161</td>
</tr>
<tr>
<td>Peanuts</td>
<td>6</td>
<td>110</td>
</tr>
<tr>
<td>Salmon roe</td>
<td>7</td>
<td>87</td>
</tr>
<tr>
<td>Soy bean</td>
<td>8</td>
<td>76</td>
</tr>
<tr>
<td>Kiwi</td>
<td>9</td>
<td>75</td>
</tr>
<tr>
<td>Banana</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Crab</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Chicken</td>
<td>12</td>
<td>32</td>
</tr>
<tr>
<td>Tree nuts</td>
<td>12</td>
<td>32</td>
</tr>
<tr>
<td>Squid</td>
<td>14</td>
<td>30</td>
</tr>
<tr>
<td>Mackerel</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>Pork</td>
<td>16</td>
<td>23</td>
</tr>
<tr>
<td>Salmon</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>Gelatin</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Yam</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>Peach</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>Others</td>
<td>21</td>
<td>491</td>
</tr>
<tr>
<td>Total</td>
<td>3882</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Study report of MHLW: fiscal 2004 (M. Ebisawa)
Follow-up Nation-wide Food Allergen Survey

Egg, Milk, Wheat, Peanut, Buckwheat, Shrimp and Crab

In 2005 (n=2283)
- Egg, 40%
- Milk, 18%
- Wheat, 9%
- Peanut, 5%
- Shrimp, 3%
- Buckwheat, 3%
- Kiwi, 2%
- Soy, 2%
- Banana, 1%
- Others, 12%

In 2008 (n=2501)
- Egg, 39%
- Milk, 21%
- Wheat, 12%
- Peanut, 5%
- Shrimp, 3%
- Buckwheat, 3%
- Kiwi, 1%
- Soy, 2%
- Banana, 1%
- Others, 9%
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Food Allergen Labeling regulation in Food Sanitation Act

- Enforced on April 1\textsuperscript{st}, 2002
- Food subjected to this regulation
  - Pre-packed processed food
  - Food additives
- Labeling is mandatory at all stages of food distribution.
- “May contain” labeling (Indication for possible inclusion) is forbidden.

\textcolor{red}{This product may contain Peanuts.}

However, providing the food allergen information to the consumer is allowed (as Marginal alert).

- This product is manufactured by the facility using Peanuts.
- Corn shares the conveyance facility with Soybean and Wheat.

\textit{“Japan Food Allergen Labeling Regulation - History and Evaluation”}
\textit{H. Akiyama et al. Advances in Food and Nutrition Research 62, 139-171 (2011)}
### Food required labeling

Food containing the designated food ingredients listed below is required to label.

#### Mandatory by ministerial ordinance

- **Egg, Milk, Wheat, Buckwheat, Peanut, Shrimp and Crab.**

  (7 ingredients)

#### Recommended by ministerial notification

- **Abalone, Squid, Salmon roe, Orange, Kiwifruit, Beef, Walnut, Salmon, Mackerel, Soybean, Chicken, Banana, Pork, Matsutake mushroom, Peach, Yam, Apple, and Gelatin.**

  (18 ingredients)

*(Ingredients are defined according to “Japan Standard Commodity Classification”)*
Labeling rule

- “Mandatory labeling ingredients” shall be labeled mandatory, regardless of the quantity (even in an extremely small level), if it is added intentionally.

- “Recommended labeling ingredients” are recommended to be labeled, if it is added intentionally.

How much is “an extremely small level”?

Regulatory limit
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Process to establish Regulatory limit

Establishing Regulatory limit, there was a severe controversy in the government. In consequence, MHLW decided to establish Regulatory limit in order to minimize the impending health hazard, and to revise Regulatory limit if in case established Regulatory limit is found out inadequate.

But still, establishing Regulatory limit was the complicated issue as making terms between the stakeholders. The food allergy sufferers consider Regulatory limit should be Zero in order to prevent allergic reactions, meanwhile the food industry considers Zero tolerance is impractical for manufacturing food products.

Finally, the study group, including the stakeholders as food industry, food scientists, medical experts etc., were organized, and finally reached the conclusion.
As for the amount of antigen inducing allergic symptoms, the study group consensus was reached that when the amount of total protein is at the mg/mL concentration (weight within mL of solution in the food load test) level, the symptoms almost always appear in general, while when it is at the few μg/mL level, whether the symptoms appear or not varies between individuals, and at the ng/mL level, the symptoms almost never appear.

“an extremely small” is presumed a few (5-6) μg protein in g food.

10 μg protein / g food
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Regulatory process of Food Allergen Labeling Regulation

Periodical inspection

Labeling inspection

Consumer claim

Quantitative test by ELISA

Inspection of Manufacturing records

Confirmatory tests by Western blot/PCR

Executive action
Labeling inspection

Name: Biscuit
Ingredients: Wheat flour, Milk, Wheat starch, Shortening, Butter oil, Whole milk powder, Vegetable oil, Margarine, Liquid sugar, Salt, Whey powder, Emulsifier (Soybean origin), Flavor
Contents: 24 pcs. (3 pcs./pack x 8 packs)
Best before: Indicated under this column
Storage: Avoid sun light, high temperature, and high humidity.
Manufacturer: Morinaga and Co., Ltd.

This product is manufactured by the facility using Egg and Peanuts.
Best before 2007.06.13

Legal indication
Voluntary Marginal alert
Regulatory process of Food Allergen Labeling Regulation

Periodical inspection

Labeling inspection

Quantitative test by ELISA

Inspection of Manufacturing records

Confirmatory tests by Western blot/PCR

Consumer claim

Executive action
Verifying the product labeling

- Examination of products by **Quantitative test**
  - Official ELISA tests (Egg, Milk, Wheat, Peanut, Buckwheat, Shrimp and Club)

  The “Positive” result is $\geq 10 \, \mu g$ protein/g food.

- Investigation of Manufacturing records
  - Raw material lists
  - Manufacturing process records

- Examination of products by **Confirmatory test**
  - Western blot test (Egg and Milk)
  - PCR test (Wheat, Peanut, Buckwheat, Shrimp and Club)
Decision Tree

Investigation of Labeling

Quantitative ELISA Test

Egg, Milk, Wheat, Buckwheat and Peanuts

*1 + or +/− Shrimp and Crab

Manufacturing Records

Confirmatory Test

Confirmatory Test

Questioning

Mandatory Labeling

Recommended labeling

Prohibited Labeling

Mandatory Labeling

Mandatory Labeling

Recommended labeling

Unnecessary Labeling

Unnecessary Labeling

OK

OK

guidance

OK

OK

guidance

OK

OK

guidance

OK

OK

OK

MORINAGA INSTITUTE OF BIOLOGICAL SCIENCE, INC.
Executive action

- Execution of regulatory action: Although the product contains \( \geq 10 \, \mu g \) “Mandatory ingredient” protein/ g food, the label does not indicate the concerned “Mandatory ingredient”.

- Regulatory actions
  - The governor gives the instruction to improve the labeling.
  - The governor can suspend or cancel the business permission of the offender if the offender does not obey the instruction.
  - If the governor's guidance is not abided by, the offender is subjected to imprisonment or fine according to Food Sanitation Act.
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10 μg/g

an extremely small amount

1 μg/g

0.1 μg/g

10 μg protein weight / g weight

Regulatory limit

A few μg protein weight / g weight

LOD of Lateral flow method

LOD of PCR method

0.1-1.0 μg protein weight / g weight

LOD of ELISA method

The detection method is required quantitative and sensitive enough to determine 10 μg protein / g food.
In 2002, Egg, Milk, Wheat, Peanuts, and Buckwheat ELISA tests were authorized as the quantitative detection method by Ministry of Health, Labor, and Welfare, Japan.

The tests were generally used for examining raw material QC, line cross-contamination, confirmation of final product etc. among the food industry, analytical lab, and inspection agency.

However, one critical drawback was emerged.
Egg ELISA failed to detect Egg in Egg containing foods

<table>
<thead>
<tr>
<th>Commercial processed food</th>
<th>Observation [μg/ g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread (Egg)</td>
<td>N.D.</td>
</tr>
<tr>
<td>Retorted soup (Egg)</td>
<td>N.D.</td>
</tr>
<tr>
<td>Instant noodle (Egg white)</td>
<td>N.D.</td>
</tr>
<tr>
<td>Grattan (Egg white)</td>
<td>N.D.</td>
</tr>
<tr>
<td>Spaghetti (Egg white)</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D.: Not detected

Creating confusion in data interpretation!

Emerged problem of ELISA detection

Typical Egg ELISA data
What is food processing?

During food processing, food ingredients are exposed to heating, pressurizing, acidifying/alkalizing etc.

Raw egg → Boiling/Heating → Homogenized boiled egg

In consequence, egg proteins are denatured.
Solubilization process

Homogenized boiled egg

Solubilization

Before centrifugation

After centrifugation

Egg protein → Heating → Protein denaturation → Insoluble even after solubilization → Can not detect by ELISA → Insoluble precipitate
In order to increase the extraction efficiency of insoluble protein, improved extraction solution containing surfactant (SDS) and reducing agent (2-Mercaptoethanol) is developed.

Y. Watanabe et al: Journal of Immunological Methods 300(2005) 115-123
### Improvement of solubilization

**After centrifugation**

<table>
<thead>
<tr>
<th>Homogenized boiled egg</th>
<th>Conventional solution</th>
<th>Improved solution</th>
</tr>
</thead>
</table>

- **Solution containing surfactant and reducing agent**
- **Protein solubilized**
## Protein recovery after solubilization

<table>
<thead>
<tr>
<th>Food sample</th>
<th>Conventional solution</th>
<th>Improved solution</th>
<th>Japanese Food composition official table</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw egg</td>
<td>69</td>
<td>117</td>
<td>125</td>
</tr>
<tr>
<td>Boiled egg</td>
<td>20</td>
<td>101</td>
<td>129</td>
</tr>
<tr>
<td>Raw peanut</td>
<td>90</td>
<td>300</td>
<td>250</td>
</tr>
<tr>
<td>Roasted peanut</td>
<td>35</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>Millet</td>
<td>0.4</td>
<td>118</td>
<td>105</td>
</tr>
<tr>
<td>Corn</td>
<td>1.8</td>
<td>78</td>
<td>150</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>7.8</td>
<td>114</td>
<td>135</td>
</tr>
<tr>
<td>Rice</td>
<td>1.2</td>
<td>64</td>
<td>61</td>
</tr>
</tbody>
</table>

The protein solubilizing efficiency is remarkably improved.
Egg allergen recovery from Model processed food

- Model processed food added 10 ppm egg protein

**Improvement of Egg recovery**

<table>
<thead>
<tr>
<th></th>
<th>1st</th>
<th>2nd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange juice</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Biscuit</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Strawberry jam</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

1st: generation ELISA using conventional extraction solution.
2nd: generation ELISA using improved extraction solution.
### Examination of commercial processed food

#### Egg ELISA

<table>
<thead>
<tr>
<th>Commercial processed food</th>
<th>Observation (microgram/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Labelled</td>
</tr>
<tr>
<td>Bread</td>
<td>Egg</td>
</tr>
<tr>
<td>Retorted soup</td>
<td>Egg</td>
</tr>
<tr>
<td>Instant noodle</td>
<td>Egg white</td>
</tr>
<tr>
<td>Grattan</td>
<td>Egg white</td>
</tr>
<tr>
<td>Spaghetti</td>
<td>Egg white</td>
</tr>
</tbody>
</table>

N.D: Not detected

Labeled egg can be detected as it labeled.
Recovery from Model processed food in various ELISAs

<table>
<thead>
<tr>
<th>ELISA kit</th>
<th>Orange juice</th>
<th></th>
<th>Biscuit</th>
<th></th>
<th>Strawberry jam</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
<td>1st</td>
<td>2nd</td>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>Egg</td>
<td>N.D.</td>
<td>92.8</td>
<td>N.D.</td>
<td>47.2</td>
<td>N.D.</td>
<td>46.9</td>
</tr>
<tr>
<td>Milk (Casein)</td>
<td>57.8</td>
<td>74.8</td>
<td>14.2</td>
<td>26.1</td>
<td>11.1</td>
<td>72.7</td>
</tr>
<tr>
<td>Milk (Beta-lacto globulin)</td>
<td>4.36</td>
<td>82.7</td>
<td>N.D.</td>
<td>30.8</td>
<td>5.33</td>
<td>60.8</td>
</tr>
<tr>
<td>Wheat</td>
<td>15.4</td>
<td>96.6</td>
<td>—</td>
<td>—</td>
<td>N.D.</td>
<td>20.6</td>
</tr>
<tr>
<td>Peanut</td>
<td>87.6</td>
<td>175</td>
<td>—</td>
<td>—</td>
<td>22.5</td>
<td>88.7</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>N.D.</td>
<td>105</td>
<td>54</td>
<td>79.7</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Recovery (%) from 10 ppm incurred model processed food.

Recoveries of 2nd generation ELISAs are significantly higher than those of 1st generation ELISAs.
The MHLW authorized ELISAs using improved extraction solution as the official detection method of Mandatory ingredients (Egg, Milk, Wheat, Buckwheat, Peanut, Shrimp and Crab.)
Confirmatory test

Due to the matrix effect, ELISA can not exclude the false result completely, therefore the confirmatory test is required to verify ELISA result, especially before taking the regulatory action.

Regulation prescribes two confirmatory methods;
a. Western Blot method for egg and milk: The Western Blot method is a protein-based qualitative method, and is highly specific due to the target protein is separated by the molecular mass and detected by the immuno-staining using the specific antibody.
b. PCR method for wheat, buckwheat, peanut, shrimp and crab: The PCR method is a DNA-based qualitative method, and highly specific because the target gene is detected by the specially designed primers for allergen protein.
Closing

Japan is the challenging country for food allergy, *i.e.* Japan is;

– enforced Food allergy labeling regulation in 2002.
– established the regulatory system,
  • having practical regulatory rules (as Decision tree) with the penalty of violation.
  • having Regulatory limit (10 μg protein/g food).
  • having the official detection methods.
Thank you for your attention!