Safety Assessment of Foods Produced Using Recombinant DNA Techniques

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ABSTRACT : The introduction of genetically modified crops has raised concerns regarding safety issues over the insertion of foreign genes into plant genomes using recombinant DNA technology. Since 1991 in Japan, 29 foods and 6 food additives have been evaluated, based on the "Guideline for Safety Assessment", before these foods were marketed. The MHW, however, decided that safety assessment of such foods and food additives should be legally imposed, because soon such foods and food additives are expected to circulate globally and a new system for assessing safety of such foods and food additives at a pre-market stage is necessary, in order to avoid the distribution of any genetically modified foods that have had no safety assessment. The MHW published relevant announcements to amend existing regulations on 1 May 2000. "Standards for safety assessment of seed plant" is established based on a concept of substantial equivalence, and applicable to the products which are regarded as equivalent to the existing products used as foods and food additives. The characterization of the food products entails consideration of the molecular characterization, phenotypic and compositional characteristics, key nutrients and toxicants, and toxicity and allergenicity of the introduced proteins, and if there are indications of unintended effects of the modification, whether further safety testing (animal studies etc.) is needed should be considered. Safety and wholesomeness studies with whole foods should be carefully designed in order to avoid nutritional imbalances causing artifacts and uninterpretable results as was the case of Dr. Pusztaiis report. A case study of genetically modified soybeans (glyphosate-tolerant soybeans) on the immune system of rats and mice is shown. Chemical compositions were also compared with those of the non-GM soybeans. The studies failed to detect any differences in immuno-toxic activity.

Key Words : Safety assessment, Foods, Genetic modification, Allergenicity, Marker gene, Bt protein

I. INTRODUCTION

The introduction of genetically modified crops has raised concerns regarding safety issues over the insertion of foreign genes into plant genomes using recombinant DNA technology. The Japanese MHW wants to confirm whether the safety assessment of foods and food additives produced through biotechnology comply with established guidelines before these foods are marketed.

Since 1991 in Japan, 29 foods and 6 food additives have been evaluated based on the "Guideline for Safety Assessment" (Table 1). The safety assessment of such foods and food additives, that have been con-

Table 1. Foods evaluated based on Japanese guidelines

Food	Number	Trait
Soybean	1	Glyphosate tolerant
Rapeseed	13	Glyphosate tolerant
Potato	2	Insect resistant
Corn	7	Insect resistantGlufosinate tolera Glyphosate tolerant
Cotton	4	Insect resistantGlyphosate toleran Bromoxynil tolerant
Tomato	1	Flavor saver
Sugar bea	1	Glufosinate tolerant

ducted based on the Guideline, has been operating on a voluntary basis at this stage.

II. AMENDMENT OF EXISTING REGULATIONS

The MHW, however, decided that safety assessment of such foods and food additives should be legally

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List of abbreviations : MHW, Ministry of Health and Welfare; EPSPS, 5-enolpyruvylshikimate-phosphate synthase; NPT II, aminoglycoside 3'-phosphotransferase II; PAT, phosphinothricin acetyltransferase; GNA, Galanthus nivalis agglutinin; Bt, *Bacillus thuringiensis*; GOX, nitrilase

imposed, because soon such foods and food additives are expected to circulate globally and a new system for assessing safety of such foods and food additives at pre-market stage is necessary, in order to avoid the distribution of any genetically modified foods that have had no safety assessment. The MHW published relevant announcements to amend existing regulations on 1 May 2000 (MHW, 2000). According to these announcements, any foods and food additives produced by recombinant DNA techniques that no safety assessment shall be neither imported nor sold in Japan on and after 1 April 2001.

According to the amended Specifications and Standards for Foods and Food Additives and Other Related Products, "Procedure of application for safety assessment of foods and food additives produced by recombinant DNA techniques" and "Standard for manufacturing foods and food additives produced by recombinant DNA techniques" were published.

III. STANDARDS FOR SAFETY ASSESSMENT IN JAPAN

"Standards for Safety Assessment of Seed Plants (Whole) produced by recombinant DNA techniques or food produced utilizing a portion of such" include sections regarding similarities between produced food and existing food, purposes and usage of recombinants, host, vector, inserted gene and its gene product and recombinants as shown in Table 2.

In a host plant, the production of harmful physiologically active substances, such as trypsin inhibitor,

Table 3. Examples of donors of an inserted gene

Donor microorganisms	Inserted gene
Agrobacterium sp.	CP4 CP4EPSPS
Achromobacter sp.	LBAA GOX
Klebsiella pneumaoniae subsp. *Ozaenae*	oxy gene (nitrilase)
Escherichia coli k12	NPT II
Bacillus thuringiensis subsp. *tenebrio*	Cry III A
Bacillus thuringiensis subsp. *Kuristak*	Cry I A(b), Cry I (c)
Streptomyces viridochromogenes	PAT
Streptomyces hygroscopicus	PAT
Bacillus amyloliquefaciens	barstar and barnase

lectin, isoflavones, phytate, stachyose and raffinose in soybeans, allyl- and indolyl-glucosinolates, erucic acid, sinapine and phytate in rape seeds, glycoalkaloids (solanine and chaconine) in potatoes, glycoalkaloids (tomatine) in tomatoes, and gossypol and cyclopropenoid fatty acids in cotton seeds, should be taken into consideration.

For the donor of an inserted gene and its gene product, the name, origin and taxonomy must be provided. The donor of the inserted gene must not be pathogenic nor have the ability to produce toxins, and its history of safe consumption must be described. Moreover, if there is a strain that is known to be pathogenic, such as *E. coli*, it must be stated that the donor originates from a non-pathogenic strain. As shown in Table 3, many kinds of donor microorganisms have been used as a source of an inserted gene, and they are known to be universally present and frequently found in soil.

For the item of construction of an inserted gene and its gene product, it should be checked that the pres-

Table 2. Standards for safety assessment of seed plants (whole) produced by recombinant DNA techniques or food products utilizing portion of such

Section 1: Similarities between produced food and existing food
Section 2: Purposes and usage of recombinats
Section 3: Host
Section 4: Vector
    1. Name and origin 2. Properties 3. Drug resistance 4. Transmission 5. Host dependency
    6. Expression vector preparation method 7. Insertion method and site of the expression vector insertion
Section 5: Inserted gene and its gene product
    1. Donor 2. Method of gene insertion 3. Construction 4. Properties 5. Purity 6. Stability
    7. Number of inserted gene copies 8. Site, timing and level of gene expression
    9. Safety of antibiotic-resistant marker gene
    10. Presence or absence of exogenous open reading frames and the possibility of their transcription and expression
Section 6: Recombinants
    1. New properties acquired by recombinant DNA techniques 2. Allergenicity of recombinant products
    3. Toxicity of recombinant products 4. Effect of recombinant products on metabolic pathways
    5. Difference from the host 6-11. Others
Section 7: Matters related to assessment scores when safety cannot be confirmed based on sections 2-6

ence of hazardous DNA sequences encoding a known harmful protein must not exist. For the number of inserted gene copies, the insertion and copy numbers must be stated, and the DNA sequence that is adjacent to the inserted DNA must be described, thereby identifying the event that contains the inserted gene(s). This information is very useful for correct labelling of genetically modified food. For example, Monsanto Co. recently submitted a new sequence data showing the DNA sequence adjacent to the inserted DNA in glyphosate tolerant soybeans. In this report, the company showed that one copy of CP4-EPSPS was included, but two small fragments of it were also included at the 3' terminal end and other site of soybean DNA.

For recombinants, new properties and allergenicity of recombinant products are thought to be very important for evaluation. An allergenicity test of recombinant products include whether the products are known to be an allergen or not, consumption history and volume of total protein intake, structural homology of gene products with known food allergens, and sensitivity against enzyme and heat treatment.

Table 4 shows some main gene products used in genetically modified foods to date. The amounts of gene products in these genetically modified foods were not so high except CP4-EPSPS in round-up soybeans; also, the estimated daily intake amounts of these products per day in Japan were very small and no more than 18.5 mg.

In the safety assessment of antibiotic-resistant marker genes, the items most concern are changes by cooking or processing, changes in gastrointestinal fluids and allergenicity. As one example of safety assessment of antibiotic-marker genes, it is known that the NPT II protein has been used safely in human gene

therapy; there is no evidence that the NPT II protein is toxic or allergenic to humans, and it does not have significant homology with known toxins and allergens. The enzyme is inactivated by pepsin in simulated gastric fluids and by simulated intestinal fluids, and also the enzyme would be inactive in the absence of ATP (WHO, 1993).

The results of safety assessment of PAT carried out by Bremmer (1997) show that diets containing up to 5% PAT did not cause adverse effects in a 14-days toxicity study on rats. PAT has no structural similarity to known food allergens. PAT is not stable at above 50°C or outside the pH-range 5.5-10, and is rapidly inactivated and degraded in gastric fluids.

Concerning the safety assessment of Cry1A(b) protein, Kuiper and Noteborn reported (1996) that no specific receptors for the protein are present along the gastrointestinal tract of mammals including human. No histopathological effects of the protein have been observed in the digestive mucosa cells lining the gastrointestinal tract of mammals. The Cry1A(b) protein degrades rapidly under simulated gastrointestinal conditions to smaller fragments below 10 kDa. Upon high dosage oral feeding to rats, the Cry1A(b) protein digested extensively in the gastrointestinal tract to smaller peptides. The protein orally administered to mice and rabbits does not exert signs of systemic adverse effects. No indications were found for immunotoxic effects as judged from the histological examination of spleen, lymph nodes and Peyer's patches of treated animals. No specific antibodies against the protein could be detected in serum of treated rabbits.

From the results of our own examination for degradation of CP4-EPSPS protein in simulated gastric and intestinal fluids, we found that in pepsin solution the protein was digested within 30 min and in pancreatin

Table 4. Estimated daily intake of main gene products from genetically modified foods

Foods		CP4-EPSPS (mg)	NPT II (mg)	PAT (mg)	Cry I A(b) (mg)	Cry III A (mg)	GOX (mg)
Soybean		18.5	-				
Rapeseed oil		<N.D.	<N.D.	<N.D.			<N.D.
Potato	A		0.021			0.042	
	B		0.12			0.054	
Corn	A			<N.D.	0.0092		
	B				0.0059		
	C			<N.D.	<N.D.		
	D			0.0046			
Cotton oil		<N.D.	<N.D.				
Tomato			0.000057				

solution was degraded within 3 hours.

For the assessment of allergenicity, if the safety cannot be confirmed relative to already described items, safety must be assessed through data such as the binding ability of patient IgE antibody and the gene product relative to allergens with structural homologies to the gene product, and the binding ability of patient IgE antibody and the gene product relative to major allergens. This latter test would require using blood serum from patients with allergies toward items such as egg, milk, soybean, rice, wheat, buckwheat, cod, shrimp and peanuts.

In the final part of the safety assessment, when safety cannot be confirmed based on main sections 2-6, acute toxicity study, subacute toxicity study, chronic toxicity study, reproduction study, mutagenicity, carcinogenicity study and other necessary studies (immunological toxin, nutrition, etc) should be carried out.

IV. CASE STUDY FOR SAFETY ASSESSMENT

In the latter part of this report, I'll explain the results of some case studies carried out by several researchers for safety assessment of genetically modified foods.

In 1998, Dr. Pusztai reported the effect of diets containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine. He showed that diets containing genetically modified potatoes expressing the lectin GNA had variable effects on different part of the rat gastrointestinal tract such as mucosal thickness or crypt length. The proliferation of the gastric mucosa was mainly due to the expression of the GNA transgene. Other parts of the construct or the genetic transformation could also have contributed to the overall biological effects of the GNA-genetically modified potatoes. In a commentary on the research, Kuipers et al. (1998) showed that the diet of the rats is so low in protein that they are being starved; then short-term protein stress and starvation could impair the growth rate, development, hepatic metabolisms and immune function of rats. Data on the individual and total glycoalkaloid content of the tubers is not available. There is no statistical difference in the growth rate of rats fed cooked trans-

genic GNA potatoes over the 110 days when compared to the parental controls. As did other expert researchers, they reached a conclusion that the results are difficult to interpret and do not allow the conclusion that the genetic modification of potatoes accounts for adverse effects in animals.

Kuiper et al. (1997) carried out a 90-day feeding trial with transgenic tomatoes expressing Bt(Cry1A(b)) endotoxin. They used three types of diets for male and female Wister rats: 1) control, semisynthetic animal diet, 2) the control diet supplemented with 10% (w/w) of lyophilized tomato material of the parental line, and 3) the control diet supplemented with 10% (w/w) of lyophilized transgenic Bt-toxin tomatoes. In this experiment the intake amount of tomatoes by rats corresponded to 200 g/kg of body weight. The experimental results showed that feed intake, body and organ (liver, kidney, spleen and thymus) weights were normal, clinical parameters (such as serum chemistry, hematology and urinalysis values) were also normal, gross and histopathological examination of organs and tissues failed to uncover any significant macroscopic abnormality, and no indications were found for an immunotoxic effect.

Hashimoto et al. (1999) showed the results of safety assessment of soybean glycinin-transgenic potatoes by a feeding study in rats. Rats fed 4 kinds of diets: 1) a commercial diet, 2) the diet plus non-genetically modified potatoes, 3) the diet plus genetically modified potatoes with native glycinin, 4) the diet plus genetically modified potatoes with designed glycinin for 28 days by oral administration. The designed glycinin has four additional methioninyl residues in the middle of the glycinin molecule. Dose level was 2 g/kg body weight/day (3% glycinin content of total protein). Experimental results showed that rats grew well without marked differences in appearance, feed intake, body weight or cumulative body weight gain. Significant differences were not found in blood count, blood composition, and in internal organ weights. Neither pathologic symptoms in all rats tested nor histopathological abnormalities in liver and kidney were indicated.

V. IMMUNOTOXIC TEST

Teshima et al. (2000) carried out immunological

Table 5. Soybean-specific IgE and IgG production in BN rats and B10A mice fed with ground GM soybeans or non-GM soybeans

Group	BN rats ELISA- titer (n=5)		B10A mice ELISA- titer (n=5)	
	Soybean IgE	Soybean IgG	Soybean gE	Soybean IgG
GM-soybean	143±100	1576±1207	61±12	100±112
Non-GM-soybean	152±149	1950±348	88±71	57±16
Control	66±37	643±511	51±0.9	173±226

feeding trial using genetically modified soybeans. The purpose was to study the effect of genetically modified and genetically unmodified soybeans on the immune system of rats and mice. Subchronic animal feeding studies to examine the effect of glyphosate-tolerant soybeans, which contain the bacterial EPSPS from *Agrobacterium* sp. strain CP4, on the immune system were conducted with BN rats and B10A mice. The two animal groups were fed control diet, 30% genetically modified soybean diet or 30% genetically unmodified soybean diet for 15 weeks. As remarkable compositional differences in fatty acids or amino acids were not found between glyphosate-tolerant soybeans and soybeans of closely-related and one-parent same cultivar, the genetically modified soybeans seem to be equivalent to genetically unmodified soybeans. The experimental results showed that significant difference in growth, food intake, or weights of the liver and the spleen was not found between animals fed the two soybean diets. The histopathology of immune-related organs such as thymus, liver, spleen, mesenteric lymph node, Peyer's patches, and small intestine was similar in animals fed the two soybean diets. The production of soybean-specific IgE was not detected in the sera of either group, and the increase in soybean-specific IgG was the same in both groups fed genetically modified soybeans and unmodified soybeans as shown in Table 5. We concluded that no immunotoxic acitivity was found in genetically modified soybean-fed rats or mice.

REFERENCES

Bremmer, J.J. (1997): Glufosinate tolerant crops, test methods to evaluate possible health hazards, in Report of the OECD workshop on the toxicological and nutritional testing of novel foods, 22, OECD, Paris.

Even, S.W.B. and Pusztai, A. (1999): Effect of diets containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine. Lancet, **354**,1353-1356.

Hashimoto, W., Momma, K., Yoon, H.-J., Ozawa, S., Ohkawa, Y., Ishige, T., Kito, M., Utsumi, S. and Murata, K. (1999): Biosci. Biotechnol. Biochem., **63**, 1942-1946.

Kuiper, H.A. and Noteborn H.P.J.M. (1996): Food safety assessment of transgenic insect-resistant Bt tomatoes, in Food safety evaluation, pp. 50-57, OECD, Paris.

Kuiper, H.A., Noteborn, H.P.J.M. and Peijnenburg, Ad A.C.M. (1999): Adequacy of methods for testing the safety of genetically modified foods. Lancet, **354**, 1313-1316.

MHW (2000): Ordinance No. 95, 1 May 2000, Announcement No. 232-234, 1 May 2000, Notice No. 825-1, 1 May 2000.

Teshima, R., Akiyama, H., Okunuki, H., Sakushima, J., Goda, Y., Onodera, H., Sawada, J. and Toyoda, M. (2000): J. Food Hyg. Soc. Japan, **41**,188-193.

WHO (1993): Health aspects of marker genes in genetically modified plants, report of a WHO workshop, Annex III, WHO Food safety unit.