BfR Symposium (2021 10/15-17)

The perspective: Application of New Approach Methodologies to Food Risk Assessment by Food Safety Commission of Japan

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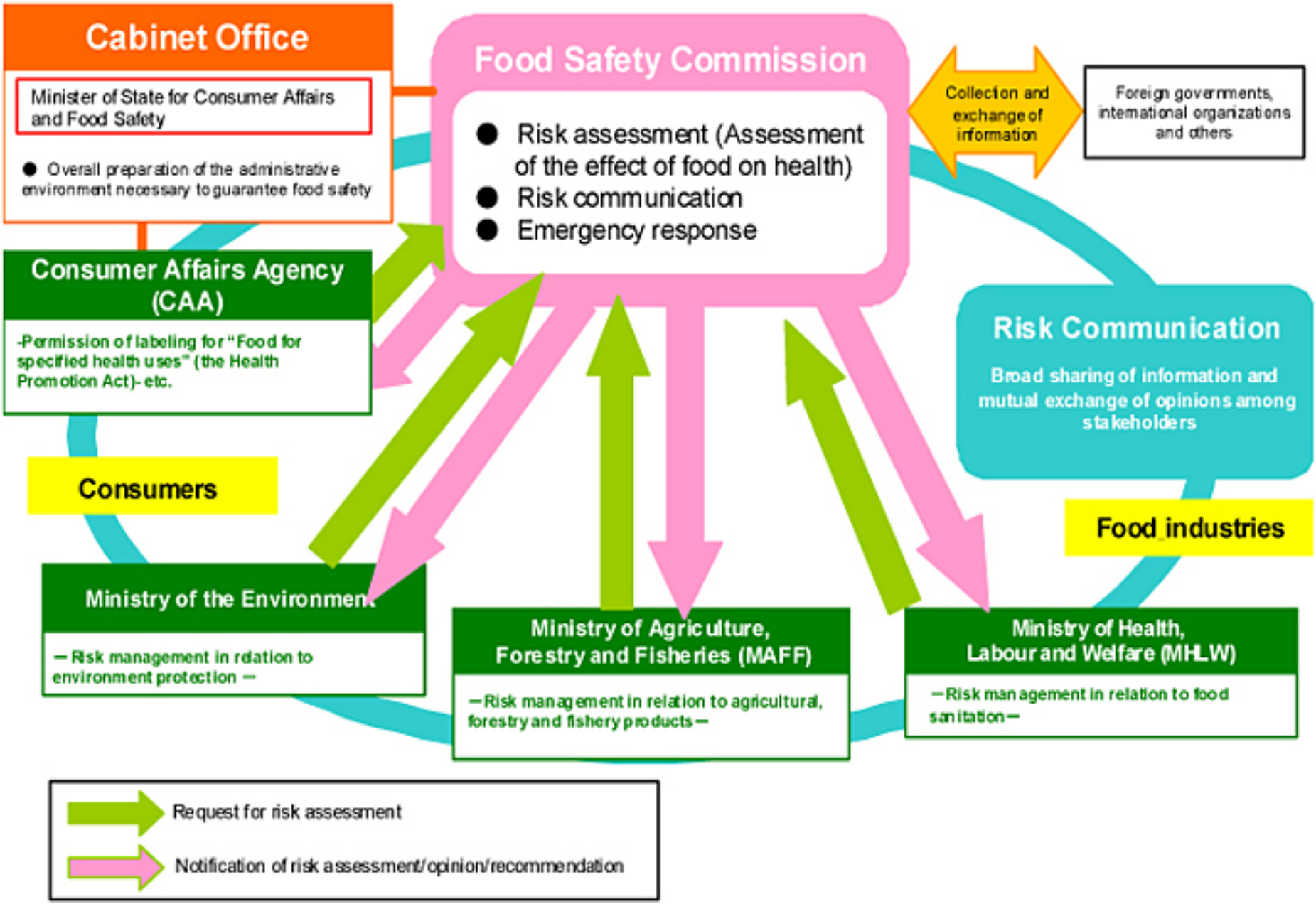
a commissioner

内閣府 食品安全委員会
Food Safety Commission of Japan

Outline of the presentation

- Food Safety Commission of Japan (FSCJ)
- NAM application to the present food risk assessment carried out in the FSCJ
- NAM which will be applied to the food risk assessment in Japan in the near future
- In the future
  - Candidates of NAM applicable to the food risk assessment
  - Hurdles to regulatory use of NAM in food risk assessment
  - Possibility of introduction of NAM
- Summing-up and conclusion

Role of the Food Safety Commission of Japan (FSCJ)



Organizational Structure of FSCJ

FSCJ: 7 Commissioners

Chairperson: Shigeki Yamamoto,

Acting chairperson: Satoshi Asano, Toru Kawanishi, and Masako Waki,
Midori Kasai, Waki Matsunaga, Mitsuru Yoshida

12 Expert Committees (over 200 experts, 1 Chair for each committee)

- Planning
- Food additives
- Pesticides
- Veterinary medical Products
- Apparatus and containers/packages
- Chemical and Contaminants
- Microorganisms and Viruses
- Prions
- Natural toxins and mycotoxins
- Genetically modified foods
- Novel foods
- Feed, fertilizers

Secretariat (DG, DDG and 4 Divisions, 100 staff)

- Director-General
- Deputy Director-General
- Director, General affairs Div.
- Director, First Risk Assessment Div.
  - Assessment Methodology Development Office
- Director, Second Risk Assessment Div.
- Director, Information, Recommendation and Public Relations Div.
- Director, Information Analysis
- Director, Risk Assessment Coordination
- Director, Risk Communication

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NAM-related approaches implemented in "Guidelines for the Risk Assessment of Food Additives" and the revision

- Flavoring substances : Exposure is assumed to be low ⇒ Threshold of Toxicological Concern (TTC) approach and Quantitative Structure-Activity Relationship (QSAR)
- Processing aids : Exposure is assumed to be low ⇒ TTC approach and QSAR
- Allergenicity : *in vitro* (OECD 442C, 442D, and 442E); Integrated Approaches to Testing and Assessment (IATA) based on adverse outcome pathway (AOP)
- Allergenicity of enzymes : *in vitro* physicochemical stability tests and *in silico* methods（homology search of amino acid sequence）; IgE-binding activity

Estimated intake classes and test items required in processing aids#

Estimated intake class		Test items
Class a	90μg/human/day or less	Genotoxicity
Class b	more than 90μg/human/day 2,000μg/human/day or less	Genotoxicity Sub-chronic toxicity*
Class c	more than 2,000μg/human/day	ADME Genotoxicity Repeated dose toxicity Carcinogenicity Reproductive toxicity Developmental toxicity Allergenicity

In addition to the above test items, available information of subject substances (especially, toxicity other than that required in the above table) should be submitted

*in principle, repeated dose toxicity tests for 90 days

NAM-related approaches implemented in “Guidelines for the Risk Assessment of Food Apparatus, Containers and Packaging” (May 2019)

- Tiered approach based on dietary concentrations, which calculated from migration tests into food stimulants, applying TTC approach and QSAR

Dietary concentreation (mg/kg Food)

Tier of Dietary Concentration	Tier Ⅰ No toxicity test	Tier Ⅱ No animal test except *in vivo* genotoxicity test	Tier Ⅲ Limited animal tests	Tier Ⅳ
		0.0005	0.05	1
Test items	- Consideration of genotoxicity and carcinogenicity based on available information	Genotoxicity	Genotoxicity Sub-chronic toxicity	Genotoxicity Sub-chronic toxicity Reproductive toxicity Developmental toxicity Chronic toxicity Carcinogenicity ADME

3Rs approach in “Basic approach of the Food for Specified Health Use”

- In principle, the following test data are required in non-clinical toxicity tests
  - Genotoxicity study
  - Single-dose oral toxicity (acute toxicity)
  - Repeated-dose 28-day or 90-day oral toxicity (sub-chronic toxicity)
  - When needed, data on 1-year chronic toxicity, carcinogenicity, reproductive and developmental toxicity, antigenicity, allergenicity and teratogenicity

3Rs approach in “Standards for the Safety Assessment of Genetically Modified (GM) Foods”

- The traits that have been intentionally or unintentionally added or eliminated are assessed comparing the GM crop with its non-GM counterpart
  - Eating experience of host and production ability of harmful substances
  - Safety of inserted genes and their products
    - Characteristics of inserted genes are apparent
    - Transgene products are harmless（by *in vitro* tests and/or *in silico* search）
      - allergenicity : *in vitro* physicochemical stability tests and *in silico* homology search of amino acid sequences ; if needed, ability tests of IgE binding
    - Gene transfer method are apparent.
  - Safety as GM Foods
    - Nutrients, deleterious components, etc. in GM crops are comparable with those in its non-GM counterpart
- Additional studies including animal toxicity tests are required, <u>when safety cannot be confirmed based on the above assessment</u>.

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“Research Grant Program for Risk Assessment on Food Safety” and “Survey Program for Collecting Data on Food Safety” sponsored by the FSCJ

- The FSCJ has implemented “Research Grant Program for Risk Assessment on Food Safety” and “Survey Program for Collecting Data on Food Safety”, in order to support research and survey for accumulating the scientific findings and knowledge, and developing and improving risk assessment methodologies.

- The FSCJ has made the Roadmap naming “Strategic direction for promoting research and survey to ensure food safety” to clarify and define overall research and survey directions with an eye to 10 years ahead, and revised it every 5 years.

Category of Research and Survey which the FSCJ supports (1)

- Accumulation of scientific findings and knowledge necessary for hazard characterization and exposure assessment

  (1-1) The research-survey for collecting scientific findings and accumulating knowledge needed for hazard characterization, and for refinement of exposure estimation, such as the estimation method using biomarkers;

  (1-2) The research-survey for collecting and analyzing scientific findings and accumulating knowledge needed for risk assessment of foods, food additives, raw materials of food apparatus, containers and packages, etc. which the cutting-edge technologies (genome-editing, micro and nano-technology, etc.) are used for their development, manufacturing, and/or processing.

Category of Research and survey which the FSCJ supports (2)

- Elucidation of expression mechanism of effects of food-related hazards on human health

  (2-1) Research-survey for elucidating expression mechanism of effects on human health regarding hazards derived from Japanese dietary habits;

  (2-2) Research-survey on mechanisms of adverse effect resulting from ingestion of foods, which are normally considered to be harmless, in a particular group;

  (2-3) Research-survey for verifying validity of extrapolation of toxicological findings in animal experiments to health effects on humans;

  (2-4) Research-survey for elucidating expression mechanism of effects on human health regarding food-related microbiological and chemical hazards, which is needed for the risk assessment.

Category of Research and survey which the FSCJ supports (3)

- Utilization of new methods in food risk assessment

  (3-1) Research-survey for introduction of new risk assessment methods, taking into account of the domestic and overseas trends: TTC and QSAR;

  (3-2) Research-survey for use of preexisting toxicity data of substances into risk assessment of the other chemicals: Grouping/Read-Across;

  (3-3) Research-survey for introduction of new risk assessment methods from the standpoints of "3R principles" and/or for improvement of experimental methods: *in vitro* and *in silico* methods, and the others, such as *in vitro* and *in vivo* computational modeling;

  (3-4) Research-survey for the correct understanding of risk assessment results and its dissemination to the people: For risk communication.

Recent NAM-related researches supported by the Grants from the FSCJ (1)

- (FY2017-2019) Study on migration test in risk assessment for synthetic resin for apparatus, containers and packaging (3-1) ⇒ “Guidelines for the Risk Assessment of Food Apparatus, Containers, and Packaging” (May 2019) and its revision (October 2020)
- (FY2018-2019) Study on application of in silico evaluation method to risk assessment of trace level chemicals unintentionally contained in food (3-1) ⇒ “Guidelines for the Risk Assessment of Food Apparatus, Containers, and Packaging” (May 2019)
- (FY2019) Study on risk assessment for food additives focusing on transferring to the body (3-1) ⇒ Revision of “Guidelines for the Risk Assessment of Food Additives” (September 2021)
- (FY2019) Study on risk assessment of food additives – Risk assessment method in infant – (3-1) ⇒ Revision of “Guidelines for the Risk Assessment of Food Additives” (September 2021)

NAM-related researches supported by the Grant from the FSCJ (2)

- (FY-2016-2017) Construction of the database of *in vivo* toxicity tests and its application to the *in silico* prediction and evaluation of *in vivo* toxicity (3-2, 3-3)
- (FY2018-2019) Development of new evaluation support technology: Examination of database utilization method for toxicity prediction (3-2, 3-3)
- (FY2020-2021) Research for refinement of prediction approach of hepatotoxicity by introducing *in silico* methods (3-3)
- (FY2020-2021) Study on risk assessment methods of metabolites from pesticide residues (3-1, 3-2)

"Construction of the database of *in vivo* toxicity tests and its application to the *in silico* prediction and evaluation of *in vivo* toxicity" (FY-2016-2017)

- The principal research investigator: Kouichi YOSHINARI(University of SHIZUOKA)

(1)Expansion of the Hazard Evaluation Support System Integrated Platform (HESS) database* by combining the database constructed using repeated dose toxicity test data (224 substances (678tests)) in the risk assessment reports of FSCJ ⇒ *In silico* analysis of Hepatocellular hyperplasia

(2)Collection of the data on reproductive and developmental toxicity tests from the risk assessment reports and literatures, and construction of the database for the reproductive and developmental toxicity ⇒ *in silico* analysis of toxicological characteristics of phthalates

* The database was established on the results of repeated dose toxicity studies of chemicals in rats, subject to the Chemical Substance Control Law in Japan.

“Development of new evaluation support technology: Examination of database utilization method for toxicity prediction” (FY2018-2019)

- The principal research investigator: Masahiro Tohkin (Nagoya City University)

(1)Extraction of toxicological findings from the repeated-dose toxicity study database of HESS;

(2)A case study of the prediction of hepatotoxicity, nephrotoxicity, and hematologic toxicity from the molecular descriptions using the (Q)SAR model based on machine leaning;

(3)Verification of the toxicity database, and evaluation of the support tools suitable for *in silico* evaluation from the case study.

Study on risk assessment methods of metabolites from pesticide residues (FY2020-2021)

- The principal research investigator: Atsushi ONO (Okayama University)

(1)In risk assessment of pesticide residues in food, it is important to evaluate not only the active ingredient (parent compound) but also the metabolites produced in plants and animals.

(2)The purpose of this study is to investigate the evaluation methods of metabolites from pesticide residues, using NAM such as TTC approach, QSAR and Grouping/Read-across, in order to implement these methods in the evaluation of pesticide residues in the near future in the FSCJ.

“Research for refinement of prediction approach of hepatotoxicity by introducing *in silico* methods” (FY2020-2021)

- The principal research investigator: Takashi Yamada (National Institute of Health Sciences)

(1)Estimation of plasma concentration of coumarin and its metabolites in human using PBPK model

(2)Estimation of hepatotoxicity of coumarin and its related substances using FDA DILI score model

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Applicable tools to NAM replacing animal tests in the future

- Human cell culture systems including ES cells, iPS cells, etc.
- Organ-on-chips
- *In vitro* & *In vivo* computational modeling
- High-throughput methods for Mode of Action (MoA) assessment

Hurdles to regulatory use of NAM replacing animal tests in food risk assessment

- Test methods used in food risk assessment are standardized and their reliability is assured by validation.
- Assessment using alternative methodologies should be equal to or better than that using present animal tests
  - → Combination of tests depending on Mode of Action (MoA)
- Regulations are formed based on criteria used in animal tests, and the criteria may not fit alternative methods

International articles for animal tests and their alternatives

- Movement of 3Rs in animal tests started in the field of cosmetics at first. The movement has been enlarged in the field of safety tests of medicines（ICH）, medical devices（ISO）, and chemicals（REACH）, and also to medical research（OIE, EU Directive, ILAR Guide, CIOMS）;
- International articles for animal tests and their alternatives have been become effective in OECD, ISO, EU, OIE, ILAR, CIOMS, etc, and basic concept and methods for animal experiments are shown in them;
- However, concrete steps are usually not described in these international standards and guidance; and
- At present concrete methods of animal tests and their alternatives in OECD guidelines are widely cited

Modified from the original by Dr H-Kojima (National Institute of Health Sciencesa)

Non-animal alternative test methods in the OECD TG(2021) (1)

Class	Test methods
Corrosion	*In vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER) :TG430
	In vitro Skin Corrosion: Reconstructed Human Epidermis (RHE) test method :TG431
	CORROSITEX Skin Corrosivity Test :TG435
Skin irritation	*In vitro* Reconstructed Human Epidermis (RhE) Test methods, EpiDerm, EPISKIN, SkinEthic, LabCyte EPI-Model: TG439
Phototoxicity	3T3 NRU Phototoxicity Test :TG432
	ROS(Reactive Oxygen Species) Assay for Photoreactivity: TG495
	In vitro Phototoxicity: Reconstructed Human Epidermis Phototoxicity test Method : TG498
Eye irritation	Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage:TG437
	Isolated Chicken Eye Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage:TG438
	Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants : TG460
	Short Time Exposure In Vitro Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage : TG491
	Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage : TG492
	Vitrigel-Eye Irritancy Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage: TG494
	In vitro Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage : TG496

Modified from the original of Dr H-Kojima

Non-animal alternative test methods in the OECD TG(2021)

(2) Class	Test methods
Skin sensitisation	*In chemico* Skin Sensitisation:TG442C
	In vitro Skin Sensitisation:TG442D
	In vitro Skin Sensitisation: TG442E
	Guideline on Defined Approaches for Skin Sensitisation
Endocrine disrupter screening	Performance-Based Test Guideline for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists and Antagonists : TG455
	H295R Steroidogenesis Assay :TG456
	Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals: TG458
	Performance-Based Test Guideline for Human Recombinant Estrogen Receptor (hrER) In Vitro Assays to Detect Chemicals with ER Binding Affinity : TG493
Genotoxicity	Bacterial Reverse Mutation Test : TG471
	In vitro Mammalian Chromosome Aberration Test : TG473
	In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes : TG476
	In vitro Micronucleus Test : TG487
	In Vitro Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene : TG490
Skin absorption	Skin Absorption: *In vitro* Method :TG428

Modified from the original of Dr H-Kojima

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Possibility of alternative methods for animal tests？

- Corrosion, skin and eye irritation, and skin sensitization ----- very promising
- Acute toxicity ----- difficult
- Repeated dose toxicity ---- very difficult
- Reproductive and developmental toxicity ----- difficult
- Genotoxicity（mutagenicity）---- very promising
- Carcinogenicity ----- difficult
- Allergenicity ----- promising

Summing-up & Conclusion

- 3Rs to animal tests for risk assessment in food area are expected as well as in medicines, medical devices, chemicals, etc. from the standpoint of not only animal welfare but also their limited predictability in human.
- TTC, QSAR, and grouping/read-across have been already applied to risk assessment, and development of innovative technology applicable to the assessment is continuing.
- There are hurdles to regulatory use of alternative methodologies in food risk assessment.
- Application of NAM to qualitative safety assessment would be promising; however, substitution of animal tests by NAM in quantitative assessment deciding health-based guidance values would not be easy.
- It is important to advance the movement and clear the hurdles utilizing the new technology in step-by-step way.

Thank you

Gracias

Merci

Danke schön

Grazie

Obrigado

спасибо

شُكْرًا

Tesekkur ederim

متشكرم

谢谢（xièxie）

고맙습니다

Terima kasih

有難うございます