Development of safety assessment methods for cellulose nanofibers

AUTHORS:

Hideo Kajihara, Katsuhide Fujita, Isamu Ogura, Tomoko Oguri

PRESENTED BY:

Hideo Kajihara

Group Leader

National Institute of Advanced Industrial Science and Technology

Background

- To accelerate the practical use of CNFs in society, the development of safety assessment methods for CNFs is required.
- Such methods have not yet been established for CNFs because their properties that are different from those of other nanomaterials.
- Hazard and exposure assessment methods have been developed for other nanomaterials such as CNTs.

Differences between conventional nanomaterials and CNFs ⇒ New development subject

- Viscosity (slurry)
- Diversity of physicochemical properties (surface modification, impurities, fibrillation states)
- Low thermal stability
- Low absorbance
- Biological origin
- Degradability (be converted to sugar)
- High dispersibility (fineness)
- Diversity of consumer product applications

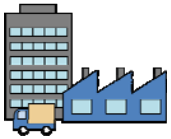
Development of safety assessment methods for cellulose nanofibers

Difficulty for assessing with conventional methods

Development of new methods

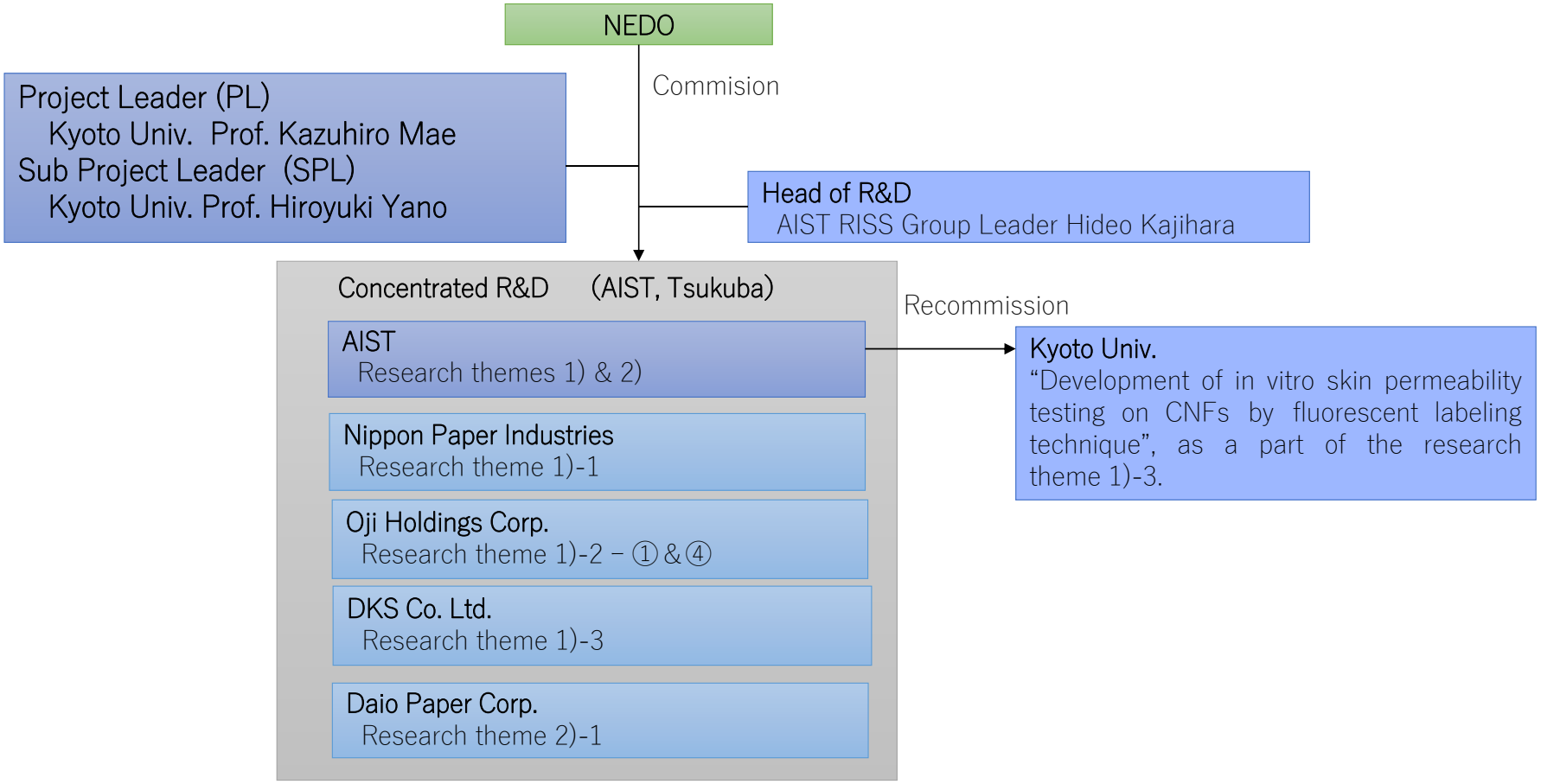
Goal

Establishment of hazard and emission/exposure assessment methods for CNFs



Safety assessments by CNF-related companies

NEDO Project “Development of safety assessment methods for cellulose nanofibers” (FY2017 ~FY2019)



Research themes

1) Development of analysis and hazard assessment methods for CNFs

1)-1 Development of methods for detection and quantification of CNFs
AIST, Nippon Paper Industries

1)-2 Development of intratracheal instillation method for CNFs
AIST

1)-3 Development of in vitro skin permeability testing for CNFs
AIST, DKS, Kyoto University

2) Development of emission/exposure assessment methods for CNFs

2)-1 Development of methods for measuring CNF emission/exposure and accumulation of evaluation cases
AIST, Daio Paper Corp.

2)-2 Case study for CNF application products by using exposure scenario
AIST

AIST

Target CNFs

Surface-modified CNF

TEMPO-oxidized CNF

Nippon Paper Industries,
DKS,
The University of Tokyo

Microfibrils of 3-4 nm in width, having a carboxyl group

Phosphoric acid-esterified CNF

Oji Holdings Corp.

Microfibrils of 3-4 nm in width, having a phosphate group

Non-surface-modified CNF

Mechanically fibrillated CNF

Microfibril bundles of >10 nm in width

Daio Paper Corp.,
Chuetsu Pulp & Paper,
Sugino Machine Limited
etc.

Three types of CNFs are chosen as main target materials

Ligno-CNF composites

Kyoto University,
Seiko PMC Corp.

As a target for exposure assessments

In collaboration with the related project "the development of production process for high performance lignocellulosic nanofibers and their applications," we receive composite samples and provide evaluation result information.

1)-1 **Development of methods for detection and quantification of CNFs**

① Development of methods for detection and quantification of CNFs

◆Issue: Establishment of detection and quantification method of trace amount CNFs in consideration of the diversity of CNFs and separability from coexisting substances

Determination as saccharides

Other methods

- Thermal analysis
- Microscopic observation

【Goal】Establish methods to detect and quantify 1 μg of CNFs for three main types of CNFs (level required for grasping trace CNF in biological tissue and environment)

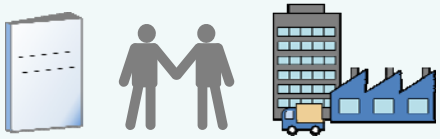
Use

Issue identification

Collaboration

Research themes 1)-2, 1)-3, 2)-1: Application to biological tissues, cells, environmental samples

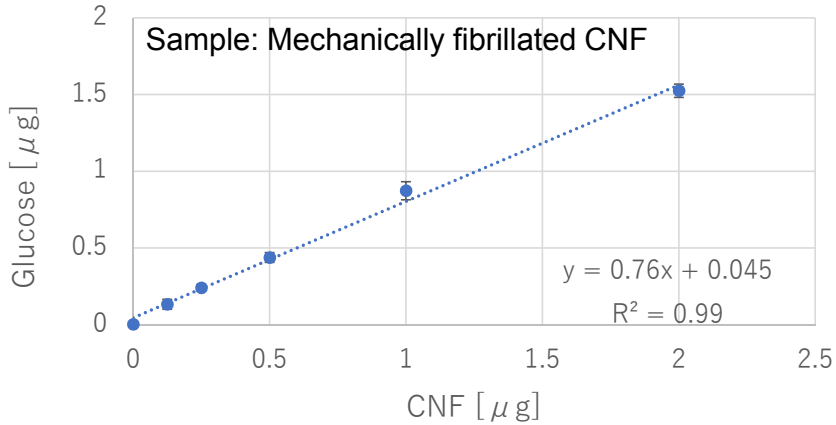
② Publication of a guidance document
→ **Support for voluntary safety assessment in companies**



1)-1 **Development of methods for detection and quantification of CNFs**

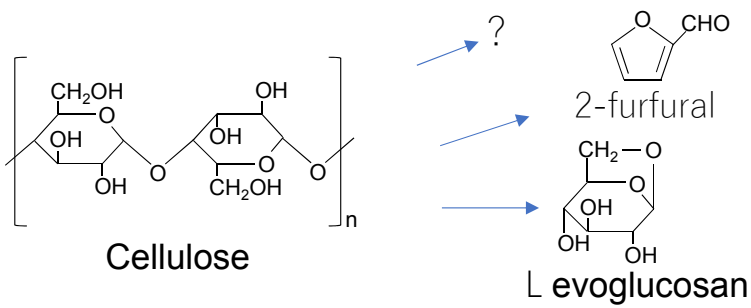
Enzymatic decomposition

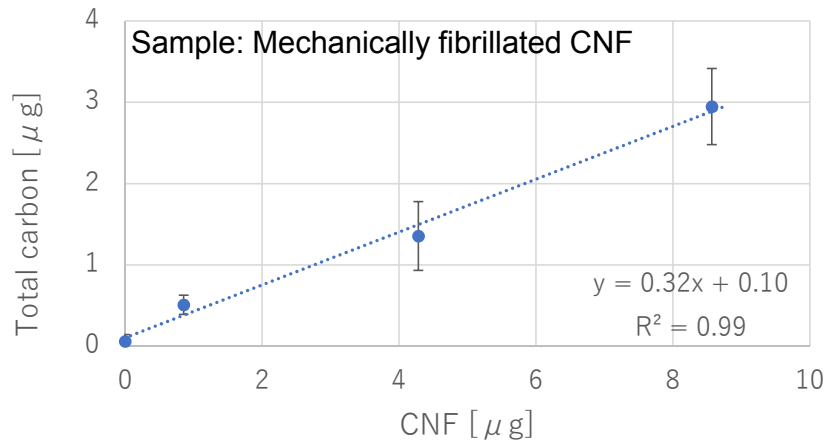
CNFs are enzymatically decomposed and quantified as saccharides.



Thermal decomposition

CNFs are thermally decomposed and quantified as decomposition products.

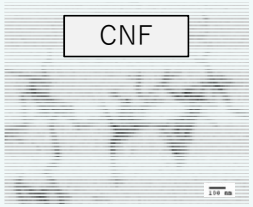




1)-2 Development of intratracheal instillation method for CNFs

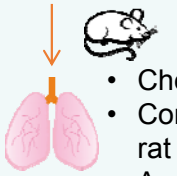
① Preparation and measurement of CNF sample for intratracheal instillation test

- Establishment of sample preparation method maintaining CNF physico-chemical properties.
- Investigation of the relationship between CNF sample preparation conditions and physico-chemical properties (concentration, cohesiveness, viscosity, etc.) in the dispersion.
- Confirmation of spray distribution of CNFs from oral dosing needles
- Establishment of CNF sterilization method for animal test.
- Development of CNF staining method.



② Intratracheal instillation test using CNF sample

CNF



◆Issue: Establishment of appropriate intratracheal instillation method

- Check for spray distribution of CNF samples.
- Confirmation of the state of intratracheally instilled rat (asphyxiation, behavioral abnormalities, etc.).
- Assessment of CNF inhalation toxicity by medium- to long-term rat intratracheal instillation test.

③ Measurement of CNFs in pulmonary sample after intratracheal instillation for assessing the distribution of CNFs in lungs

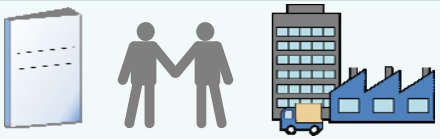
CNF

◆Issues:

- Extraction of CNFs from lung samples
  - ・Ultrasonic-enzymatic degradation method
- Appropriate CNF analysis
  - ・Application of methods developed in 1)-1
  - ・Spectroscopic analysis
  - ・TEM observation

【Goal】Establishment CNF sample preparation condition and CNF measurement method in lung sample for developing intratracheal instillation method on CNFs

④ Publication of a procedure manual
→ Support for voluntary safety assessment in companies



Plan for rat intratracheal instillation (IT) test

	2017	2018	2019
Test 1	CNF ・Development of CNF sample preparation Short-term IT test Feeding period：7days after instillation ・Confirmation of dose of CNF	・Development of CNF quantification method in lung	
Test 2		CNF ・Establishment of sterilization method ・Sample preparation of dyed CNF Medium- to long-term IT test Feeding period：90 days after instillation ・Establishment of CNF quantification method in lung ・Assessment of inhalation toxicity of CNF	

1)-3 Development of in vitro skin permeability testing for CNFs

Investigation of the relationship between CNF sample preparation conditions and physico-chemical characteristics such as viscosity in dispersion

① Verification of human skin model

CNF

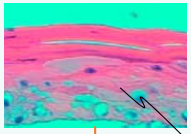
◆Issues:
- CNF sample conditions for human skin models
- Difference such as CNF permeability among 3D reconstituted human epidermis (RHE) models

- Selection of suitable 3D RHE models for CNF skin permeability test.
- CNF permeability test using synthetic membrane.

② Development of CNF detection method in skin model

・CNF
・Dyed CNF
・Fluorescence-labeled CNF

3D RHE model



CNF

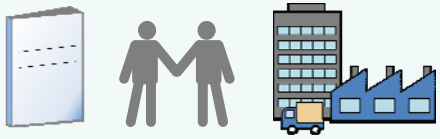
CNF distribution

◆Issue:
Microanalysis of CNFs in human skin models

- Application of methods developed in 1)-1
- Spectroscopic analysis
- Fluorescent labeling technique
- TEM observation

【Goal】Establishment CNF detection method in human skin model for development of skin permeability test method

③ Publication of a procedure manual
→ Support for voluntary safety assessment in companies



Test Outline

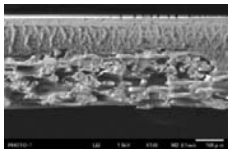
Development of skin permeability test method

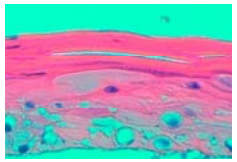
(1)CNF (2)Fluorescence-labeled CNF (3)Dyed CNF

High performance synthetic membrane

3D reconstituted human epidermis (RHE) models

Strat-M®





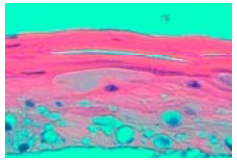
Samples	Test materials	Analysis
receptor fluid	(1) CNF	Sugar analysis, GC/MS, HPLC *etc.*
	(2) Fluorescence-labeled CNF	Quantitative analysis of fluorescent labels
	(3) Dyed CNF	Spectroscopic measurement
Inside of synthetic membrane or RHE models	(2) Fluorescence-labeled CNF	Qualitative analysis of fluorescent label by confocal microscope
	(3) Dyed CNF	Microscopic observation

Skin irritation test

OECD TG439

CNF

3D RHE models



(1) EpiDerm™ (EPI-200SIT), MatTek

(2) SkinEthic™ RHE, SkinEthic

- MTT assay
- Cytokines
- Comprehensive gene expression

Development of skin permeability test method while comparing the results of skin irritation test

2)-1 Development of methods for measuring CNF emission/exposure and accumulation of evaluation cases

① Development of methods for measuring CNF emission and exposure

◆Issue: Establishment of measurement method of trace amount CNFs in the presence of coexisting substances

Application of methods developed in 1)-1

Evaluation of utility of existing methods

- Aerosol measuring instrument
- Thermal analysis
- Microscopic observation

【Goal】Establishment of measurement method for CNFs (target value: 1 μg/m^3)

Use

Issue identification

② On-site surveys and emission tests

◆Issue: Lack of CNF emission/exposure information

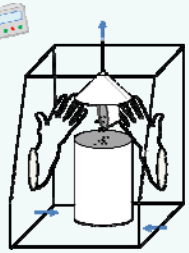
On-site surveys

- Factories for CNF production
- Research laboratories

Emission tests

Examples:

- Aerosolization of CNF slurry as droplets
- Aerosolization of CNF slurry after drying
- CNF emission during handling of CNF dry powder
- CNF emission during machining of CNF composites



【Goal】Accumulate CNF emission/exposure information: amount (number, mass), size, morphology

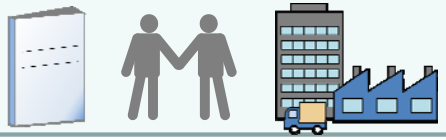
(Type of CNF × type of processes = 20 or more)

Measurement methods

Evaluation cases

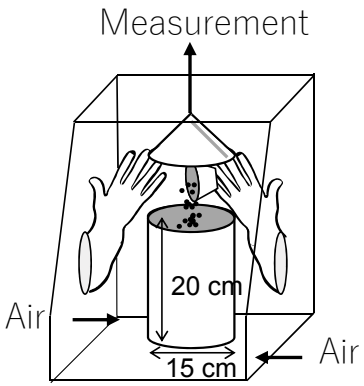
③ Publication of a guidance document

→ Support for voluntary safety assessment in companies



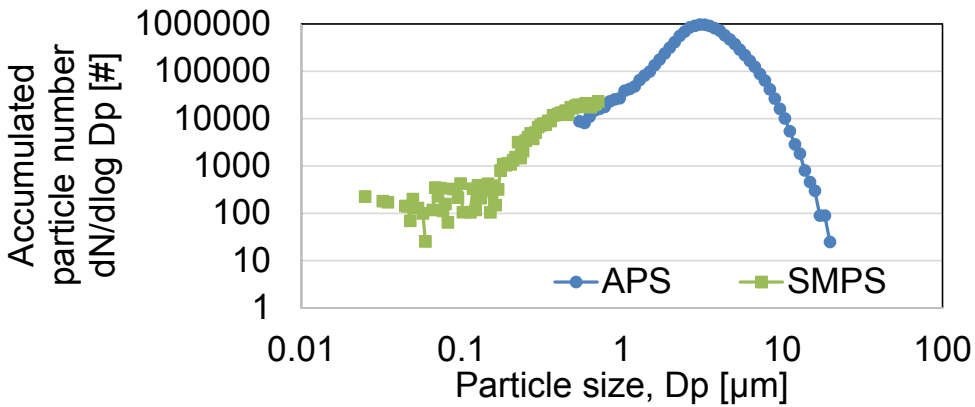
2)-1 Development of methods for measuring CNF emission/exposure and accumulation of evaluation cases

Handling of CNF powder

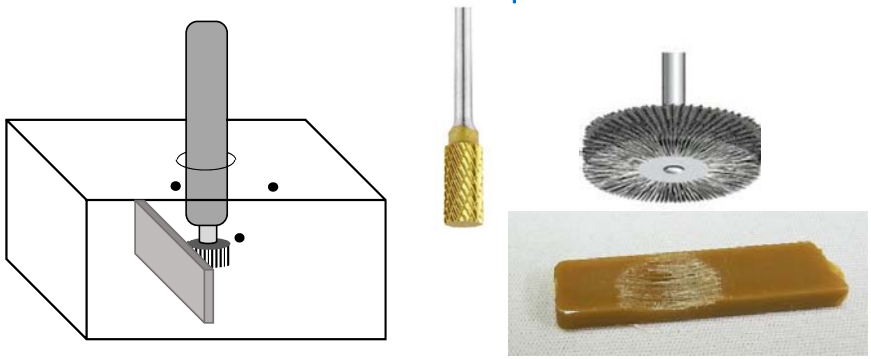


CNF powder

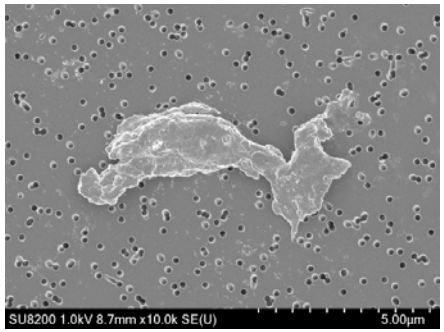
Particle size distribution of aerosolized CNFs

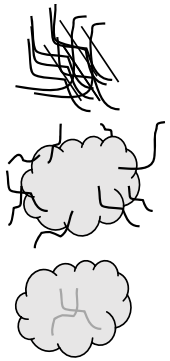


Grinding and abrasion of CNF composites



Particles released during grinding of CNF composites



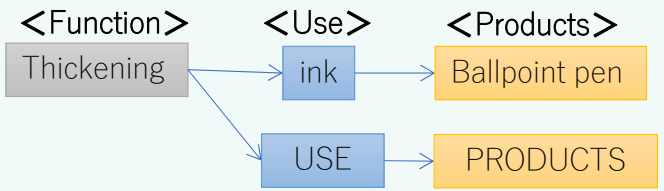


2)-2 Case study on CNF application products by using exposure scenario

① Extraction of the typical products cases

◆Issue: Lack of exposure information on CNF application products

Extraction of the typical product cases in the context of function and use from the present and future CNF application products



【Goal】 Extraction of typical products cases

② Obtain biodegradability data for CNFs

◆Issue: Stability/degradability data for usage and disposal

Biodegradability test according to OECD TG 301C

【Goal】Obtain biodegradability data for typical CNFs

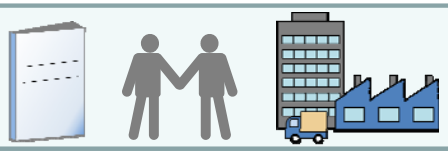
③ Extraction of exposure scenarios

◆Issue: Exposure scenario through the life cycle of CNF application products

Life Cycle	Production	Use	End of Life
Receptor	Worker	Consumer	Environment
Article	Dry powder, Slurry, composite production process	Degradation and ablation of ligno-CNF composite	Biodegradation of ligno-CNF composite material
Mixture	Dry powder, Slurry	Exposure when using products	Biodegradation of typidal CNFs

【Goal】Extraction of exposure scenarios containing 2 exposure routes (inhalation, dermal) and 2 products categories (article, mixture)

④ Publication of an emission/exposure scenario documents
→ Support for voluntary safety assessment in companies



Biodegradability test of CNF

Objective：Confirmation of whether CNF is rapidly decomposed (degradable) when discharged into the environment

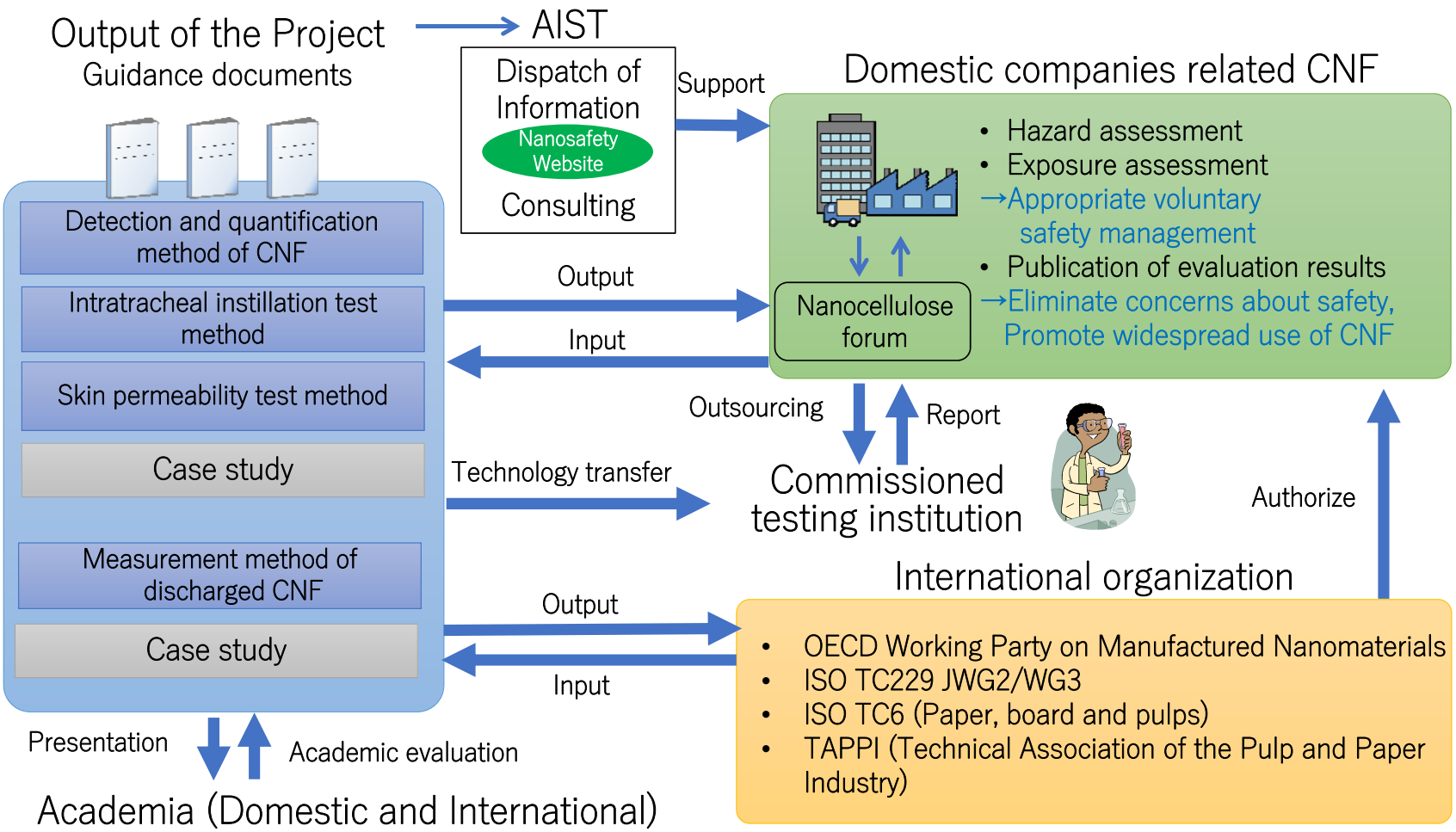
Method：OECD-301C method (Modified MITI test) , which is a 28 day respirometry test that predominantly measures oxygen consumption.

CNF Type	Manufacturer	Result
TEMPO-CNF	**DKS**	Ready biodegradable*
Phosphoric acid-esterified CNF	**OJI Holdings**	Ready biodegradable*
Mechanically fibrillated CNF	**Daio Paper Corp.**	Ready biodegradable*
Acetylated CNF**	**Kyoto Univ.**	Ready biodegradable*

All four CNFs subjected to the test are considered to be rapidly degraded after environmental discharge.

* “Ready biodegradable" means that the biodegradability of chemical substances is 60% or more.
** "Acetylated CNF" means a raw material for ligno CNF composite used in "Kyoto Process", and the degree of acetylation (DS) of the **sample** is 0.69.

Impacts and Ripple effects



Thank you for your attention

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