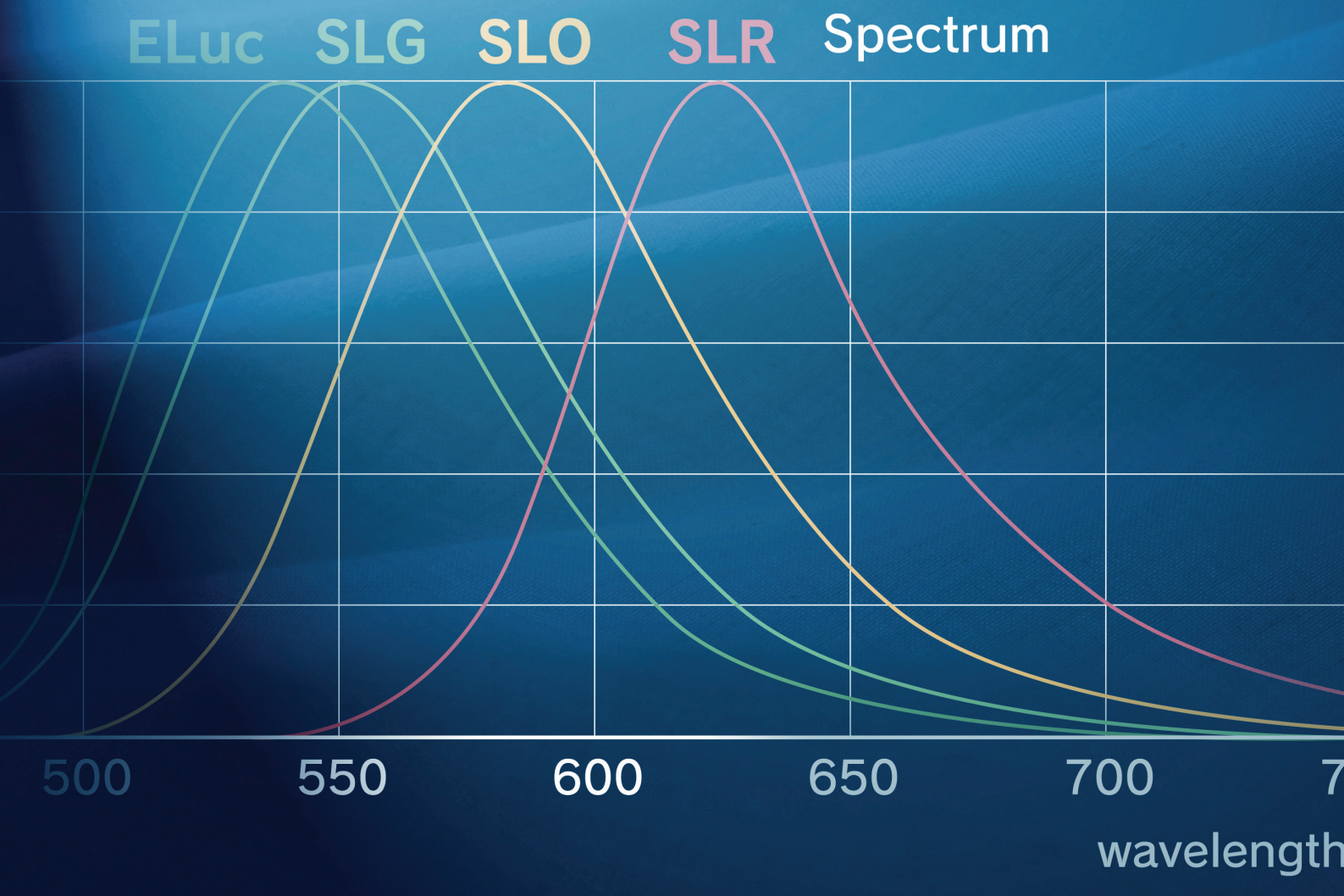
R&D of In Vitro and In Silico Alternatives to Animal Testing under the Strategic R&D of Chemical Risk Analysis Technologies by NEDO, Japan



NEDO

PREFACE

Approximately 5,000 high production volume (HPV) chemicals are now used by industries around the world although there are insufficient safety data for most of them. The Ministry of Economy, Trade and Industry (METI) of Japan shares responsibility for collecting biodegradation, bioaccumulation and health/ecological effect data for such chemicals.

To accelerate HPV data collection, and more generally, to promote chemical risk analysis by industry, the New Energy and Industrial Technology Development Organisation (NEDO), an R&D management organisation supervised by METI, started strategic R&D of chemical risk analysis technologies in 2000.

The chemical risk analysis technologies under development include high-throughput (HTP) assay systems as alternatives to animal testing. This R&D targets carcinogenicity, teratogenicity and immunotoxicity tests due to the technological difficulty involved and the long development period required. A cell transformation assay (CTA) using Bhas 42 cells has been being developed to detect both initiators and promoters among nongenotoxic carcinogens. HTP assay systems for teratogenicity and immunotoxicity have also been being developed using a multicolour luciferase reporter gene assay method. They are simple and mechanism-based alternative assay systems.

In addition, R&D of a QSAR *in silico* system and gene expression profile datasets, based on 28-day repeat dose oral toxicity studies, has progressed through NEDO projects.

This brochure outlines NEDO's R&D on chemical risk analysis technologies, including the project organisation for R&D on *in vitro* and *in silico* alternatives.

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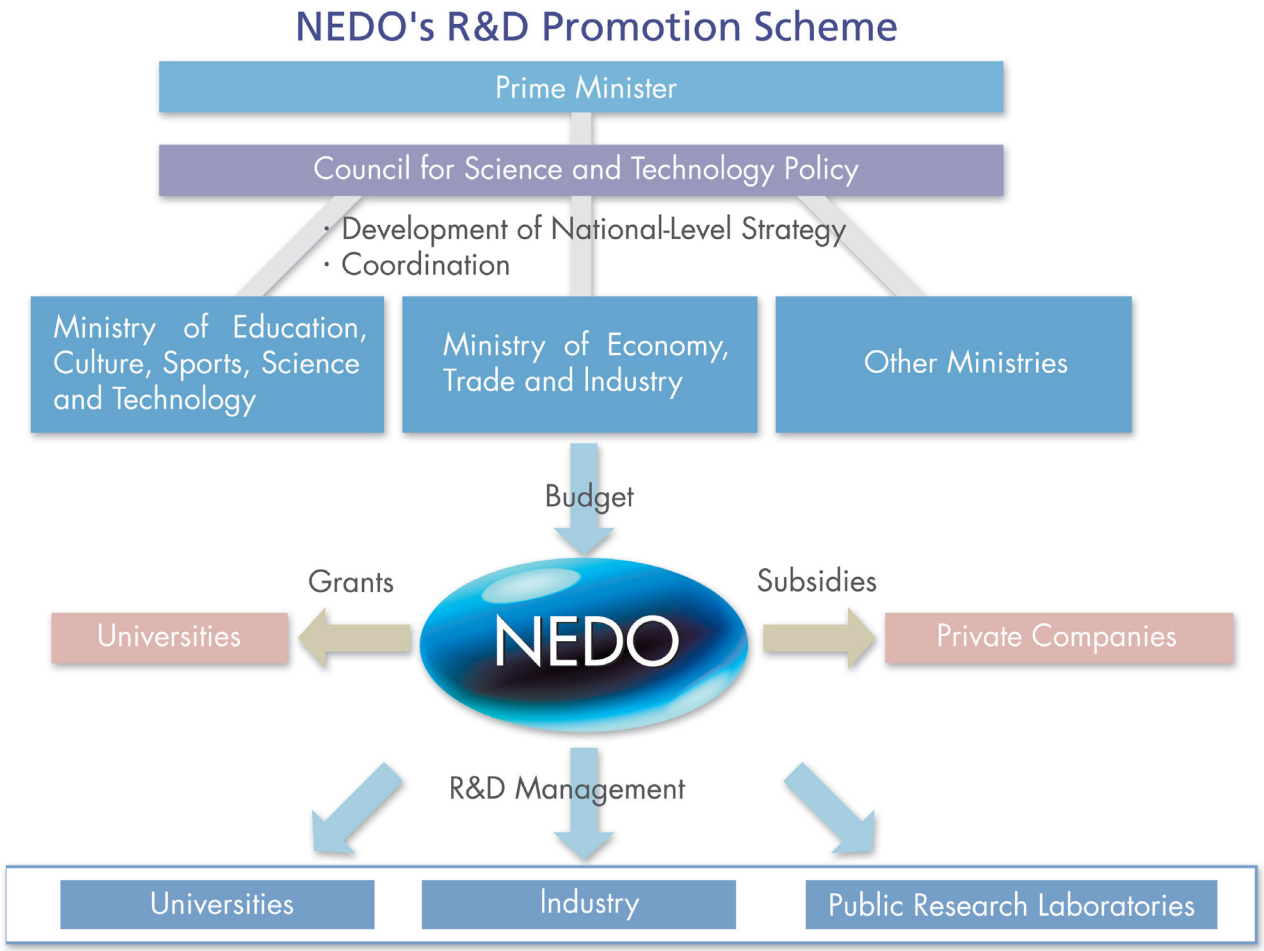
WHAT IS NEDO?

The New Energy and Industrial Technology Development Organisation (NEDO) is an incorporated administrative agency, under the Ministry of Economy, Trade and Industry of Japan.



NEDO's primary activities are:

1) Development of industrial technology;

2) Development and promotion of new energy and energy conservation technologies; and

3) Acquisition of emission reduction credits through the Kyoto Mechanisms.

For the development of industrial technology, NEDO promotes private sector participation in national technology development projects, supports the private sector in pursuing its own research and development efforts, and disseminates newly developed technologies.

By facilitating the practical application and commercialisation of advanced new technologies, NEDO endeavours to ensure a stable and efficient supply of energy under fluctuating domestic and international socio-economic conditions and to assist in the development of Japan's economy and industry.

At the same time, NEDO strives to contribute to the fulfilment of Japan's Kyoto Protocol commitment without overly restricting energy use and industrial activities in Japan through, for example, obtaining emission credits via the Kyoto Mechanisms.

For further information, please visit NEDO's Website at http://www.nedo.go.jp/english/.

Strategic R&D of Chemical Risk Analysis Technologies by NEDO

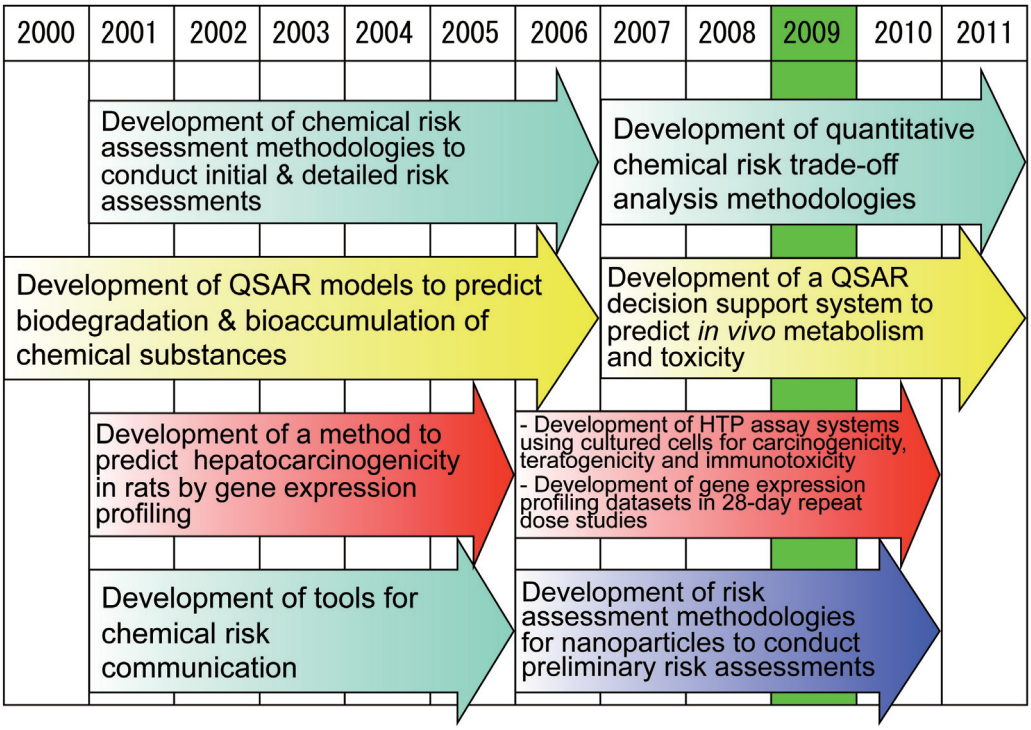
In conjunction with the following international movements in the field of chemical management policy, the NEDO has been promoting R&D of chemical risk analysis technologies as shown in the figure below:

1) Risk-based management is replacing hazard-based management;

2) Companies are replacing governments as the major players in collecting hazard data;

3) In animal testing for collecting hazard data, the 3Rs of animal welfare (Reduction, Refinement and Replacement) are carefully observed; and

4) Risk analysis is deemed to be the foundation for prosperous nanotechnology industry development.



The OECD Environment, Health and Safety (EHS) Programme is the global centre of chemical risk analysis. Information regarding this programme is available at www.oecd.org/ehs, and a May 2009 brochure can be downloaded at http://www.oecd.org/dataoecd/18/0/1900785.pdf (2.4MB).

The above NEDO projects are fully contributing to the EHS Programme activities.

- To propose new or supplimental Emission Scenario Documents (visit http://www.oecd.org/document/46/0,3343,en_2649_34373_2412462_1_1_1_1,00.html)
- To provide data and methodologies to the QSAR Application Toolbox, Phase II (visit www.oecd.org/env/existingchemicals/qsar)
- To propose new Test Guidelines for non-animal testing (visit http://www.oecd.org/document/48/0,3343,en_2649_34377_40695856_1_1_1_1,00.html)
- As a sponsor of Sponsorship Programme, taking tasks of testing C60, SWCNT and MWCNT (visit www.oecd.org/env/nanosafety)

1. Toxicogenomics

Project Name: Development of Simple and Highly Functional Hazard Assessment Methods

1.1 In Vitro Alternatives to Animal Testing - HTP Assay Systems Using Cultured Cells

R&D Theme: Development of Assessment Methods Using Cultured Cells

1.1.1 Project Overview

In 2006, NEDO initiated R&D to establish alternative, non-animal test systems to supplement and/or to replace animal toxicity tests. The R&D theme has focused on carcinogenicity, reproductive toxicity, and immunotoxicity to screen a large number of existing chemicals.

The R&D period is five years from FY2006 to FY2011.

Final Goal of the R&D

The final goal of the R&D is to establish cell-based high throughput (HTP) screening systems to detect chemicals having toxicity such as carcinogenicity, reproductive toxicity and immunotoxicity. The systems are expected to be internationally validated and then be used as international test guidelines, namely OECD test guidelines (see the figure below).

Carcinogenicity

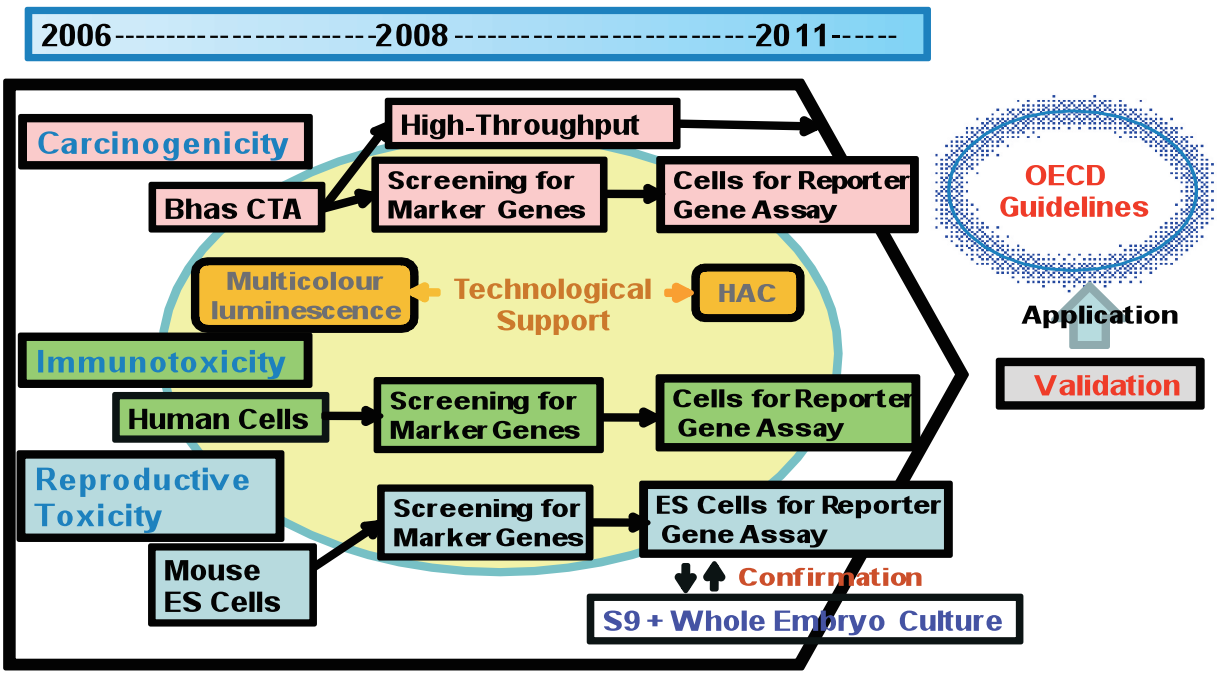
High-sensitivity transformation assay that detects both initiators and promoters using Bhas 42 cells

Reproductive toxicity

High-throughput reporter gene assay involved in target genes regulating reproductive and embryo toxicity using murine embryonic stem cells

Immunotoxicity

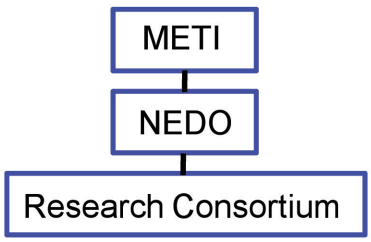
High-throughput reporter gene assay involved in target genes for immunotoxicity using human immune cells such as dendritic cells, keratinocytes and T-cells



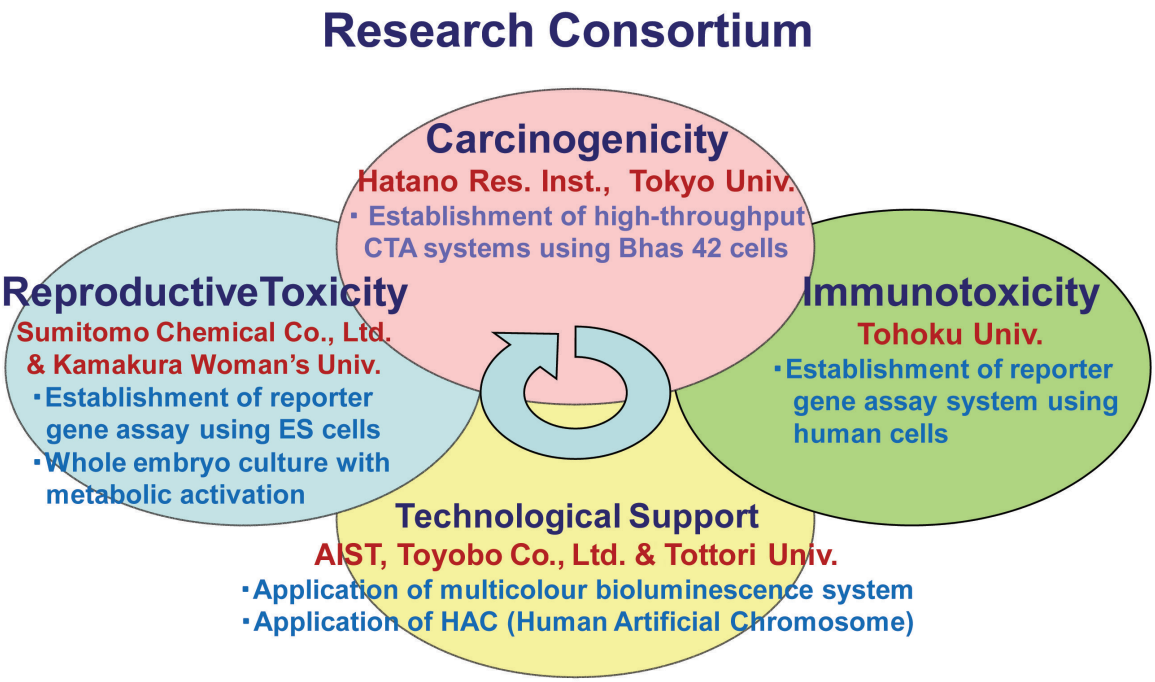
HAC: Human Artificial Chromosome

Participating Institutions

Two public institutes and six institutions from academia and industry (see the figure below) are participating in the R&D. Three main toxicity groups are supported by a technological group.

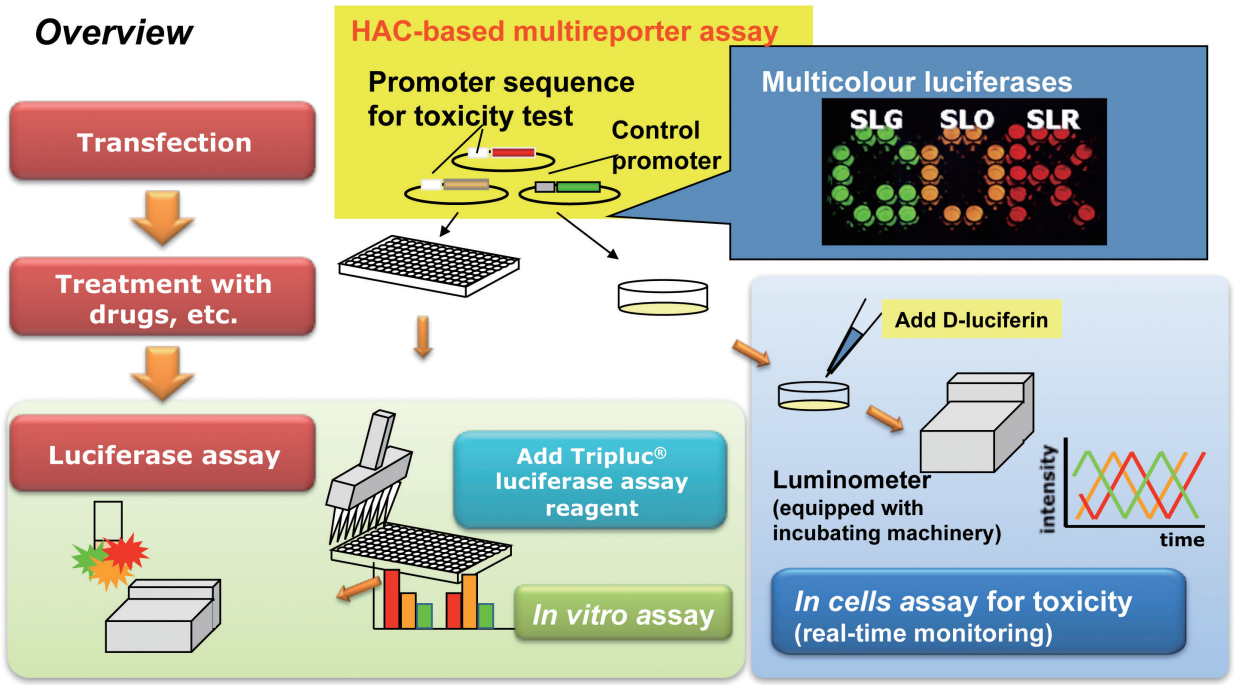


Project Leader: Dr. N. Tanaka
Chief for Carcinogenicity: Dr. K. Sasaki
Chief for Immunotoxicity: Dr. S. Aiba
Chief for Repro. Toxicity: Dr. K. Saito
Chief for Technological Support: Dr. Y. Ohmiya



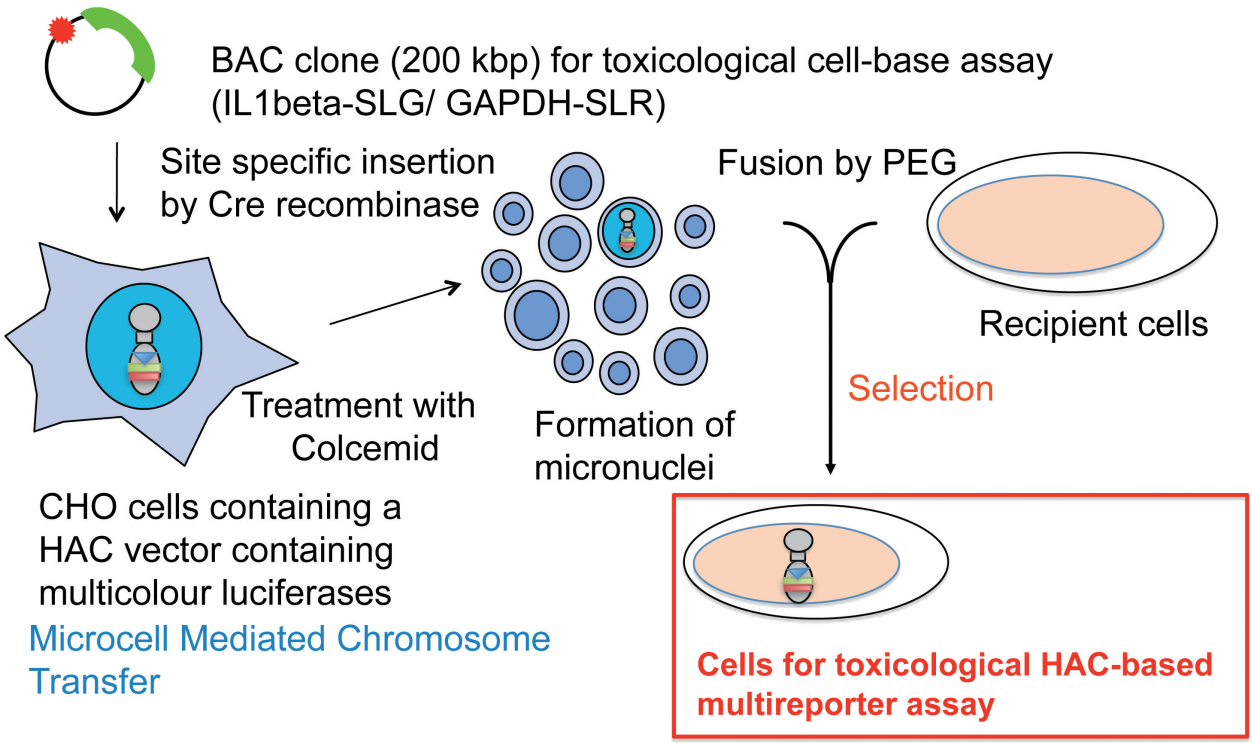
1.1.2 Multireporter Assay System and HAC System

New toxicity test methods based on gene expression analysis using cultured cells consist of multireporter assay and HAC systems.



HAC-based Multirepoter Assay Cells - R&D by AIST, Toyobo Co., Ltd. and Tottori University

Toxicity test of cultured cells using HAC and multicolour luciferases

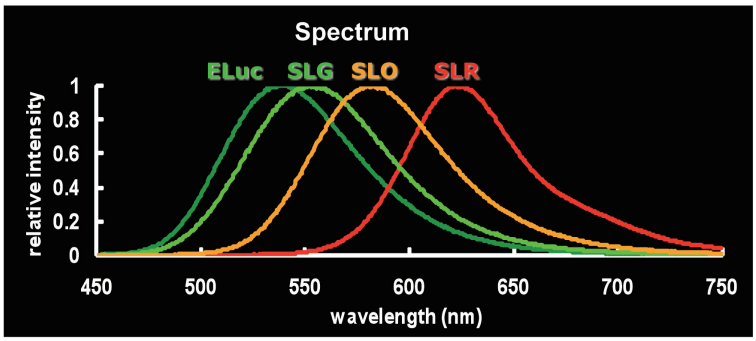


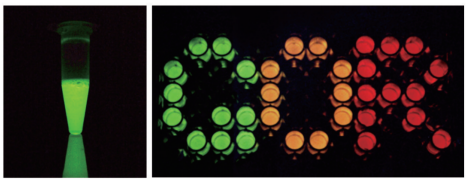
1) Overview of Multireporter Assay System - R&D by AIST and Toyobo Co., Ltd.

Luciferase reporter	Gene symbol	λmax	Origin	Amino acid sequence
Emerald Luc	ELuc	538 nm	Pyrearinus termitilluminans	Wild type
Green-emitting luciferase	SLG	550 nm	Ragophthalmus ohbai	Wild type
Orange-emitting luciferase	SLO	580 nm	Ragophthalmus ohbai	Mutant
Red-emitting luciferase	SLR	630 nm	Phrixothrix hirtus	Wild type

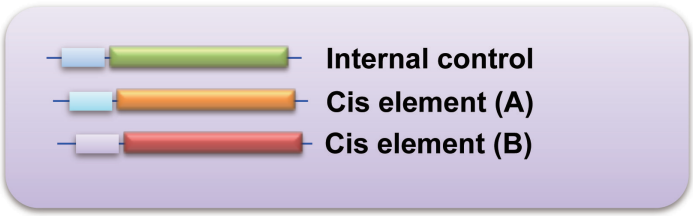
Single high-intensity reporter system
Emerald Luc System

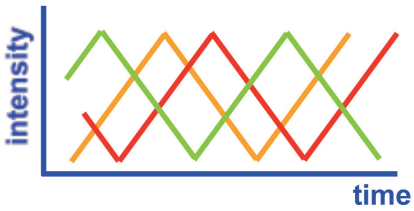
Tri-coloured reporter system
Multireporter Assay System Tripluc®



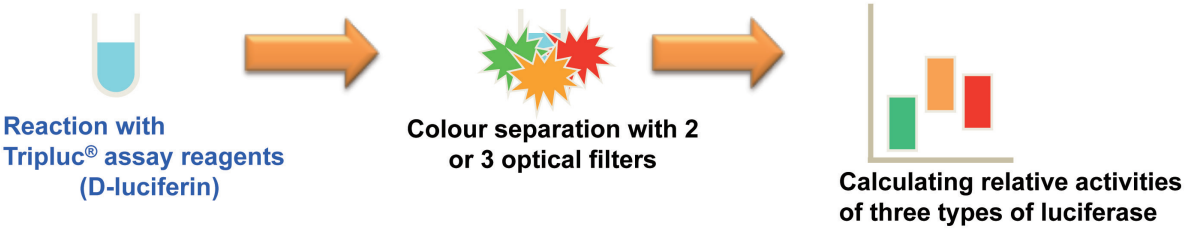


Merit 1 Simultaneous monitoring of gene expressions of two or three targets



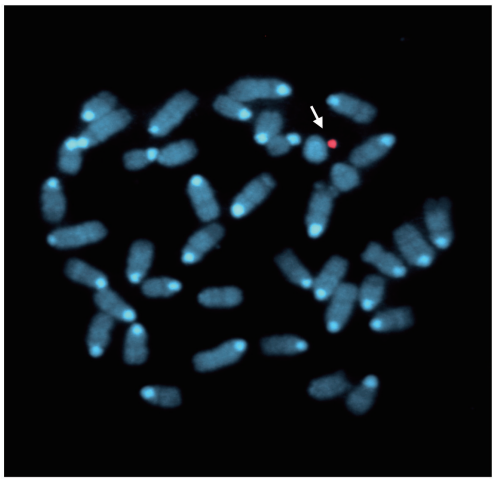


Merit 2 Luciferase assay with single substrate (single step)



2) Overview of HAC System - R&D by Tottori University

Advantages of a human artificial chromosome (HAC) vector



Metaphase chromosomes of the mouse ES cell carrying a HAC vector derived from human chromosome 21 (arrow)

It can replicate and segregate like an endogenous chromosome in host cells.

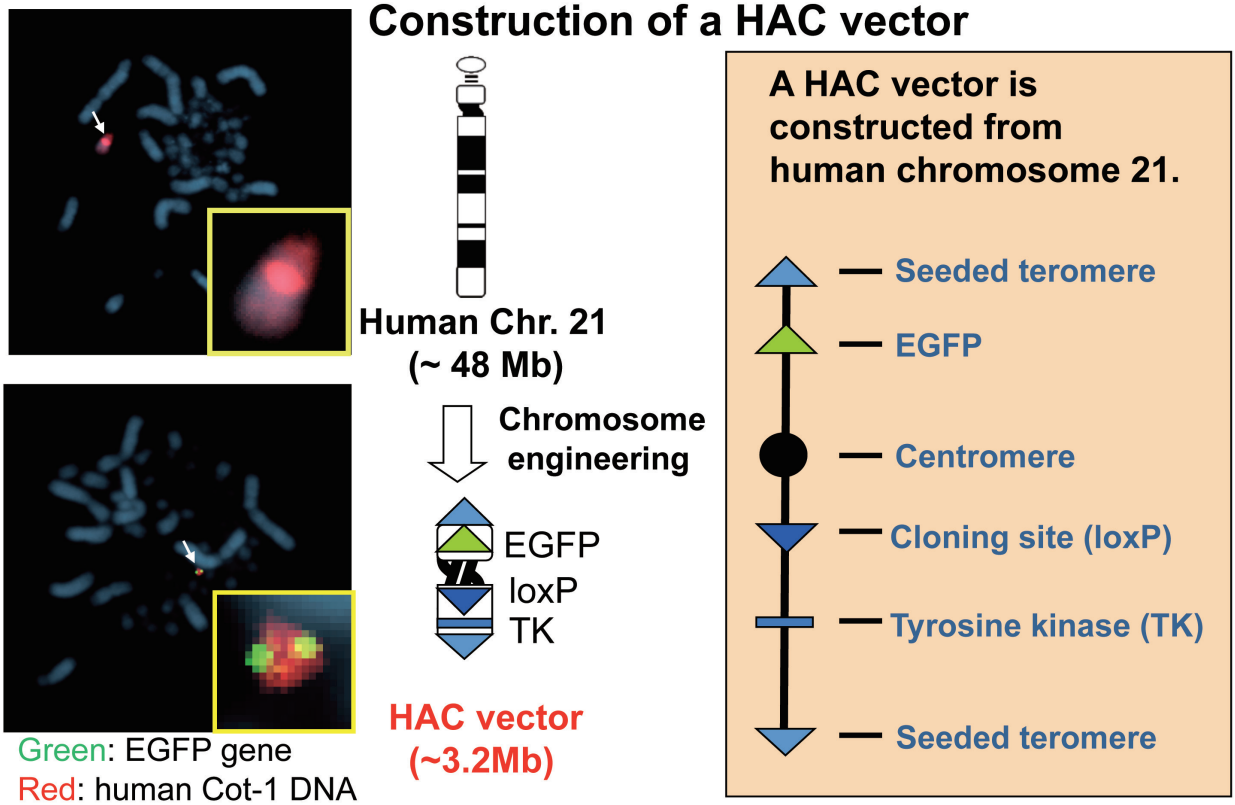
- Transgenes on HACs are expressed under physiological regulation in host cells.

It can avoid problems from random integration of transgenes into a host genome.

- Transgenes can evade silencing by the chromosomal position effect.

It can deliver large size DNA segments.

- Gene loci including transcriptional regulatory elements are transferrable.



1.1.3 HTP Assays for Carcinogenicity

Back ground

A multistage process is involved in carcinogenesis, but conventional classification of carcinogens as initiators and promoters is practical and beneficial for the screening of carcinogenic chemicals. Most initiators can be detected by various genotoxicity tests, the results of which are used for carcinogenicity prediction and regulatory purposes by governmental authorities. In the case of tumour promoters, several methods have been proposed, but none have been routinely applied for regulatory purposes.

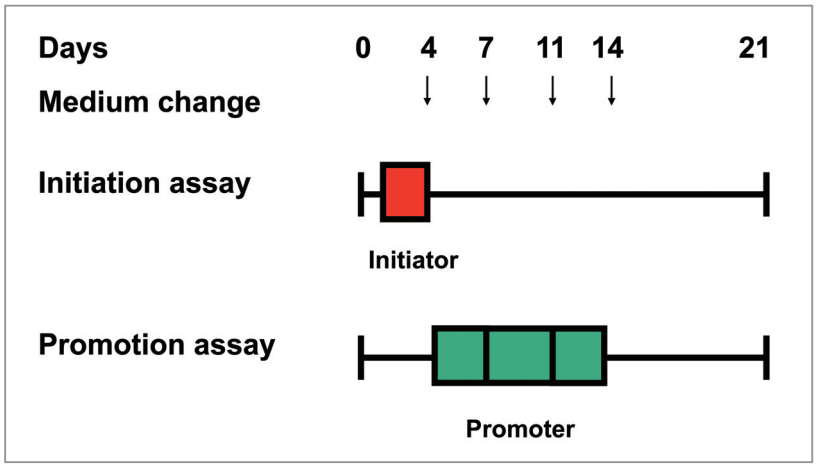
Cell transformation assays (CTA) using BALB/c 3T3 cells and C3H10T1/2 cells can stimulate the process of two-stage animal carcinogenesis. Formation of transformed foci is the consequence of the complex process of transforming cells to a malignant state.

Since CTA can detect both initiating and promoting activities, its inclusion as a screening tool is anticipated, but it has not yet been approved as a routine screening method because of the laborious and time-consuming procedure compared to routine genotoxicity assays.

In NEDO's project, a sensitive short-term CTA has been developed using Bhas 42 cells established from BALB/c 3T3 cells transfected with v-Ha-ras gene. The Bhas 42 CTA system can sensitively examine many more chemicals, including non-genotoxic carcinogens in a short period of time.

Aim

The aim of this R&D is to establish a short-term HTP assay system for carcinogens and to propose an international test guideline after evaluation by means of validation studies.

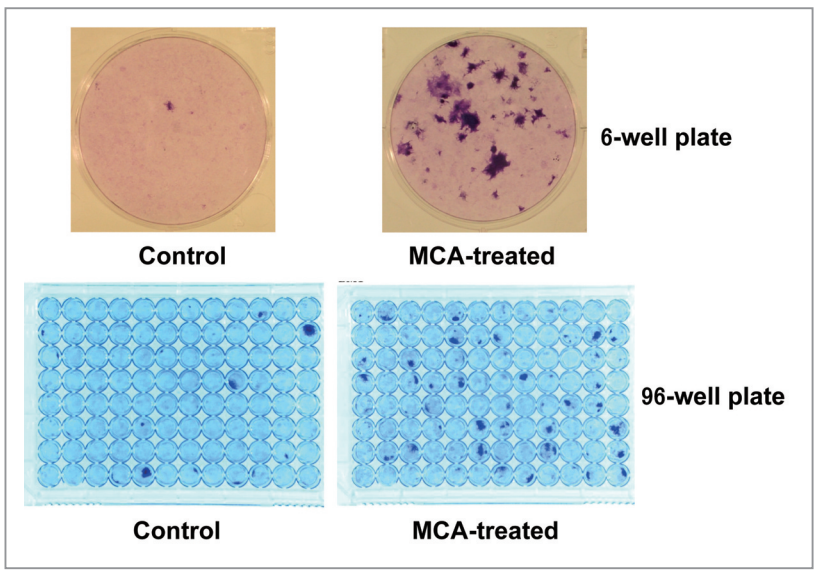


Method

Protocol of Bhas 42 CTA

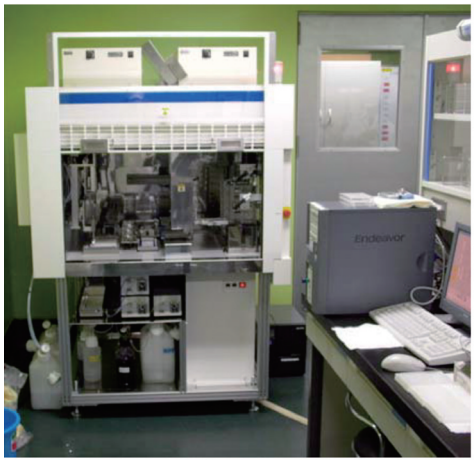
Progress

3-Methylcholanthrene induced transformed foci



Progress

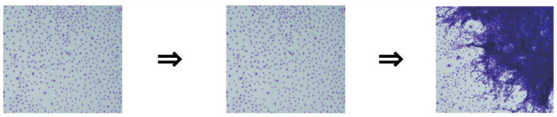
Robotic high-throughput screening system



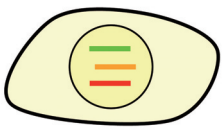
System overview

96-well plates on racks in incubator

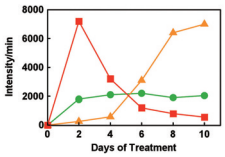
Cells	Before treatment with chemicals	In treatment with chemicals	Appearance of transformed cells
Morphology	Normal	Normal	Transformed
Transformation-related genes	−	+	+



Three-colour luciferases transfected cells



Conceptual figure



Progress

Visualisation of early stage in transformation

Future Plan

1. Propose an international test guideline for the Bhas 42 CTA system
   1) Compare results of Bhas 42 CTA with existing *in vivo* data.
   2) Proceed international validation studies using the 96-well plate method
2. Establish a high-throughput assay
   1) Develop a robotic system
   2) Exploit a luciferase reporter gene method using marker genes involved in cell transformation

Publications

1. Muramatsu D., Sasaki K, Kuroda S., Hayashi K., Tanaka N., Sakai A. Comparison of sensitivity to arsenic compounds between a Bhas42 cell transformation assay and a BALB/c 3T3 cell transformation assay. Mutat Res. 2009;675:66-70.
   ---> http://linkinghub.elsevier.com/retrieve/pii/S1383571809000527
2. Sakai A. BALB/c 3T3 cell transformation assays for the assessment of chemical carcinogenicity. AATEX. 2008;14, Special Issue:367-373.
   ---> http://altweb.jhsph.edu/wc6/paper367.pdf (0.7MB)
3. Sakai A., Suzuki C., Masui Y., Kuramashi A., Takatori K., Tanaka N. The activities of mycotoxins derived from Fusarium and related substances in a short-term transformation assay using v-Ha-ras-transfected BALB/3T3 cells (Bhas 42 cells). Mutat Res. 2007;630:103-111.
   ---> http://linkinghub.elsevier.com/retrieve/pii/S1383571807000939

1.1.4 HTP Assays for Reproductive and Developmental Toxicity

The aim of this R&D is to establish novel short-term HTP assay systems for reproductive and developmental toxicity using murine embryonic stem cells (ES cells) and whole embryo culture (WEC).

1) ES cell study

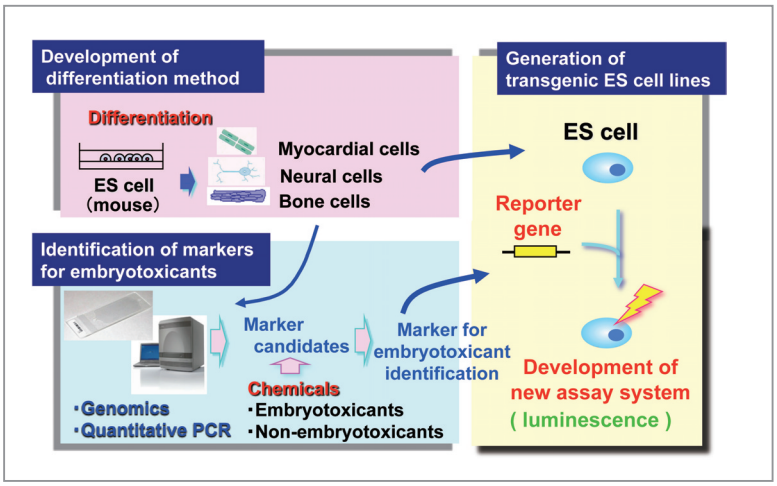
Back ground

The embryonic stem cell test (EST) has been proposed as an *in vitro* assay for embryotoxicants and their classification into three different classes of *in vivo* embryotoxicity (H. Spielmann et al., 1997). The EST was designed to predict embryotoxicity based on the inhibition of the differentiation of embryonic stem cells (ES cells) into pulsating cardiomyocytes in combination with cytotoxicity data in monolayer ES cell cultures and 3T3 cells.

The tests are reliable and transferable, however, some problems have been reported (S. Bremer et al., 2004).

Aim

The aim of this R&D is to establish novel short-term HTP assay systems for reproductive and developmental toxicity using murine ES cells.



Progress

An overview of this R&D is given below.

- Development of differentiation methods
- Identification of markers for embryotoxicants
- Generation of transgenic ES cell lines
- Development of a new assay system

In DNA microarray analysis of ES cells during differentiation into cardiomyocytes, genes which were substantially up-regulated during the differentiation process were isolated as candidate marker genes. Thirteen genes showing remarkable expressions have been identified through the comparison between embryotoxicants and non-embryotoxicants treated groups, suggesting that these genes be useful markers for predicting embryotoxicity. Stable transgenic ES cells have been developed to detect chemical-dependent changes in candidate genes easily and conveniently.

Future plan

Several embryotoxic and non-embryotoxic chemicals used in international validation studies of the original EST will be evaluated, and the usefulness of developed transgenic ES cells will be validated.

Related Publications

Japanese Patent: 2008-145433A

Japanese Patent: 2009-125077A

2) WEC study

Back ground

The European Centre for the Validation of Alternative Methods (ECVAM) in the EU currently appears to be at the forefront of the development of alternative methods for reproductive and developmental toxicity tests. Why is it difficult to develop alternative methods for developmental toxicity tests in comparison with other toxicity tests? In developmental toxicity tests, chemical substances first enter the bloodstream and then reach the placenta via the metabolism in the liver and other organs. After further metabolism in the placenta, chemical substances finally reach the fetus where they affect fetal development. The difference in the *in vivo* route of chemical substances is an important reason for the difficulty in establishing new methods for developmental toxicological tests compared to general toxicity tests.

According to the EU, the use of *in silico* techniques for developmental toxicity tests may be difficult. The *in silico* technique is basically a method for predicting toxicological effects from existing data, and it cannot predict new effects because data obtained by developmental toxicological tests are too complex.

Three techniques are now being examined to overcome the difficulty in changing the method for developmental toxicological tests. They include the embryonic stem cell test (EST), the micromass (MM) culture technique and the whole embryonic culture (WEC) technique.

Aim

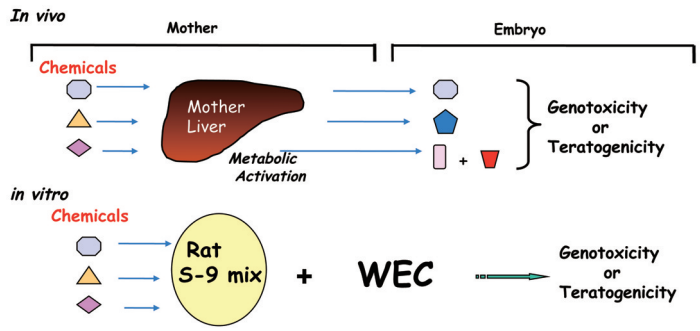
The aim of this R&D is to establish a novel HTP assay system for developmental toxicity, with two focus areas shown on the right.

Progress

Development of a novel WEC method has been attempted with metabolic activation of chemicals using rat S-9 mix. The effects of ten chemicals on cultured embryos were examined with and without S-9 mix. The S-9 mix addition enhanced the test chemical-dependent adverse effects on the cultured embryos. This data suggests that the novel WEC method with a metabolic system may be a better test method than existing ones for detecting the possibility of embryo abnormality caused by a test chemical and its metabolites. New equipment has also been developed to reduce serum volume in the WEC method.

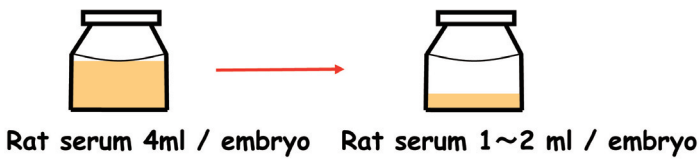
No. 1

Incorporate maternal metabolic viability into WEC to make the WEC technique more similar to the *in vivo* state



No. 2

Minimise the amount of rat serum used as a culture (WEC) medium



Future plan

An examination of the novel WEC (alternative methods) for reproductive and developmental toxicity tests will be carried out, and international validation studies of the novel WEC for reproductive and developmental toxicity tests will be conducted.

1.1.5 HTP Assays for Immunotoxicity

Background for immunotoxicity

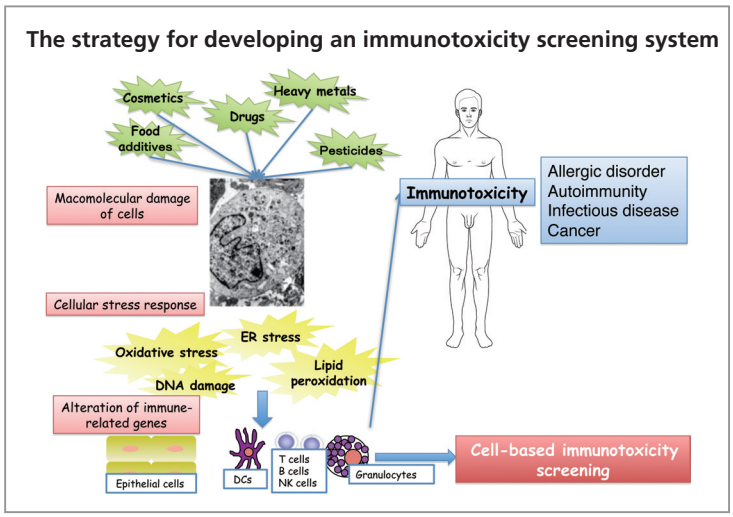
Immunotoxicity is defined as the adverse effects on the normal functioning of the immune system that result from exposure to chemical substances. An altered immune function may lead to increased incidence or severity of allergic disorders, autoimmunity, infectious diseases or cancer. Observations in humans and studies in rodents have clearly demonstrated that a number of environmental and industrial chemicals can adversely affect the immune system. Identifying immunotoxicants is difficult because chemicals can cause a wide variety of complicated effects on the immune function.

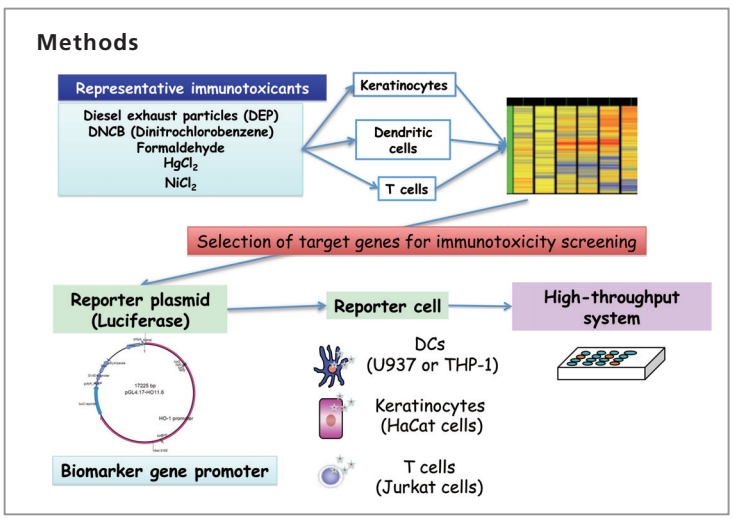
To develop a new method for immunotoxicity screening, the following was hypothesised:

1) Although each chemical has its own mechanism to assault cells, chemicals that inflict damage on the macromolecules of cells induce a defence reaction from them, which is called a cellular stress response (Kultz D, 2005). Many aspects of the cellular stress response are not stressor specific because cells monitor stress based on macromolecular damage without regard to the type of stress that causes such damage. For example, cellular mechanisms activated by DNA damage and protein damage are interconnected and share common elements.
2) The cellular stress response triggers a limited number of signal transduction pathways, which are tightly connected with the expression of immune-related genes.
3) Consequently, it is assumed that chemicals share a mechanism that regulates the expression of several immune-related genes.

Aim

The aim of this R&D is to establish a novel short-term HTP assay system for immunotoxicity using human immune cells.





Progress (1)
Biomarkers for immunotoxicity

	5 chemicals	4 chemicals	3 chemicals	2 chemicals
Dendritic cells	IL-8	C5AR1	CCL3	CCL15, 20
	PBEF1	CXCL3	CXCL2	CD55
		INHBA	HMOX1	CXCL10
		ZEB1	GADD45A	FOSL1
			HSPA4L	GADD45B
			IL-1β	SPA1A, 1B1, 1B, CB, D1, E1, H1
			IRAK2	IL-1α
			IRF1	LY9
			TNF	SLAMF7
			TNAIP2	S100A9
			TNFSP18	SOD2
				THBS1
				TREM1
Epidermal cells			IL-8	GAAD45A
			HMOX1	HSPA6
			HSP1A	IL-1b
				IL-6
				PBEF1
				TNF-a
T cells			CXCL3	FAIM3
Augmented			GADD45A	HMOX1
				IL-7R
				TXNIP
T cells	IFN-γ	IL-12b	CCL17	CCR1
Suppressed	IL-4		CCL22	CD80
			IL-5	IL-10, 22
			IL-9	CTLA4
			LIF	CXCL10, 11
				INDO
				IRF8
				KLRC4
				TBX21

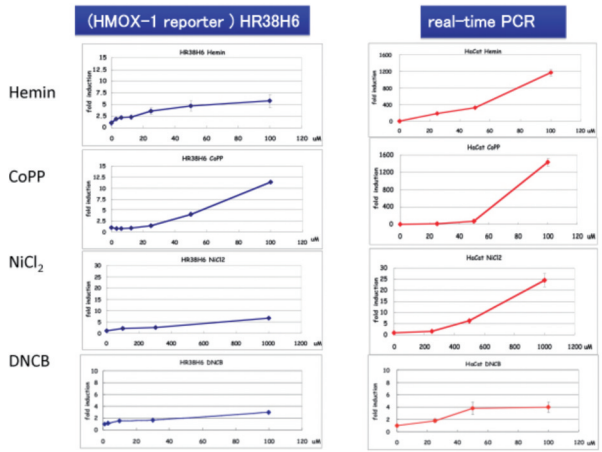
Biomarkers for immunotoxicity in dendritic cells, epidermal cells and T-cells have been determined.

Progress (2)
Reporter cells

The following cell lines have been established:

1. HMOX-1 reporter cell lines derived from HaCat cells and U937.
2. IL-1b reporter cell line derived from U937.
3. IFN-g reporter cell line derived from Jurkat cells.
4. IL-4 reporter cell line derived from Jurkat cells.

Correlation between hemeoxygenase-1 (HMOX-1) reporter assay and its mRNA expression



Future plan

Increase reporter cell lines

Construct an immunotoxicity screening platform

Conduct intra-and inter-laboratory validation studies

Publications

1. Ohtani T., Mizuashi M., Nakagawa S., Sasaki Y., Fujimura T., Okuyama R. et al. TGF-beta1 dampens the susceptibility of dendritic cells to environmental stimulation, leading to the requirement for danger signals for activation. Immunology 2009;126:485-499. ---> http://www3.interscience.wiley.com/journal/122223975/abstract
2. Ohtani T., Memezawa A., Okuyama R., Sayo T., Sugiyama Y., Inoue S. et al. Increased hyaluronan production and decreased E-cadherin expression by cytokine-stimulated keratinocytes lead to spongiosis formation. J Invest Dermatol 2009;129:1412-1420. ---> http://www.nature.com/jid/journal/v129/n6/abs/jid2008394a.html
3. Numata I., Okuyama R., Memezawa A., Ito Y., Takeda K., Furuyama K. et al. Functional Expression of Heme Oxygenase-1 in Human Differentiated Epidermis and Its Regulation by Cytokines. J Invest Dermatol 2009. ---> http://www.nature.com/jid/journal/vaop/ncurrent/abs/jid2009119a.html
4. Kagatani S., Sasaki Y., Hirota M., Mizuashi M., Suzuki M., Ohtani T. et al. Oxidation of cell surface thiol groups by contact sensitizers triggers the maturation of dendritic cells. Journal of Investigative Dermatology 2009. ---> http://www.ncbi.nlm.nih.gov/pubmed/19641517

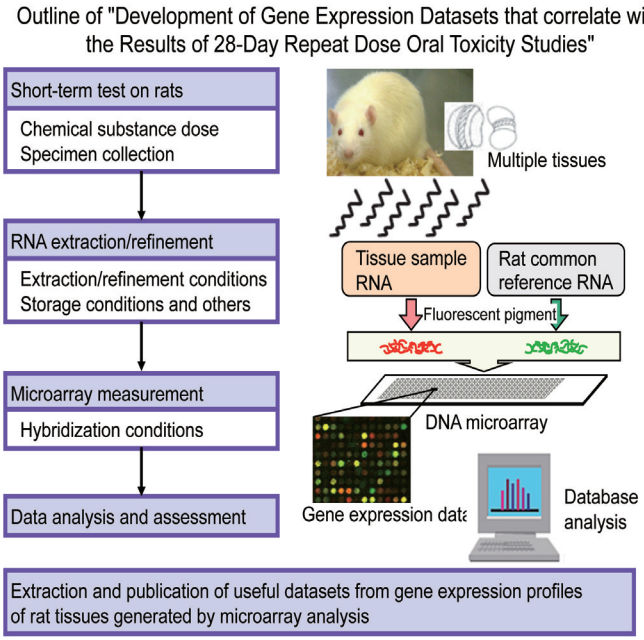
1.2 Gene Expression Profile Datasets

R&D Theme: Development of Gene Expression Datasets that Correlate with the Results of 28-day Repeat Dose Toxicity Studies

Project Leader: Professor Shinya Watanabe, Fukushima Medical University

R&D Period: FY2007-2010

Toxicology principally investigates the influence of chemical substances on living organisms by use of biological indicators detected by experimental methods including biochemical, immunological, and pathological approaches that require method-specific multiple platforms. In contrast, recently developed genomics enables the employment of thousands of genes as parameters to assess diverse biological phenomena on a single platform such as gene expression profiling. Thus, the integration of genomics into toxicology should exploit novel fields for the biological assessment of substances, describing alterations after exposure of substances to animals or cultured cells with multiple parameters in a single platform across diverse specimens. In this R&D, as a single experimental cycle, five chemical substances are independently administered repeatedly to male rats for 28 days, multiple tissue samples from each animal are prepared, and gene expression levels in the multiple tissues are comprehensively investigated with DNA microarrays containing probes representing approximately 11,000 species of rat transcripts. This single experimental cycle is going to be conducted nine times by the end of fiscal year 2010. It is expected that data obtained from this R&D may contribute to the establishment of novel accurate approaches for the assessment of current and future chemical substances through comparison with previously accumulated findings obtained by repeated dose 28-day oral administration to rats.



Published datasets can be accessed through CIBEX of the DNA Data Bank of Japan (http://cibex.nig.ac.jp/). The first dataset for five chemical substances (2-Butanone Oxime, m-Xylylenediamine, 3-Cyanopyridine, 2-(2-Aminoethylamino) Ethanol, and Tetrahydrofurfuryl Alcohol) was published on 15 July 2009 as CBX93 (see: http://cibex.nig.ac.jp/cibex2/ExperimentMiame.do?queryExperimentalDesignAccession=CBX93).

2. In Silico Alternatives to Animal Testing - QSAR Decision Support System

Based on the category approach for repeated dose toxicity

Project Name: Development of Hazard Assessment Techniques Using Structure-Activity Relationship Methods

Project Leader: Dr Makoto Hayashi, Biosafety Research Centre, Foods, Drugs and Pesticides

Project Period: FY2007- FY2011; FY2009 Project Budget: 170 million yen

In order to evaluate repeated dose toxicity (RDT) of chemical substances without depending on animal tests, it is necessary for experts to make a comprehensive judgment based on actual test data for similar chemical substances, known action mechanisms and metabolic behaviour in the body, and data on the physicochemical properties of similar chemical substances, where expert decisions can be supported by knowledge information databases that are comprehensive compilations of high-quality RDT study reports and information about mechanisms of toxic action and metabolism.

This project aims to construct a comprehensive platform for a hazard assessment support system focused on RDT, based on relevant known information concerning toxicity in humans as well as new knowledge derived from such information.

1) Development of a toxicity knowledge information database

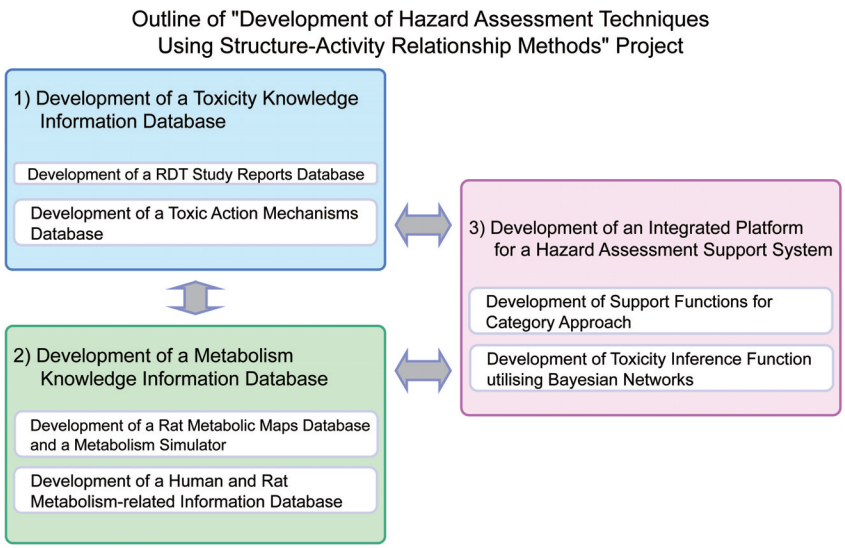
Data from high-quality RDT study reports and information on mechanisms of toxic action will be collected, analysed and systematised, and a toxicity knowledge information database with search functions linking to chemical structures will be developed.

2) Development of a metabolism knowledge information database

A metabolism knowledge information database will be developed to compile existing information on metabolism reaction in rat livers. It will include models for extrapolating metabolites and metabolic pathways in rat livers for any chemical structure.

3) Development of an integrated platform for a hazard assessment support system

An integrated platform for a hazard assessment support system will be developed to facilitate the efficient acquisition of various information required for expert decision making on hazard assessment. The platform will be linked, through categories of toxicology pathway, to the aforementioned toxicity and metabolism information databases.



3. Risk Trade-off Analysis

- Methodology and tools for companies considering replacing listed chemical substances (PRTR required) with something not listed

Project Name: Development of Methodologies for Risk Trade-off Analysis towards Optimum Chemical Substance Management

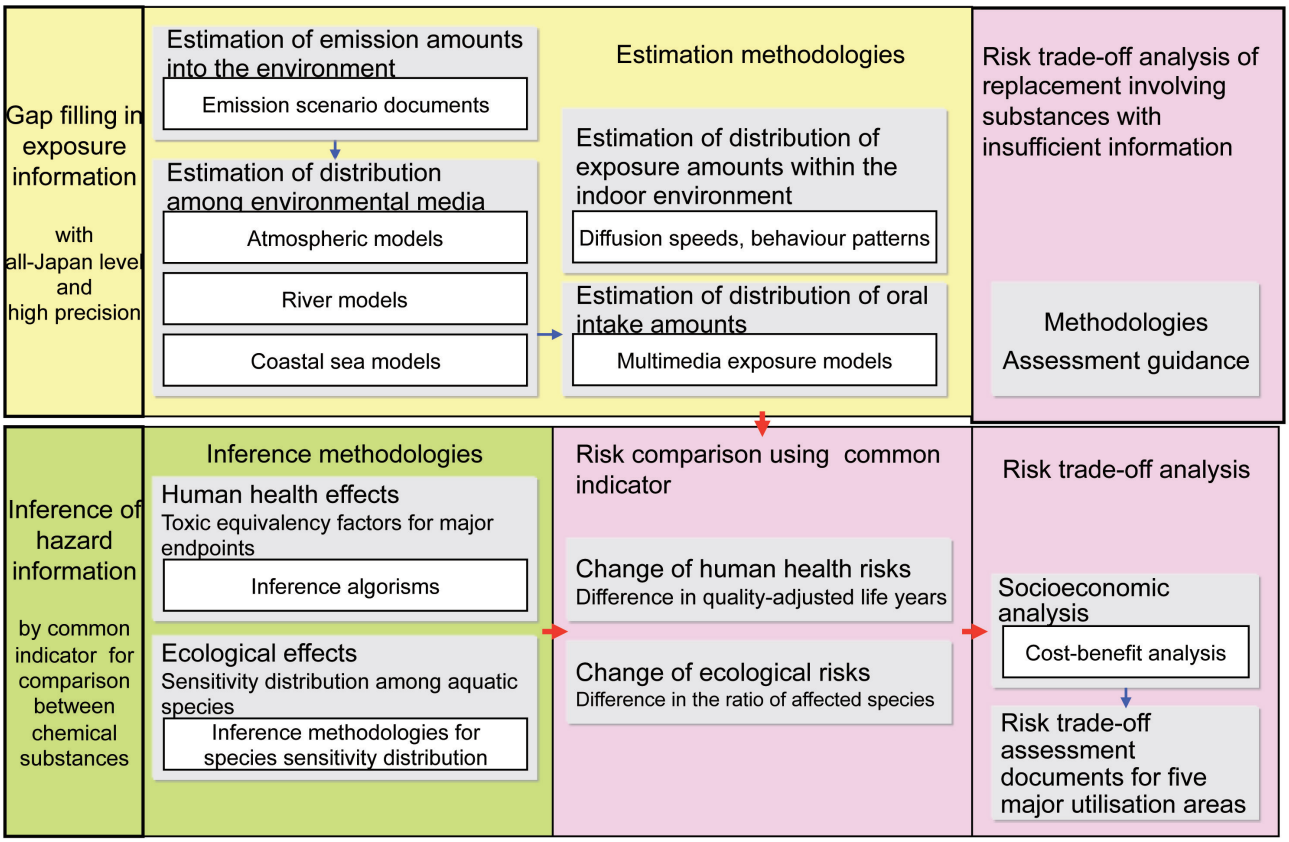
Project Leader: Dr Kikuo Yoshida, National Institute of Advanced Industrial Science and Technology (AIST)

Project Period: FY2007 - FY2011; FY2009 Project Budget: 106 million yen

Chemical substance management policy around the world is shifting from "hazard-based" towards "risk-based". Risk-based management involves not only seeking to keep risks of conventional substances within an acceptable range whilst making maximum use of their benefits, but also seeking low-risk alternative substances. In the latter case however, if an alternative substance is selected without appropriate risk assessment, a higher or different risk may emerge and counter the risk reduction effect of the replacement, which is the issue of risk trade-off.

With regard to current risk assessment methodologies, there may be some difficulties such as insufficiency of exposure information and/or hazard information for various chemical substances. This project aims to develop risk trade-off analysis methodologies and tools that businesses and governments can utilise for highly precise quantitative risk analysis of chemical substances in five major utilisation areas: industrial cleaning agents, plastic additives, solvents, metals in electronics equipment, and household goods.

Outline of "Development of Methodologies for Risk Trade-off Analysis" Project



4. Nano-Safety

- From characterisation methodologies to risk assessment methodologies

Project Name: Research and Development of Nanoparticle Characterisation Methods

Project Leader: Dr Junko Nakanishi, National Institute of Advanced Industrial Science and Technology (AIST)

Project Period: FY2006 - FY2010 ; FY2009 Project Budget: 400 million yen

Manufactured nanoparticles such as fullerenes, carbon nanotubes and titanium dioxide are considered revolutionary new materials. Even with the same chemical composition, manufactured nanoparticles can show considerably different physicochemical characteristics depending on their structures. Because of this, conventional risk-analysis methods that have been commonly used for typical chemical substances may not be directly applied to manufactured nanoparticles. Also, as nanotechnology itself is an advanced yet still developing technology field, characterisation techniques have yet to be sufficiently established for manufactured nanoparticles.

This project, therefore, focused first on the collection and compilation of relevant findings on the potential effects of manufactured nanoparticles of concern that may impact human health and/or the environment. The primary purpose of the project is to develop the following methods applicable to manufactured nanoparticles:

1) Characterisation methods to derive physicochemical characteristics required for risk assessment

2) Exposure assessment methods including analysis techniques to identify environmental concentrations, environmental emission sources, and fates and behaviours in the environment

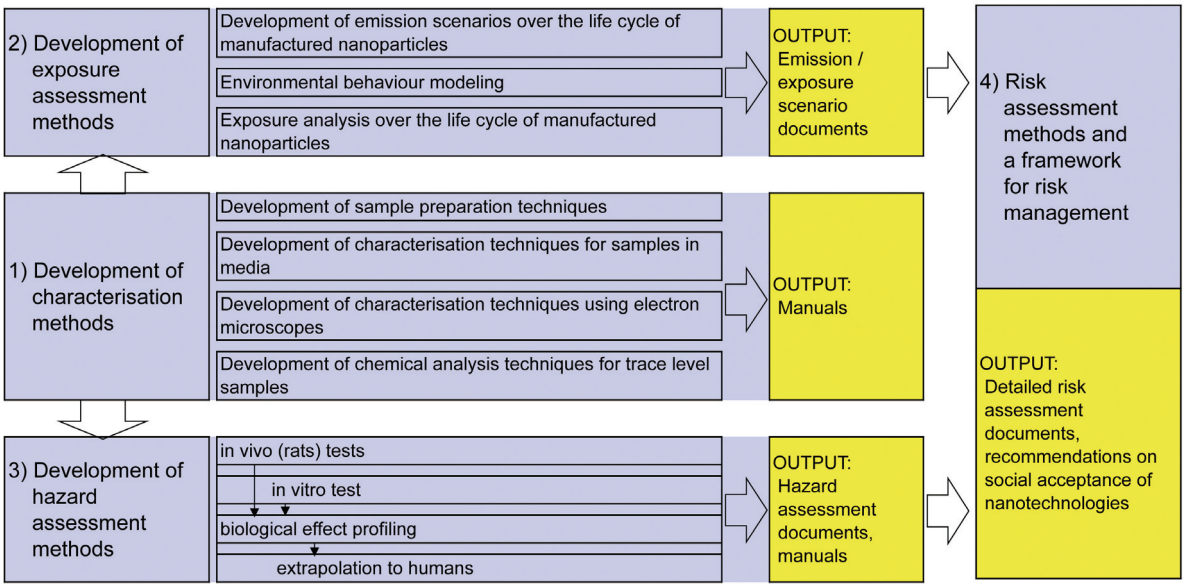
3) Basic hazard assessment methods

4) Risk assessment methods and a framework for risk management

For further information, please download the separate brochure published in March 2008: http://www.nedo.go.jp/kankobutsu/pamphlets/bio/nanoryuushi2008e.pdf (0.7MB).

You may also wish to visit AIST's Web page for this project at http://www.aist-riss.jp/projects/nedo-nanorisk/index_e.html.

Outline of "R&D of Nanoparticle Characterisation Methods" Project



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Edition
1st Edition (Editor: Takuya Igarashi), published on 25 August 2009, in commemoration of the 7th World Congress on Alternatives & Animal Use in the Life Sciences held in Rome, Italy from 30 August to 3 September 2009.
Visit http://www.aimgroup.eu/2009/WC7/ for further information.

A low resolution version (1.2MB) of this e-brochure can be downloaded from NEDO's Website at http://www.nedo.go.jp/english/publications/brochures/pdf/chemrisk.pdf. The e-brochures will be updated from time to time.

Links to Relevant Sites

Japanese Center for the Validation of Alternative Methods
Relevant explanatory materials in English:
http://altweb.jhsph.edu/wc6/paper483.pdf (1.1MB)
http://wwwsoc.nii.ac.jp/jsaae/KOJIMA.pdf (1.6MB)
http://ntp.niehs.nih.gov/files/6b_Japan_SACATAM_200706.pdf (6.7MB)
http://ntp.niehs.nih.gov/files/2-06_SACATM_200806kojima_508.pdf (0.5MB)
http://ntp.niehs.nih.gov/Ntp/about_NTP/SACATM/2009/June/Presentations/JaCVAM_Update.pdf (0.4MB)

Japanese Society for Alternatives to Animal Experiments
http://www.asas.or.jp/jsaae/e_index.html