Current status of alternative study in NITR

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NITR
(National Institute of Toxicological Research)
A specialized national research institute responsible for conducting scientific research to secure the safety and efficacy of food & drugs.

The National Institute of Toxicological Research is a national organization for toxicological test and research. Under the Korea Food & Drug Administration, the Institute performs research on toxicology, pharmacology, and risk analysis of foods, drugs, and their additives.
Establish national research management system for toxic substances and strengthen research activities on them.

Establish the infra-structure of risk assessment on harmful substances in foods and drugs.

Strengthen research on safety and efficacy evaluation of drugs.

Promote specialization and globalization in the field of laboratory animal care.

Develop a specialized research institute with international cooperative networks.

Vision
2. Alternative studies of NITR
Alternative studies of NITR

NITR make an effort to facilitate development, validation and regulatory acceptance of new and revised regulatory test methods that reduce, refine, and replace the use of animals in testing while maintaining and promoting scientific quality and the protection of human health, animal health, and the environment 10 years ago.
Alternative studies of NITR

✓ To establish the foundation international institute for coordinating validation of alternative methods, KSAAE (Korean Society for Alternatives to Animal Experiment) is inaugurated by effort cosmetic industries, manufacture of medicines, academics and the governments.

✓ From now on, we try to establish an infrastructure about alternative for the intensity of life ethics and improvement to international level.
Laboratory Animal Resources team is maintaining various qualified species and strains of animals under the controlled circumstances of the established animal facilities in order to supply the laboratory animals and to support the animal management at KFDA, NITR and NIH.

The animal facilities have been already accredited by AAALAC International (the Association for Assessment an Accreditation of Laboratory Animal Care).

In accordance with AAALAC International policy, animal care and use program are provided and Institution Animal Care and Use Committee (IACUC) are supported.
Toxicological Research Department

Acute Oral Toxicity Test

- Acute Oral Toxicity-Fixed Dosed Procedure (TG420)
- Acute Oral Toxicity-Acute Toxic Class Method (TG423)
  ⇒ decision of the defined exposure ranges which is expecting fetal
- Acute Oral Toxicity-Up-and Down Procedure (TG425)
  ⇒ calculation of accurate $LD_{50}$

Reproductive & Developmental Toxicity Test

- Whole embryo culture system
- Midbrain micromass culture system
- Limb buds micromass culture system
- Limb buds organ culture system
Genetic Toxicity Test

- Bacterial Reverse Mutation Test
- In vitro Mammalian Chromosomal Aberration Test
- Mammalian Erythrocyte Micronucleus Test
- Mammalian Bone Marrow Chromosomal Aberration Test
- In vitro Mammalian Cell Gene Mutation Test
- Sex-Linked Recessive Lethal Test in Drosophila melanogaster
- In vitro Sister Chromatid Exchange Assay in Mammalian Cells
- DNA Damage and Repair Unscheduled DNA Synthesis in Mammalian Cells in vitro
Alternative short/medium term carcinogenicity Study

- Multi organ carcinogenicity study
- RasH2 transgenic mouse model

Endocrine Toxicity Test

- ER (estrogen receptor) binding affinity assay
- AR (androgen receptor) binding affinity assay
- TR (thyroid hormone receptor) binding affinity assay
- Aromatase and steroidogenesis in vitro assays
- Aryl hydrocarbon receptor recognition/binding assay
■ Skin sensitization Test

- non-radioactive LLNA (local lymph node assay)
- In vitro alternative skin sensitization assessment (THP-1, MC/9 cell line)

■ Skin irritation Test

- Artificial skin model (3D Culture): KeraSkin™ etc
- Fibroblast, HaCaT cell use
Phototoxicity Test

- 3T3 NRU Phototoxicity assay
- RBC Hemolysis assay

Eye irritation Test

- HET-CAM (Hen’s Egg Test on the Chorioallantoic Membrane)
- BCOP (Bovine Corneal Opacity assay)
NITR Budget for Research on Alternatives to safety evaluation of cosmetics

Budget (US $)

Research on Alternative study
(eye toxicity, skin irritation, skin sensitization)

Skin sensitization

Phototoxicity, Skin irritation

30,000
50,000
70,000
80,000
280,000


Fiscal year
Priority of issues in Korea

- Prepare organization like as KoCVAM (Korean Center for the Validation of Alternative Method) in NITR
- Increase the budget for support validation and research on new alternatives
- Construct intra- and international cooperation structure
- Collect and distribute information on alternatives
3. Inter-laboratory validation in Korea
Inter-laboratory validation in Korea

- Cytotoxicity tests (NITR) 1991-
- Eye irritation tests (NITR, SNU, AP co.) 2000-2006
  - Hen’s egg methods,
  - Bovine corneal opacity assay
  - Neutral red uptake
- Phototoxicity tests (NITR) 2004-
- Skin irritation and corrosivity tests
  - KeraSkin® (MCTT, NITR) 2006-
  - EpiDerm® (NITR, KTRI) 2004-
Skin sensitization test

- Measurement of lymph node weight
- BrdU incorporation index by Immunohistochemistry
  SI (stimulation index)

\[
SI = \frac{\text{BrdU Labelling index of test chemical}}{\text{BrdU Labelling index of vehicle control}}
\]

√ LLNA cell proliferation assay by BrdU Immunohistochemistry

Day 1 2 3 4 5 6
Daily exposure

BrdU injection Collection

Skin sensitization test

Image of histological sections.
Eye irritation test

\[ \sqrt{\text{HET-CAM (Hen’s Egg Test on the Chorioallantoic Membrane)}} \]

\[ \text{Value (Q) = score text / score reference (Texapon)} \]

\[ \text{Evaluation: Slight irritating; <0.8, Mod. Irritating; 0.8~1.2, Irritating; 1.2~2.0, Sev. Irritating; >2.0} \]
Bovine Corneal Opacity assay

Ex vivo model

The corneal irritancy of test material is known to be related to their propensity to promote denaturation and disruption of corneal protein.

Cutting the cornea fit into 96well

Measured absorbance at 570nm

Final opacity = (post-treatment O.D.) - (initial O.D.)

15% SLS  Saline  test material

Saline treat- normal

SLS 15% treat
– Stroma denaturation

Test material treat
- Stroma swelling
√ Neutral red uptake assay

SLS treated cell
(C1: the highest conc., C8: the lowest conc.)

Control 3T3 cell – NR uptake

Treated 3T3 cell – cytotoxic effect

NR50 (%)
4. Future validation plan in Korea
Skin irritation and corrosion tests:

KeraSkin model™ (2006- )

Short/medium term carcinogenicity study:

HBx Tg mouse model for hepatocellular carcinoma(HCC) (2006- )
Road to regulatory acceptance of new toxicity tests

Development of new methods

\[\Downarrow\]

Validation

(Confirm reproducibility, sensitivity, relevance, etc.)

Intra-laboratory validation: establish basic method

\[\Downarrow\] \[\Uparrow\]

Inter-laboratory validation: confirm reliability

\[\Downarrow\]

Construct data base

\[\Downarrow\]

Evaluation → Acceptance
Skin irritation and corrosion test

In vitro Human skin model test
Epiderm, KeraSkin

Artificial skin model (3D Culture)

Chemical exposure (Sodium Lauryl Sulfate, 1,1,1-trichloroethylene etc.)

In vitro end point (MTT, CCK-8 etc.)

In vivo correlation

Human skin model: Epiderm™
Short/medium term carcinogenicity study

HBx Tg mouse carcinogenesis model

Hepatitis B virus (HBV)

X-gene construct microinjection in fertilized egg of C57BL/6 X DBA F1

product of HBV X-gene, increase of several heterogeneous viral promoter and cellular promoter function

Alterative foci (A) and hepatocellular carcinoma (B) at 6 month
5. KSAAE
1. Purpose

- Protection of human health, welfare and scientific development

- animal experiments are inevitable

- minimizes animal number and reduces pain

- guarantee ultimately welfare of animals on experiment

- accomplish foundation and application research for technique developments to replace the use of animals
Alternating currents of scientific information and education

Contributes relative industrial development and national health promotion
2. Organizing committee

• **President**
  Professor Jae-Hak Park (Seoul National Univ., pjhak@snu.ac.kr)

• **Vice-President**
  Professor Yong Heo (Catholic Univ. of Daegu, yheo@cu.ac.kr)
  Dr. Sung-Jun Moon (Amore-Pacific Corp., sjmoon@amorepacific.com)
  Dr. Ha-Jung Sung (KTRI, hjsung@kotric.or.kr)

• **Auditing**
  Dr. Kui-Lea Park (NITR, parkkl@kfda.go.kr)

• **Secretary**
  Dr. Bae-Hwan Kim (Amore-Pacific Corp., bhkim@amorepacific.com)
  Dr. Jong-Kwon Lee (NITR, jkleest@kfda.go.kr)

• **Members** 80
3. Progress report

Assembly record

1) 2006. 01. 20. Establishment of a study group
2) 2006. 03. 17. Improvement of organization, give a name WC6 Seoul satellite meeting
3) 2006. 03. 30. Decision of Society official name -> KSAAE
4) 2006. 06. 22. Discussion about summer symposium
5) 2006. 07. 21. The inaugural meeting of KSAAE
6) 2006. 08. 22. Set up website of WC6 Seoul satellite meeting
   - http://eanimal.snu.ac.kr/ksaae
7) 2006. 11.14. Discussion of
   - KSAAE home page, logo, emblem manufacture
   - Publication of J. Alternatives to Animal Experiment The official journal of the KSAAE
   - Conference 23 Feb. 2007
Korean Society for Alternative to Animal Experiments

News

2008년동계학술대회 개최
-일시: 2007년 2월 23일(금)
-장소: 국립복싱연구원

WC6-Seoul Satellite Symposium
-Title: "New Era of Korean Alternative Research for World Harmonization"
-장소 및 일시: 2007년 8월 20일 서울대학교 수의과대학 스코필드홀

Office of Extramural Research

Phone/Fax: +82-2-880-1256

http://eanimal.snu.ac.kr/ksaae
1) 2006. 01. 20.  
1\textsuperscript{st} Conference  
Special seminar for experimental animals

2) 2006. 05.11-12.  
2\textsuperscript{nd} Conference  
Alternative Toxicology Methods

3) 2006. 07. 21.  
3\textsuperscript{rd} Conference  
Summer Symposium of KSAAE

4) 2007. 02. 23.  
4\textsuperscript{th} Conference (plan)  
Winter Symposium of KSAAE

5) 2007. 08. 20.  
WC6 Seoul Satellite meeting (plan)
January 2006, 1st Conference
Special seminar for experimental animals

✓ Date: 20. Jan. 2006

✓ Venue: Amore-Pacific R&D Center

✓ Organizer: Korean Association for Alternatives
1. The worldwide status of alternative researches nowadays, including Japan status
   - Dr. Yasuo Ohno, National Institute of Health Sciences

2. Alternative method for safety test of cosmetics
   - Dr. Bae-Hwan Kim, Amore-Pacific Co. R & D Center

3. Alternative method for skin sensitization test
   - Dr. Jong-Kown Lee, NITR.

4. In Vitro model for modulation of helper T cell differentiation and activation
   - Professor Yong Heo, Catholic University of Daegu

5. Alternatives in Korea
   - Professor Jae-Hak Park, Seoul National University
May 2006, 2nd Conference
Alternative Toxicology Methods

Date: 11-12. May, 2006.
Venue: Catholic University of Daegu Hayang Campus
Organizer: KSOT/KEMS
Laboratory animal welfare going to the alternative road
- Professor Jae Hak Park, Seoul National University

Current activities on alternative research in Japan
- Dr. Hajime Kojima, JaCVAM, NIHS.

In silico toxicity evaluation and mode of action predition based on reference compendia
- Dr. Hans Gmuender, Genedata AG, Basel, Switzerland

Toxicogenomics data generation for predictive biomarker Discovery
- Dr. Seokjoo Yoon, Korea Institute of Toxicology

In vitro evaluation methods for development of cosmeceutical ingredients
- JaeSung Hwang, Amore-Pacific Corp. R & D Center
July 2006, 3rd Conference
Summer Symposium of KSAAE

- **Venue**: NITR Conference room
- **Organizer**: KSAAE
What is alternative to animal experiments?
- Professor Jae Hak Park, Seoul National University

Current status of alternative study
- Dr. Kui Lea Park, NITR immunotoxicology division

In silico prediction of ADME/Tox profile
- Seung-Hun Choi, In silicotek

Alternative Approach to Test Immune-Toxicity for Xenobiotics Safety – focusing on immune-sensitization
- Professor Yong Heo, D.V.M. Catholic Univ. of Daegu

Toxicity test and alternative study of cosmetic ingredients
- Dr. Kyung Hoon Sohn, KFDA

Alternative method application for safety test of cosmetics
- Dr. Byung-Whee Seo, Amore-Pacific R & D Center
6. WC6 Seoul satellite meeting
6th World Congress on Alternatives and Animal Use in the Life Sciences

- 21-25, Aug. 2007 in Tokyo
- One day for satellite symposium in Kyoto
- Another satellite symposium at Seoul in KOREA
WC6 Seoul Satellite Meeting

- **Homepage:**
  - [http://eanimal.snu.ac.kr/ksaae](http://eanimal.snu.ac.kr/ksaae)
  - (tentative)

- **Specific theme:**
  - New Era of Korean Alternative Research for World Harmonization

- **Date:** 20. Aug. 2007

- **Venue:** Seoul National University, College of Veterinary Sciences Auditorium

- **Organizer:** KSAAE
Invited Speakers overseas

- Dr. Noriho Tanaka (FDSC, Japan) :
  Introduction of Alternatives in Japan

- Dr. Thomas Hartung (ECVAM) :
  Overview of Alternatives in Europe

- Dr. Kathryn Bayne (AAALAC, USA) :
  AAALAC’s view on Alternative Research

- Dr. David Basketter (UK) :
  Alternative tests details focusing on skin sensitization

Five presentations by Korean scientists
Thank you for your attention