Toxicological data requirements for agrochemical registration in Taiwan and opportunities for 3Rs

Wei-Ren Tsai [*1] , Yueh-Yi Lee[1], Wen-Chuan Chiang[2], Chun-Lin Liao[1], Jin-Yi Chang[1]

Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Council of Agriculture, Executive Yuan, Taichung 41358, Taiwan (R.O.C.)[1]
Animal Protection Section, Department of Animal Industry. Council of Agriculture Executive Yuan, Taipei 10014, Taiwan (R.O.C.)[2]
*Email: sftsai@tactri.gov.tw (chief of applied toxicology division)

1

Outline

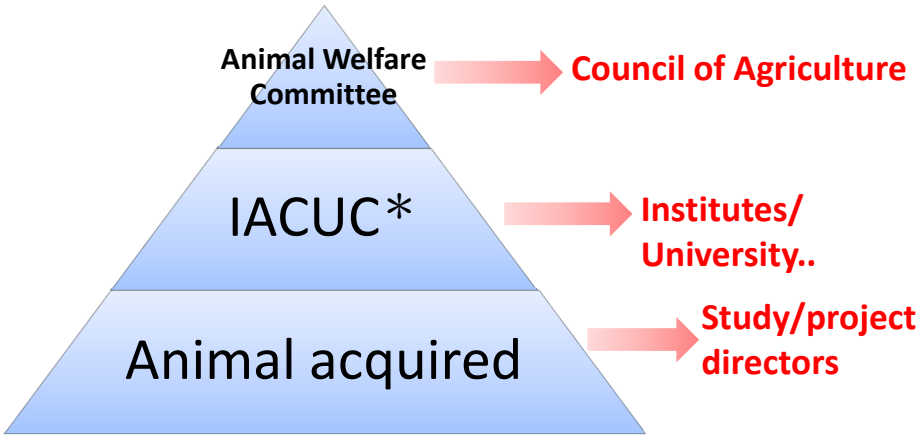
- Scientific application of laboratory animals in Taiwan
- Toxicological data requirements for agrochemical (pesticides) registration in Taiwan
- 3Rs policies for pesticides registration (how to reduce animal with the case of LLNA)
- Future aspects for 3Rs

2

Laws and statistics of scientific application of laboratory animals in Taiwan

3

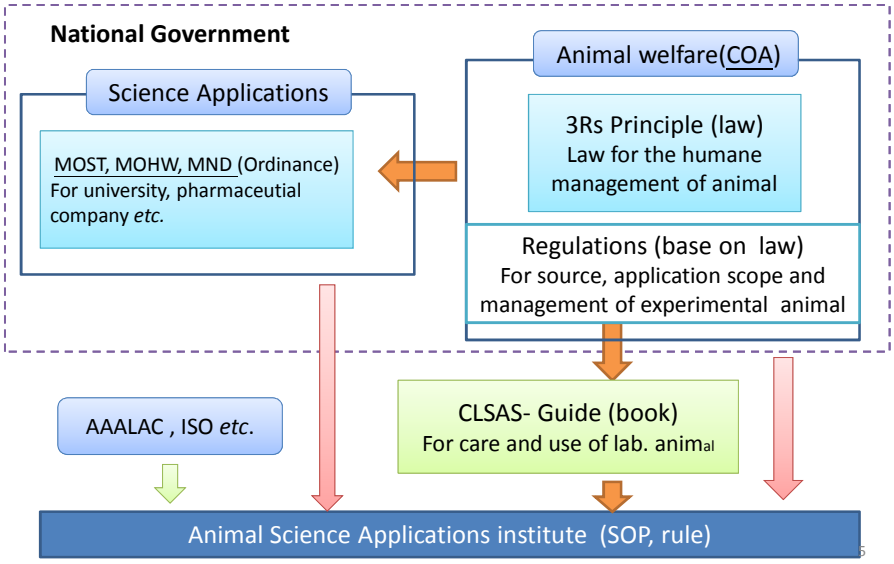
Laws of scientific application of laboratory animals in Taiwan



Institutional Animal Care and Use Committee, IACUC*

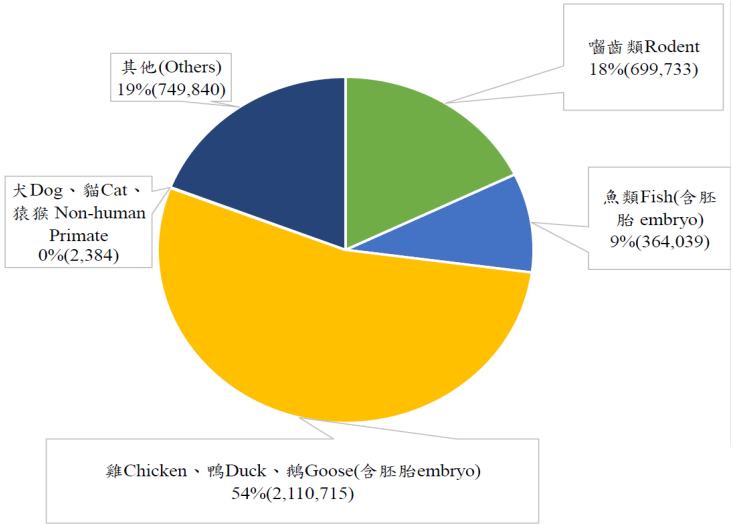
4

Framework of Animal Law in Taiwan



Scientific application of laboratory animals in Taiwan

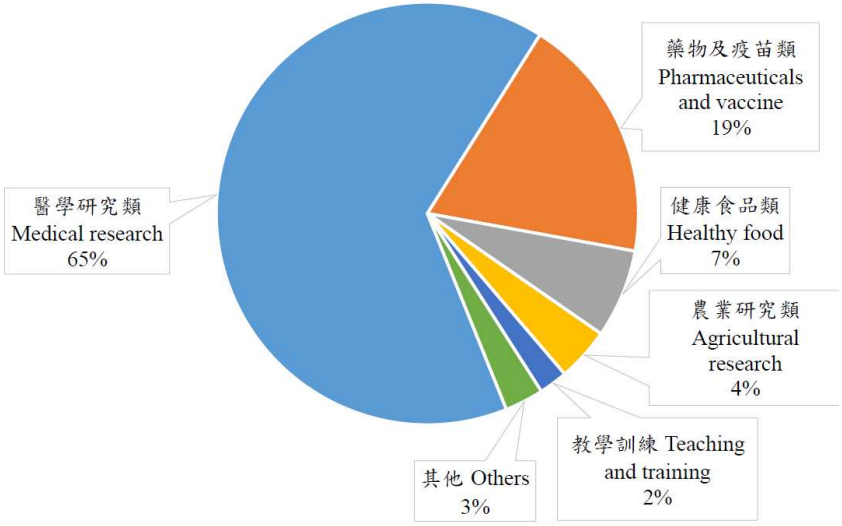
Distribution of laboratory animal species used in the institutions



Data source: Council of Agriculture, Executive Yuan, Taiwan (R.O.C.). Humane care of laboratory animals. Annual report 2016.

Scientific application of laboratory animals in Taiwan

Category of protocols for various scientific research



Data source: Council of Agriculture, Executive Yuan, Taiwan (R.O.C.). Humane care of laboratory animals. Annual report 2016.

Institutions conduct toxicology tests for pesticides registration in Taiwan

- Government
  - TACTRI
- Companies (Contract laboratory)
  - Level Biotechnology Inc.
  - Super Laboratory Co., Ltd.
  - Medgaea Life Sciences, Ltd.
- Non government organization
  - Agricultural Technology Research Institute

Approximate: 2,500 animals /year
(not including fish for aquatic toxicity test)

8

Toxicological data requirements for pesticides registration in Taiwan

9

Toxicological data requirements for agrochemical in Taiwan

Toxicological testing and animal numbers estimate

Acute toxicity testing	Subchronic toxicity testing	Chronic toxicity testing
Oral toxicity	90-day feeding	Feeding
Dermal toxicity	21-day dermal	Oncogenicity
Inhalation toxicity	90-day inhalation	Reproductive
Eye irritation	90-day neurotoxicity	Prenatal development
Dermal irritation	**Mutagenicity testing**	**Non-target organism toxicity testing**
Dermal sensitization	In vivo cytogenetics	Aquatic toxicity
Neurotoxicity	**Metabolism studies**	Fish or aquatic invertebrate life cycle study
	Metabolism studies	Avian toxicity

Animal numbers acquiring estimating

- Acute toxicity testing: 200
- Subcronic toxicity testing: 260
- Mutagenicity testing: 50
- Chronic toxicity testing: 1500
- Metabolism study: 20
- Non-target organism toxicity testing: 200

10

Toxicological data requirements for agrochemical in Taiwan

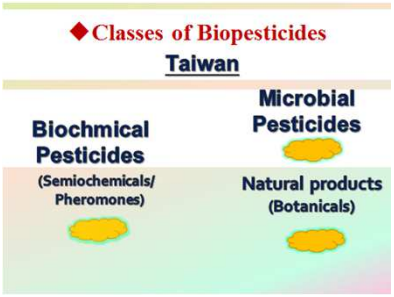
Pesticides registration categories

By end-use

- New active ingredient
- New formula/Mix types
- Extension use
- 'Me too' product (commercialized products with granted active ingredients and active ingredient impurities)

By active ingredient

- Organic
- Inorganic
- Biopesticides
  - Natural
  - Microbial
  - Biochemical (pheromones)

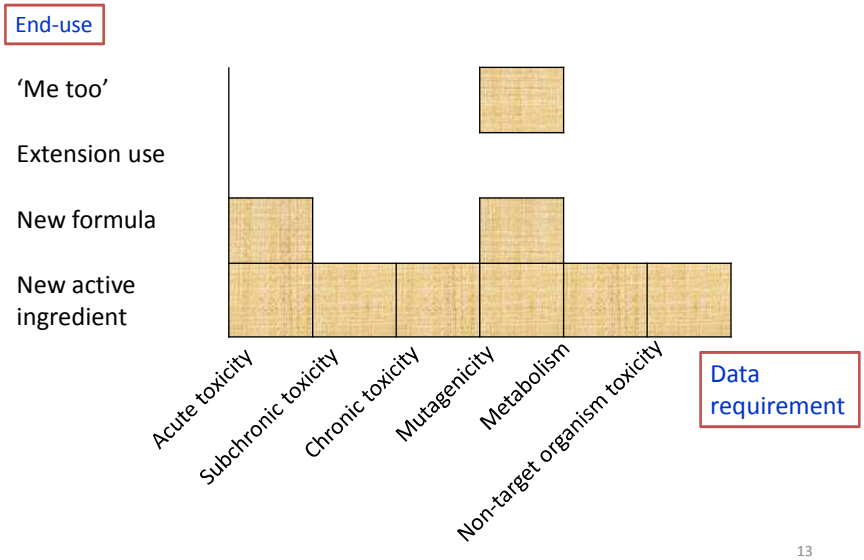


11

BIOPESTICDE

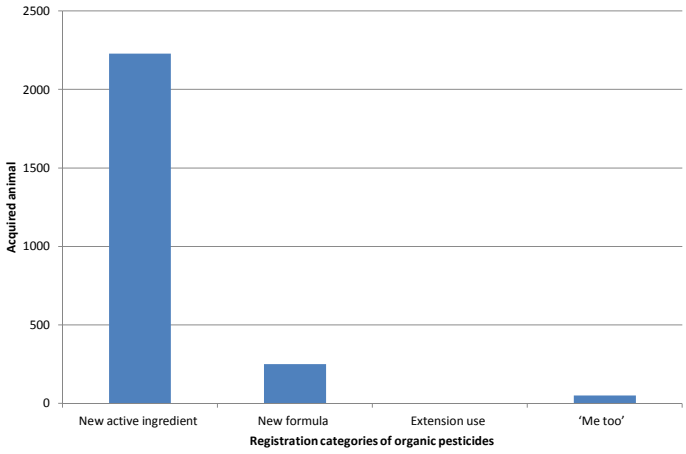
- **To encourage the development and use of low risk biological pesticide**
- **Biopesticide (microbial pesticide) registrations may require a much less data set compared to conventional chemical pesticides. (reduce animal use)**
- **There are less timeframe and fees for faster registration processes.**

Toxicological data requirements for agrochemical (organic chemical) registration in Taiwan



13

Toxicological data requirements for agrochemical (organic chemical) registration in Taiwan



14

3Rs policies for pesticides registration

(case study by local lymph node assay)

15

3Rs policies for pesticides registration

Different substances, different amounts of toxicology data

1. **According to different pesticide categories, different amounts of data is required**
2. **Different substance characters are also considered (For example, more safety formulation, less toxicology data)**

International trends

1. **International consistency-GLP reports**
2. **EU SANCO 1987 for registration reference to reduce toxicology data requiring for me too pesticides, which is only impurities concentration different from the original registration product.**

16

3Rs policies for pesticides registration- International trends always on-trend

	Toxicological testing (Before policy adopting)	Toxicological testing (Now)	Animal use reduction /Registration
More safety formulation (same AI%)	• Oral toxicity • Dermal toxicity • Inhalation toxicity • Eye irritation • Dermal irritation • Dermal sensitization	• Oral toxicity • Dermal toxicity • Eye irritation	Less
Me too product (SANCO 1987-)	• Oral toxicity • Dermal toxicity • Inhalation toxicity • Mutagenicity (in vivo)	• Mutagenicity (In vivo-cytogenetics)	Less

17

3Rs policies for pesticides registration

Test methods

1) OECD 401 «OECD 425
2) OECD 429 «OECD 442B
3) OECD 405
Humane anesthesia and analgesic method introduce

18

3Rs by Test methods

3Rs

Reduction

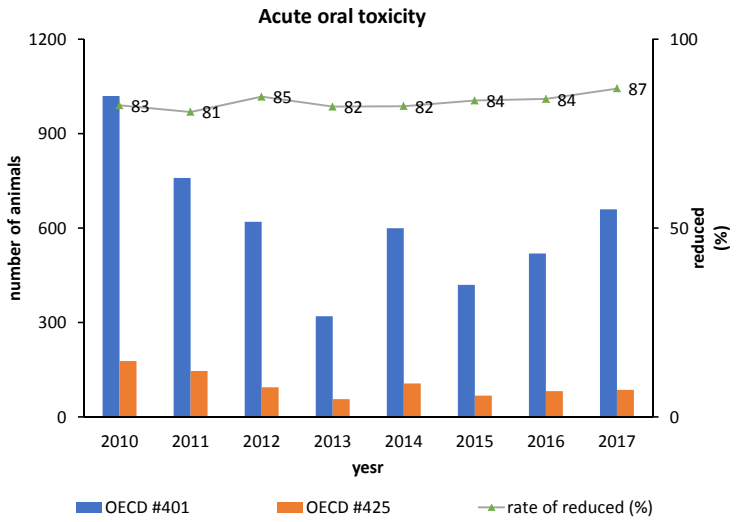
- **Acute oral toxicity-2010**
  ~~OECD 401(1998)~~ ➡ OECD 425(2008) — **80%**
- **Skin sensitization-2013**
  OECD 429 (Guinea pig) ➡ OECD 442B (mouse) — **50%**

Refinement

- **Eye irritatation-2015**
  (OECD 405, 2012) — **50%**

19

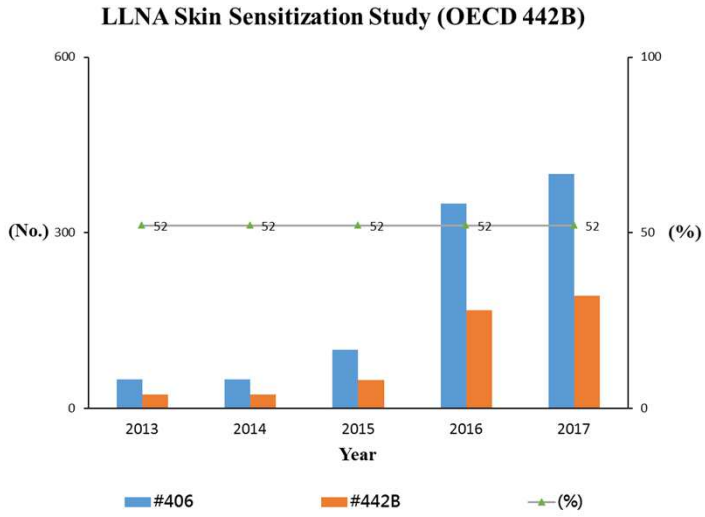
Animal use reduction by acute oral toxicity (OECD 425)



8 year 152case 4100 animals NT 200 million

20

Animal use reduction by LLNA



5 years,19 cases, 500 animals

21

3Rs policies for pesticides registration-always look ahead

The progress of skin sensitization test (case study by local lymph node assay)

2002 Buehler Test

2004 GPMT

2005 MEST and LLNA setup

2009 LLNA conduct

2010 OECD guideline 442A/442B

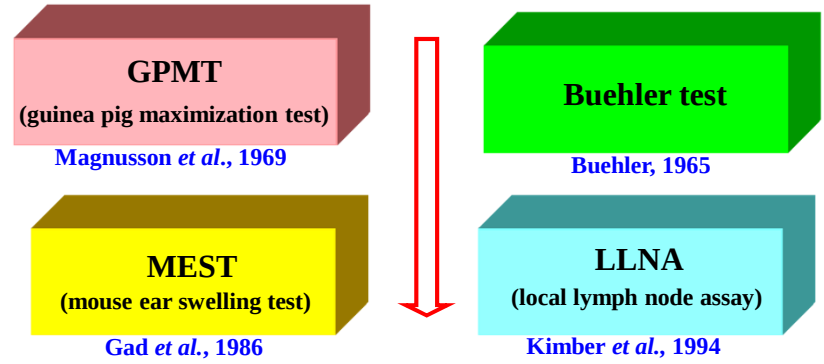
2010 LLNA conduct

2010 Report accept

Some obstacles in LLNA process

22

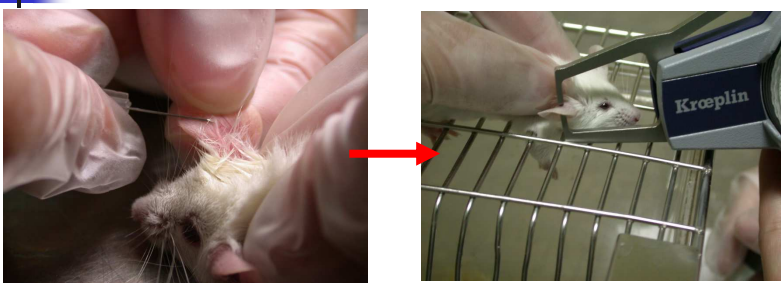
LLNA advantages



- measured objectively/Quantitative data
- Short test duration (1 week)
- Reduce the pain and distress to lab. animals
- minimal animal treatment (50%)
- Stimulated index (SI)

23

MEST (mouse ear swelling test)



The degree of ear swelling was expressed as % ear swelling =test/control ear thickness x 100

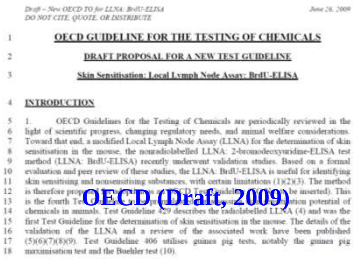
24

LLNA (local lymph node assay)

obstacles

[3H]thymidine (3H-TdR) detetion

5'-bromo-2'-deoxyuridine (BrdU) instead 3H-TdR (Takeyoshi *et al*., 2001)


Draft – New OECD TG for LLNA: BrdU-ELISA
DO NOT CITE, QUOTE, OR DISTRIBUTE
June 26, 2009

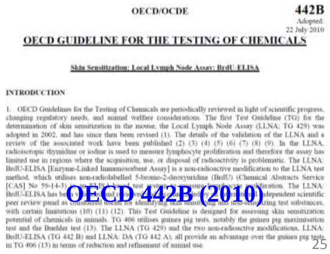
OECD GUIDELINE FOR THE TESTING OF CHEMICALS

DRAFT PROPOSAL FOR A NEW TEST GUIDELINE

Skin Sensitisation: Local Lymph Node Assay: BrdU-ELISA

INTRODUCTION

OECD (Draft, 2009)


OECD/OCDE 442B
Adopted: 22 July 2010

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

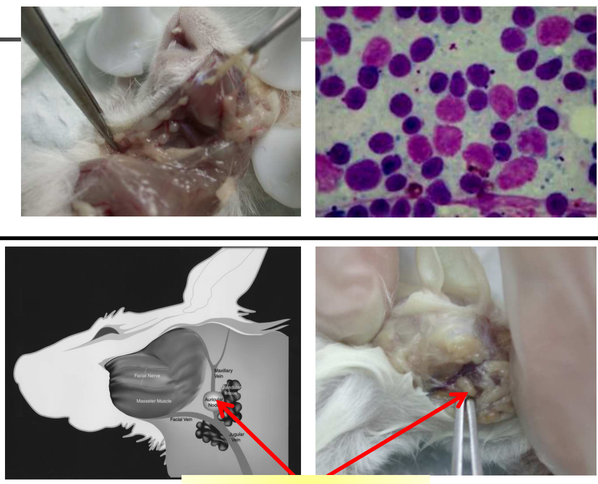
Skin Sensitization: Local Lymph Node Assay: BrdU-ELISA

INTRODUCTION

OECD 442B (2010)

25

Obstacles in LLNA

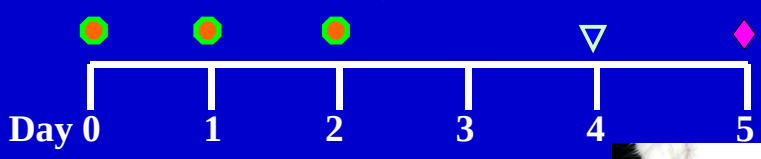


Lymph node site

26

Local lymph node assay, LLNA

Balb/c mice 6-8 weeks (female)



applied to the back of the ears (25μl)

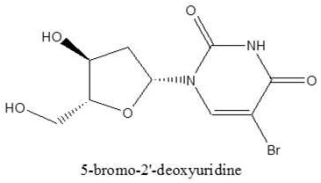
BrdU (5-bromo-2'-deoxyuridine) IP

sacrificed and the lymph nodes c --BrdU (ELISA)

-- Calculation of stimulation index (SI)

27

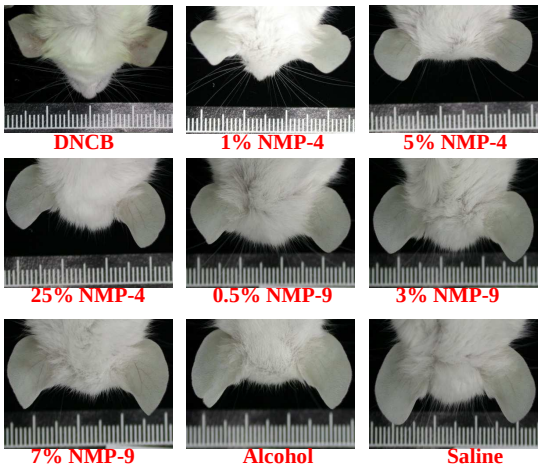
Ear irritative appearance of mice treated with pyrrolidinone pesticide other ingredients in LLNA assay



5-bromo-2'-deoxyuridine

BrdU (labelling index, LI):

stimulation index (number x proliferation) SI>1.6



28

Skin sensitization evaluation standards (reference)

Categories		Moderate ~ serious	Mild or none
Reactive animals (%)		>29	≤ 28
stimulation index	isotope	≥3	< 3
	DA	≥ 1.8	< 1.8
	BrdU	≥ 1.6	< 1.6
Warning signs			none
Warning words		Skin sensitization	none

29

3Rs policies for pesticides registration-amazing calculate

Different substances Different amounts	International trends	Test methods
• Application categories • Characters 1) Chemical 2) Formulation 3) More safety formulation	• International consistency-GLP reports • SANCO 1987- me too product • Anesthesia and analgesic method introduce	• OECD 401 «OECD 425 • OECD 442B «OECD 429
1,061,500 animals/year	31,500 animals/year	600 animals/year (in our Institute)

30

Future aspects for 3Rs

Different substances Different amounts	International trends	Test methods
• Case by case study • High risk pesticides reduction (registration reduction) • Label discrimination	• In silico simulations • Testing duration: 12 months to 3 months for dog feeding test. (In law making process)	• OECD402(2017)-acute dermal toxicity • In vitro tests Reducing, replacement and refinement

31

Summary-3Rs

Toxicological data requirements for agrochemical registration

- Low risk biological pesticide (waive test)
- Same AI (impurities content lower than 0.1%)

The methods-according new test guideline

- Reduction (acute dermal toxicity-OECD 402 2017)
- Refinement

32

Thank for your attention!

Thank you my co-worker and study team

Dr. Lu SY
Dr. Tsai BL
Dr. Yang JH
Mr. Wu WJ, Lu CP
Ms. Chang SM

33