【S-10】

Strategies for Evaluating the Safety of Genetically Modified Crops

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Genetically modified (GM) crops with agricultural traits including herbicide resistance and insect tolerance have been commercialized. The safety testing strategies conducted for food and feed ingredients from GM crops differ from those applied to food ingredients in that they are conducted to demonstrate similarity between the GM food and the appropriate non-GM comparator rather than for quantitative risk assessment. However, there are similarities in the design and conduct of the safety assessment studies between these types of studies that should be readily recognized by toxicologists. The current presentation reviews some of the basic principles of safety assessment of typical dietary ingredients and compares and contrasts them with the testing strategies applied to GM foods and products obtained from them.

APPLICATION OF GOOD LABORATORY PRACTICES TO *IN VITRO* TOXICOLOGY STUDIES:

Perspective from a Toxicologist

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Key Issues – *In Vitro* Systems

- *In vitro* studies will only be accepted by regulators if data allow classification of effects the same way that animal studies allow
- Must be predictive of anticipated outcome in animals or humans
  - Liebsch and Spielmann (2002) Toxicol Lett 127:127-134.

Key Issues – GLP

- Document!
- **Document!!**
- **DOCUMENT!!!**
- (If you can't document what happened...it didn't happen)

OUTLINE

I. Why *In Vitro* Toxicology Studies?
II. Types of *In Vitro* Toxicology Studies
III. Why GLP?
IV. GLPs for Toxicology Studies
V. GLPs for *In Vitro* Studies
VI. Perspectives from a Toxicologist
VII. Next?

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Why *In Vitro* Toxicology Studies?

- Recent legislation to reduce animal use
- Public opinion about animal use
- Benefits vs. *In vivo* Toxicology Studies
  - Cheaper
  - Faster
  - Less test substance required
  - Validated substitute for longer term studies
    - Example: Mutagenesis → Carcinogenicity

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Types of *In Vitro* Toxicology Studies

- **Genotoxicity**
- Host mediated assay
- Sister chromatid exchange
- Unscheduled DNA synthesis
- Cell transformation assay
- Many others

Types of *In Vitro* Toxicology Studies

- Genotoxicity:
  - Carcinogenicity studies detect genotoxic substances, but so do:
    - Bacterial systems
      - Ames
    - Mammalian systems
      - MLA
      - Chromosome aberration
      - Micronucleus

Types of *In Vitro* Toxicology Studies

- Genotoxicity
  - Clearly associated with carcinogenesis and heritable effects
    - Direct
      - Point mutations
      - Frameshift mutations
      - Chromosome rearrangements and deletions
      - Aneuploidy
    - Indirect
      - DNA Adducts
      - Strand breakage

Types of *In Vitro* Toxicology Studies

- In relation to **Key Issues**, the results from *in vitro* genotoxicity studies:
  - Are accepted by regulatory agencies
  - Allow for classification of results the same way that animal studies would
  - Are predictive of anticipated outcome in humans or animals
  - Typically are conducted according to GLP

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Why GLP?

- To promote confidence in toxicology data submitted to regulatory authorities
  - Reliability
  - Integrity
  - Reproducible
  - Traceability of data
  - International acceptability

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GLPs for Toxicology Studies

- Basics:
  - GLP is a **MANAGEMENT SYSTEM** that defines organization, process and conditions under which studies are:
    - Planned
    - Conducted
    - Reported
    - Documented
    - Archived

GLPs for Toxicology Studies

- For *in vivo* toxicology studies, GLPs apply to three primary areas:
  - Facilities
  - Management
  - Study Plan

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GLPs for *In Vitro* Studies

- Intent is the same as *in vivo* studies:
  - To give high degree of confidence in toxicology data submitted to regulatory authorities
  - Reliability
  - International acceptability

Facilities

- General GLP issues:
  - *Building*
    - Design
    - Structure
    - Size
    - Maintenance
    - Archive facilities
  - Proper identification/labeling of materials
    - Manufacturer or supplier
    - Name and address
    - Lot number

Facilities

- *In vitro* specific GLP issues:
  - Equipment controls rather than environmental
    - Incubators
    - Laminar flow hoods
    - Continuous monitoring
    - Calibration
  - Sufficient number of rooms
  - Appropriate separation of concomitant studies
  - *Minimize contamination*

Facilities

- *In vitro* specific GLP issues:
  - Cell culture
    - Media
    - Flasks, Petri dishes, etc.
  - Proper storage of materials to prevent:
    - Damage
    - Infestation
    - Contamination and spoilage
  - Unique to *in vitro* studies
    - Cell counters
    - Incubators
    - Micropipettors
  - Electronic data recording and analysis
    - Microplate readers
    - PCR machines

Management

- General GLP issues:
  - Organization
  - Training and Qualification of Personnel
  - Master Schedule
  - QA Program

Management

- *In vitro* specific GLP issues:
  - Ensure:
    - Qualified personnel
      - Training records
      - Aseptic procedures
      - Biohazard handling
    - *Equipment*
      - Calibration
      - Maintenance
      - Proper function
      - Training of operators

Study Plan

- General GLP issues:
  - Protocol
  - Test Substance Information
  - Principle Investigator
  - Sponsor
  - *Study Director*
  - Report
  - Archiving

Study Plan

- *In vitro* specific GLP issues:
  - Protocol
  - Test Substance Information
  - **Test System → Study Performance Criteria**
  - Principle Investigator
  - Sponsor
  - Study Director
  - Report
  - Archiving

Study Performance Criteria

- Treatments
  - Concentrations of test substance (rather than dose)
- Performance acceptance criteria
  - Negative controls
  - Positive controls

Study Performance Criteria

- Test system
  - Justification
  - Characterization of *in vitro* system
    - Species
    - Tissue origin
    - Source
    - Culture conditions

Study Performance Criteria

- Key feature for *in vitro* studies
  - Performance criteria
  - Basis for determining acceptability of an assay
  - Reliance on historical control data and positive and negative controls
    - Assumes laboratory has extended previous experience and database
  - Should perform within predetermined limits
  - Tracking of performance over time

Study Performance Criteria

Bacterial mutagenicity of a standardized mixture of citrus PMFs (-S9)
Revertants/plate

mg/plate	TA98	TA100	TA102	TA1535	TA1537
DMSO	45 ± 16	166 ± 124	255 ± 111	13 ± 12	5 ± 11
0.0005	42 ± 128	141 ± 115	191 ± 159	9 ± 11	2 ± 13
0.0050	45 ± 123	200 ± 153	259 ± 147	12 ± 16	3 ± 10
0.0500	34 ± 16	170 ± 113	285 ± 16	13 ± 14	5 ± 14
0.5000	40 ± 11	172 ± 137	220 ± 114	14 ± 11	3 ± 13
5.000	19 ± 12	163 ± 16	105 ± 17**	10 ± 11	1 ± 11
Control	563 ± 156	341 ± 114	393 ± 117	352 ± 16	385 ± 15
	2-Nitrofluorene (2.0 µg/plate)	Sodium azide (1.5 µg/plate)	Mitomycin C (2.0 µg/plate)	Sodium azide (1.5 µg/plate)	9-Aminoacridine (100 µg/plate)

Delaney et al (2002) Food Chem Toxicol 40:617-624

Study Performance Criteria

Bacterial mutagenicity of a standardized mixture of citrus PMFs (-S9)
Revertants/plate

mg/plate	TA98	TA100	TA102	TA1535	TA1537
DMSO	31 ± 11	164 ± 17	283 ± 116	10 ± 16	4 ± 13
0.0005	44 ± 125	176 ± 13	319 ± 18	11 ± 14	3 ± 11
0.0050	33 ± 11	167 ± 11	318 ± 15	17 ± 15	7 ± 14
0.0500	33 ± 11	194 ± 11	312 ± 18	13 ± 11	14 ± 10**
0.5000	26 ± 13	155 ± 11	282 ± 129	14 ± 11	7 ± 14
5.000	20 ± 11	172 ± 118	174 ± 19**	6 ± 14	4 ± 11
Control	1503 ± 1362	1108 ± 1174	880 ± 158	60 ± 19	52 ± 119
	2-Aminoanthracene (10.0 µg/plate) →				

Delaney et al (2002) Food Chem Toxicol 40:617-624

Test Systems

- Justification
- Types of cells
  - Source (Supplier)
  - Origin
    - Species, organ, etc.
  - Nomenclature (ATCC)
- Method by which they were obtained
- Chronology of custody

Test Systems

- Culture conditions
  - Environmental
  - Define and maintain:
    - Cell passage number
    - Population doubling time
- Labeling
- Study records
- Storage
- Performance requirements

Performance of the Study

- Predetermined acceptance criteria
  - Positive control
  - Negative
  - Solvent
- Historical control data

QA Unit

- Inspect critical phases of study
  - Independent from the conduct of the study
  - Can't be done by your "lab buddy"
- Equipment
  - Cleaning and decontamination
  - Maintenance
  - Calibration
- SOPs

GLPs for *In Vitro* Toxicology Studies

- Important Publications:
  - OECD:
    - Application of GLP Principles to *In Vitro* Studies (http://appli1.oecd.org/olis/2004doc.nsf/linkto/env-jm-mono(2004)26)
    - Application of GLP Principles to Short Term Studies (http://www.olis.oecd.org/olis/1999doc.nsf/LinkTo/env-jm-mono(99)23)

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Perspectives from a Toxicologist

- Remember the Basics of Toxicology:
  - Exposure to some substances can cause adverse effects
  - Toxicology studies are conducted to understand risks from exposure to substances
  - Whether from an *in vivo* or *in vitro* study, data from toxicology studies should be:
    - Accurate
    - Reproducible
    - Documented

Perspectives from a Toxicologist

- Limits of *in vitro* studies (not related to GLPs)
  - Dose extrapolation
  - Pharmacokinetics
  - Not for quantitative risk assessment
  - Carcinogens
    - Genotoxic – OK
    - Non-Genotoxic – ???

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Next???

- New in vitro technologies being applied to evaluate the effects of chemicals *in vitro*:
  - Transcript profiling
  - Proteomics
  - Metabolomics

Thank you!!!

감사합니다!