益诺思 INNOSTAR

国家上海新药安全评价研究中心
National Shanghai Center for
New Drug Safety Evaluation and Research

Genetic Toxicology: Progress on International Test Guidelines and New Methods

Yan Chang
Ph.D. , DCST
Shanghai NCDSER .

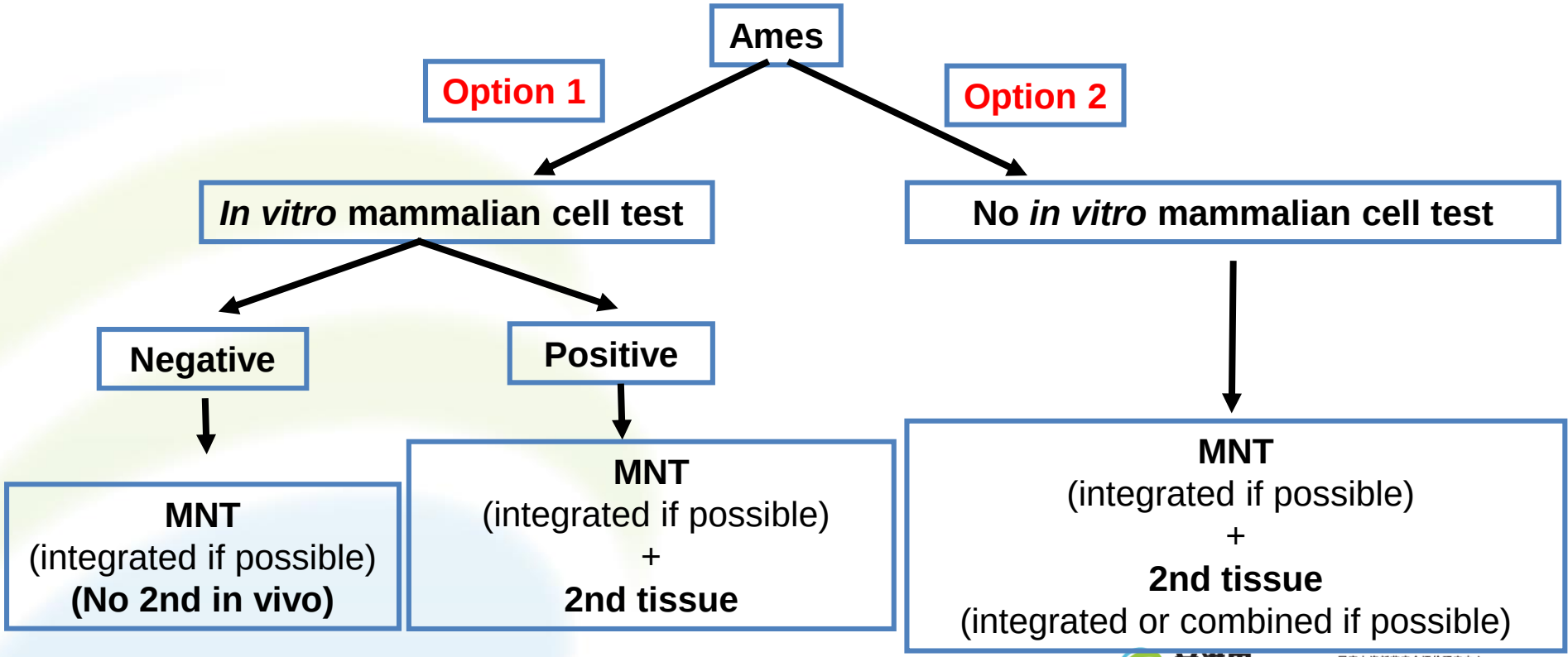
2018年5月31日

Contents

- Test Guidelines in Genetic Toxicology
- New Technologies in Genetic Toxicology

ICH S2(R1)

— Adopted on Nov. 2011



OECD Test Guidelines-*In Vitro*

- TG 471 Bacterial Reverse Mutation Test

- TG 473 *In Vitro* Chromosome Aberration
- TG 487 *In Vitro* Mammalian Cell Micronucleus Test
- TG 490 *In Vitro* Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene
- TG 476 *In Vitro* Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes

OECD Test Guidelines-*In Vivo*

- TG 474 Mammalian Erythrocyte Micronucleus Test
- TG 475 Mammalian Bone Marrow Chromosomal Aberration Test
- TG 487 *In Vivo* Mammalian Alkaline Comet Assay
- TG 488 Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays
- NEW: *In Vivo* Pig-a Gene Mutation Assay

  In Vivo Liver Micronucleus Assay

CFDA: Guideline on Genotoxicity Tests for Pharmaceuticals

— Updated on 15 Mar. 2018

药物分子结构的安全性评价
- 安全预测 - 小分子

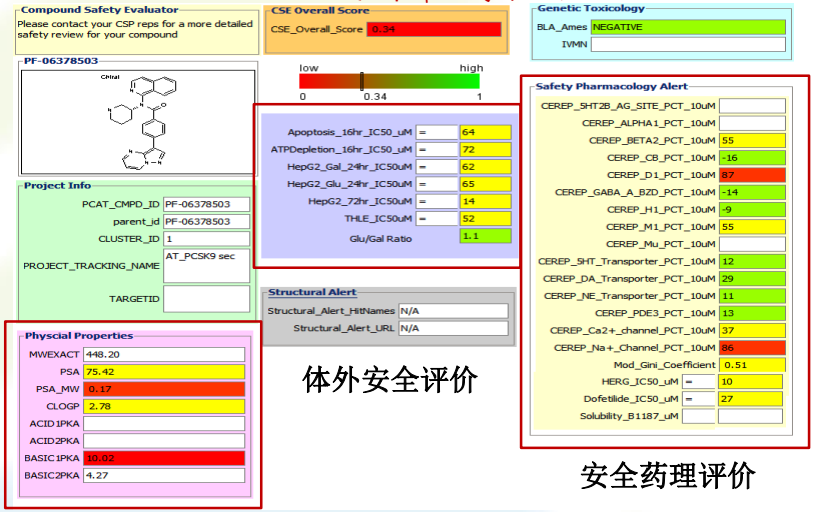
早期安全评价

- 分子安全预测

复合安全评估 - 通过整合其理化性能、安全特性和药理学特性，来预测药物分子的化学性质和安全性。

靶点筛选 | 分子筛选 | 先导化合物 | 候选药物筛选 | 一期临床 | 二期临床 | 三期临床 | 四期临床/投放市场

小分子计算机毒性预测

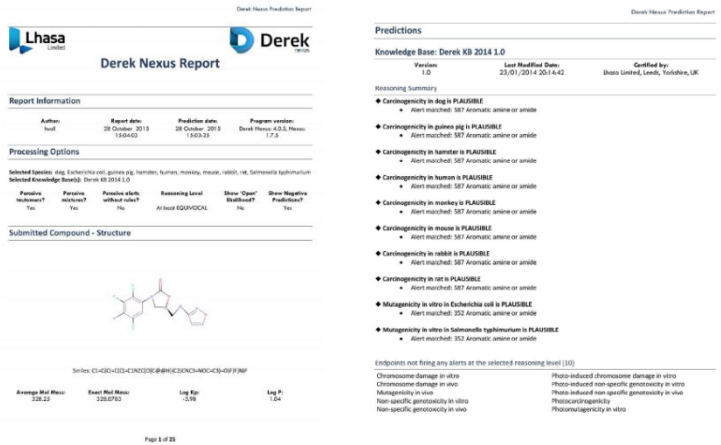


体外安全评价

安全药理评价

物理化学特征

预测报告和化合物得分

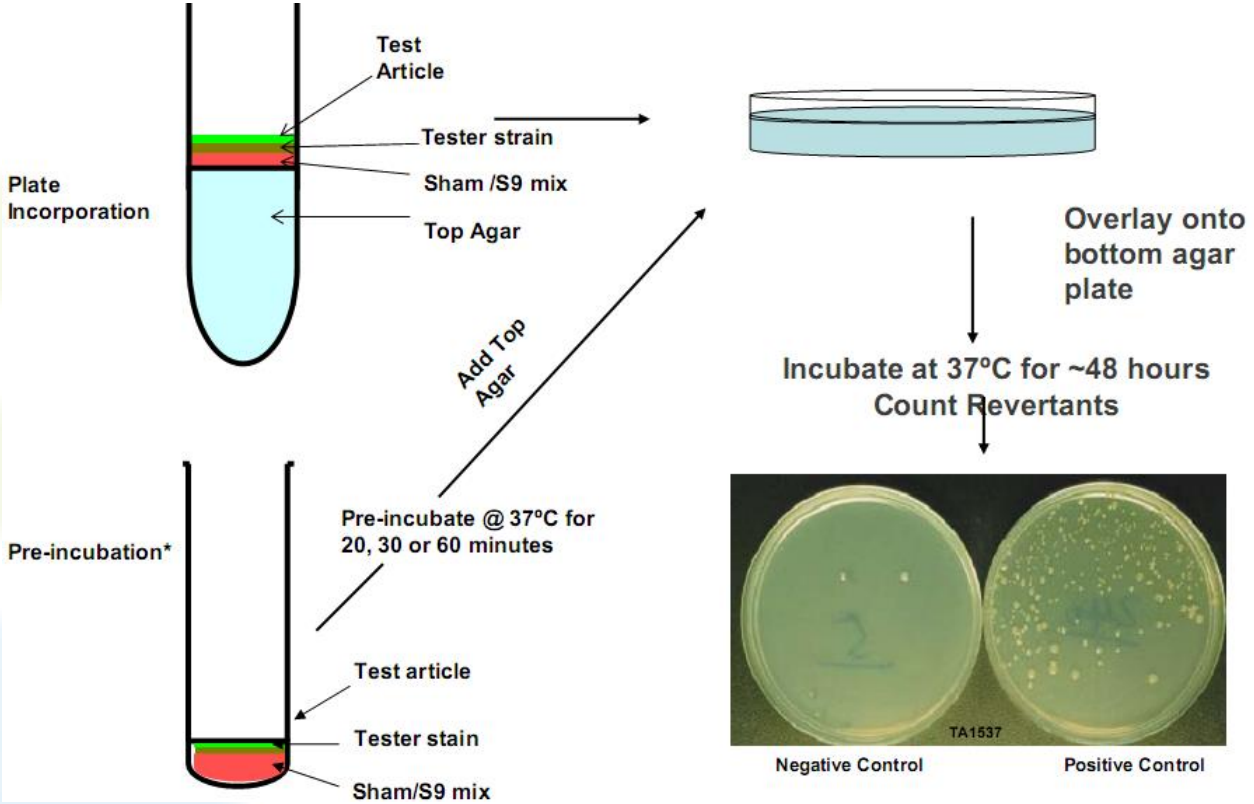


Ames Assay

Strains	*his*/*trp* Mutation	Deletion Mutation				Reversion Event
		Repair	LPS	VIT	Plasmide	
TA 1535	G46	urvB-	rfa-	bio-	-	Base-pair Substitution
TA 1537	C3076	urvB-	rfa-	bio-	-	Frameshift
TA97	D6610	urvB-	rfa-	bio-	pKM101	Frameshift
TA 98	D3052	urvB-	rfa-	bio-	pKM101	Frameshift
TA 100	G46	urvB-	rfa-	bio-	pKM101	Base-pair Substitution
TA 102	G428	urvB+	rfa-	bio-	pKM101	Base-pair Substitution
WP2uvrA	tryp E	uvrA-	-	-	pKM101	Base-pair Substitution

Ames Assay

Plate Incorporation Methodology



In Vitro Chromosome Aberration

Day 0

CHL/CHO cell seeding

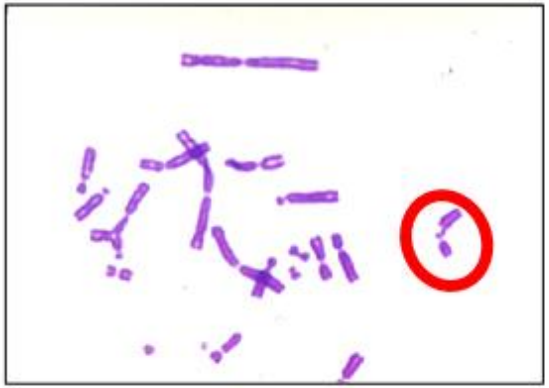
Day 1

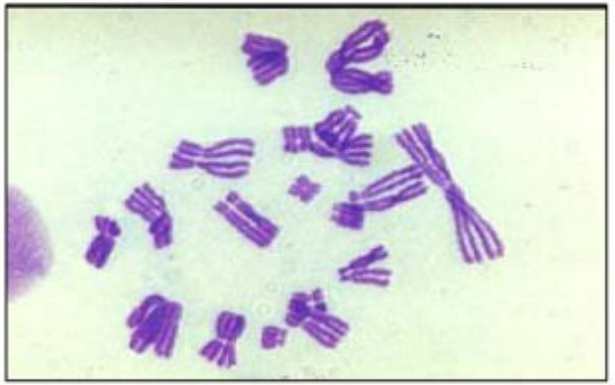
Exposing culture of cells to the dose formulations (4h+/-S9; 24h-S9)

Day 2

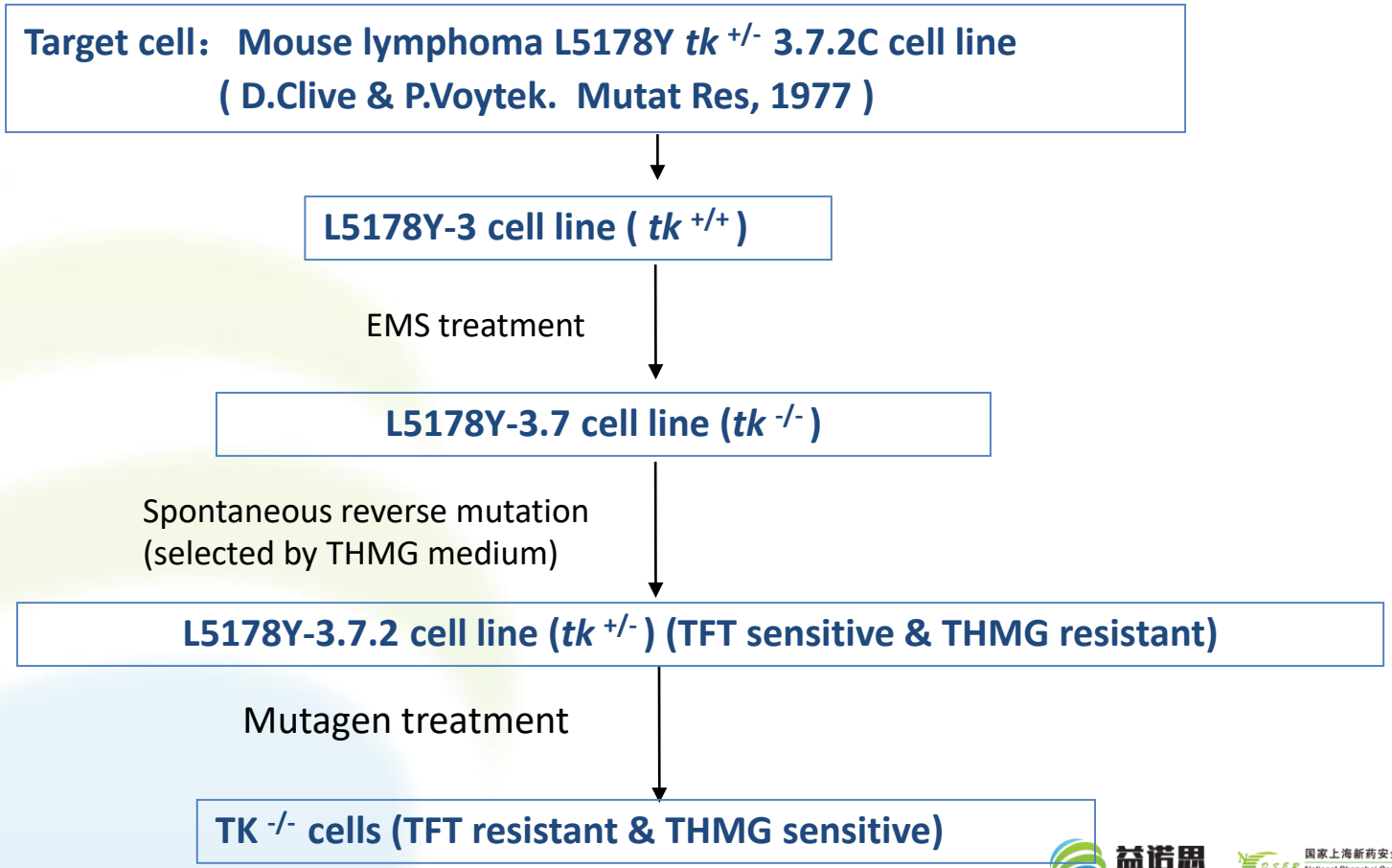
- Adding colchicine 2~6 hrs prior to cell harvest
- Harvest
- Preparing slides

Reading slides with blind codes (150 cells/slide).





Mouse lymphoma Assay (MLA)



MLA

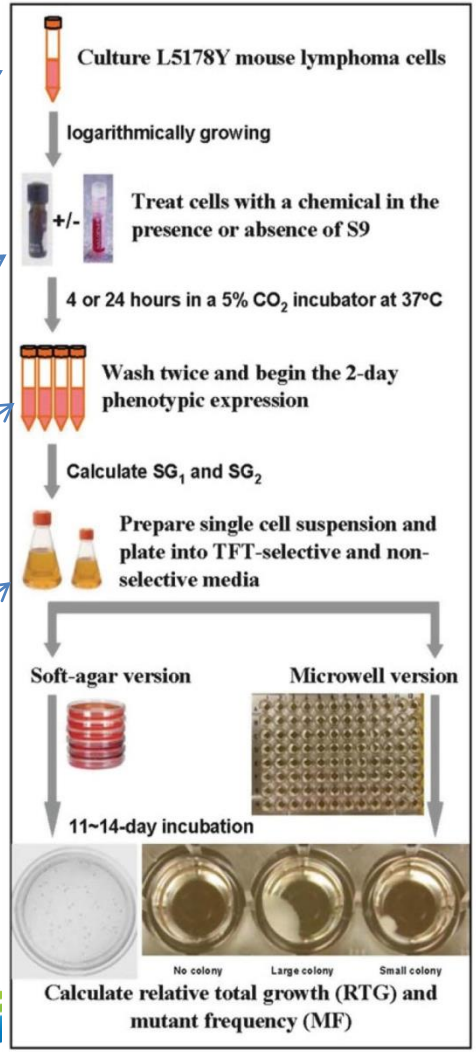
MLA microwell version:

Culture

4 h/24 h

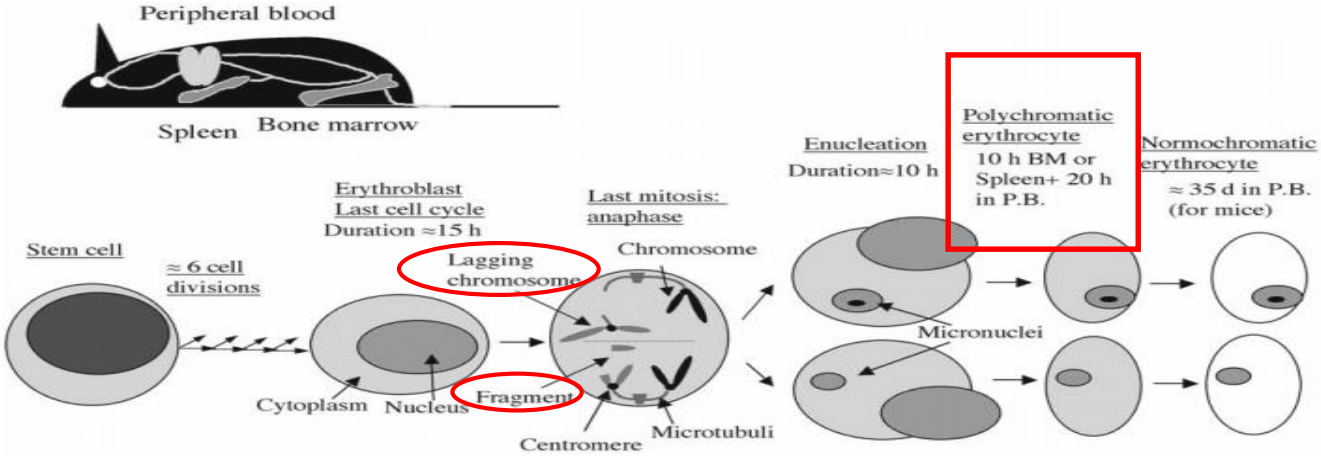
2-day expression

11-14 day incubation

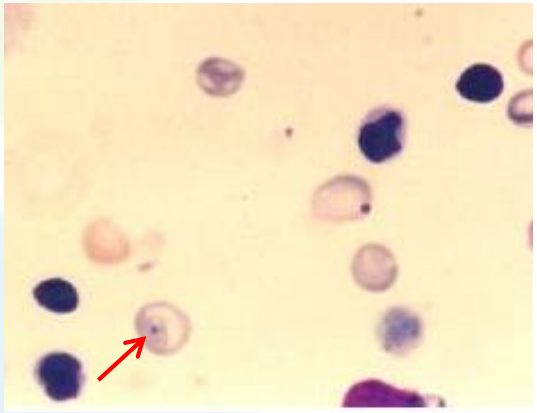


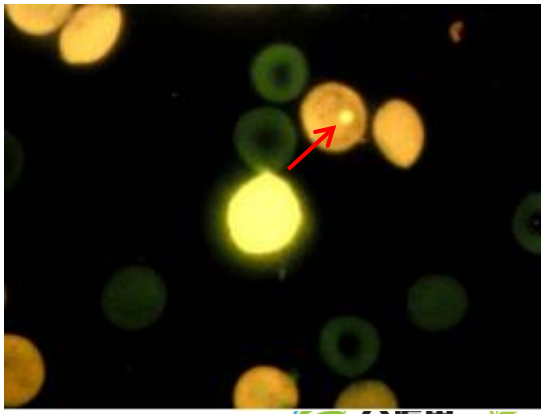
Mei et al., Optimization in Drug Discover: In Vitro Methods, 2014, pp.561.

Mammalian Erythrocyte Micronucleus Test



Giemsa





Acridine Orange

Contents

- Test Guidelines in Genetic Toxicology
- New Technologies in Genetic Toxicology

FCM Analysis Micronuleus – *In Vivo*

Dosing

Blood Collection（～100μl）

－80℃ fixed for at least 3d

Buffer washout

Centrifuge(300*g*,10min,4°C)

Labeling

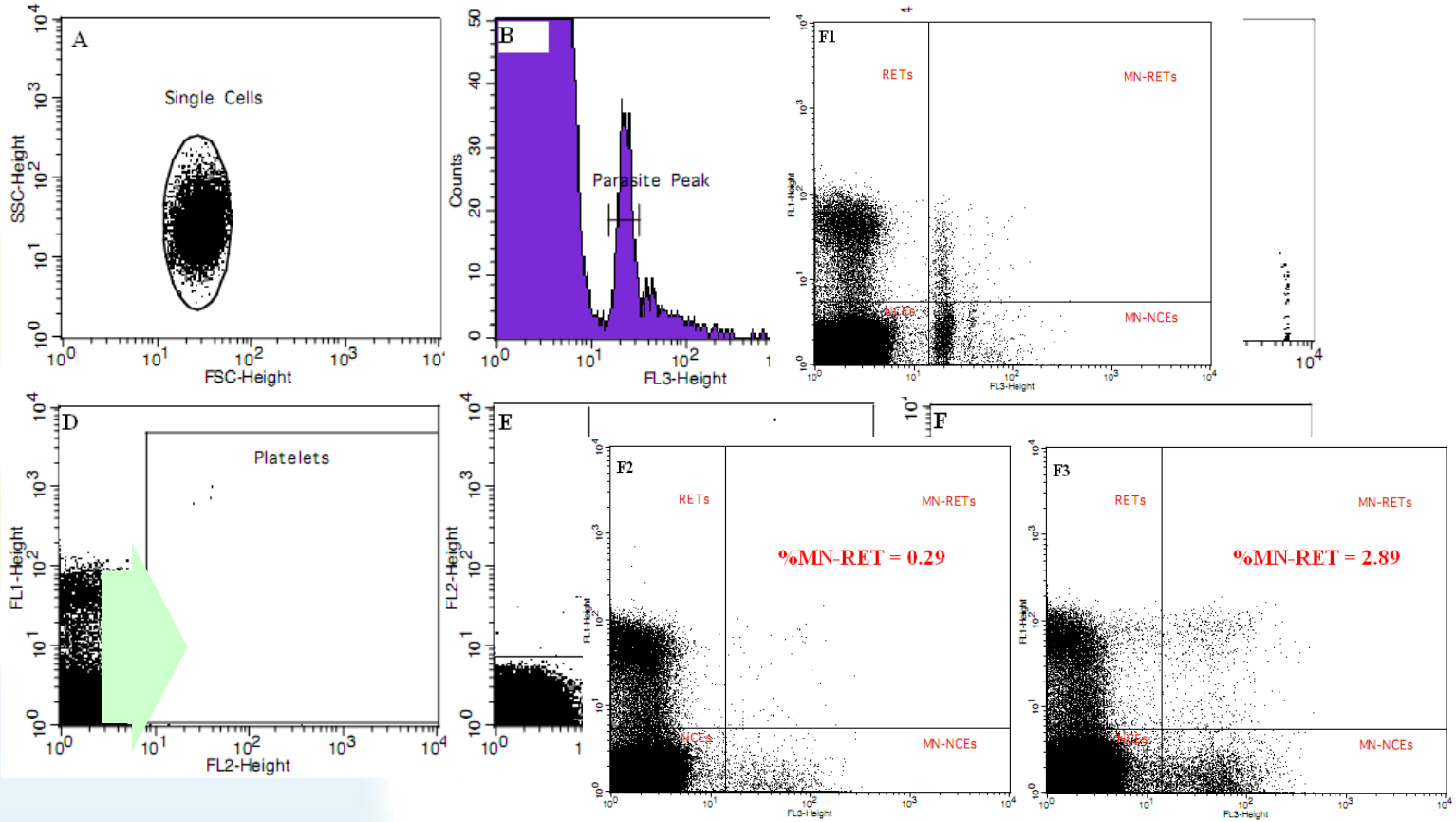
4℃, 30min

RT/37℃, 30min

FCM Analysis

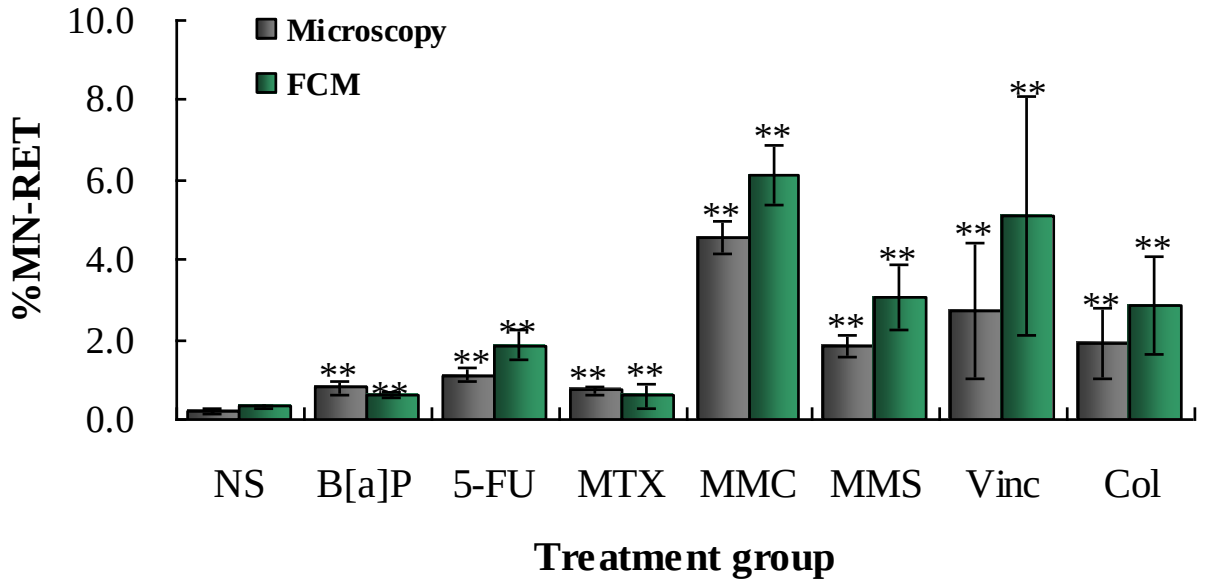
FCM Analysis Micronuleus – *In Vivo*

Template



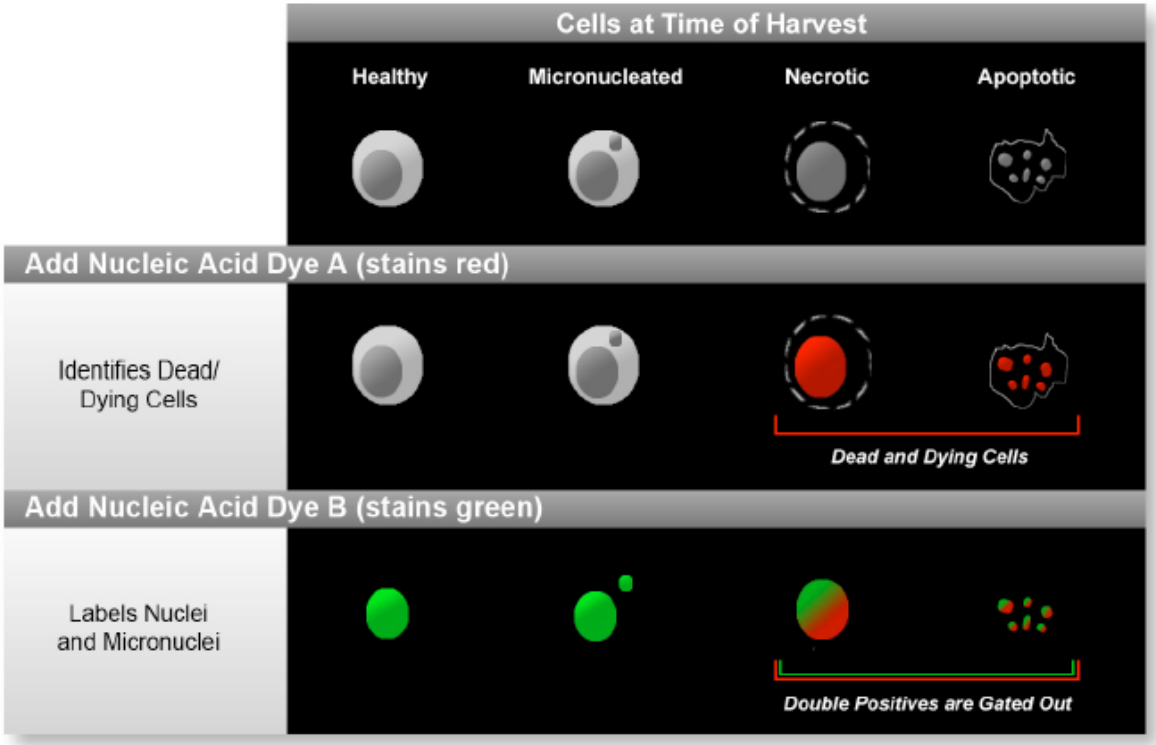
FCM Analysis Micronuleus – *In Vivo*

Case Study in NCDSER



FCM Analysis Micronuleus – *In Vitro*

Principle



Advantages

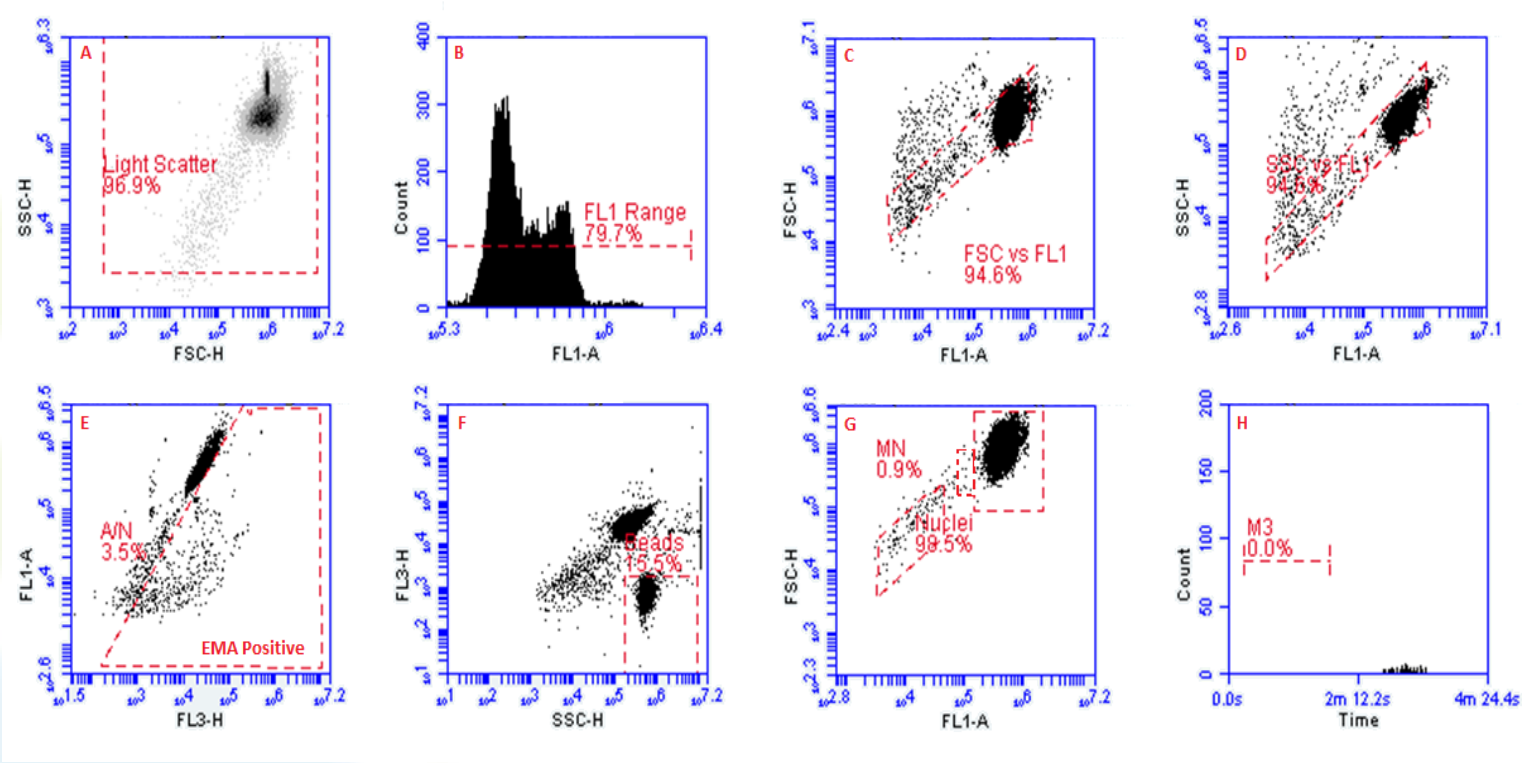
- Elimination of interference of toxicity
- Descrimination of clastogen and auengen
- Prompt the risk of polyploid

益诺思 INNOSTAR

国家上海新药安全评价研究中心 National Shanghai Center for New Drug Safety Evaluation and Research

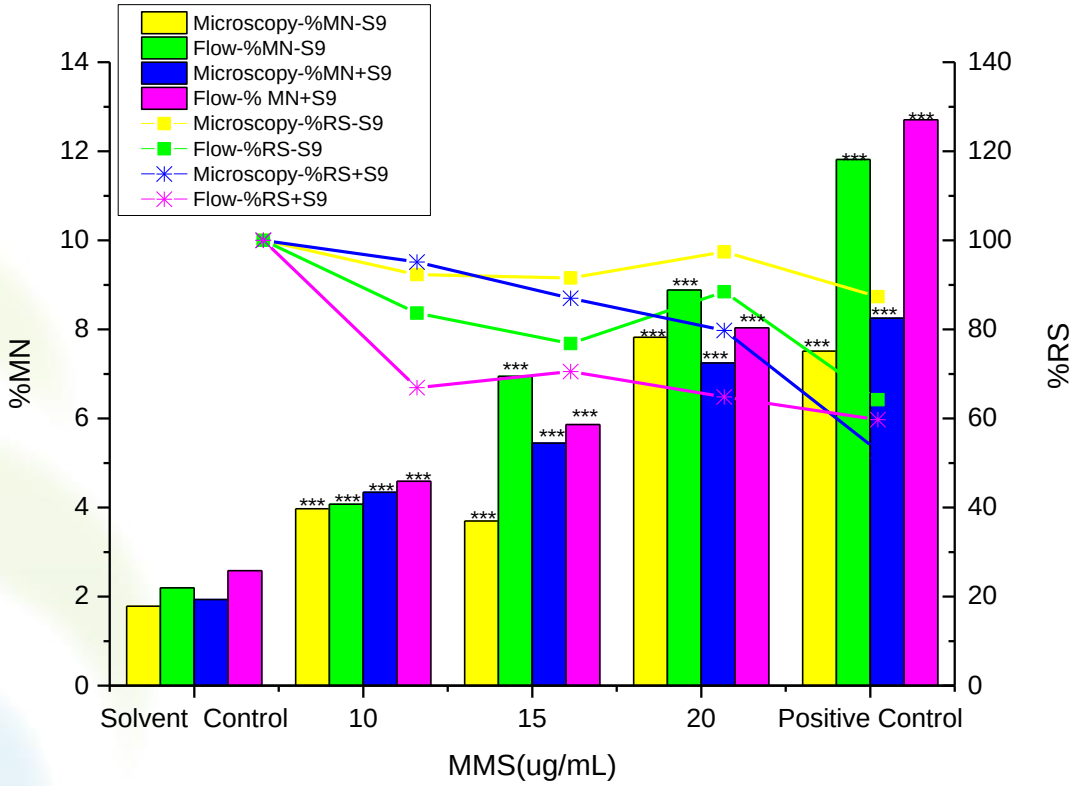
FCM Analysis Micronuleus – *In Vitro*

Template



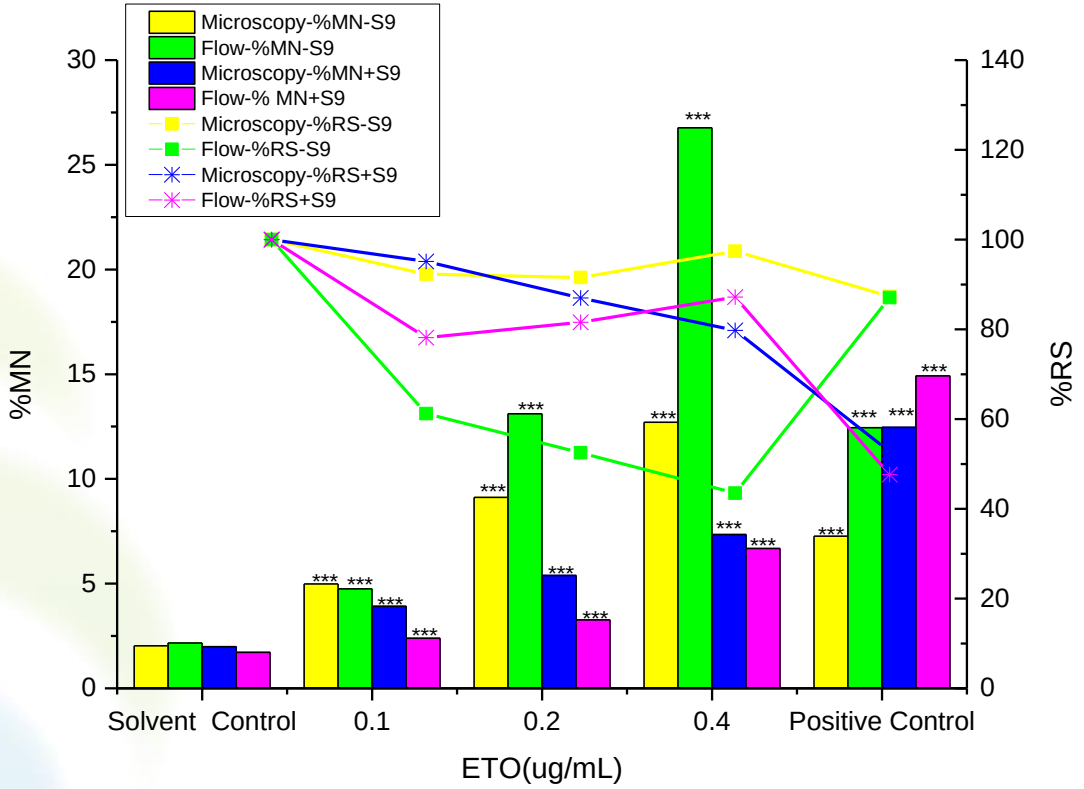
FCM Analysis Micronuleus – *In Vitro*

Case Study in NCDSER



FCM Analysis Micronuleus – *In Vitro*

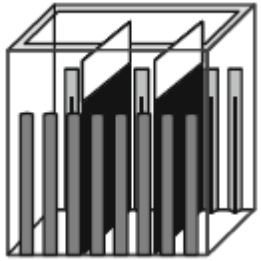
Case Study in NCDSER



Mammalian Alkaline Comet Assay

Tissue Sampling

Slide Preparing



Lysis

Unwinding & Electrophoresis

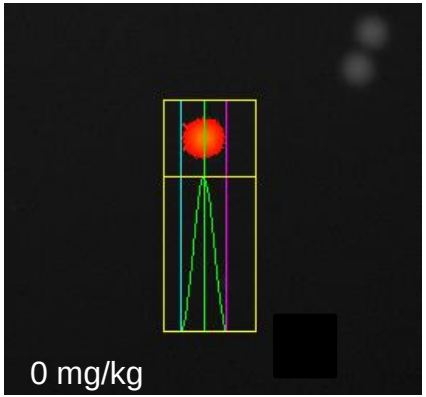
Comet Assay IV Analysis

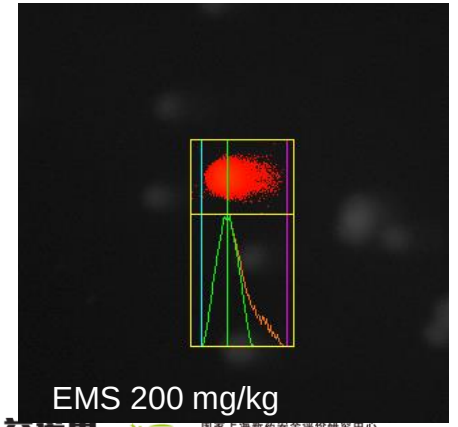
Mammalian Alkaline Comet Assay

Case Study in NCDSER

Group	Dosage (mg/kg)	$\overline{x}_{median} \pm s$		
		Liver	Stomach	Kidney
0.9%sodium chloride injection	0	0.04±0.02	1.05±1.29	0.05±0.03
EMS	50	5.53±2.42*	8.87±1.33*	6.09±4.77*
EMS	100	18.76±3.71*	22.25±6.07*	20.03±3.03*
EMS	200	22.87±1.19*	24.56±3.73*	25.32±2.29*

*: *P<0.05, compared with the vehicle control.*





Pig-a Mutation Assay

ICH M7(R1)(adopted on 31 Mar 2017) indicated the selection of other in vivo genotoxicity assays should be scientifically justified based on knowledge of the mechanism of action of the impurity and expected target tissue exposure.

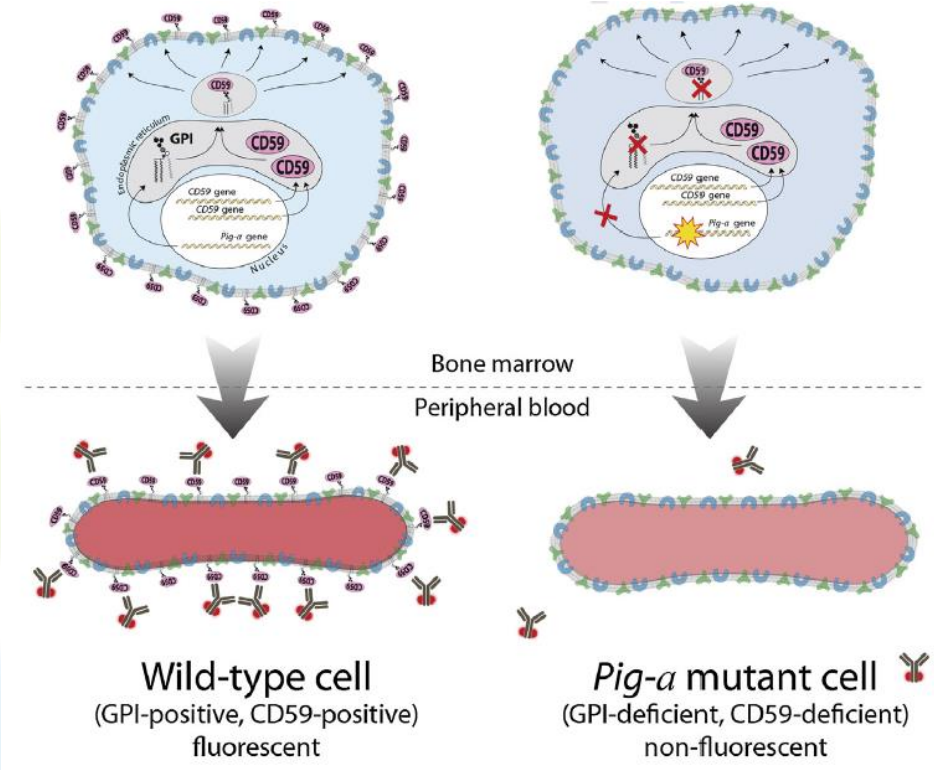
In vivo test	Factors to justify choice of test as fit-for-purpose
Transgenic mutation assays	• For any bacterial mutagenicity positive. Justify selection of assay tissue/organ
Pig-a assay (blood)	• For directly acting mutagens (bacterial mutagenicity positive without S9)*
Micronucleus test (blood or bone marrow)	• For directly acting mutagens (bacterial mutagenicity positive without S9) and compounds known to be clastogenic*

Rat liver Unscheduled DNA Synthesis (UDS) test	• In particular for bacterial mutagenicity positive with S9 only
	• Responsible liver metabolite known
	  ○ to be generated in test species used
	  ○ to induce bulky adducts

Comet assay	• Justification needed (chemical class specific mode of action to form alkaline labile sites or single-strand breaks as preceding DNA damage that can potentially lead to mutations • Justify selection of assay tissue/organ
Others	• With convincing justification

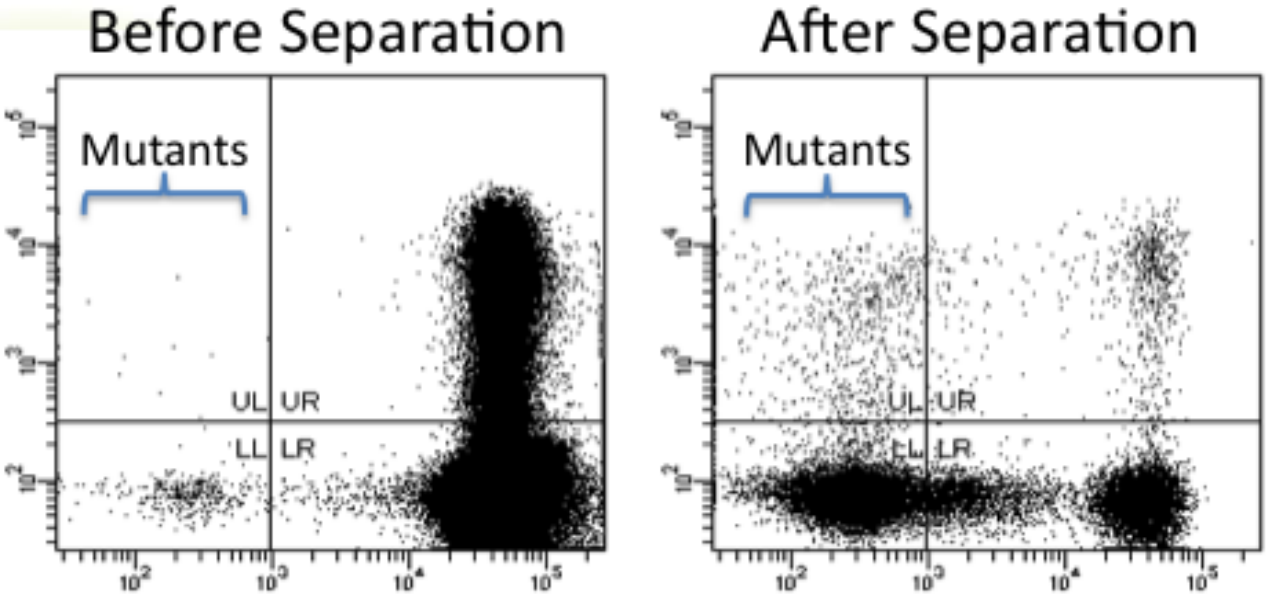
Pig-a Mutation Assay

Principle



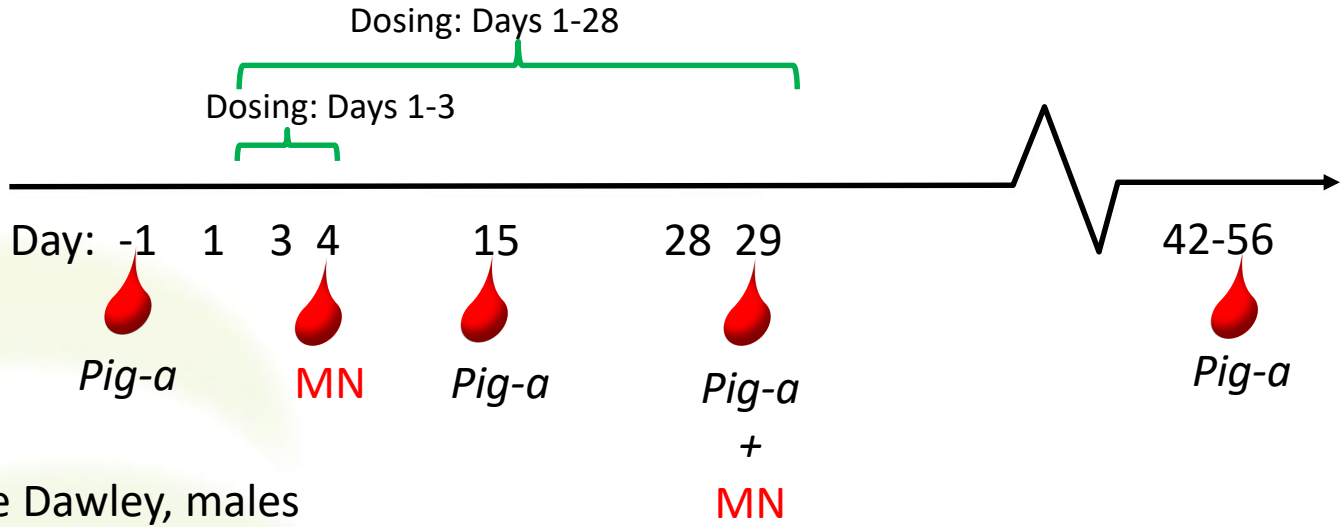
Pig-a Mutation Assay

Immunomagnetic Separation ("HTS" Method)



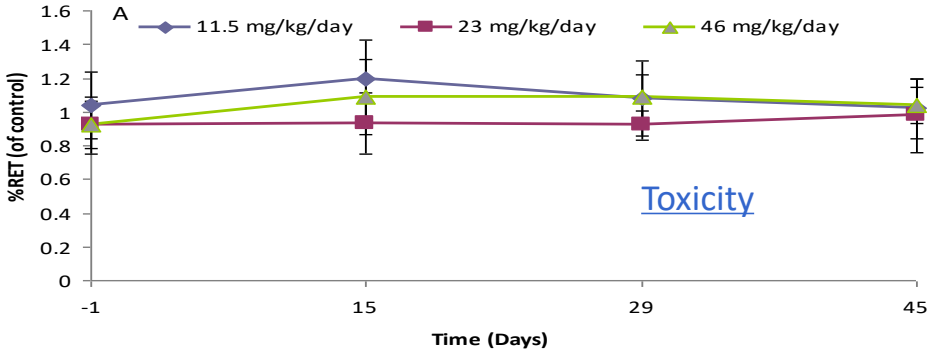
Pig-a Mutation Assay

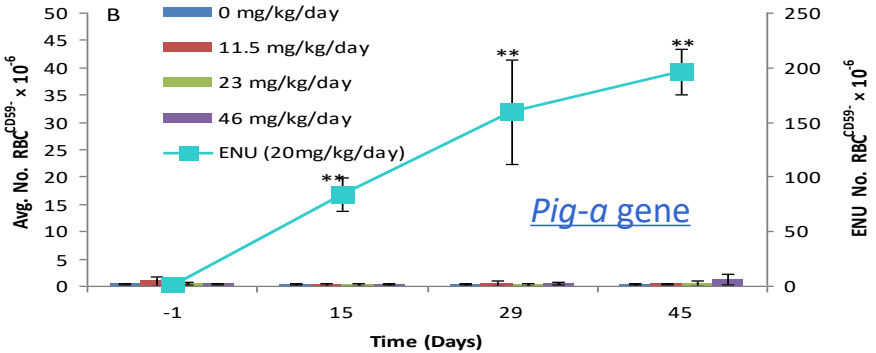
Dose Regimen

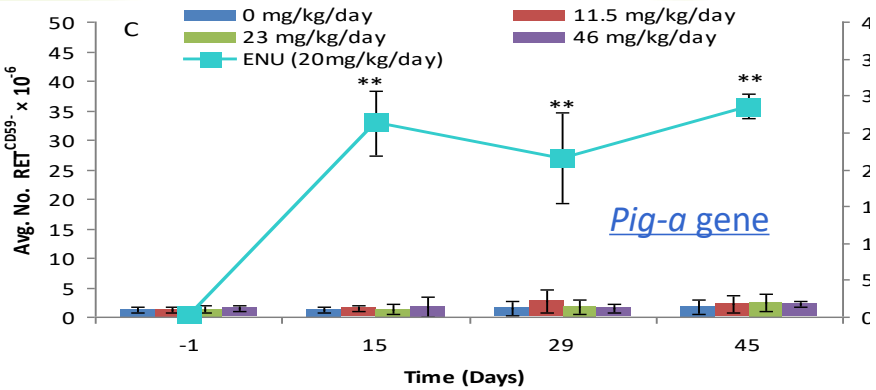


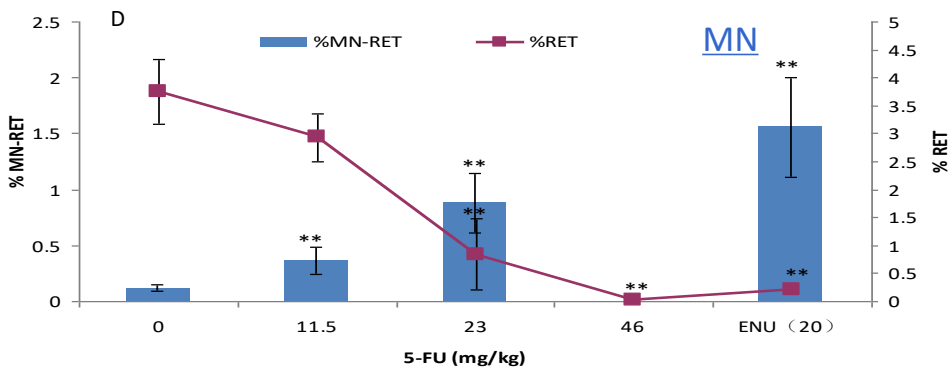
- Sprague Dawley, males
- n = 5 per group
- Oral gavage
- Vehicle + 3 dose groups +Pos Ctrl

Pig-a Case Study in NCDSER— *5-FU*









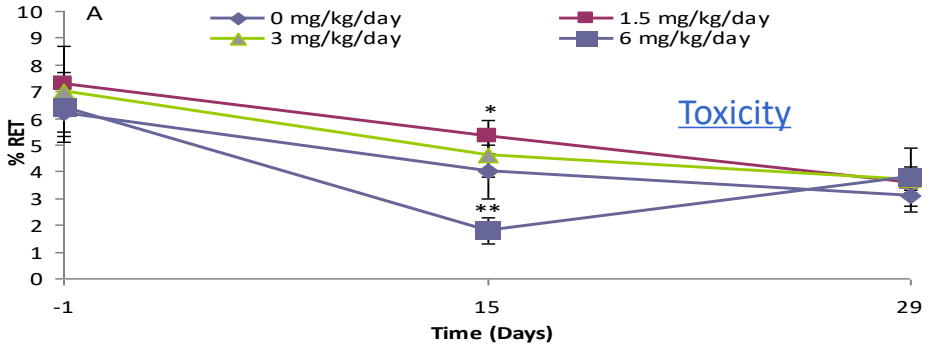
3 Days Dosing:

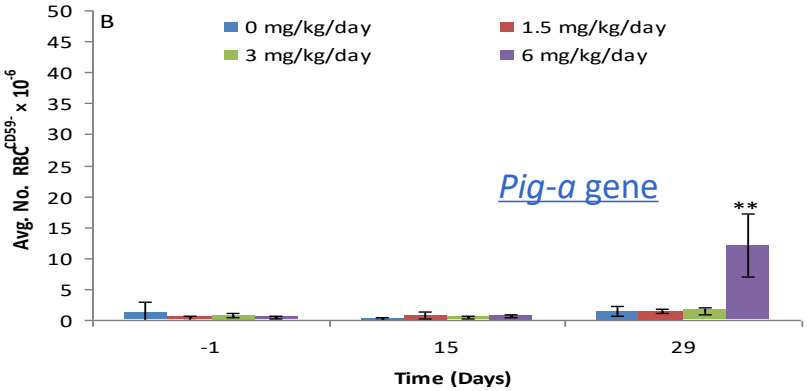
Pig-a: - MN: +

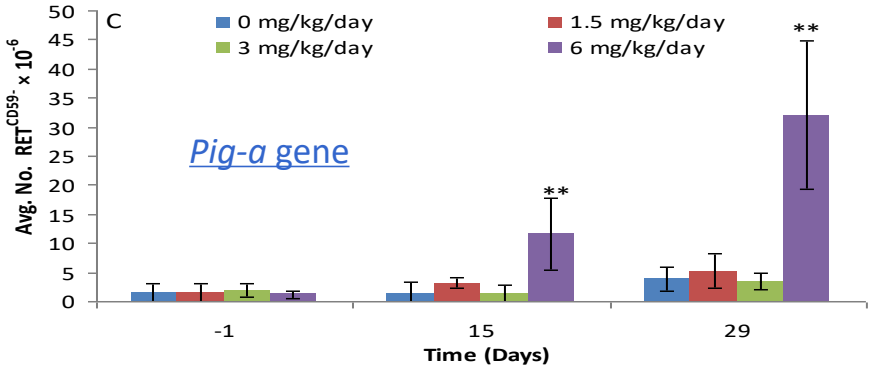
益诺思 INNOSTAR

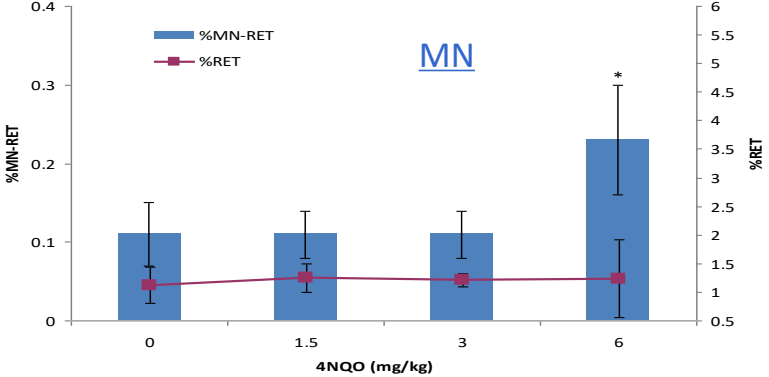
国家上海新药安全评价研究中心 National Shanghai Center for New Drug Safety Evaluation and Research

Pig-a Study in NCDSER— *4-NQO*





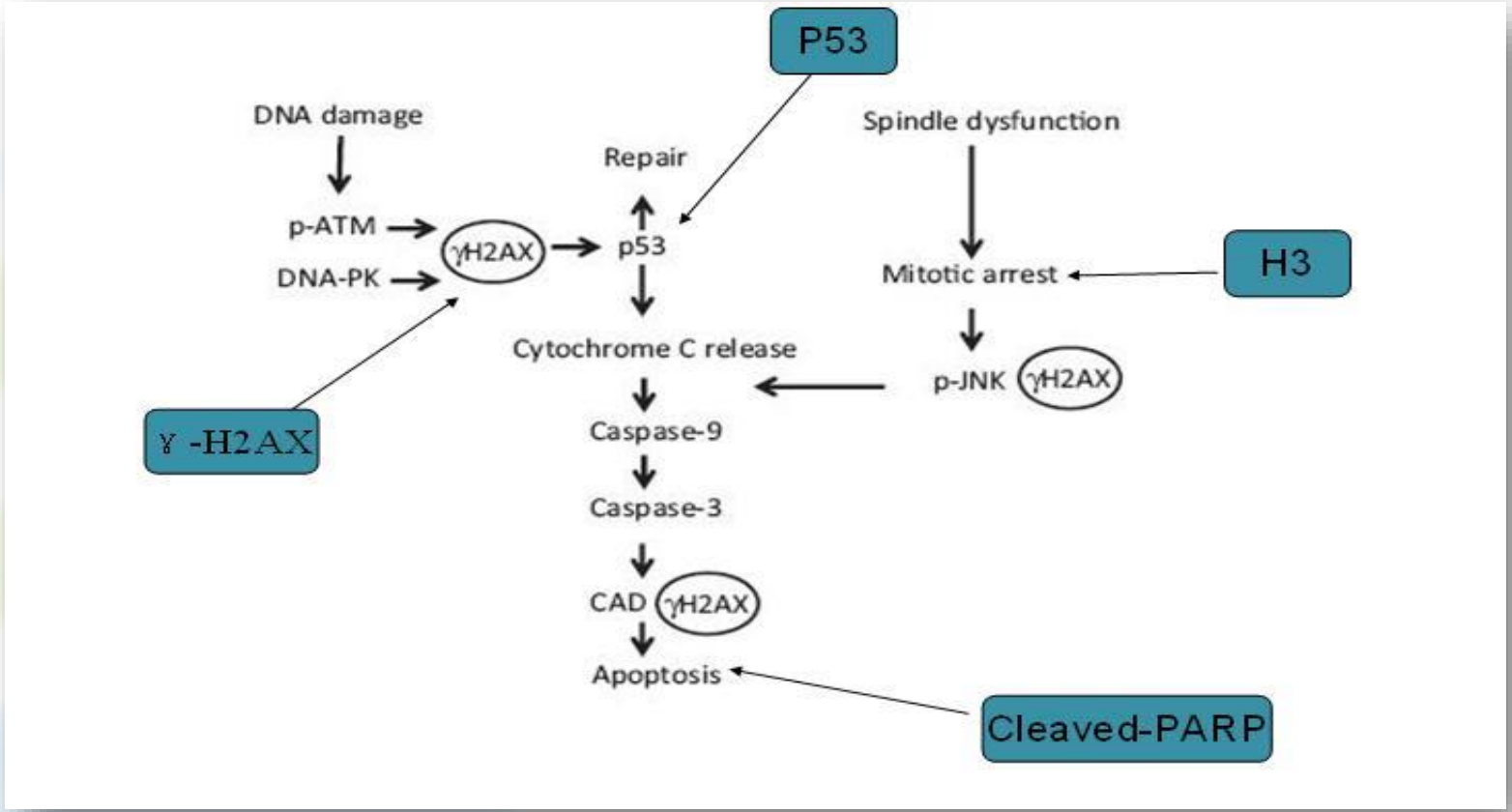




28 Days Dosing:

Pig-a: + *MN: +/-*

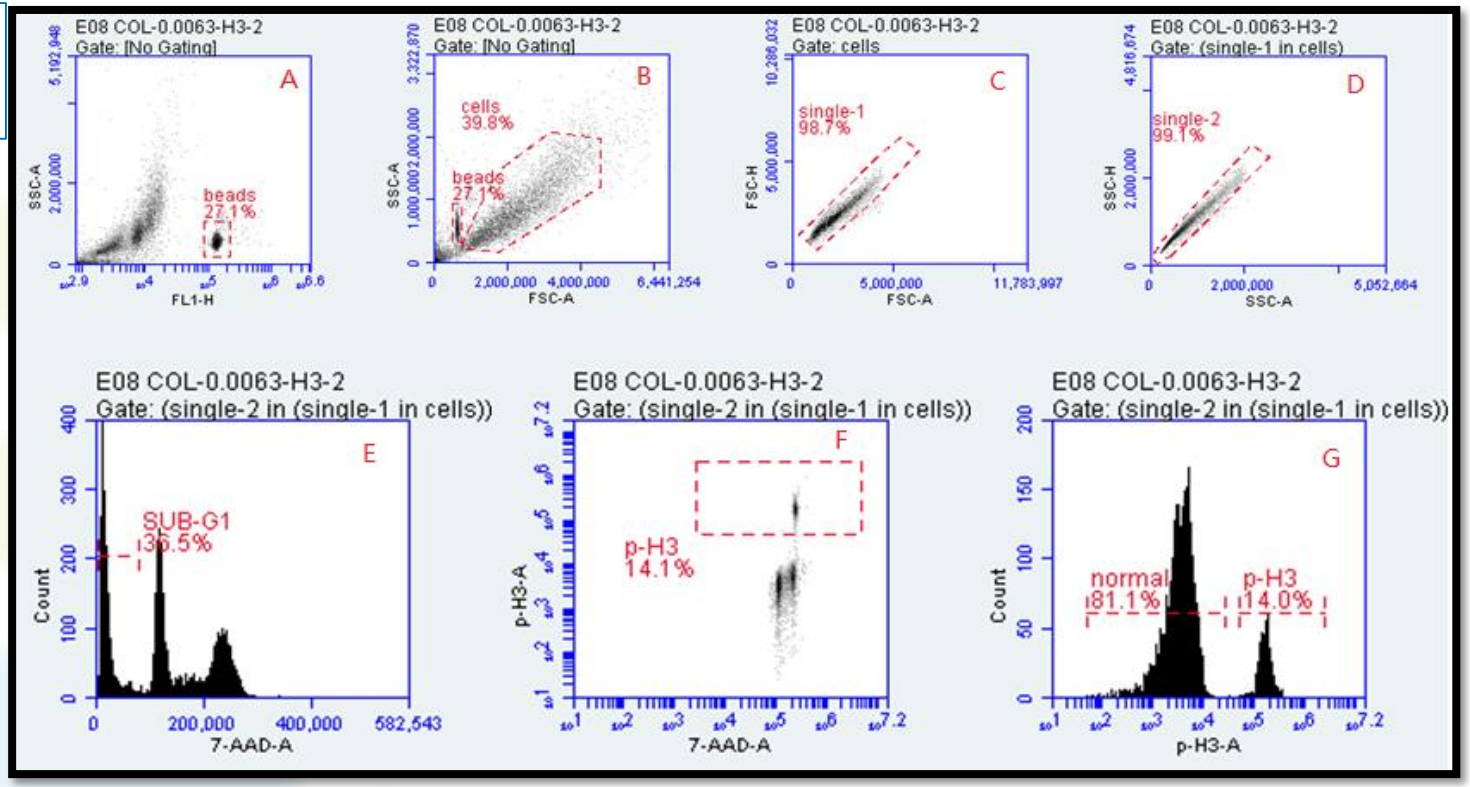
In Vitro Multi-biomarker Genotoxicity



TOXICOLOGICAL SCIENCES 140(1), 103–117 2014

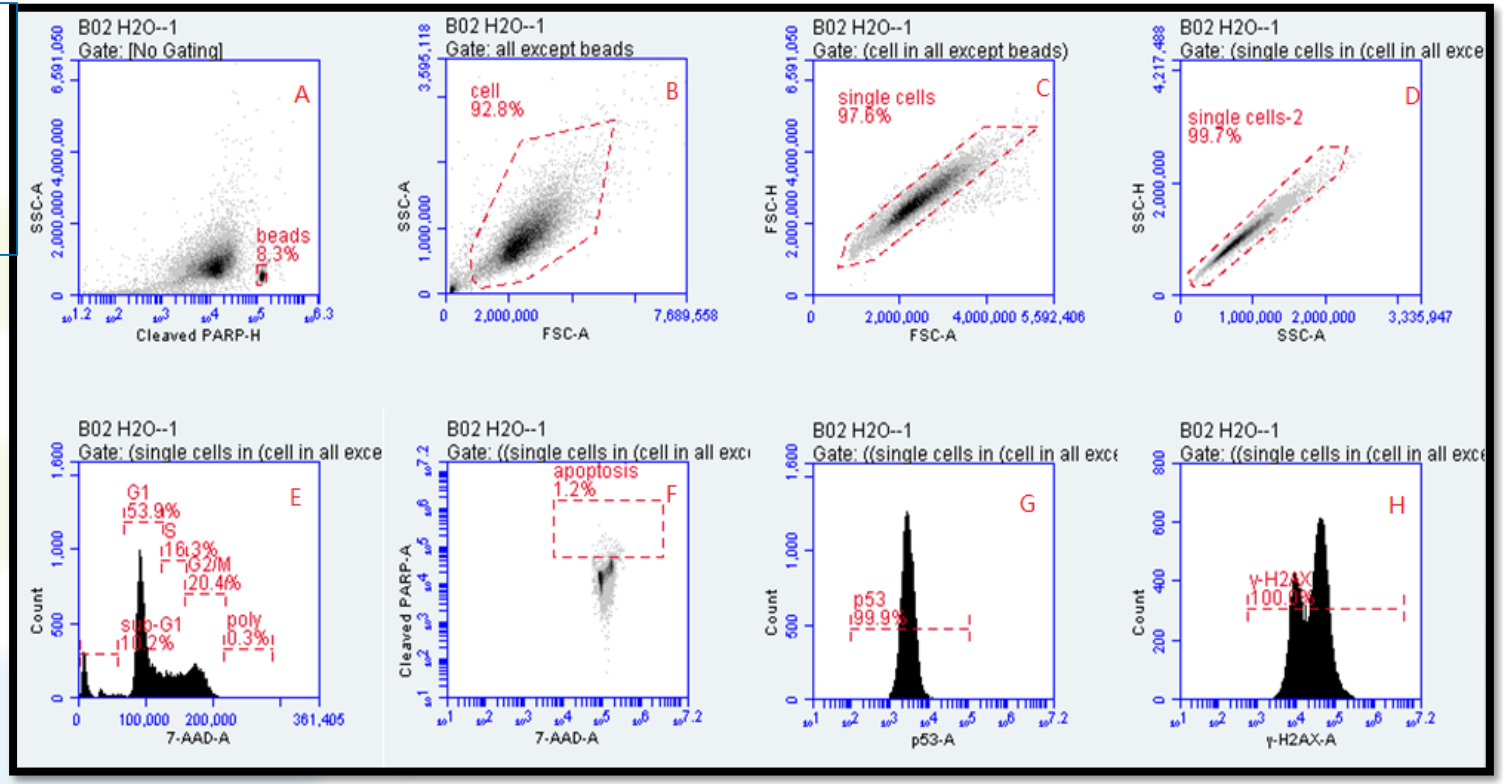
In Vitro Multi-biomarker Genotoxicity

Template 1:
p-H3

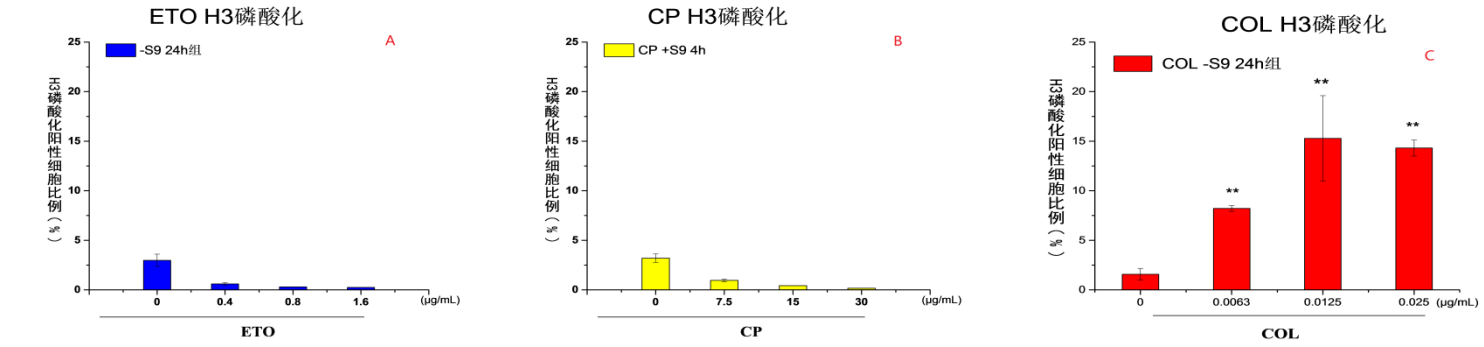


In Vitro Multi-biomarker Genotoxicity

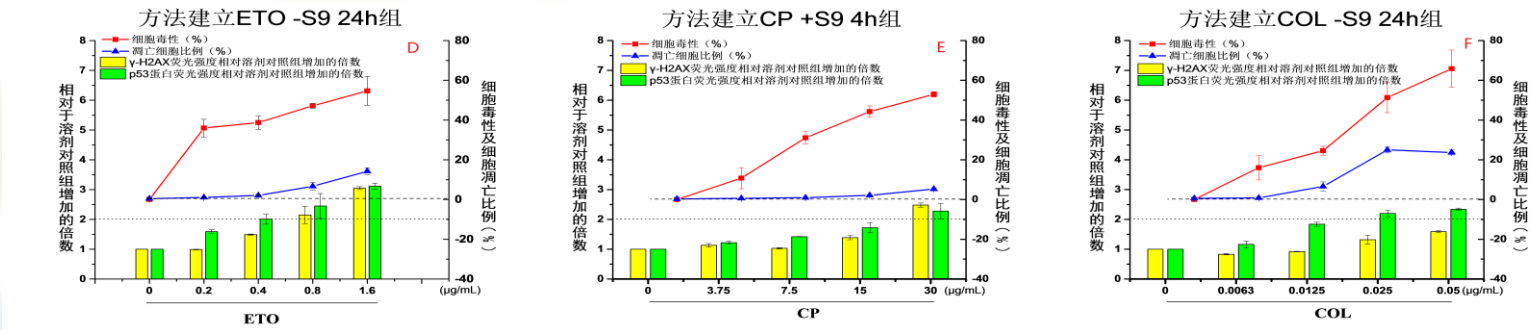
Template 2:
γ-H2AX/ p53/ Cleaved PARP /Cell cycle



In Vitro Multi-biomarker Genotoxicity



Figs A~C: p-H3 profiles for ETO、CP and COL- treated TK6 cells



Figs D~F: γ-H2AX, p53, cytotoxicity and apoptotic profiles for ETO、CP and COL- treated TK6 cells

In Vitro Multi-biomarker Genotoxicity

Case Study in NCDSER

1.Clastogen

2.Aneugen

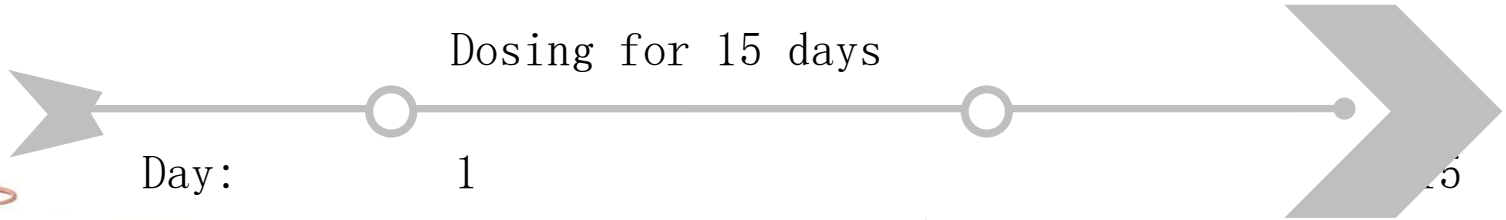
3.Nongenotoxicant

Compouds（Abbr.）	γ-H2AX	p53	p-H3	Classification
MMC				Clastogen
MMS				Clastogen
B[α]P				Clastogen
cDDP				Clastogen
PT				Aneugen
VCR				Aneugen
NaCl				Nongeno-
Amp G				Nongeno-
Prop				Nongeno-

Positive

Negative

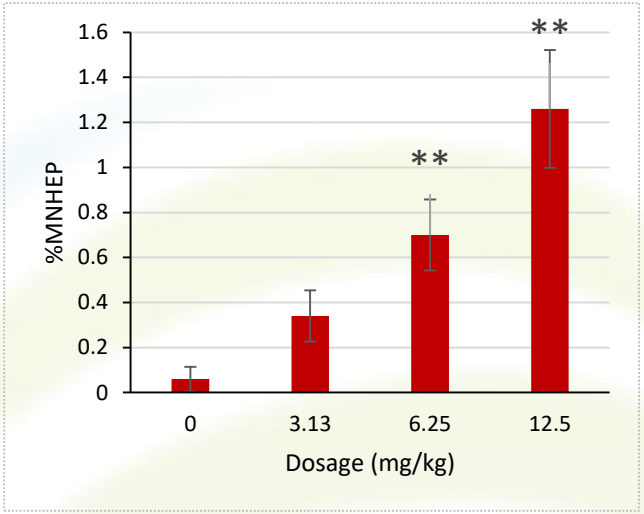
Liver Micronuleus Test



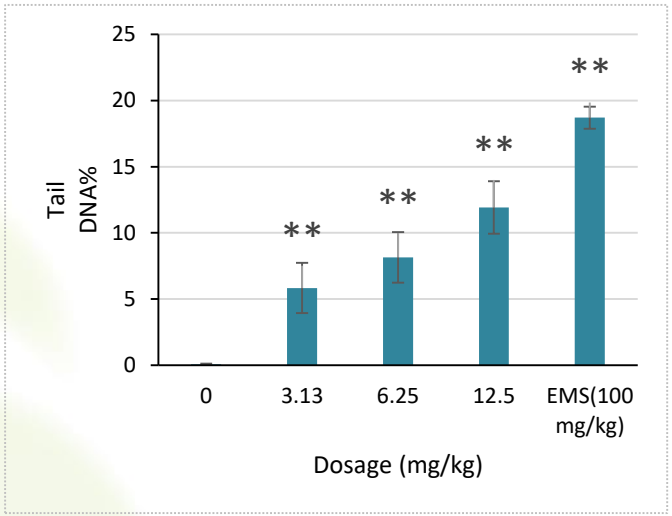
PBMN, LMN、Comet

- Sprague Dawley, males
- n = 5 per group
- Oral gavage
- Vehicle + 3 dose groups(DEN: 3.13, 6.25, and 12.5 mg/kg/day) +Pos Ctrl

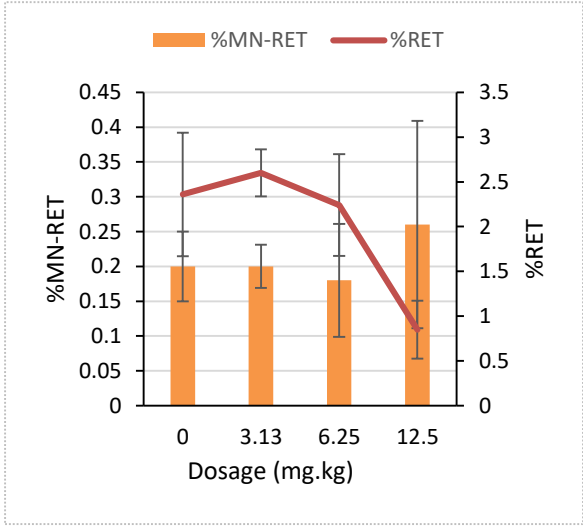
Liver Micronuleus Test



DEN MNHEP



DEN Comet



DEN PBMN

Liver Micronuleus Test

Case Study in NCDSER

Compounds	Expected Outcomes of Liver MNT	Results of Liver MNT	Published data for Multi-genotoxicity Tests	Results for Multi-genotoxicity Tests
Auramine O	Positive	Positive	PBMN:- Comet:+	PBMN:- Comet:+ Pig-a:-
O-aminoazotoleuene	Positive	Negative	PBMN:+ Comet:+	PBMN:+ Comet:- Pig-a:-
1,3-Propanesultone	Positive	Positive	PBMN:+ Comet:+ Pig-a:+	PBMN:+ Comet:+
Methyl Carbamate	Negative	Negative	PBMN:- Comet:- Pig-a:-	PBMN:- Comet:-

Thank You!

E-mail: ychang@ncdser.com