108學年度國立嘉義大學
「動物實驗使用者及動物飼養管理人員教育訓練」
主辦單位：國立嘉義大學實驗動物照護及使用委員會IACUC）

實驗動物於保健食品安全性評估與經驗分享
(The food safety evaluation in the laboratory animals)

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毒性(Toxicity)

•「藥即是毒」

- **毒性(toxicity)**
  ⇒ 描述物質特性的**形容詞**，如同描述顏色或沸點等
  ⇒ 指任何物質(藥物, 化學物或毒物...)造成傷害的能力(potency)

- Paracelsus(1564) :
  All substances are poison; there is none which is not a poison.
  The right dose differentiates a poison from a remedy.
  「劑量決定毒性」

藥劑毒性 (toxicity) + 暴露量 (exposure) = 危害 (hazard)

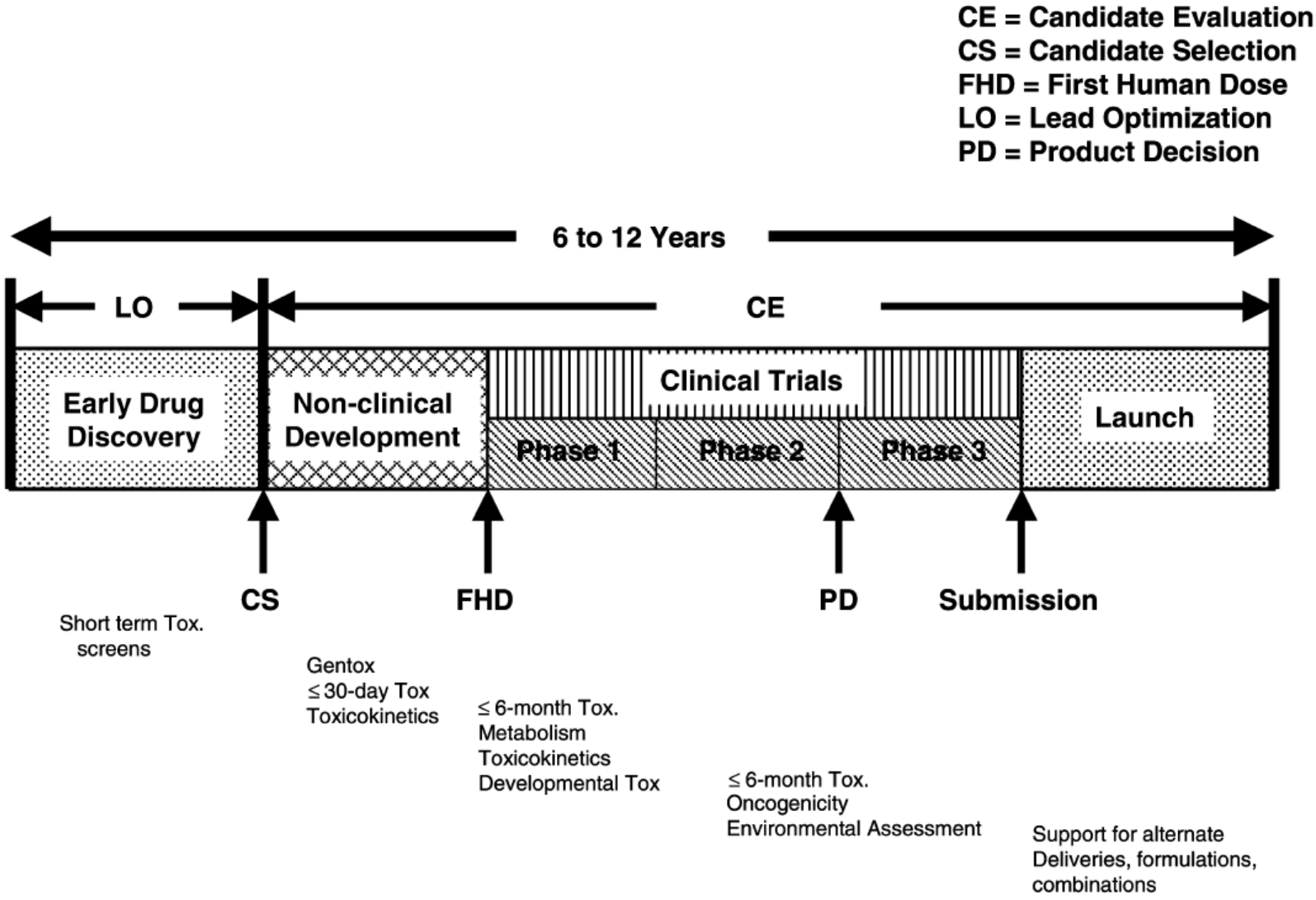


Fig. 2. High-level relationship of toxicology profile to phases of drug development.

M.A. Dorato, J.A. Engelhardt / Regulatory Toxicology and Pharmacology 42 (2005) 265–274

新藥物之毒理試驗需求

•藥物研發

分析方法之建立
製造毒理測試樣品
製造臨床試驗樣品
• 代謝與藥物動力學
吸收、分佈、代謝、排泄等

純度、不純物檢測方法
臨床試驗藥物配方開發
環境影響評估.....
藥物-蛋白質結合分析..

•臨床前毒理(安全性)評估

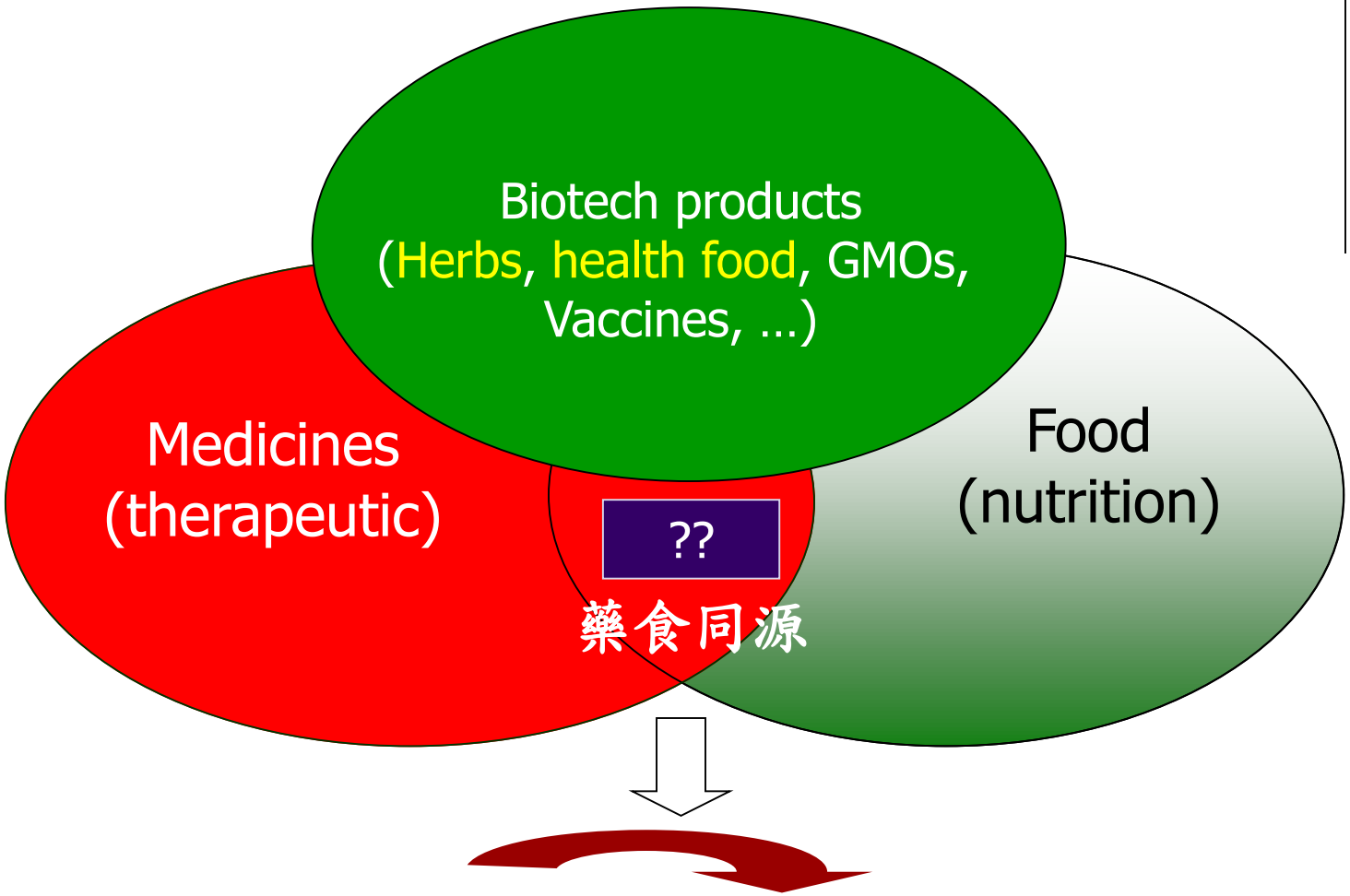
急性毒性試驗　**亞慢毒性試驗**　**基因毒性試驗**
生殖毒理試驗　**慢毒性/致癌性**　**其他毒理試驗**

Table 4.3 EPA/FIFRA Requirement for Hazard Evaluation of Pesticides

GUIDELINE NO.	REVISED 870 GUIDELINE	TYPE OF TOXICITY STUDY	TEST SYSTEM	OBJECTIVE	APPROXIMATE COST/STUDY (US$)
81-1	1100	Acute oral	Rats	Define toxic dose by ingestion	2000
81-2	1200	Acute dermal	Rabbits	Define toxic dose by absorption through skin	1500
81-3	1300	Acute inhalation	Rats	Define toxic dose by inhalation	5000
81-4	2400	Ocular	Rabbits	Assess eye irritation/injury	1500
81-5	2500	Skin irritation	Rabbits	Assess skin irritation/injury	100
81-6	2600	Sensitization	Guinea pigs	Assess allergic potential	3000
81-7	6100–6855	Neurotoxicity*†	Hens/rats	Assess nervous system injury	25,000†
84-2	5100–5915	Mutagenicity‡	In vivo/in vitro	Determine genotoxic potential; screen for carcinogenicity	5,000§
82-1	3050–3465	Range-finding‡	Rats	Determine effects following	70,000
		Subacute (28- to 90-day§)	Mice	repeated doses; set dose level	70,000
			Dogs	for longer studies	100,000
			Rabbits		75,000
			Tats	Identify target organs; set dose	190,000
			Mice levels for chronic studies		190,000
83-5	4200–4300	Carcinogenicity/ Chronic toxicity	Rats	Determine potential to induce	1,400,000
83-2			Mice	tumors; define dose-response relationships (lifetime)	800,000
83-1			Dogs	Determine long-term toxic effects (1 year)	400,000
83-3	3550–3800	Reproduction and teratogenicity	Rats	Determine potential to cause fetal	505,000
83-4			Rabbits	abnormalities and effects on development, fertility, pregnancy, and development of offspring over at least two generations	
85-1	7485	Toxicokineties	Rats Mice	Determine and quantitate the metabolic fate of a pesticide	100,000

Casarett & Doull's Toxicology. 2008

NT$:141,487,500



一般用詞

- 保健食品
- 機能性食品
- 生技產品

法定名詞

- **健康食品**
  (Health food,
  DOH, 1999)

陳樹功, 2003

需經動物試驗佐證
有效性及安全性

已知，
不需經動物試驗

http://www.fda.gov.tw/upload/122/20130625141844306 31.jpg

衛生署公告功能性項目及審核通過之健康食品 (1999/1-2006/4)

保健功效項目	評估指標	通過件數
1. 腸胃功能改善	促進消化; Probiotic; 胃黏膜細胞...	25
2. 調節血脂	TG, TC (HDL-C, LDL-C, Oxidation)...	25
3. 免疫調節	Non-specific (Con-A) and specific OVA)..	9
4. 護肝功能	GOT, GPT, TG, TC, Antioxidant, Fibrosis...	7
5. 幫助骨質保健	High serum Ca^{2+}; Bone density; low PTH...	1
6. 牙齒保健	Sucrose-induced; *Strep. Sorbrinus*....	2
7. 調節血糖	STZ-induced: Glucose level, Insulin, HbA_{1C}	2
8. 抗疲勞	運動能力; 生化值(BUN, glycogen, Lactate)...	3
9. 延緩老化	Survival rate; D-galactose-induced ROS...	1
10.減少脂肪	High energy food, BW, food intake, fat....	2
11.降低癌症發生	**致癌物致癌模式...**	
12.減輕腫瘤放療	**T細胞功能檢測...**	
總計		77

(DOH, 2006/4)

衛福部審核通過之健康食品一覽表

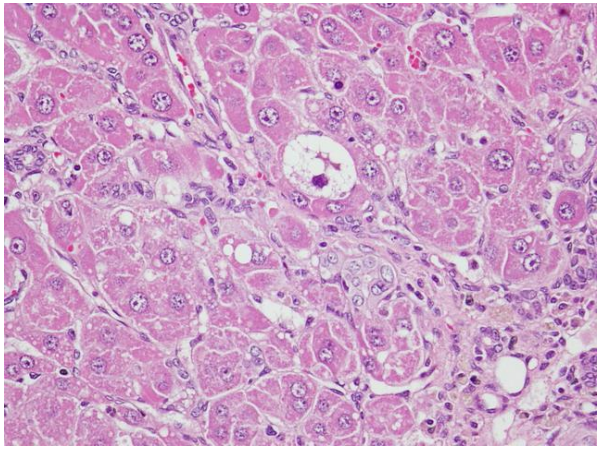
(1999/1-2018/2)

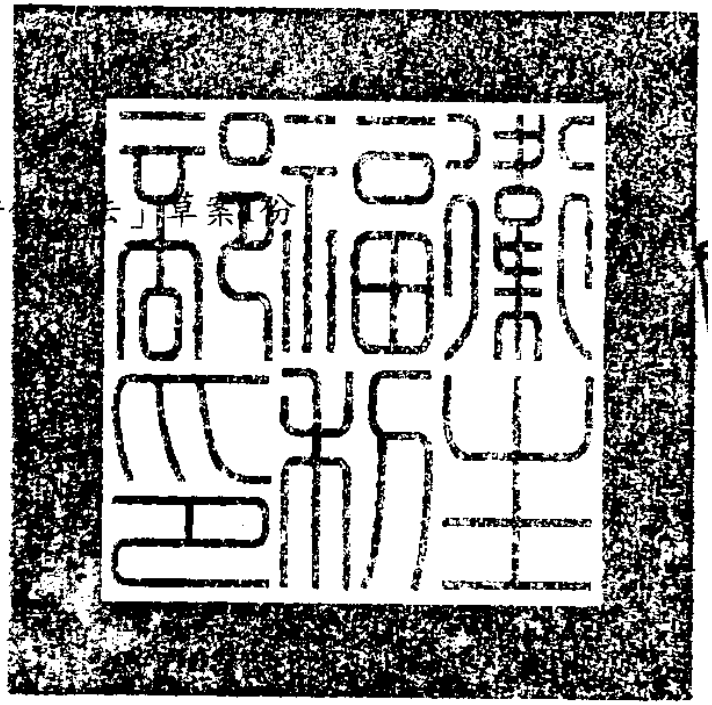
保健功效項目	評估指標	通過件數
1. 調節血脂	**TG, TC (HDL-C, LDL-C, Oxidation)...**	**155**
紅麴（規格標準）-調節血脂功能		**39**
魚油（規格標準）-調節血脂功能		**25**
2. 胃腸功能改善	促進消化; Probiotic; 胃黏膜細胞…	80
3. 免疫調節	Non-specific (Con-A) and specific OVA)..	45
4. 護肝功能	GOT, GPT, TG, TC, Antioxidant, Fibrosis…	42
5. 骨質保健	High serum Ca^{2+}; Bone density; low PTH…	20
6. 牙齒保健	Sucrose-induced; *Strep. Sorbrinus*….	6
7. 調節血糖	STZ-induced: Glucose level, Insulin, HbA_{1C}	18
8. 抗疲勞	運動能力; 生化值(BUN, glycogen, Lactate)…	15
9. 延緩衰老功能	Survival rate; D-galactose-induced ROS…	5
10.不易形成體脂肪功能	High energy food, BW, food intake, fat….	19
11.輔助調整過敏體質功能		21
12.輔助調節血壓功能		3
13.促進鐵吸收功能		5
總計		498

https://consumer.fda.gov.tw/Food/InfoHealthFood.aspx?nodeID=162#

(TFDA, 2018/2)

發文日期：中華民國103年3月4日
發文字號：部授食字第1031300396號
附件：「健康食品之護肝功能(針對化學性肝損傷)評估方法」草案1份





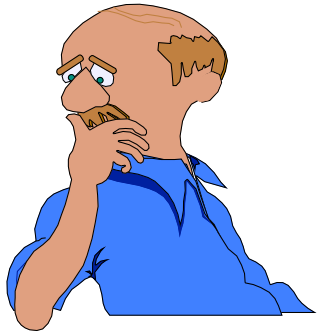
（五）**組織病理切片觀察**：

3. 病理切片之判讀

本評估方法將肝組織進行H&E 染色，以方便觀察肝細胞的受損、脂肪堆積、壞死等慢性肝損傷之變化：

....**至於病理的半定量分析評估，則應由獸醫病理醫師 (病理專科獸醫師)**，在不清楚本實驗設計的情況下進行單盲封讀，對所有切片進行評分比較（評分表如表二），最後再以統計分析方法進行各組差異性的分析

Safety ??



Function ??

健康食品安全性毒理評估

- 第一類：(免提毒性測試資料)
  - 產品之原料為傳統食用且以通常加工食品形式供食者
  - 產品具有完整之毒理學安全性學術文獻報告及曾供食用之記錄
- 第二類：(產品之原料為傳統食用而非以通常加工食品形式供食者)
  - 基因毒性
  - 28天餵食毒性試驗
- 第三類：(產品之原料非屬傳統食用者)
  - 基因毒性
  - 90天餵食毒性試驗
  - 致畸胎毒性試驗
- 第四類：(產品之原料非屬傳統食用且成份含有致癌物之類似物者)
  - 基因毒性
  - 致畸胎毒性試驗
  - 致癌性試驗
  - 90天餵食毒性試驗
  - 後代繁殖試驗

(DOH, 2002)

「健康食品安全性評估方法修正草案產官學共識會」會議

21. 28 天動物重複餵食試驗不建議以小鼠進行，以免血量不足。血液、尿液檢驗為必測：血清生化項目增加為creatine phosohokinase (CPK）等21 項：器官秤重及組織病理切片8 項。
22. 基因毒性應加入檢體溶液過濾沉澱物的說明，但實際執行困難。
23. 90 天餵食試驗為致癌性試驗的前試驗，故需進行完整的25+8 項器官病理切片，取消＊視試驗需要才進行。器官秤重8 項。

24. **粗修片方式統一比照INHAND 國際標準，以符合國際毒性病理判讀方式，毒性病理組織切片建議由從事病理相關獸醫師進行判讀及簽署，或由病理專科獸醫師判讀為佳，**以鼓勵國內獸醫師報考並通過“病理專科獸醫師”專科認證。本次會議決議此項建議尚不列入修正草案中，改以行政考量辦理。

25. 動物體內微核試驗不建議併在大鼠28 天餵食試驗進行，單獨以較敏感的小鼠進行為宜。
26. **口服急毒性試驗〔Acute oral lethal dose study〕可作為預試驗參考，但不納入評估必要項目。**
27. 安全性第二類產品除了提供相關試驗外，也應提供組成／安全性文獻／食用紀錄的佐證，以作為MOS 判斷放鬆的依據。

107年病理專科獸醫師甄審

『健康食品』安全性毒理評估

- 第一類：(免提毒性測試資料)
  - 產品之原料為傳統食用且以通常加工食品形式供食者
  - 產品具有完整之毒理學安全性學術文獻報告及曾供食用之記錄

- 第二類：(產品之原料為傳統食用而非以通常加工食品形式供食者)
  - 基因毒性 (Ames with 5 strains, CA, MN assays)
  - 28天餵食毒性試驗

- 第三類：(產品之原料非屬傳統食用者)
  - 基因毒性
  - 90天餵食毒性試驗
  - 致畸胎毒性試驗

- 第四類：(產品之原料非屬傳統食用且成份含有致癌物之類似物者)
  - 基因毒性
  - 致畸胎毒性試驗
  - 致癌性試驗
  - 90天餵食毒性試驗
  - 後代繁殖試驗

(TFDA, 2002)

毒理試驗參考規範

1. 衛福部健康食品安全性評估試驗規範，1999

2. 衛福部藥品非臨床試驗安全規範，2014

3. OECD GUIDELINES FOR TESTING OF CHEMICALS SECTION 4 - HEALTH EFFECTS, No. 407-453, 2018
https://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm

4. Health Effects Test Guidelines, *OPPTS Harmonized Test Guidelines,* Series 870, EPA 712-C-98-201
http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series870.htm

基因毒性試驗
(Genetic assays, *in vitro*)

1. Ames test:

1.1. Mortelmans K, and Zeiger E. 2000. The Ames Salmonella/microsome mutagenicity assay. Mutat Res. 455(1-2):29-60.

1.2. USEPA, Office of Prevention, Pesticides and Toxic Substances. 1998. Bacterial Reverse Mutation Test. In: *OPPTS Harmonized Test Guidelines*, Series 870.5100, EPA 712–C–98–247, 13 pp. Washington, DC.

1.3. Organization for Economic Cooperation and Development. 2002. Bacterial Reverse Mutation Test. In: *OECD Guideline for the Testing of Chemicals.* Section 4: Health Effects, No: 471, 14 pp., Adopted: 21th July, 1997.

2. Micronucleus test (MN):

2.1. Hayashi, M., Sofuni, T., and Jr, M.I. 1983. An application of acridine orange fluorescent staining to the micronucleus test. Mutation Research. 120, 241-247.

2.2. USEPA. 1998. Mammalian Erythrocyte Micronucleus Test. In: OPPTS *Harmonized Test Guidelines*, Series 870.5395, EPA 712-C-98-226. 10 pp.

2.3. Organization for Economic Cooperation and Development. 2016. Mammalian Erythrocyte Micronucleus Test. In: *OECD Guideline for the Testing of Chemicals.* Section 4: Health Effects, No: 474, 10 pp. Adopted: 29 July 2016.

3. Chromosome aberration test (CA):

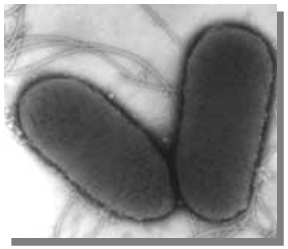
3.1. OECD. 2016. *In vitro mammalian chromosome aberration test.* Guideline for the Testing of Chemicals, No. 473, adopted 29 July 2016.

3.2. Savage, J. R. K. 1999. An introduction to chromosomal aberrations. In: *Atlas of Genetics and Cytogenetics in Oncology and Haematology*. http://atlasgeneticsoncology.org

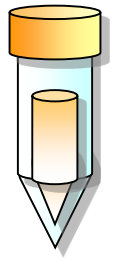
3.3. USEPA. 1998. In vitro Mammalian Chromosome Aberration Test. In: *Health Effects Test Guidelines, OPPTS Harmonized Test Guidelines,* Series 870.5375, EPA 712-C-98-223, p. 1-13.

1. 微生物基因突變分析(gene mutation in bacteria)

菌株使用下列5種菌株：*S. typhimurium* TA98, 100, 1535, 1537, 102



TA strains of *Salmonella* typhimurium

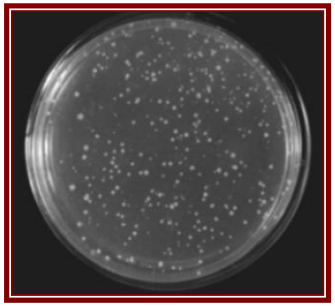


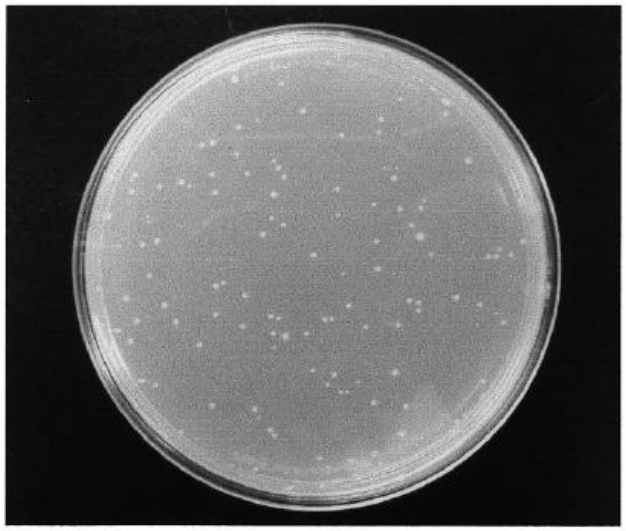
Incubated overnight to a final conc. of 1~2 × 10^9 cfu/ ml

Histidine & Biotin 0.2 ml
with or without S9 0.2 ml
Samples 0.1 ml

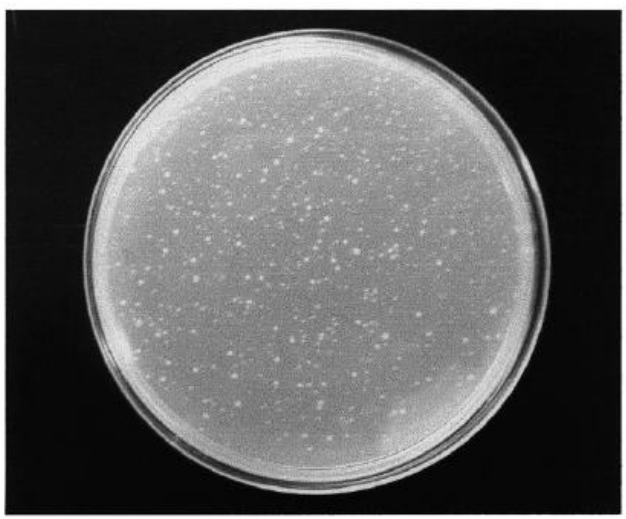
2 ml soft agar

incubation for 48 hrs

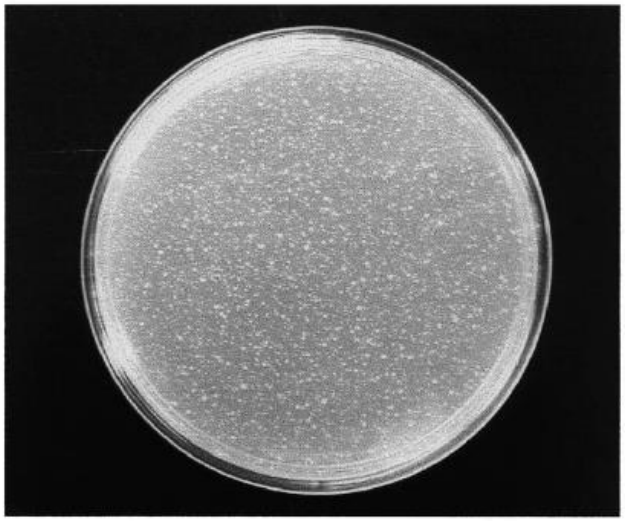




Control



Dose 1



Dose 2

Fig. 3. Mutagenic dose response with strain TA100 and sodium azide. Control: spontaneous revertants; dose 1:2.5 mg/plate; dose 2:5 mg/plate.

K. Mortelmans, E. Zeiger / Mutation Research 455 (2000) 29–60

Revertant changes of test article in Salmonella TA98 mutagenicity test

Group/ replicate	Blank control	4-NQO[1] (positive control)	TA98/without S9 activation xxx (mg/plate)				
			0.25	0.5	1	3	5
1	23	252	17	28	22	21	32
2	41	360	23	24	31	23	31
3	32	294	24	25	39	29	23
Mean	32	302*	21.3	25.67	30.67	24.3	28.67
SD	9	54.4	3.8	2.1	8.5	4.2	4.9

Group/ replicate	Blank control	2-AA[2] (positive control)	TA98/with S9 activation xxx(mg/plate)				
			0.25	0.5	1	3	5
1	37	1736	38	34	33	28	32
2	38	1872	42	39	42	32	22
3	40	2168	42	29	34	32	37
Mean	38.3	1925.3*	40.67	34	36.3	30.67	30.3
SD	1.5	220.9	2.3	5	4.9	2.3	7.6

Data are expressed as the mean±SD (n = 3).

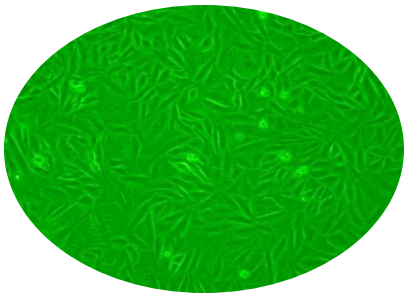
[1] 4-nitroquinoline-N-oxide, 1μg/plate, as positive control in assay without S9 activation.

[2] 2-aminoanthracene, 5 μg/plate, as positive control in assay with S9 activation.

* Significant difference of colonies more than two folds of blank control and treated groups at $p < 0.05$.

2. 體外哺乳類細胞染色體異常試驗

(Chromosomal Aberration Test with Mammalian Cell)



CHO-K1：

2×10^5 cells/25T flask

- Positive control:
- - Mitomycin (2.5 ug/ml) (-S9 mix)
  - CP (25 ug/ml (+S9 mix)
- Negative control：SDW
- xxx: 1.25, 2.5, 5 mg/ml

Incubated for 21 hrs

Add 100 μl colcemid/flask

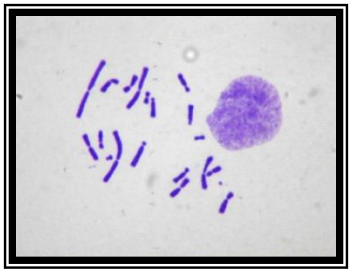
Incubated for 3 hrs

Cells are harvested by 0.25% trypsin

Incubated for 1 min

0.5% KCl

methanol/acetic acid solution fixation



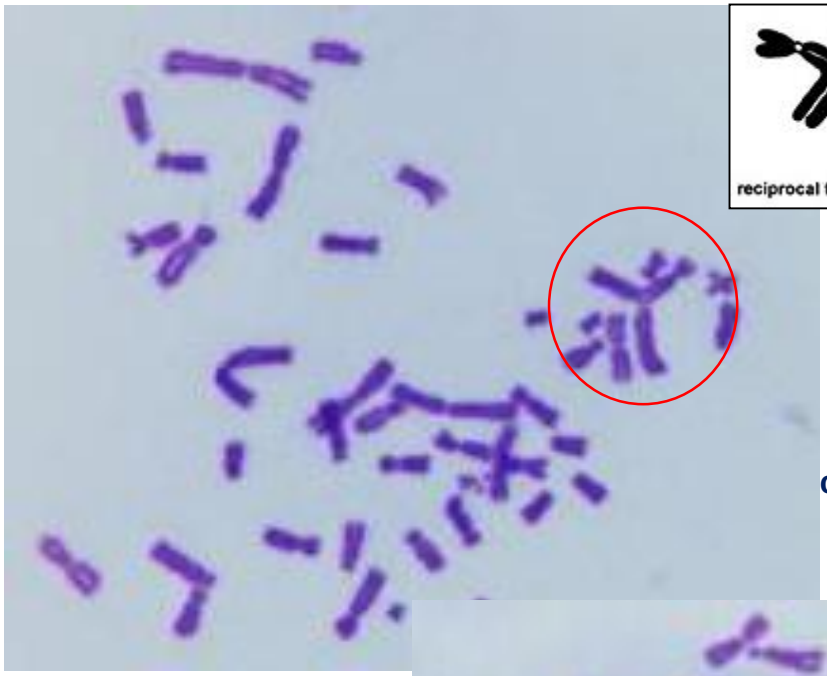
5% Giemsa stain or

Diff-Quik stain

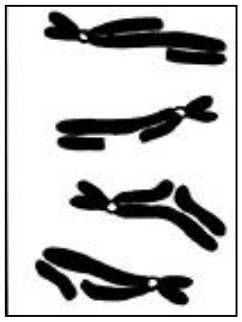
1000x

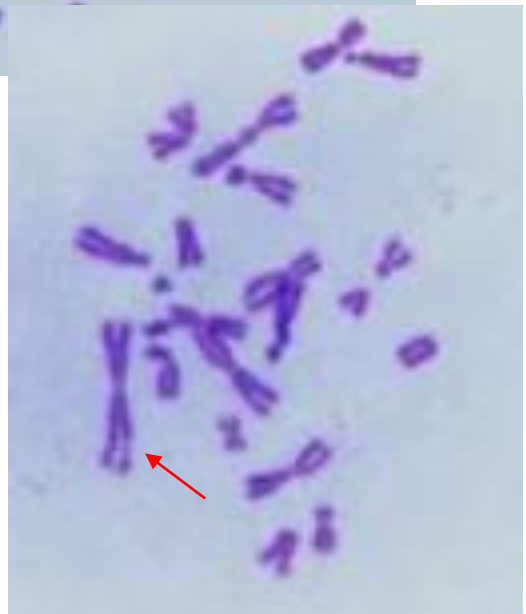
CP:Cyclophosphamide

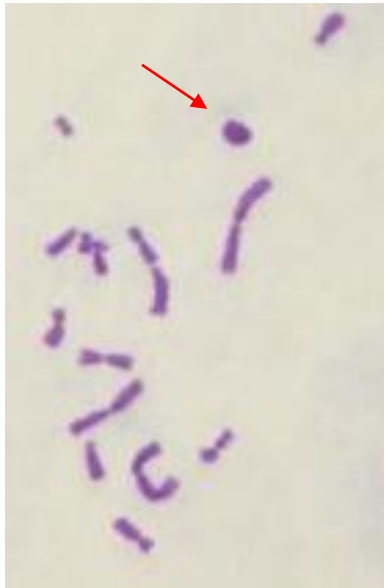
Location of chromosomal aberrations in CHO cells



Positive control-cyclophosphamide(S9)







Frequency of chromosomal aberration of xxx in cultured CHO-K1 cells

Group	Frequency of chromosomal aberration (%)[1]	
	-S9	+S9
Negative control	5.0±3.6	2.3±2.5
Mitomycin C (2.5 μg/ml)	20.3±3.8*	-
Cyclophosphamide (25 μg/ml)	-	11.0±2.0*
T101-146 (mg/ml)		
1.25	6.7±3.5	3.7±1.2
2.5	3.3±0.6	3.7±1.5
5	6.0±1.7	4.0±1.0

1 Two slides were prepared and stained with Diff Quik Kit for 3 steps and a total number of 300 metaphases were counted for each dosage. All results were expressed in number of aberration per plate.
2 The number of cells with damage chromosomes was recorded from which the rate of mutation was calculated. Aberration rate (%) = (number of cells with damage chromosomes/100) × 100.
- Not done.
* Significant difference between the negative control and treated groups at $p < 0.05$.

Locations of chromosomal aberrations of test article in CHO-K1 cells (-S9)

Group	Frequency (%) of aberrations[1]	Chromatid-type			Chromosome-type	
		Deletion	Intra-change	Inter-change	Gap	Ring
Control	2.3±2.5[2]	0.3±0.6	0.0±0.0	0.3±0.6	0.7±1.2	1.0±1.0
Cyclophosph-amide (25 μg/ml)	11.0±2.0*	2.7±1.5	0.3±0.6	1.0±1.0	1.3±2.3	5.7±1.2*
T101-146 (mg/ml)						
1.25	3.7±1.2	1.0±1.0	0.3±0.6	0.7±1.2	0.3±0.6	1.3±2.3
2.5	3.7±1.5	1.0±1.0	0.0±0.0	0.0±0.0	1.0±1.0	1.7±1.5
5	4.0±1.0	0.3±0.6	0.7±1.2	0.7±0.6	0.7±1.2	1.7±2.1

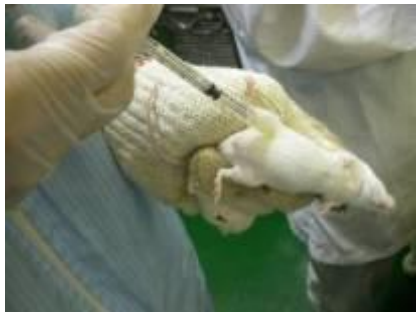
1 Two slides were prepared and stained with Diff Quik Kit for 3 steps and a total number of 300 metaphases were counted for each dosage. All results were expressed in number of aberration per plate. Frequency of total aberrations (%) = (total aberrations/ total counted cells) × 100.

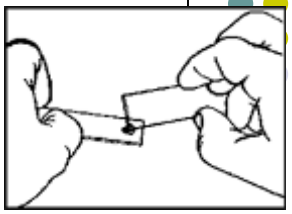
2 Data were expressed as mean±SD, n=3

* Significant difference between negative control and treated groups at $p < 0.05$

3. 活體動物週邊血液微核測試法
(Micronucleus test via peripheral blood collection in mice)

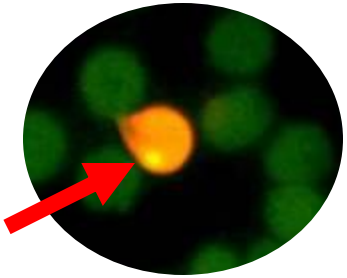
Positive control:
Cyclophosphamide
40(50) mg/kg, ip





Acridine orange stain

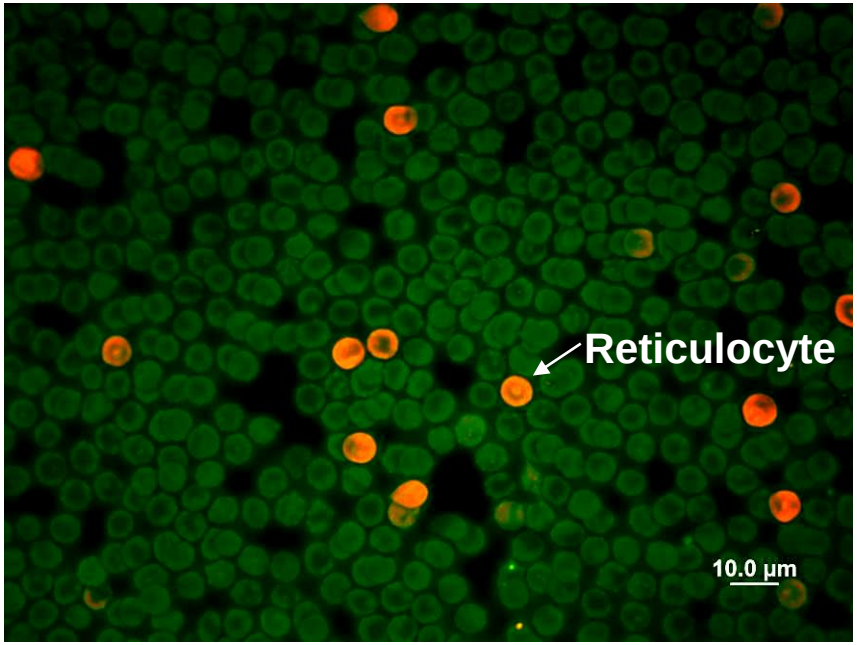
Blood collected via orbital sinus venipuncture after 48, 72 hr



Micronuclei in reticulocytes

Negative control: DW, gavage

xxx: 2 g/kg, gavage



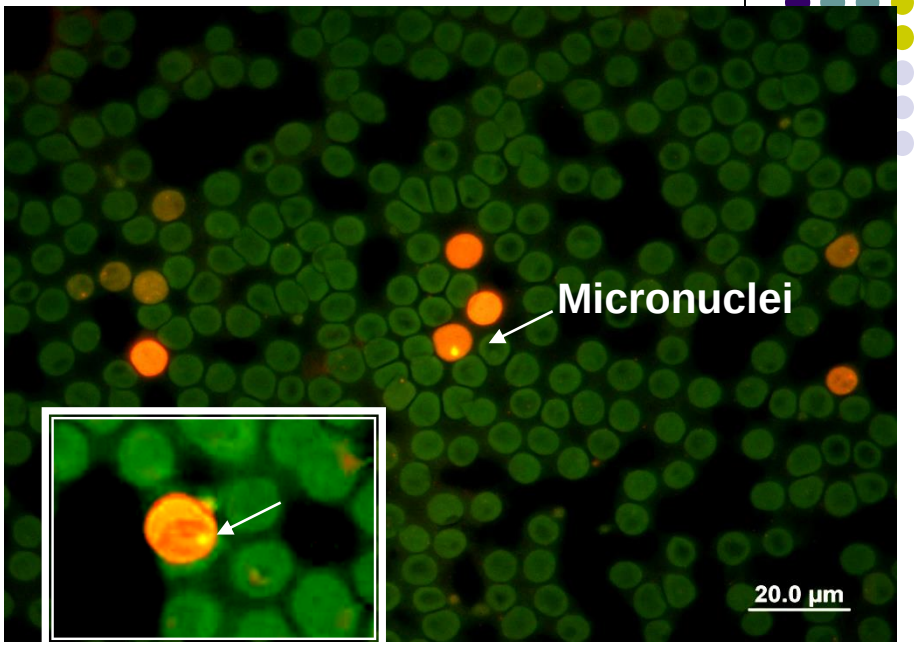


Fig.1. Photomicrographs of reticulocytes and micronuclei in reticulocytes in mice. A. Reticulocytes of peripheral blood displayed orange-red color after staining with acridine orange and were observed under fluorescence microscope in a negative control mouse. B. A micronucleated reticulocyte exhibited yellow-green fluorescence with a diameter of about 1/20-1/5 of an erythrocyte (1000X). (Animal No. 201, post 48 h treatment)

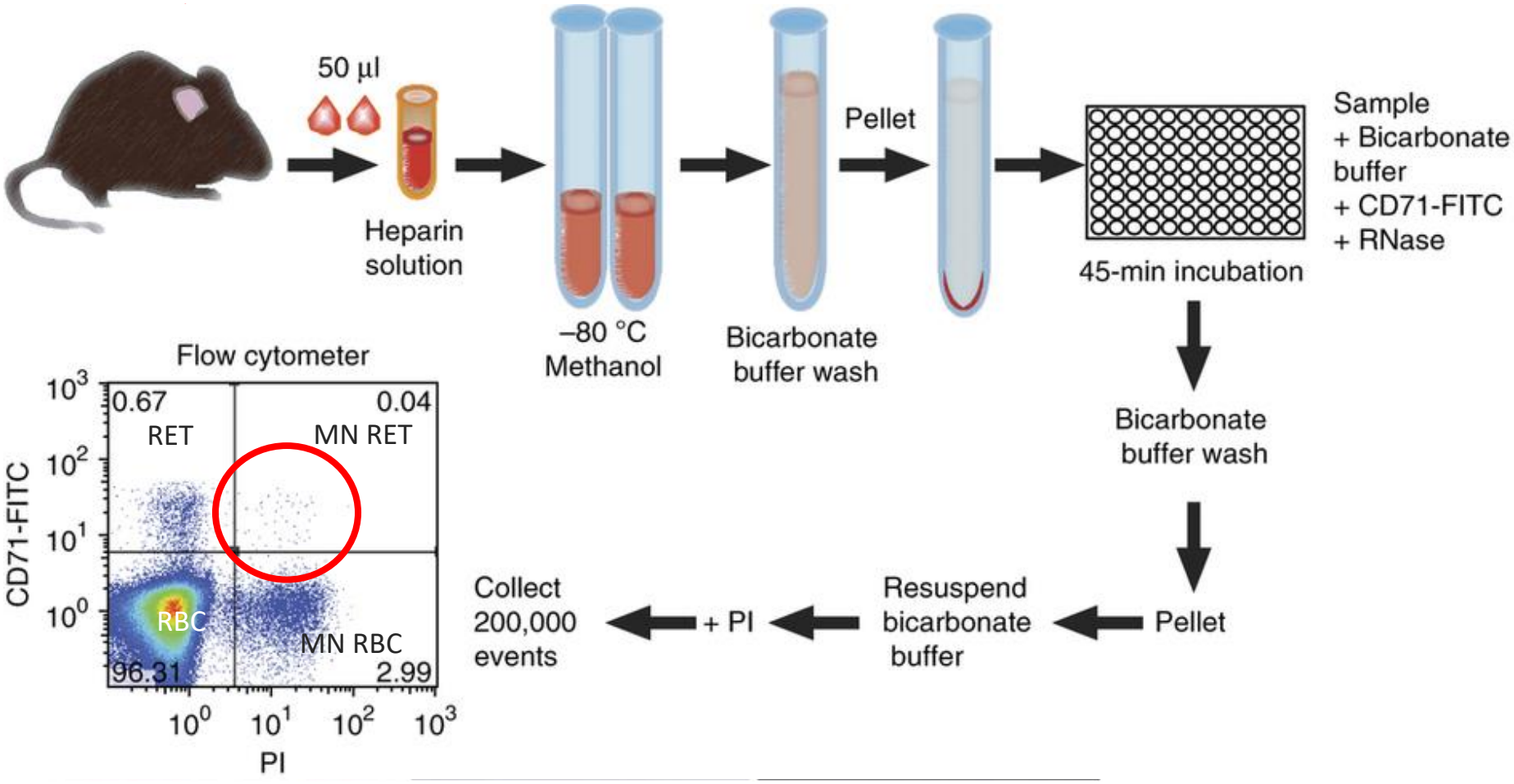
Micronuclei assay of test article in the peripheral red blood cells in male mice

Sampling intervals/ Group[a]	Dose (g/kg)	RETs/1000 NCEs (‰)	MN-RETs/ 1000 RETs (‰)
48 h			
C	0	36.2 ± 6.1	1.4 ± 0.9
CP	0.05	19.8 ± 3.3*	15.6 ± 5.7*
xxx	1	37.6 ± 5.5	1.2 ± 1.3
xxx	3	39.0 ± 5.7	1.4 ± 0.5
xxx	5	39.0 ± 2.4	1.8 ± 0.8
72 h			
C	0	34.8 ± 4.3	1.4 ± 0.5
CP	0.05	20.2 ± 2.3*	8.0 ± 3.1*
xxx	1	32.8 ± 3.0	1.6 ± 0.9
xxx	3	37.4 ± 4.9	2.6 ± 1.5
xxx	5	36.6 ± 8.0	1.2 ± 1.3

OECD 474: 2000 erythrocytes for peripheral blood
TFDA: 1000 erythrocytes for peripheral blood

Protocol of micronucleus assay in Mouse Peripheral Blood

Test sample



http://www.nature.com/nprot/journal/v10/n1/abs/nprot.2015.010.html

Reticulocytes and micronucleated reticulocytes counts in the peripheral blood at intervals of 48 and 72 hours of male mice

Group/ Intervals	Dose (mg/kg)	RETs/1000RBCs (‰)	Mn-RETs/1000RETs (‰)
Male			
48hrs			
NC	0	19.1±5.2	2.6±0.8
PC	60	3.6±2.1*	22.9±12.2*
xxx	Low	20.4±2.4	2.0±0.9
	Middle	21.8±2.5	1.7±0.8
	High (2 g/kg)	20.8±1.9	1.6±0.2
72hrs			
NC	0	23.7±5.2	2.2±0.3
PC	60	3.9±2.1*	6.8±3.7*
xxx	Low	30.0±6.1	3.0±1.0
	Middle	29.9±5.0	2.5±0.5
	High (2 g/kg)	29.5±4.6	2.6±0.5

NC: negative control; RETs : reticulocytes ; RBCs: erythrocytes ; Mn-RETs : micronucleated reticulocytes;
PC: positive control (Cyclophosphamide 60 mg/kg bw. ip)
* Significant difference in compared with the negative control and treated groups at $p < 0.05$.
200,000 erythrocytes for peripheral blood

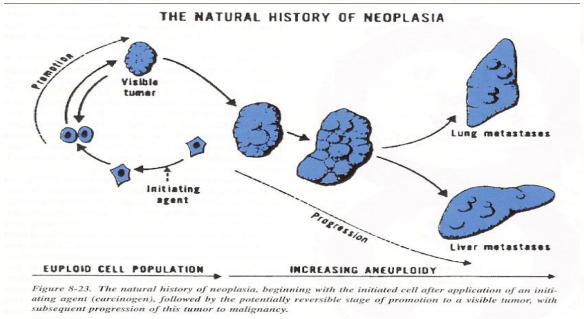


Figure 8-23. The natural history of neoplasia, beginning with the initiated cell after application of an initiating agent (carcinogen), followed by the potentially reversible stage of promotion to a visible tumor, with subsequent progression of this tumor to malignancy.

Figure 8-32. Graphic representation of mutagens and nonmutagens in relation to their known carcinogenic potential in animal tests.

The labeling of the quadrants using the classification of Table 8-18 is a further potential extrapolation of these data. [After Sugimura et al. (1976), with permission of authors and publishers.]

Casarett & Doull's Toxicology. 2002

動物毒理試驗
(Animal toxicity tests, *in vivo*)

1. 衛福部健康食品安全性評估試驗規範，1999

2. 衛福部藥品非臨床試驗安全規範，2014

3. OECD GUIDELINES FOR TESTING OF CHEMICALS SECTION 4 - HEALTH EFFECTS (pink pages), No. 407-453, 2018
https://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm

1. Health Effects Test Guidelines, *OPPTS Harmonized Test Guidelines,* Series 870, EPA 712-C-98-201
http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series870.htm

OECD Test Guidelines for the Chemicals

Section 4: Health Effects (Software for TG 455, TG 432 and TG 425)

OECD Test Guidelines for the C

oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm

應用程式 國立中興大學(Nati... (您有 23 封新信) M... 國立中興大學-獸醫... 網站管理系統 - 登... 台灣高鐵網路訂位... CSVP網站管理系統... 教師網站管理系統 -... 其他書籤

BETTER POLICIES FOR BETTER LIVES

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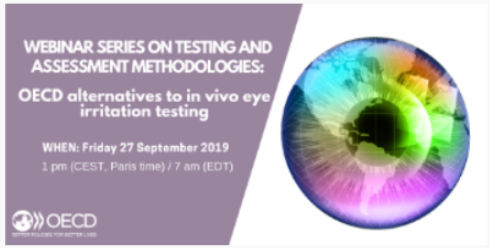
OECD Home > Chemical safety and biosafety > Testing of chemicals > OECD Test Guidelines for the Chemicals

- Testing of chemicals
- Assessment of chemicals
- Risk management of chemicals
- Chemical accident prevention, preparedness and response
- Pollutant release and transfer register
- Safety of manufactured nanomaterials
- Agricultural pesticides and biocides
- Biosafety - BioTrack

OECD Test Guidelines for the Chemicals

Register for our webinar on OECD alternatives to in vivo eye irritation testing

*The **OECD webinars on Testing and Assessment Methodologies** focus on selected ongoing projects that will result in the short to medium term in new methodologies to test and assess chemicals. These free webinars are open to potential end users among regulators and contract research organisations who want to get familiar with new standards and approaches. Video recordings of our webinars are made available online afterwards.*



When: Friday 27 September 2019 @ 1 pm (CEST, Paris time) / 7 am (EDT)
Where: The convenience of your computer

This webinar will present an overview of the various alternative test methods developed as OECD Test Guidelines and relevant guidance material to address eye irritation and serious eye damage for hazard classification of chemicals.

Presentations by:

- Anne Gourmelon from the OECD Environment Directorate
- Chantra Eskes from the Swiss Centre for 3 Rs, and one of the lead authors of the OECD IATA Guidance document on eye irritation
- Bertrand Desprez from Cosmetics Europe, who will present a project under way to develop Defined Approaches to better determine the eye irritation potential of chemicals
- Dave Allen from Integrated Laboratory Systems (ILS), who will speak about applicability/limitations of the current alternative methods.

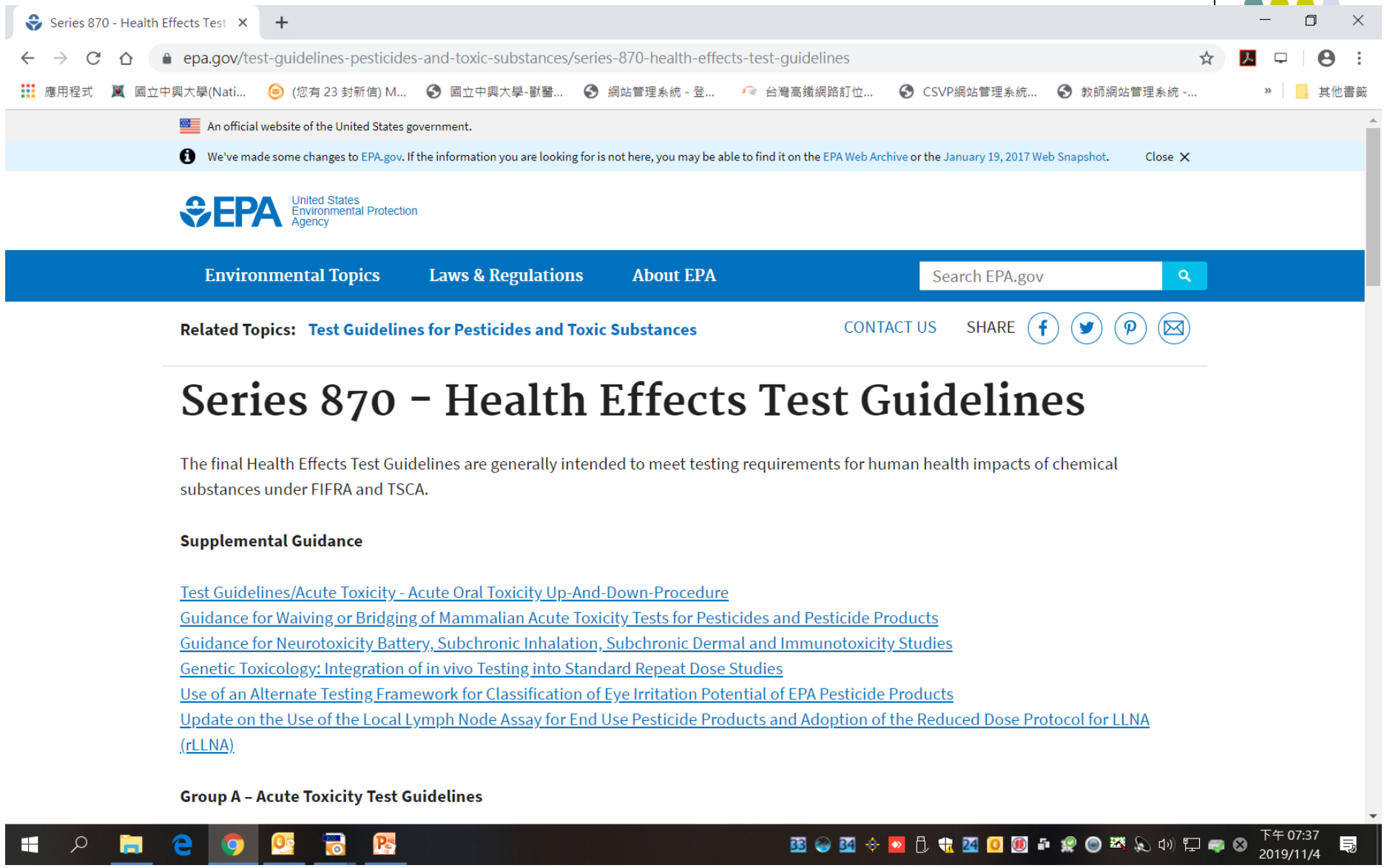
Duration: 1 hour 15 (Presentations of 60 minutes followed by 15 minutes of Q&A).

To register for the free online webinar:

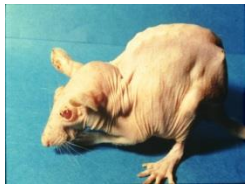
1. Go to the online registration.
2. Enter the requested information and click on "Register now". There is no password required for registration.

下午 07:02
2019/11/4

Series 870 - Health Effects Test Guidelines



Animals Used in the Toxicological Studies



Nude mice

Guinea pig

Hamster

Laboratory animals:

- mouse, rat, hamster, guinea pig, gerbil, rabbit, dog, cat...

Domestic animals:

- fish, chicken, quail, sheep, swine, cattle, horse...

Primate animals:

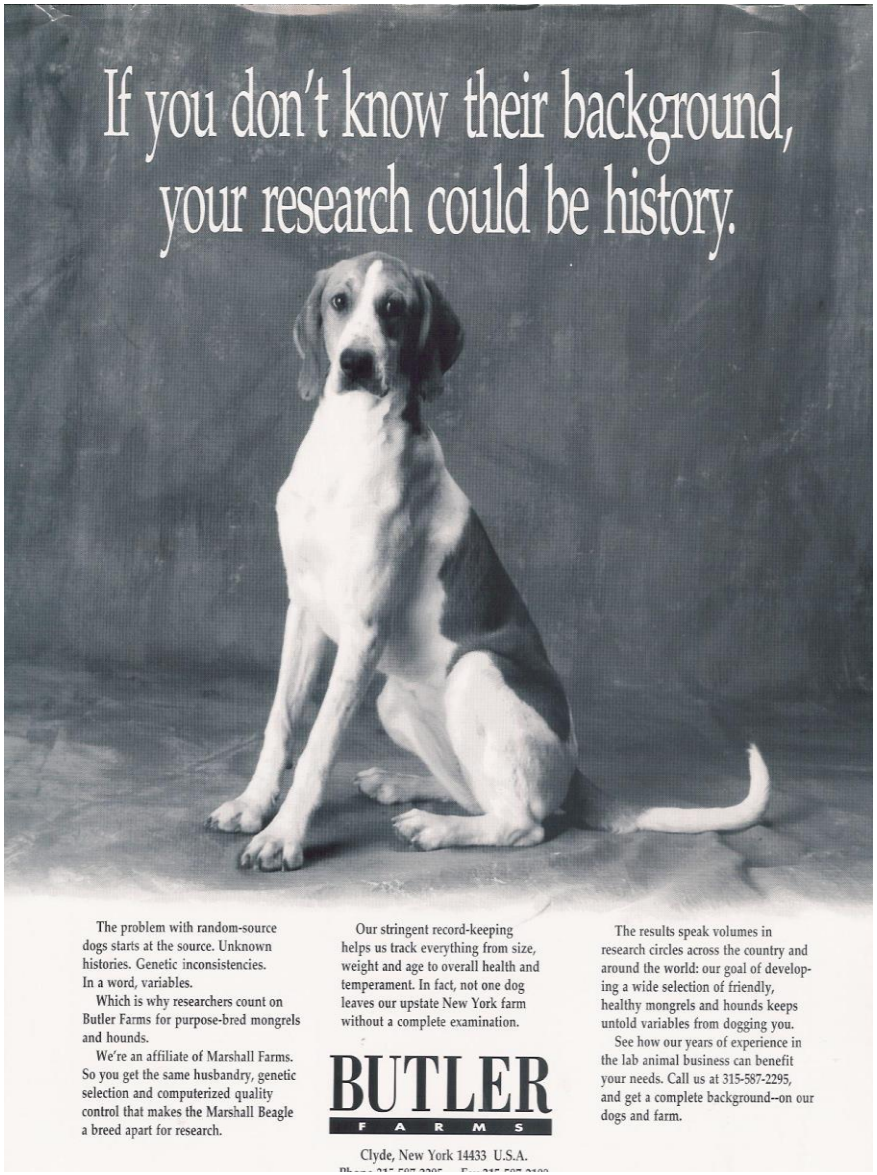
- monkey..

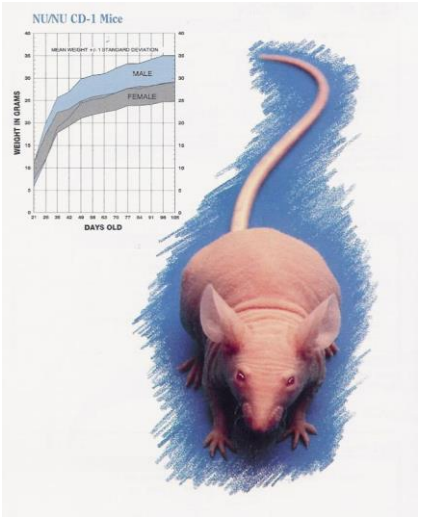
ICR mouse

SD rat

Wistar rat

Animals Used in the Toxicological Studies





Experimental Animals

- Same species and same strain in a sequence of toxicity studies

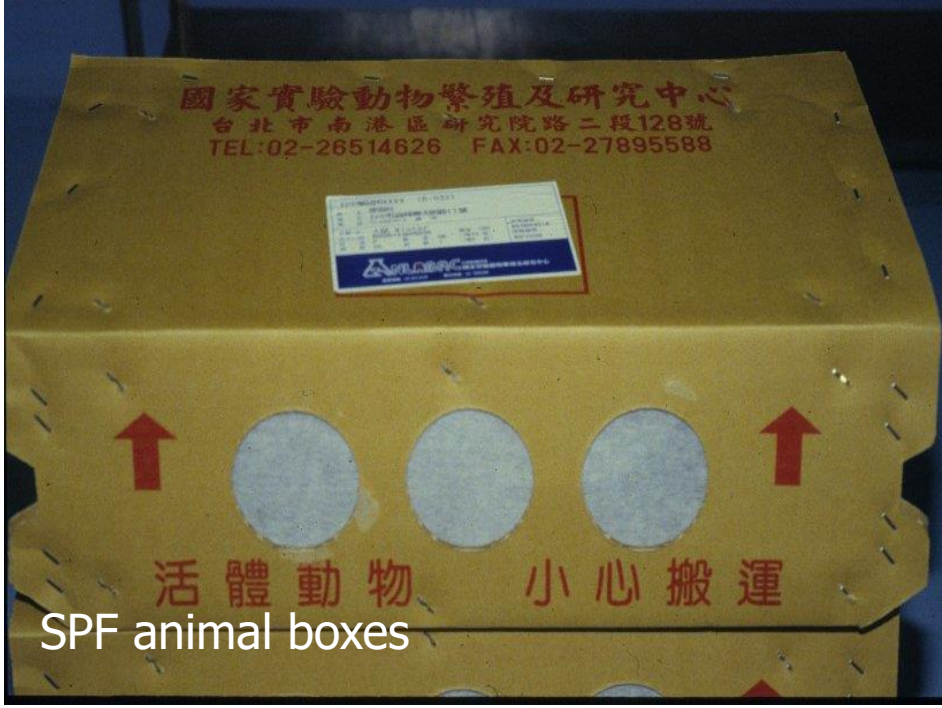
- Healthy young adult animal well known origin international standard strains: SD/Charles River IGS, Wistar/Hanover

- Animal welfare (reduction, refinement, replacement, 3R)

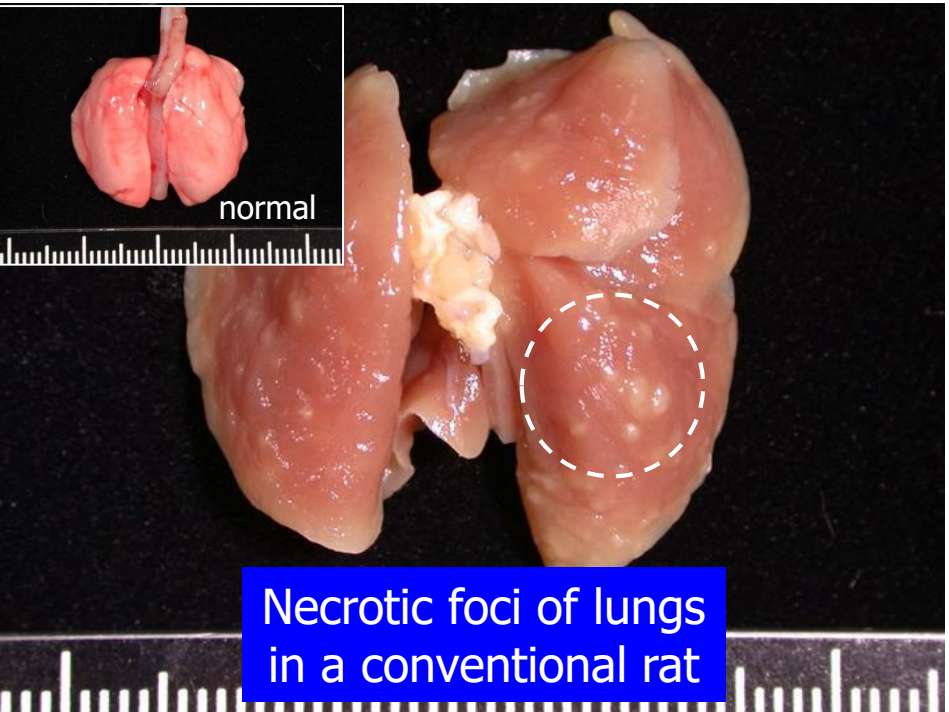
(動物保護法, 1998; Guide for the Care and Use of Laboratory Animals. 7th ed. 1996. Institute of laboratory Animal Resources Commission on Life Sciences, National Research Council.)

Casarett & Doull's Toxicology. 2002

Specific Pathogen Free (SPF) Animals and House



SPF animal boxes



Necrotic foci of lungs in a conventional rat


Bronchopneumonia in a conventional rat

無特定病原實驗動物之標準

一、定義:

(一) 無菌動物 (Germfree)

無菌動物體內沒有任何微生物，但可能有感染垂直感染的腫瘤病毒。

(二) 無病原動物 (Gnotobiotics)

動物在隔離箱 (isolator) 中長期用無菌飼養，並用實驗室方法檢定該動物無寄生或無共生微生物存在。即該動物僅可能存有現今實驗技術無法分離到的微生物，或者存在的常在菌。

(三)無特定病原動物 (Specific pathogen free, SPF)

無特定病原動物是由無菌或無病原動物繁殖生產，並符合國家動物中心所定無特定病原動物標準為SPF實驗動物。

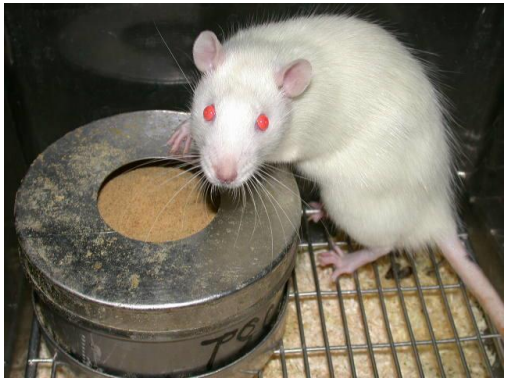
二、實驗動物之品質保證:

(一)細菌監控

(二)黴漿菌監控

(三)病毒監控

(四)寄生蟲監控



無特定病原動物 (Specific pathogen free, SPF)

	小鼠(鼷鼠)	大鼠	倉鼠	天竺鼠(豚鼠)
應無下列病原體之感染	Mouse	Rat	Hamster	Guinea Pig
A. 病毒 (Virus)	×	×	×	×
肺炎病毒Pnermonia Virus of Mice (PVM)	×	×	×	×
理奧病毒 Reo 3	×	×	×	×
台病毒 Sendai	×	×	×	×
淋巴球脈絡炎病毒 Lymphocyte choriomeningitis (LCM)	×	×		
鼠腦脊脽腦脊隨炎病毒 (GD V11) Theiler's Encephalomyelitis	×	×		
鼠小病毒 Minute Virus of Mice (MVM)	×	×		
鼠肝炎病毒Mouse Hepatitis Virus (MHV)	×	×		
鼠腺病毒 Mouse Adenovirus	×			
鼠痘 Ectromelia	×			
囊腫病毒 Polyoma	×			
K Virus	×			
小病毒 Toolan H-1		×		
小病毒 Kilham Rat Virus (KRV)		×		
大鼠冠狀病毒 Cornavirus (RCV)		×		
猴病毒 Simian Myxovirus (SV5)			×	×
B.細菌				
黴漿菌 Mycoplasma pulmonis	×	×	×	
Corynebacterium kutsheri	×	×	×	×
Bordetella brochiseptica	×	×	×	×
Salmonella spp.	×	×	×	×
Yersinia pseudotuberculosis	×	×	×	×
C.寄生蟲	(不得有體內、外之寄生蟲之感染)			

The classification of toxicity

♣ Acute toxicity

- The effects of a single dose or multiple doses during a 24-hour period..
  - ⇒ Half of lethal dose (LD_{50}, mg/kg body weight)
  - ⇒ extremely, highly, moderately, slightly hazardous, practically non toxic.

♣ Subchronic toxicity

- The effects produced by prolonged exposure with the duration of exposure being 3 months or longer.

⇒ NOAEL (no observed adverse effect levels, mg/kg/day)

♣ Chronic toxicity

- The effects produced by prolonged exposure with the duration of exposure being 1 year or longer.
  - ⇒ NOAEL (no observed adverse effect levels, mg/kg/day)
  - ⇒ ADI (acceptable daily intake, mg/kg/day) = NOAEL/(UF*MF)
  - ⇒ RfD (Reference dose, mg/kg/day) = NOAEL/(UF*MF)

Casarett & Doull's Toxicology. 2002

Acute Toxicity and Irritation Studies

- Acute Oral Toxicity Study-- (rat or mouse)
- Acute Dermal Toxicity Study-- (rat or rabbit)
- Acute Inhalation Toxicity Study-- (rat or mouse)

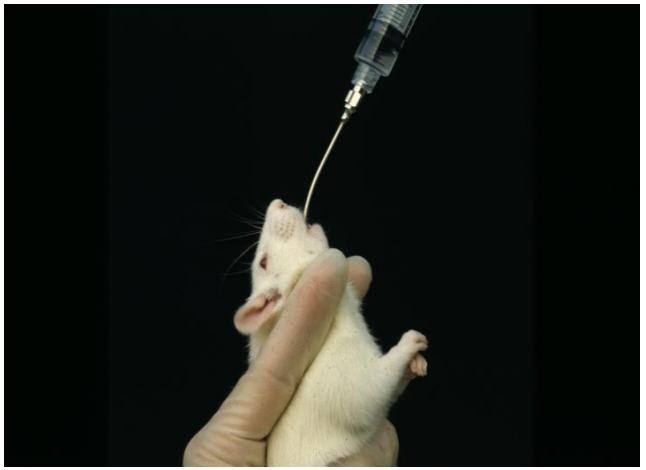
- Primary Eye Irritation Study-- (rabbit)
- Primary Dermal Irritation Study-- (rabbit)
- Dermal Sensitization Study-- (guinea pig)

- Delayed Neurotoxicity of Organophosphorus Substances Study-- (hen or rat)

Acute Oral Toxicity Test

Environmental Protection Agency. Health Effects Test Guidelines OCSPP (formerly OPPTS) 870.1100: Acute Oral Toxicity, EPA 712·-C- 02- 190. 2002.

- Animal: rat or mouse (gavage method)
- Control, vehicle control, and treated groups
- Number: 5 males and 5 females per group
- Dosing volume: 10 ml/kg body weight



- Dosing:
  3~5 dose levels (between 10-90% mortality)
  maximal dose: 5,000 mg/kg bw; Single treatment and a 14-day observation period
- Observations:
  Clinical signs, Morbidity, Mortality, Body weight change, Gross and micro pathology
- Result analysis (oral LD_{50}, mg/kg bw)

USEPA, Harmonized Test Guidelines, 1998

Animal Welfare Considerations

13. All three Guidelines provide significant improvements in the number of animals used in comparison **to Guideline 401, which required 20 animals in a test at least**. In addition, they all contain a requirement to follow the OECD Guidance Document on Humane Endpoints (6) which should reduce the overall suffering of animals used in this type of toxicity test. Furthermore, Guideline 420 has as its endpoint evident toxicity rather than mortality and uses a sighting study to minimize the numbers of animals and Guideline 425 has a stopping rule which limits the number of animals in a test.

OECD GUIDELINES FOR TESTING OF CHEMICALS TEST
401 ACUTE ORAL TOXICITY

Information: Following the OECD Council decision, the test 401 '*Acute Oral Toxicity*' **was deleted on 17th December 2002**. Those who would like to obtain it should contact

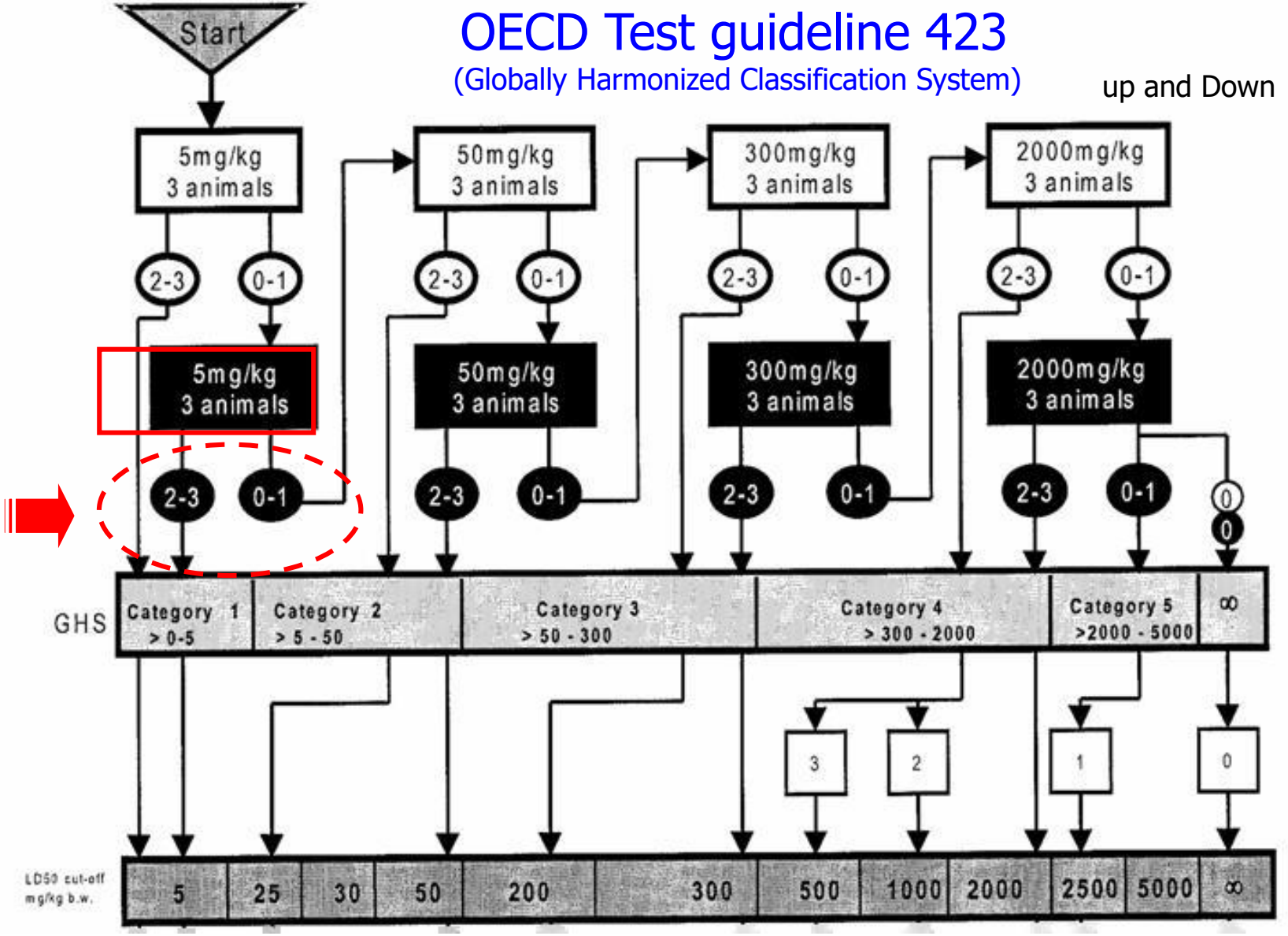
DATA NEEDS

- Guideline 420: typically 1 animal can be expected to die on test.
- Guideline 423: 2-3 animals per test can be expected to die in a full test.
- Guideline 425: the expected number of deaths is between 2 and 3.

OECD Test guideline 423

(Globally Harmonized Classification System)

up and Down



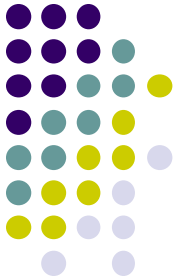
Adopted:
17th December 2001

Classification of relative toxicity of general compounds

Class	Toxicity	Volume Dose	
		Dog (20 kg)	Cow (450 kg)
Super toxic	≦ 1 mg / kg	0.004 teaspoon	0.09 teaspoon
Extremely toxic	≦ 5 mg / kg	0.04 teaspoon	1 teaspoon
Highly toxic	5-50 mg / kg	0.2 teaspoon	4.5 teaspoon
Moderately toxic	50-500 mg / kg	2 teaspoon	1 cup
Slightly toxic	0.5-5 gm / kg	0.45 cup	2.5 quarts
Practically nontoxic	5-15 gm / kg	1.34 cup	2 gallons
Relatively harmless	＞15 gm /kg	＞1.34 cup	＞2 gallons

(Gosselin et al., 1984. In: *Clinical Toxicology of Commercial Products, Acute Poisoning*)

The classification of toxicity



♣ Acute toxicity

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⇒ NOAEL (no observed adverse effect levels, mg/kg/day)

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  - ⇒ NOAEL (no observed adverse effect levels, mg/kg/day)
  - ⇒ ADI (acceptable daily intake, mg/kg/day) = NOAEL/(UF*MF)
  - ⇒ RfD (Reference dose, mg/kg/day) = NOAEL/(UF*MF)

Casarett & Doull's Toxicology. 2002

RAT'S AGE VERSUS HUMAN'S AGE: WHAT IS THE RELATIONSHIP?

Total lifespan:	13.8 rat days	= 1 human year
Nursing period:	42.4 rat days	= 1 human year
Prepubescent period:	4.3 rats days	= 1 human year
Adolescent period:	10.5 rat days	= 1 human year
Adult phase:	11.8 rat days	= 1 human year
Aged phase:	17.1 rat days	= 1 human year
Average:	16.7 rat days	= 1 human year

FIGURE 1 - Correlation days / year of age of mice against human

ANDREOLLO et al., ABCD Arq Bras Cir Dig 2012;25(1):49-51

TABLE 1 - The rat's age in months and its relationship in years with human being in social maturity phase[11]

Rat's age in months	Human's age in years
6 months	18 years
12 months	30 years
18 months	45 years
24 months	60 years
30 months	75 years
36 months	90 years
42 months	105 years
45 months	113 years
48 months	120 years

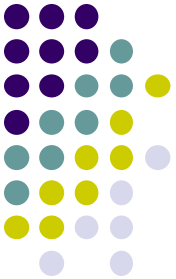
ANDREOLLO et al., ABCD Arq Bras Cir Dig 2012;25(1):49-51

毒性試驗期與動物壽命(%)及人類生活期(月)對照表

壽命(%) / 生活期(月)	Rat (%)/ Human(mo)(yr)	Dog/ Human	Pig/ Human	Monkey/ Human	Rabbit/ Human
試驗期(月)					
1	**4.1/37 (3-yr)**	0.82/6.5	0.82/6.5	0.55/4.5	1.5/12
2	8.2/74 (6-yr)	1.6/14	1.6/14	1.1/9.0	3.0/24
3	**12/108 (9-yr)**	2.5/20	2.5/20	1.6/13	4.5/36
6	25/225 (19-yr)	4.9/40	4.9/40	3.3/27	9.0/72
12	49/441 (38-yr)	9.8/81	9.8/81	6.6/53	18/45
24	**99/900 (75-yr)**	20/162	20/162	13/107	36/289

1 year=12 months

Subacute (28D) and Subchronic (90D) Toxicity Test



- Rat and mouse (oral, dermal, or inhalation)
- Control, vehicle control, and treated groups
- Number: 10 males and 10 females per group (total no.:120)
- Dosing:

  3 dose levels (low, intermediate, and high doses)

  Satellite (recovery) groups (control and high dose)

  .Repeated doses (gavage or in diet) treatment and a 28 or 90-day observation period
- Observations:

  Clinical signs, Morbidity, Mortality, Body weight change Hematology, Gross pathology, Histopathology, Urinary... circulating thyroid hormones (T4, T3, TSH; required)
- Data analysis (NOAEL, mg/kg/bw)

Test No. 408, Repeated Dose 90-Day Oral Toxicity Study in Rodents, 2018

OPPTS Harmonized Test Guidelines, Series 870.4100, EPA 712-C-98-210

Subchronic Toxicity Study in Rats

Experimental design:

1. A suspected adulteration of pet food (L-107) was fed to rats (Sprague-Dawley, SD), with 10, 20, 50% in diet for 8 weeks, then the diet was increased to reach 100% from week 9 to 12.

2. Rodent diet (Purina Mills LLC, St. Louis, MO, USA) as control diet.



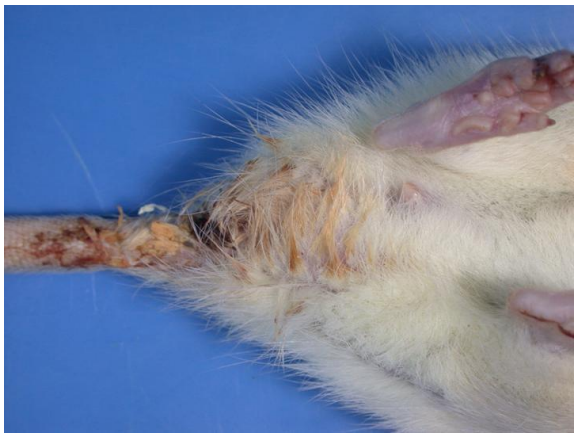

Chen, et al., 2009. *Toxicologic Pathology.* 37(7): 959-68

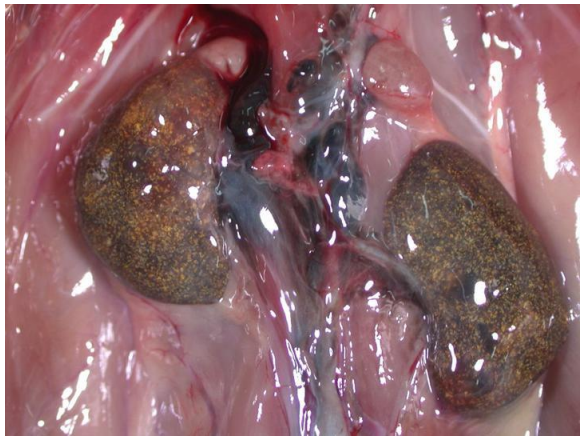
Clinical signs:

1. In clinic, rats had no significant change in the body weight and food consumption under 50% groups, until the diet was switched to 100% L-107 on week 9.

2. Rats became lethargy, anorexia, wet fur and **wet bedding**, gradually loss of body weight, where a significant decrease in these two categories became evident.

3. Two rats died of 50% diet supplement with rotten kidneys and brownish crystal formation inside in kidneys.





Chen, et al., 2009. *Toxicologic Pathology.* 37(7): 959-68

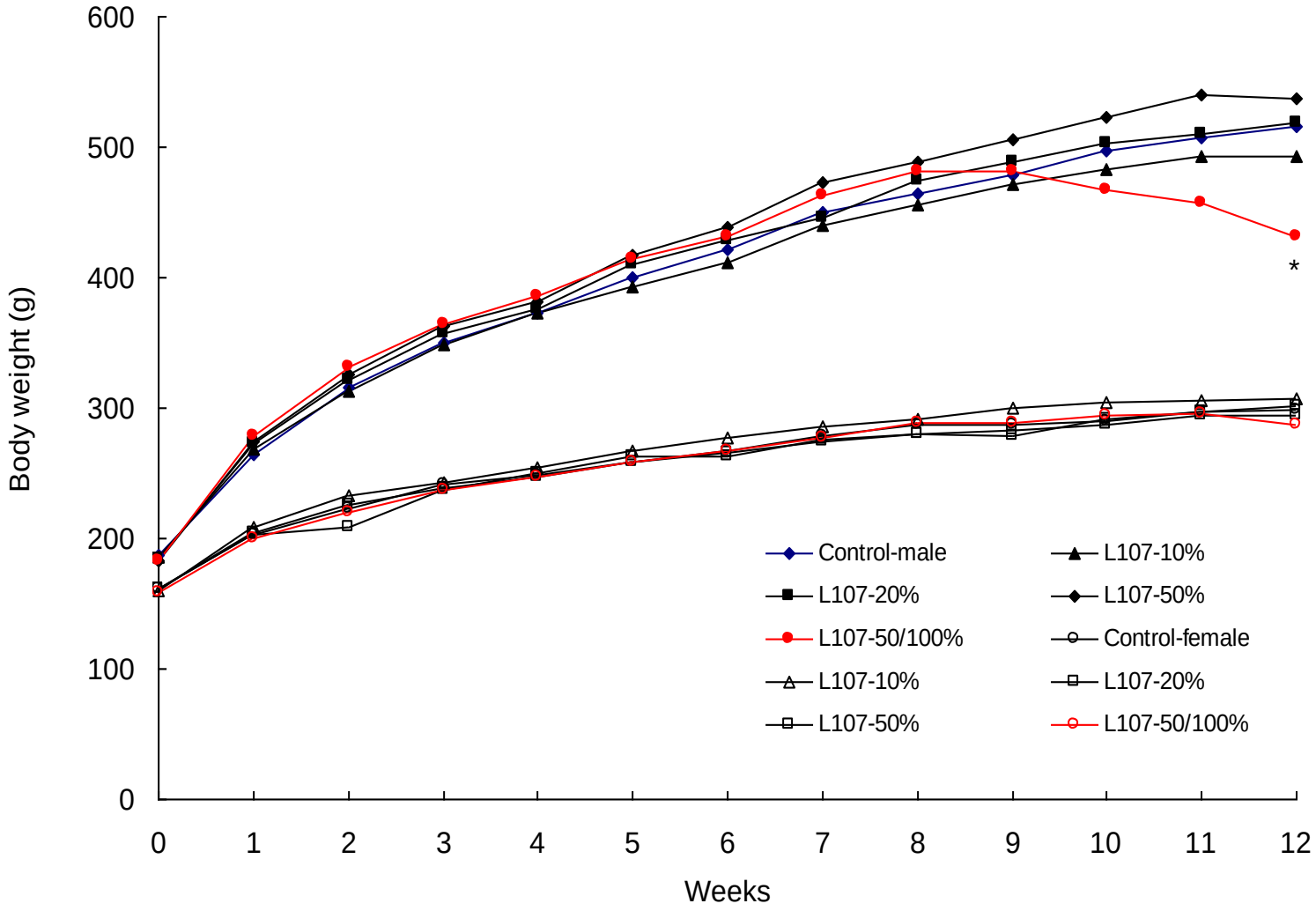


FIGURE 1A.—Changes of body weight of rats fed with various levels of L107 diet for twelve weeks. *Significant difference from the control group at $p < 0.05$.

Chen, et al., 2009. *Toxicologic Pathology.* 37(7): 959-68

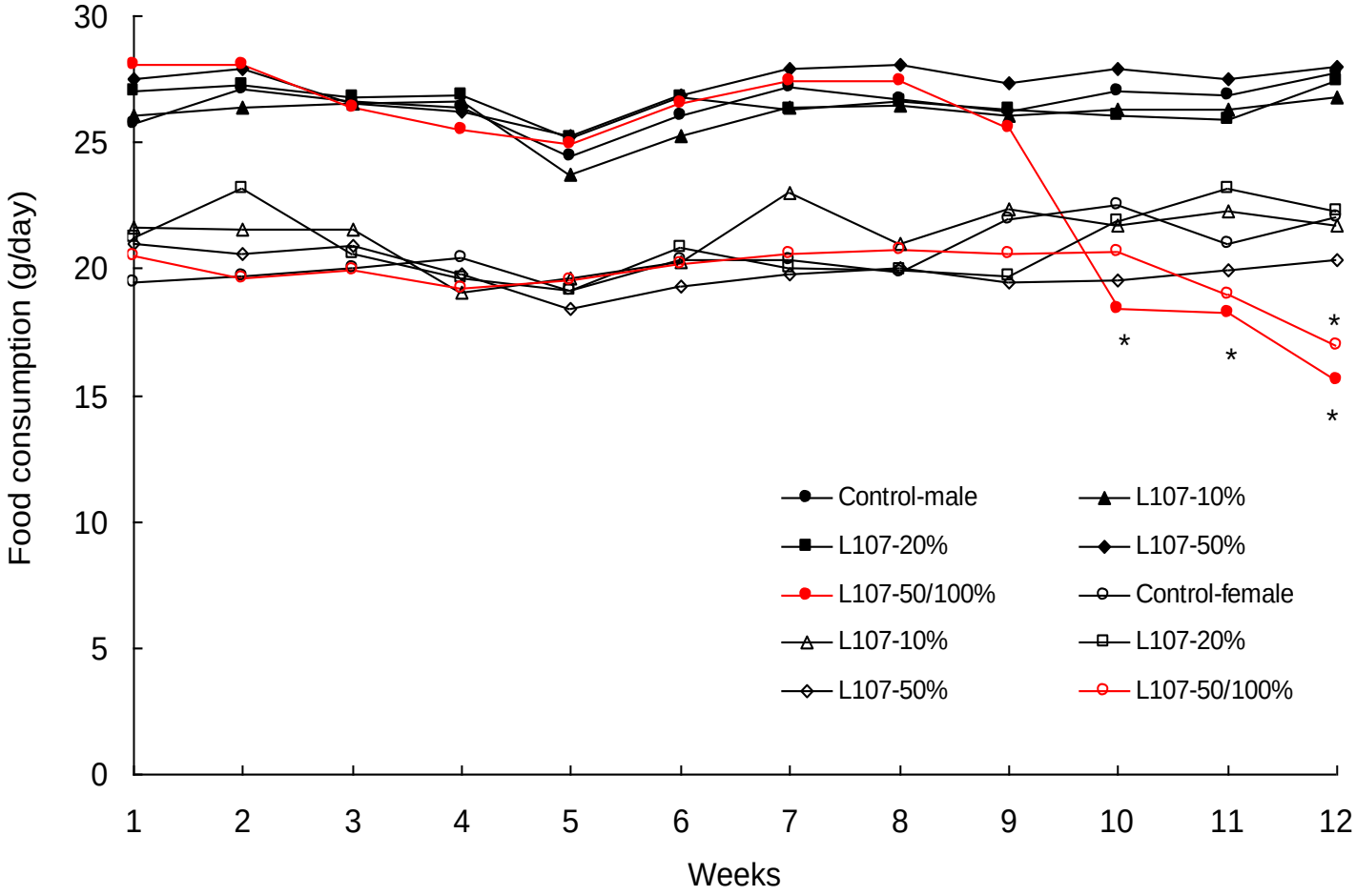


FIGURE 1B.—Changes of food consumption of rats fed with various levels of L107 diet for twelve weeks *Significant difference from the control group at $p < 0.05$.

Chen, et al., 2009. *Toxicologic Pathology.* 37(7): 959-68

Clinical Biochemistry:

CBC Abnormalities:

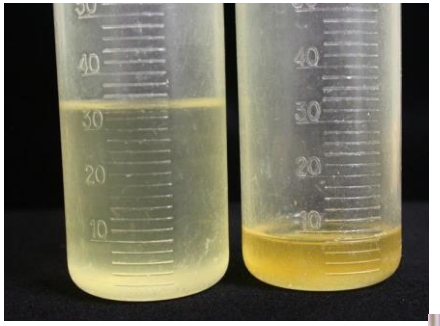
1. Elevated WBC counts (13.6 x10^3/μl) [control: 6.5 x10^3/μl], mainly in segmental neutrophils (49.6%) [control: 21%], and decreased lymphocytes (45%) [control: 73.2%] in WBC differentiation.
2. Decreased the value of MCV (54.5 fl) value [control: 59].

Clinical Chemistry Abnormalities:

1. Increased the levels of BUN (165 mg/dl) and creatinine (3.9 mg/dl) [control: 17 and 0.5 mg/dl).
2. Increased GGT (2.4 U/l), CK (145 U/l), and phosphorus (14 mg/dl) parameters [control: 0.4, 51 and 7.5 mg/dl).
3. Decreased serum chloride (128 mg/dl) [control: 151 mg/dl).

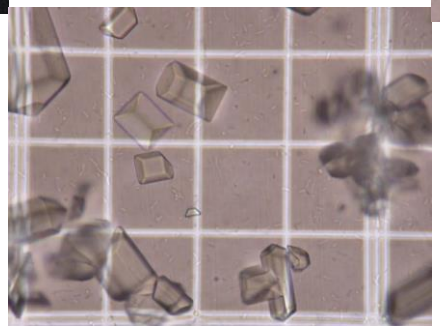
➡ **4. No effect on the levels of AST and ALT parameters.**

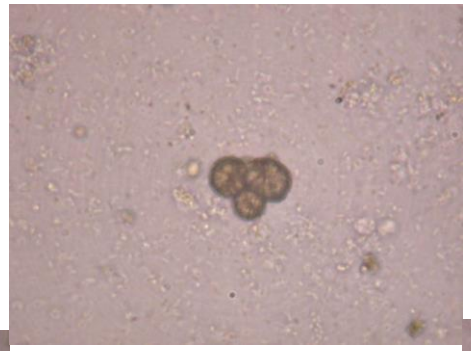
Chen, et al., 2009. *Toxicologic Pathology.* 37(7): 959-68



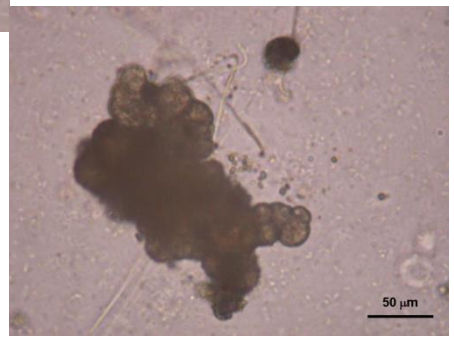
Increase of urine volume

Crystals in control





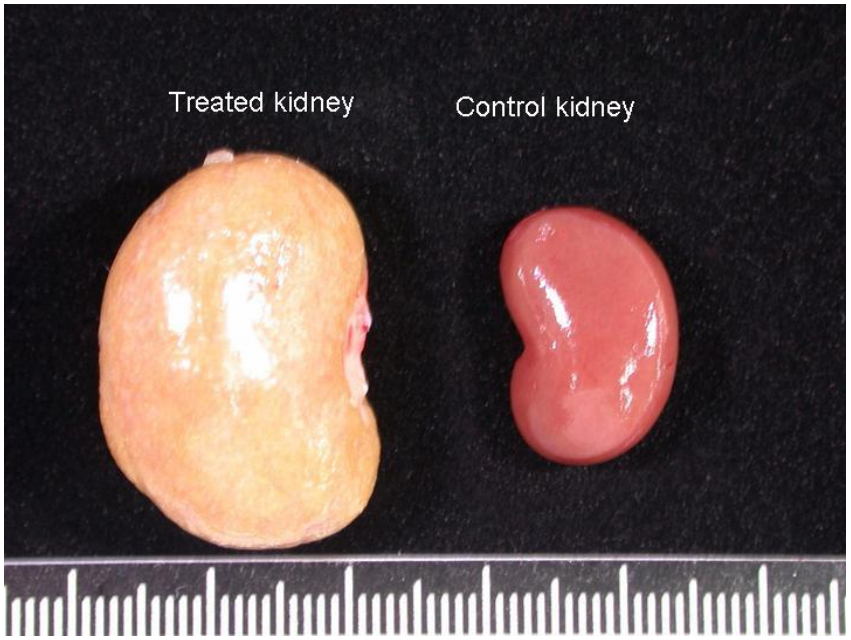
MC crystals in 100%

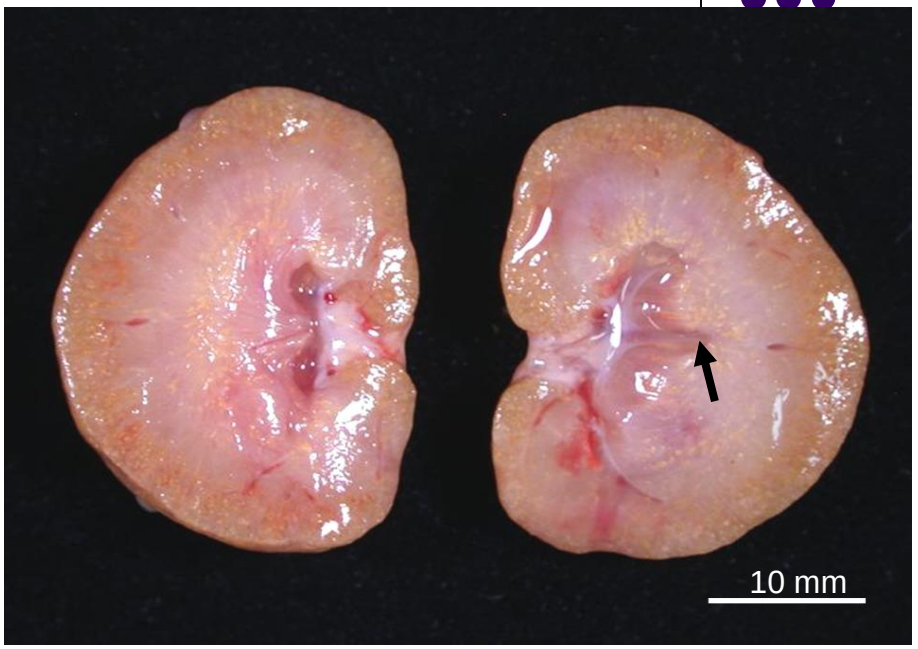


Urinary Abnormalities:

1. Increased the urine volume (30 ml) [control: 10 ml).
2. Decreased the urinary ketone, pH, protein, uric creatinine and urobilirubin (5, 6.7, 30. 0.2 mg/dl, and 36.5 E.U/dl) parameters [control: 25, 7.7, 140, 0.7 mg/dl, and 121 E.U./dl).
3. Increased the number of green to brownish aggregated largely round plate-like with radiating striations crystals in the urinary sediments.

Chen, et al., 2009. *Toxicologic Pathology.* 37(7): 959-68

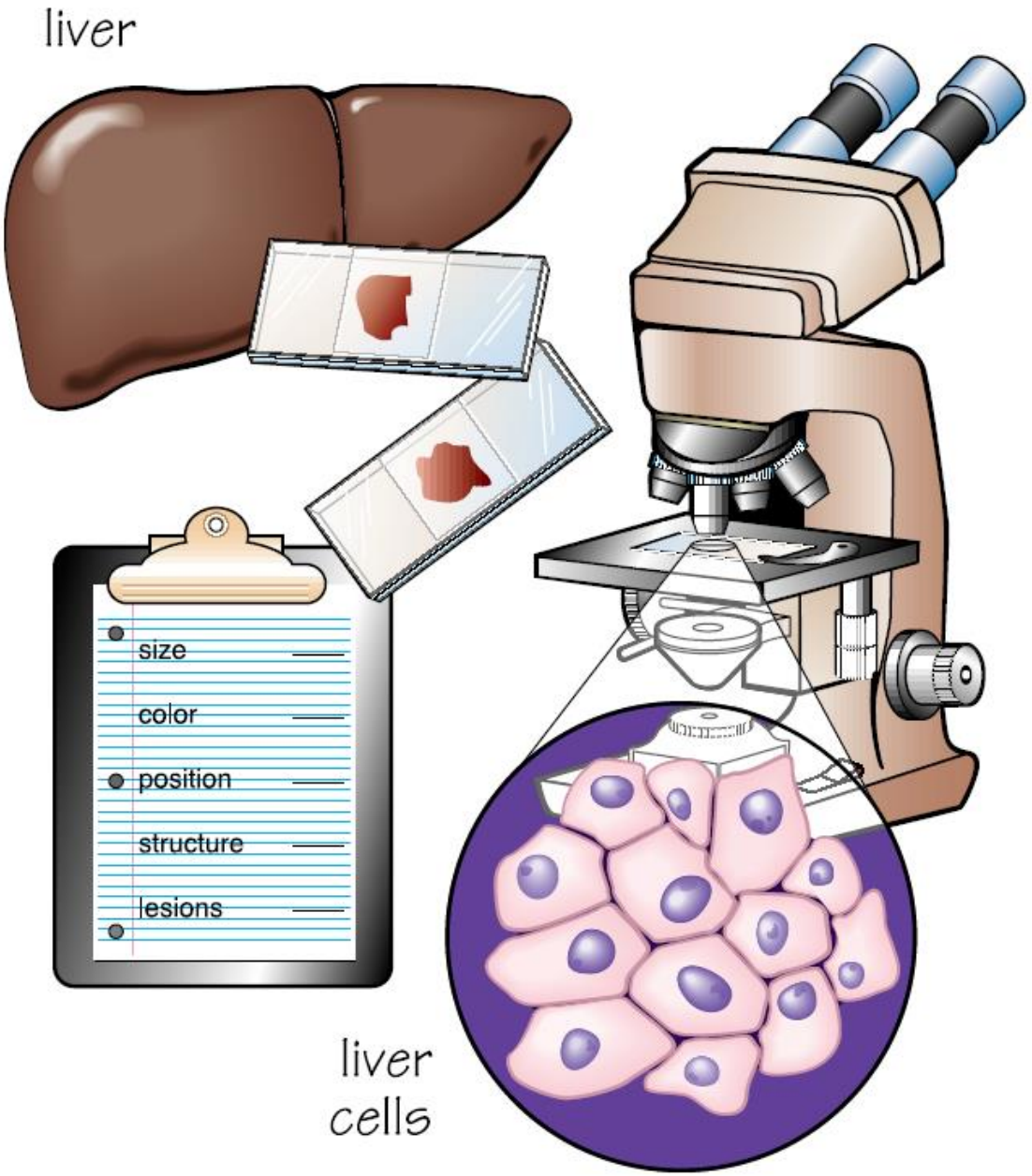




Gross finings:

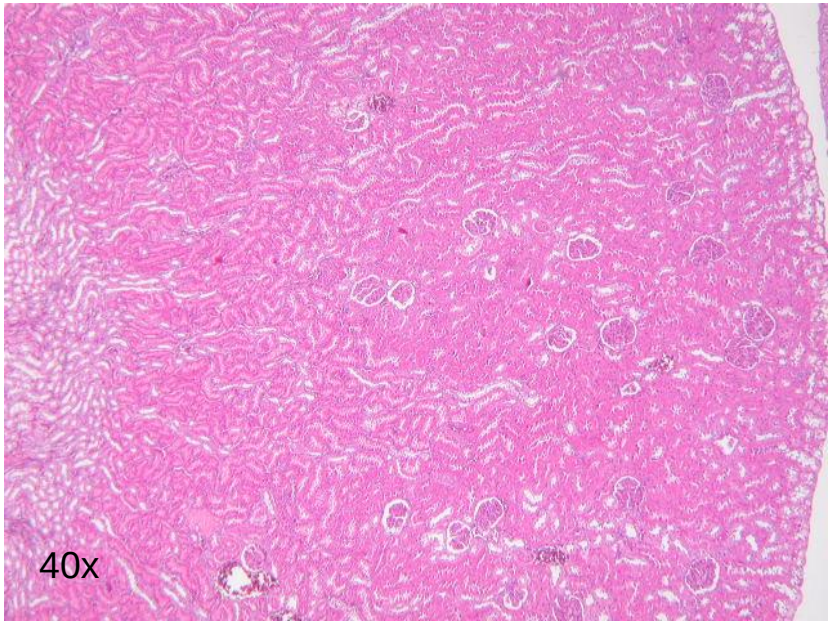
1. At necropsy, significant elevated the kidney weight (1.78%), up to 3 folds compared with the control (0.58%).
2. Gross findings of kidneys presented extremely enlargement or atrophy, brownish with hemorrhagic plaques and irregular in shapes and rough surface.
3. Dilated pelvic and variable fine greenish radiate birefringence crystals located in the cortex and medulla of kidneys.

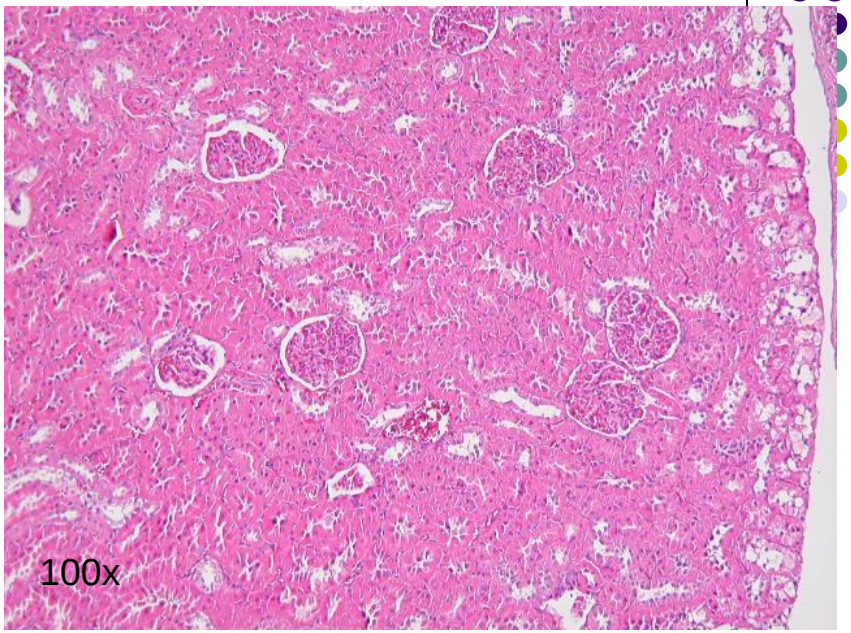
Chen, et al., 2009. *Toxicologic Pathology.* 37(7): 959-68

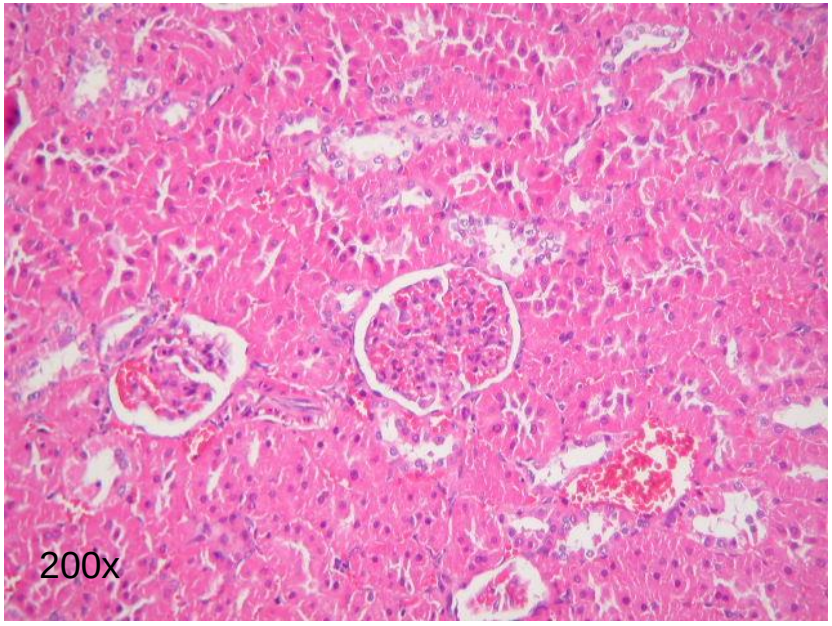


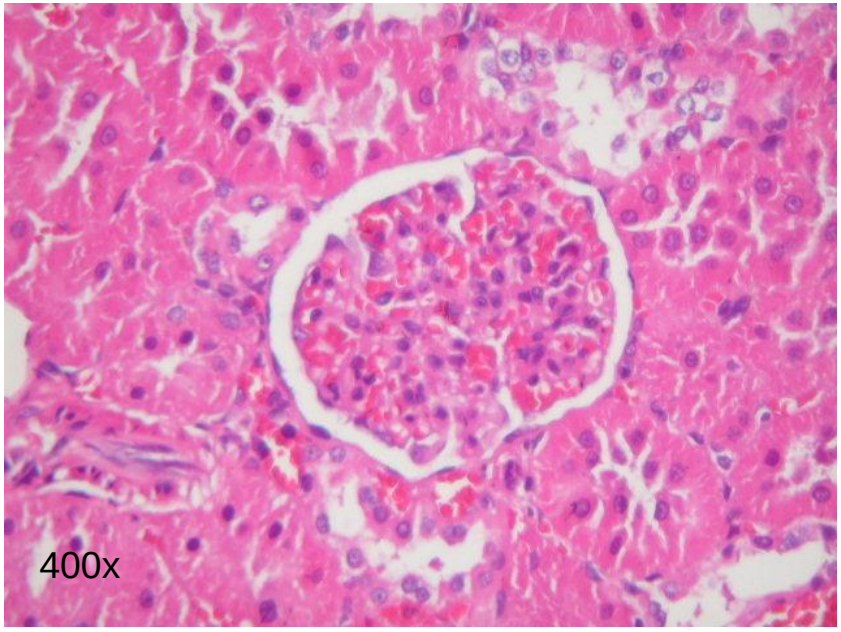
Blessing A, et al., 2002

Kidney, Control rat

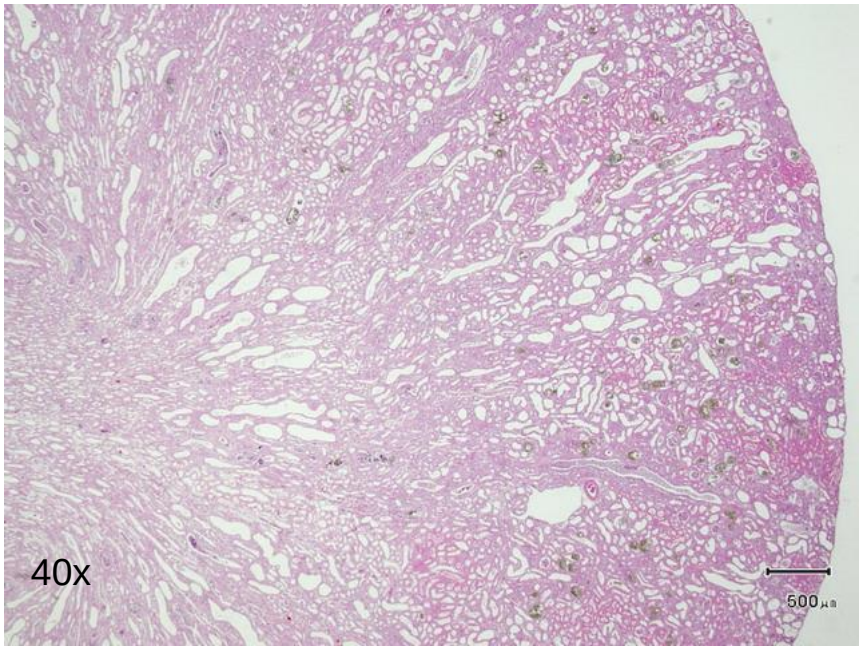


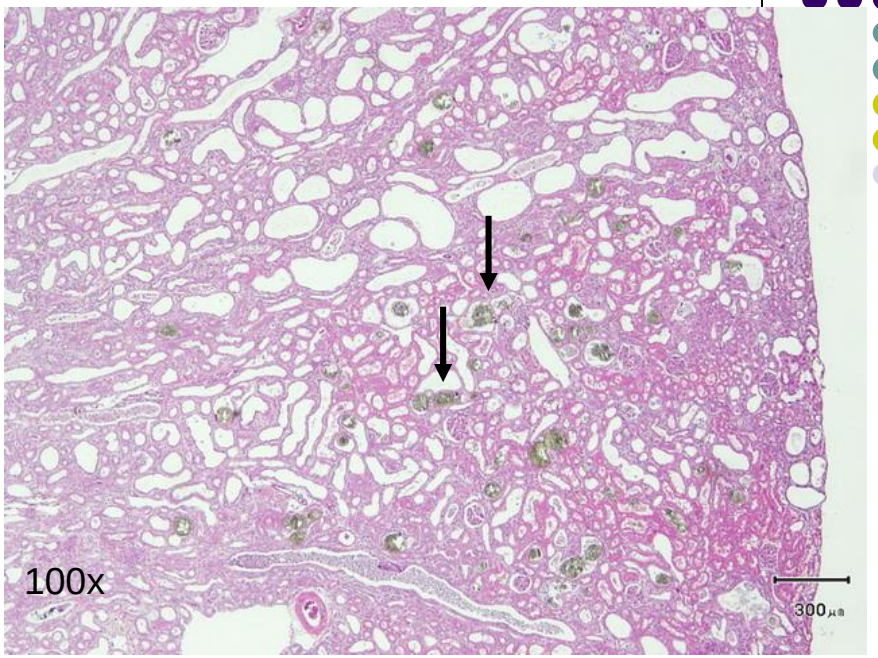


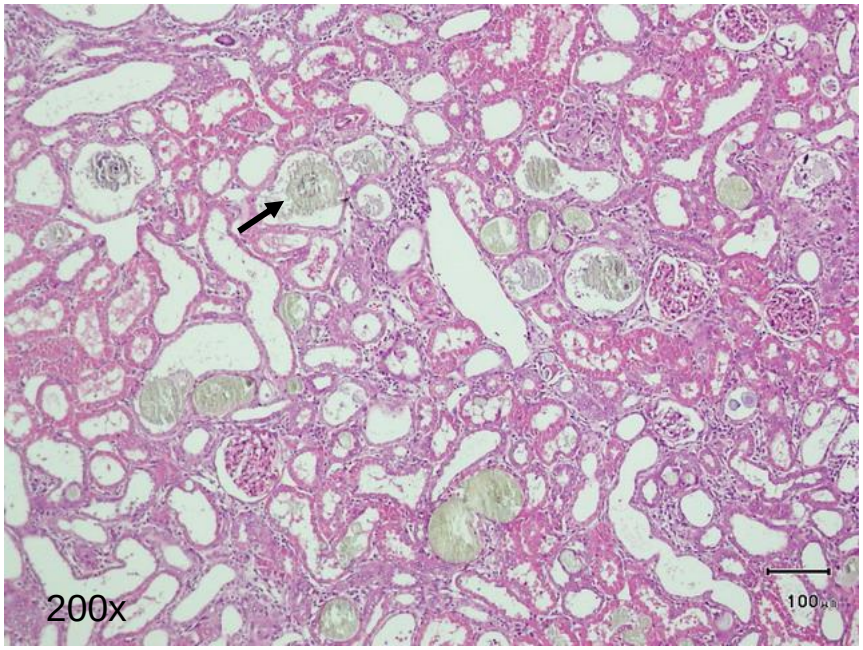


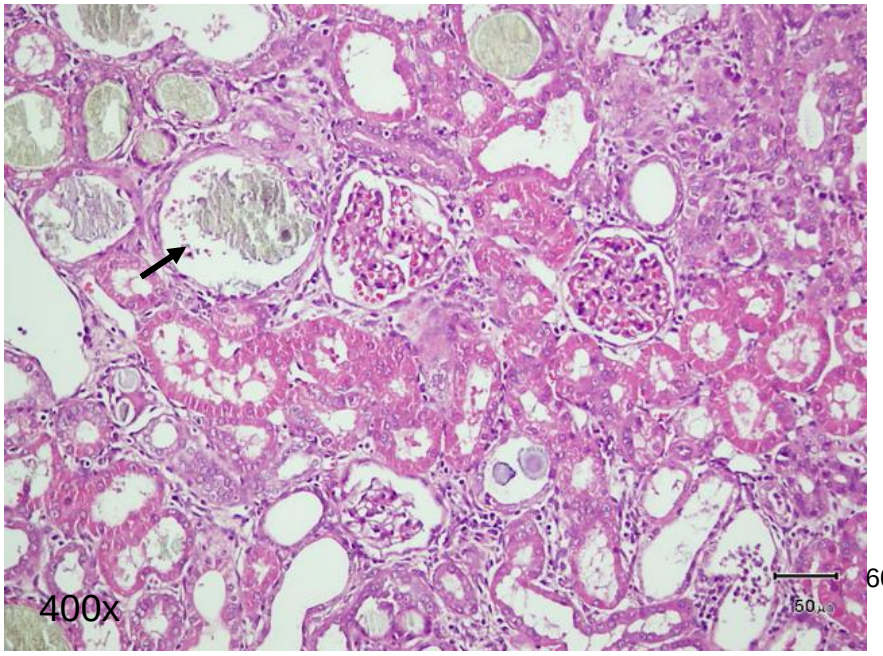


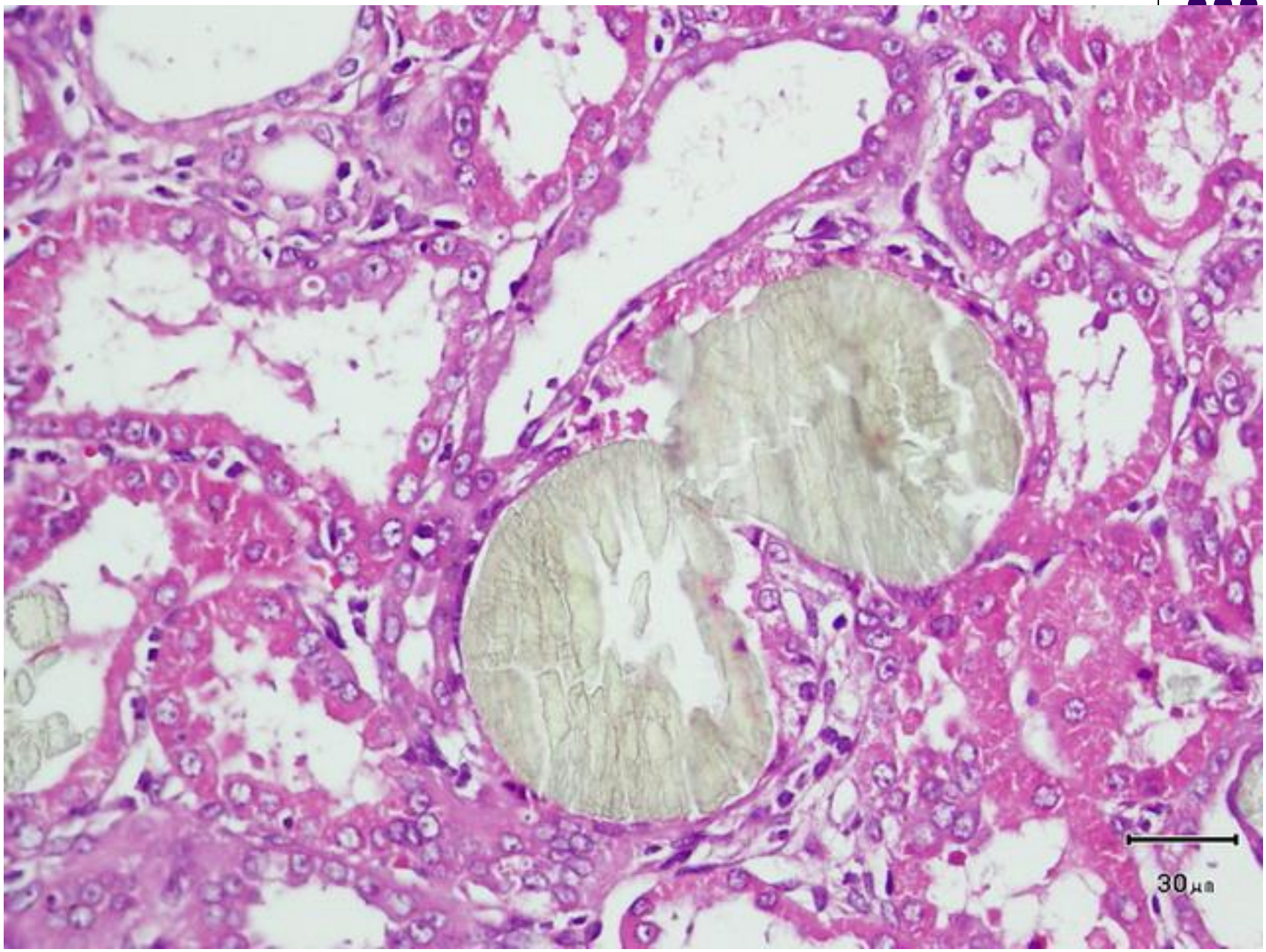
Kidney, 50-100% diet rat



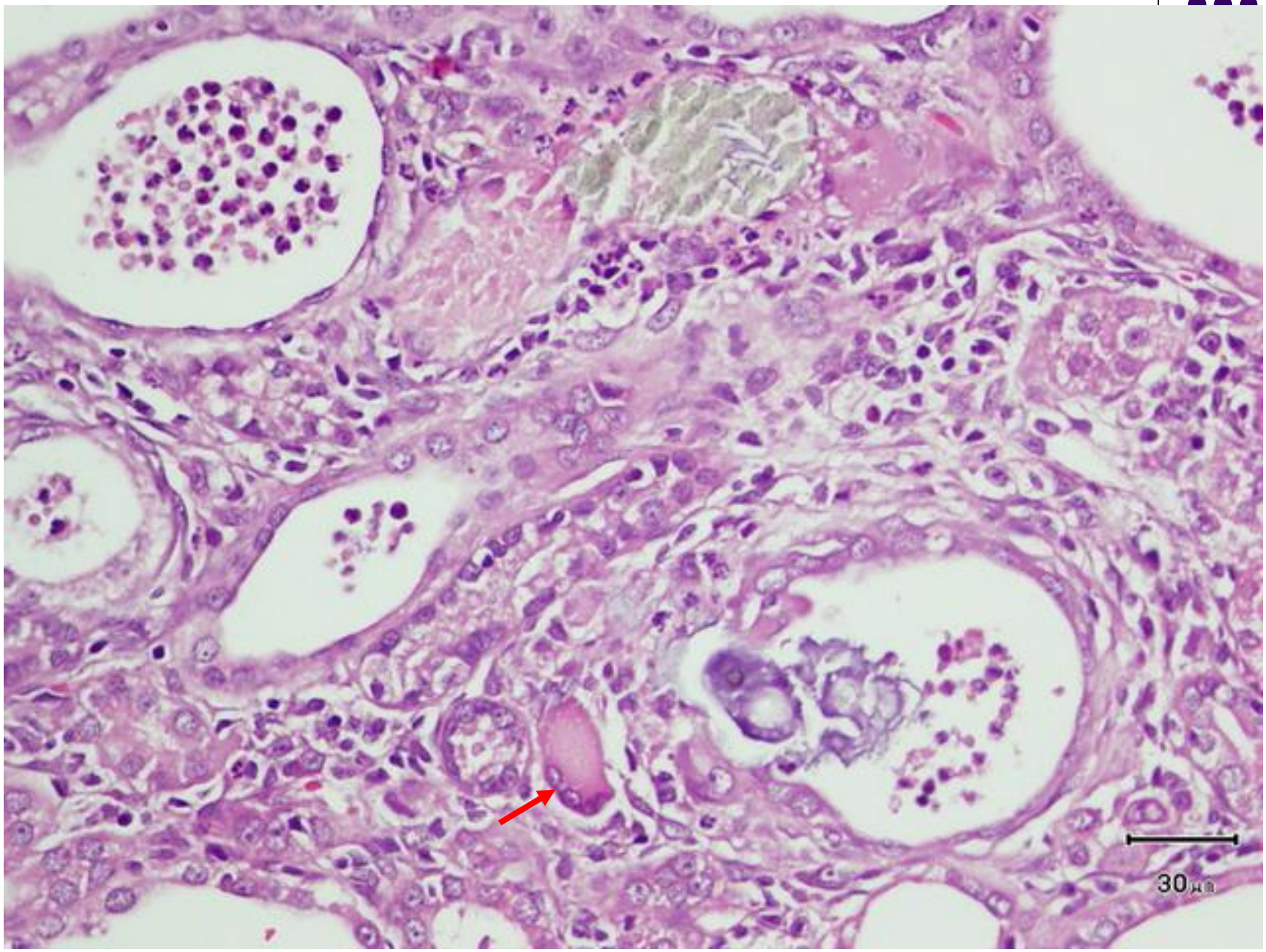








Chen et al., *Toxicol Pathol*. xxx, 2009



Chen et al., *Toxicol Pathol*. xxx, 2009

TABLE 6.—Histoscores of injured kidneys in rats fed with various dietary levels of L107 diet for 12 weeks

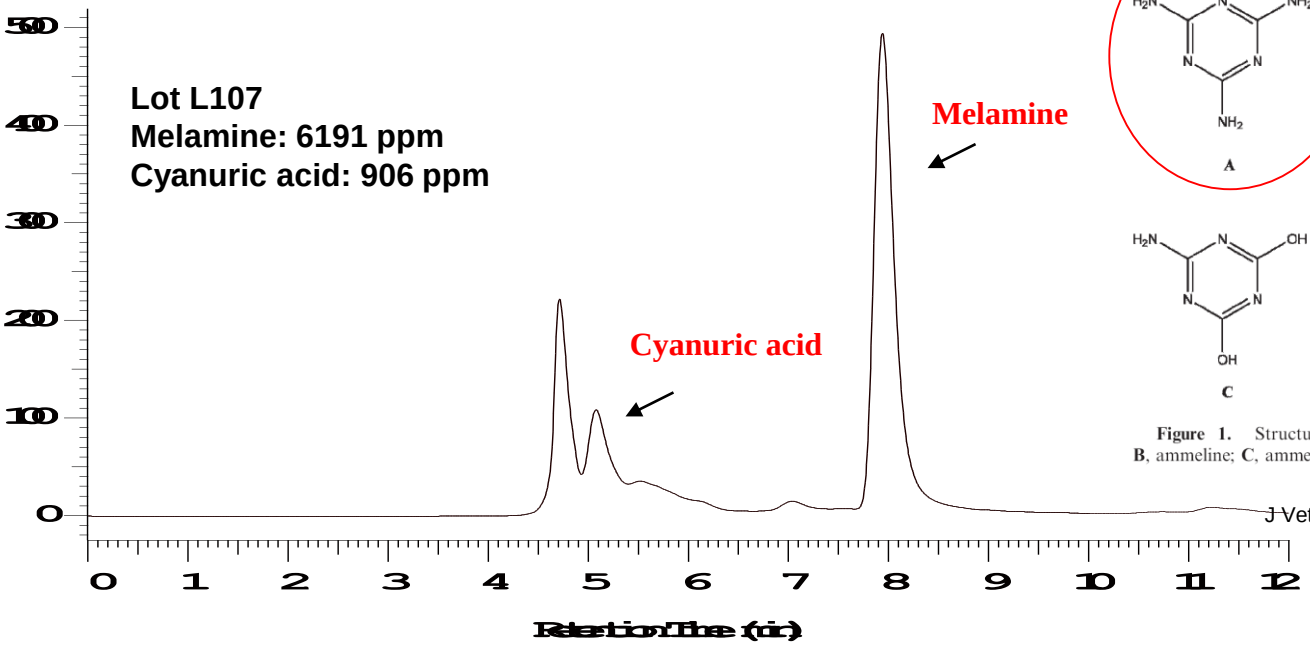
Lesions	Group (% in diet)									
	Male					Female				
	C	10	20	50	50-100[a]	C	10	20	50	50-100[a]
Tubular MC crystals	-[a]	-	-	0.8±0.7[b]	3.4±0.5*	-	-	-	-	1.4±0.8*
Tubular necrosis	-	-	-	1.6±1.6	4.8±0.4*	-	-	-	0.2±0.4	2.6±1.5*
Tubular dilation	-	-	-	2.0±1.1*	5.0±0.0*	-	-	-	-	3.0±1.9*
Tubular regeneration	-	-	-	2.8±1.7*	5.0±0.0*	-	-	-	0.4±0.8	3.0±1.7*
Inflammatory cells	-	-	-	2.8±1.7*	5.0±0.0*	-	-	-	0.4±0.8	2.6±1.4*
Interstitial fibrosis	-	-	-	1.8±1.6	2.8±0.4*	-	-	-	0.2±0.4	1.2±0.7*

- [a] -: No significant lesion.

[b] Degree of lesions was graded from one to five depending on severity: 1 = minimal (< 1%); 2 = slight (1-25%); 3 = moderate (26-50%); 4 = moderate/severe (51-75%); 5 = severe/high (76-100%). Histoscore = score of each affected kidney/ no. of rats were examined. Data are expressed as Mean ± SD (n = 5, except n = 4 in Group 4).

* Significant difference between the control and treated groups at $p < 0.05$.

Chen, et al., 2009. *Toxicologic Pathology.* 37(7): 959-68



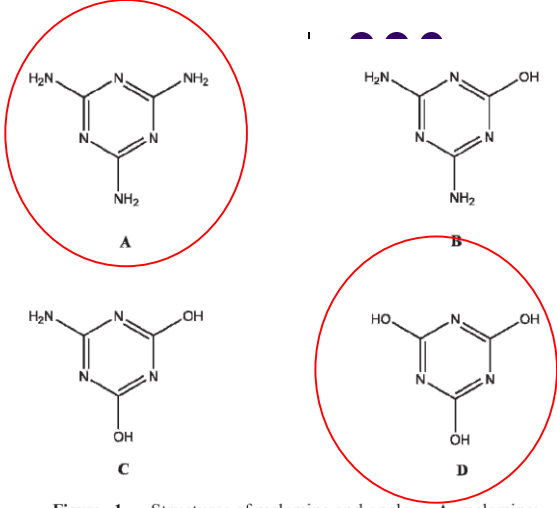
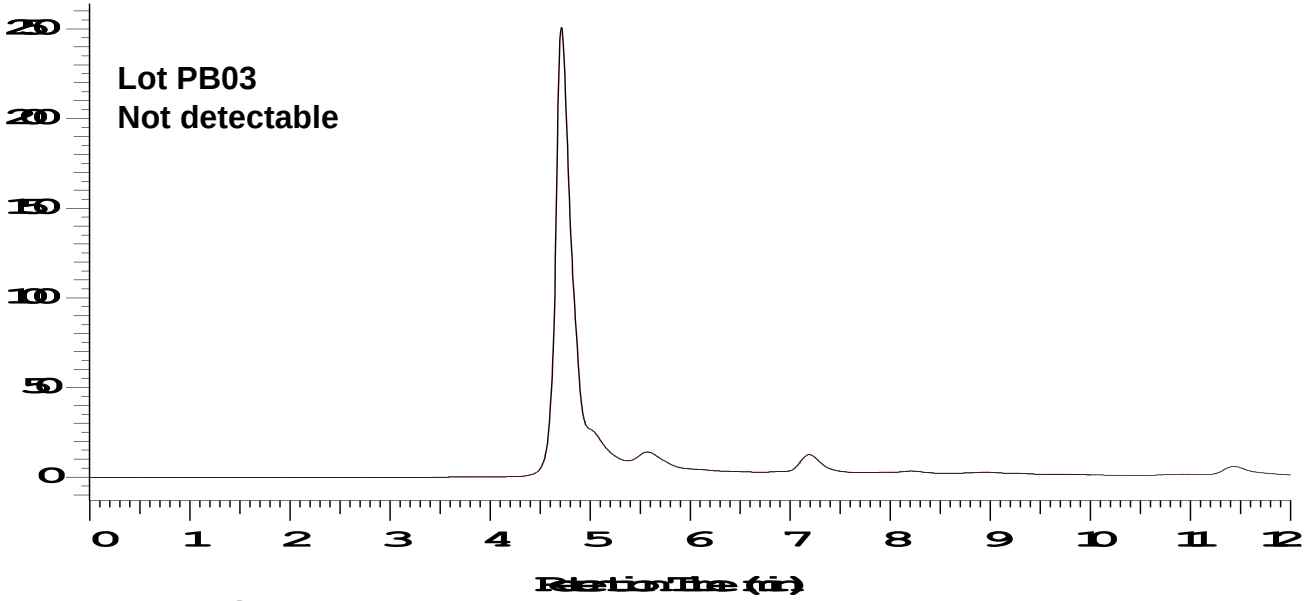


Figure 1. Structures of melamine and analogs. **A**, melamine; **B**, ammeline; **C**, ammelide; **D**, cyanuric acid.

J Vet Diagn Invest 19:616–624 (2007)



reverse phase-HPLC-UV

Chen, et al., 2009. *Toxicologic Pathology.* 37(7): 959-68

TABLE 2.— The calculated daily intake of cyanuric acid and melamine of rats fed with various dietary levels of L107 diet for 12 weeks.

Sex/(mg/kg/day)	Group			
	10%	20%	50%	50-100%
Male				
Cyanuric acid	6.0±1.2[a]	11.8±2.6	29.4±6.6	38.3±6.0[b]
Melamine	40.6±8.2	80.4±17.8	200.4±44.7	260.9±40.6
Female				
Cyanuric acid	7.2±0.9	14.6±2.0	35.2±5.4	60.3±4.4
Melamine	48.9±6.2	99.7±13.7	240.0±36.9	410.7±30.2

[a] Data are expressed as mean±SD (n = 5, except n = 4 in Group 4).
[b] The daily intake of cyanuric acid and melamine in rats fed with 100% L107 diet was calculated from week 9 to 12.

Chen, et al., 2009. *Toxicologic Pathology.* 37(7): 959-68

發育及生殖毒性
(Development and reproductive toxicity)

Toxicants → ?? → 子代發育及性成熟影響

親代毒性	胚胎毒性	畸胎毒性	後代毒性
母鼠增重	子宮增重	仔鼠畸形數	仔鼠數
肝比重	黃體數	仔鼠畸形種類	仔鼠重量
	著床數		仔鼠性比例
	著床前胚損失數		
	著床後胚死亡數		

Test No. 443, Extended One-Generation Reproductive Toxicity Study, 2018

Test No. 414, Prenatal Developmental Toxicity Study, 2018

Test No. 415, One-Generation Reproduction Toxicity Study, 1983

Test No. 416, Two-Generation Reproduction Toxicity, 2001

游碧堉, 呂水淵, 2003

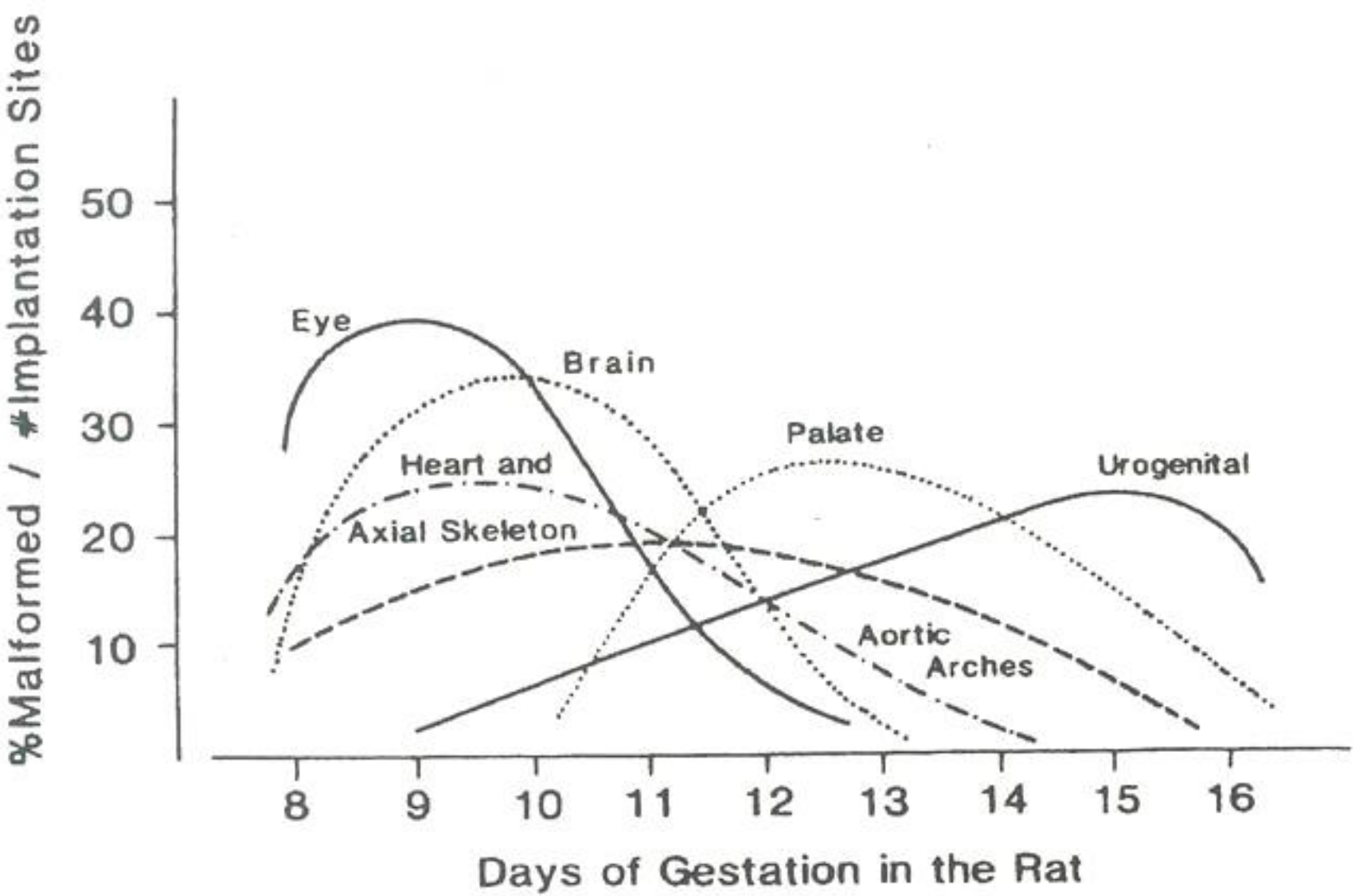


FIG. 5. Hypothetical pattern of susceptibility of embryonic organs to teratogenic insult. Adapted from Wilson (95).

發育及生殖毒性
(Development and reproductive toxicity)

- **第一階試驗[發育毒性試驗]**
  ⇒觀察藥劑對動物發育至成熟期是否影響
  [試驗前給藥60天(M) + 配種前給藥14天(F) + 懷孕期]

- **第二階試驗[畸胎性試驗]**
  ⇒觀察藥劑對懷孕期動物胎兒是否引起畸形
  [懷孕期第6至15天連續給藥，並於分娩前大鼠懷孕第20天剖腹取出胎兒]

- **第三階試驗[畸胎性試驗+發育毒性試驗]**
  ⇒為藥劑對懷孕期中期及分娩後毒性試驗
  [懷孕期第15天 + 分娩後3週]

- **生殖毒性**
  ⇒觀察藥劑對受胎、胎兒發育及後代生殖能力
  [第0代(F0) + 第1代(F1) + 第2代(F2)全期]

Test No. 414, Prenatal Developmental Toxicity Study, 2018

呂水淵, 游碧堉, 2003

2.1 Criteria for external and visceral examination of fetuses

External structure	Visceral structure
Head	Head
Cranium	Brain
Eye	Neck
Palate	Heart
Abdominal	Abdominal
Limbs	Sex
Tail	Others
Genital	
Others	

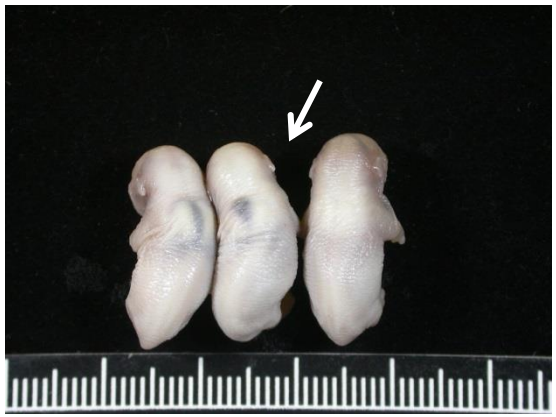
Manson and Kang, 1994.

2.2 Criteria for external and skeletal anomalies

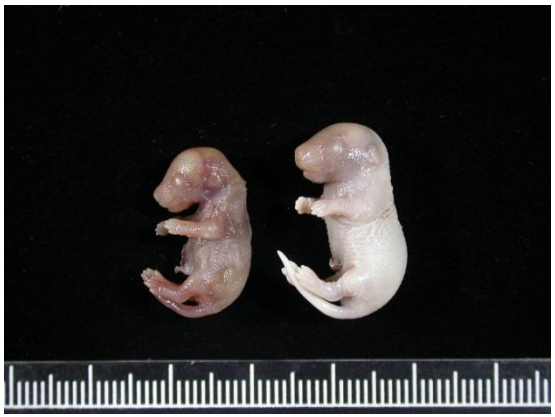
Position	Bone	Position	Bone
Pectoral Girdle	Clavicle	**Skull**	Braincase
	Scapula		Tympanic Bulla
	Humerus		Body of Hyoid
	Radius		Palate
	Ulna		Jaw
	Metacarpals		
	Phalanges	**Axial skeleton**	Sternebrae
			Vertebrae
Pelvic Girdle	Ilium		
	Ischium		Tail
	Femur		
	Tibia		Ribs
	Fibula		
	Metatarsals		
	Phalanges		

Manson and Kang, 1994.

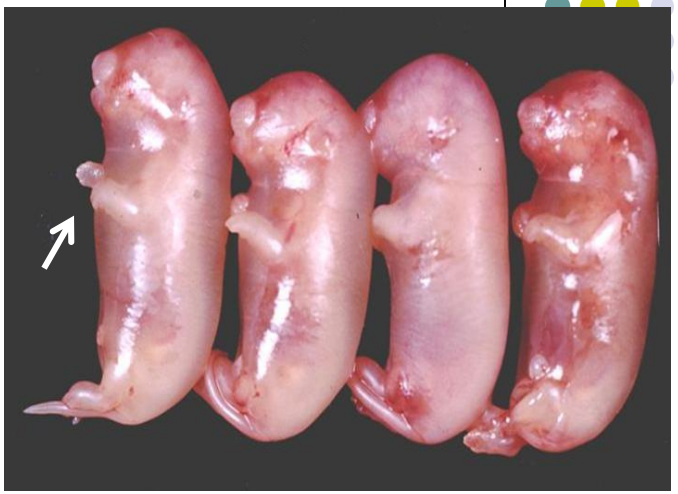
External anomalies in fetuses of mice



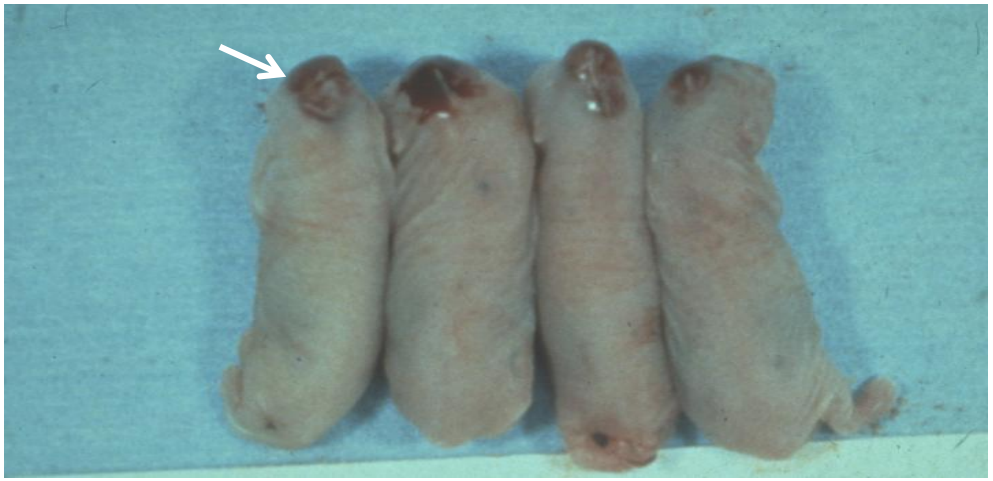
vertebra side bend



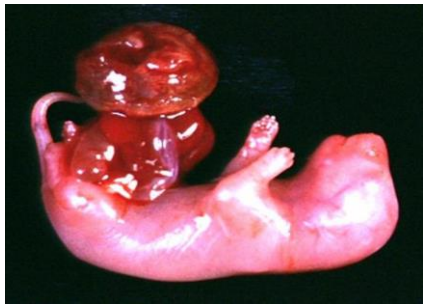
umbilical hernia



short head, Phocomelia



Exencephaly



gastroschisis

呂水淵, 2003

Table 1. External anomalies in fetuses of mice on GD 20 following maternal treatment with substances

Group	Control	Reference data
No. fetuses/litters examined	111 (21)	38900
No. litters with abnormalities (%)[1]	9 (42.8)	
No. fetuses with anomalies, N (mean %)	23 (20.7)	
Type of anomalies, N (mean %)[2]		
Head		
Hydrocephalic	1 (0.9)	0.3-1.2
Abdominal		
Umbilical hernia	4 (3.6)	0.1-1.0
Phocomelia	9 (8.2)	0.3-1.6
Tail		
Kidney	0 (0.0)	0.7-1.7
Fracture	0 (0.0)	?
Other		
Torticollis	1 (0.9)	?
Vertebra side bend	6 (5.4)	?
Anatomy closed not entire	2 (1.8)	?

[1] %= Number of litters affected/total litters examined x 100.
[2] %= Number of fetuses affected/total fetuses examined x 100.
[3] Manson and Kang, 1994.

Visceral anomalies in fetuses of mice

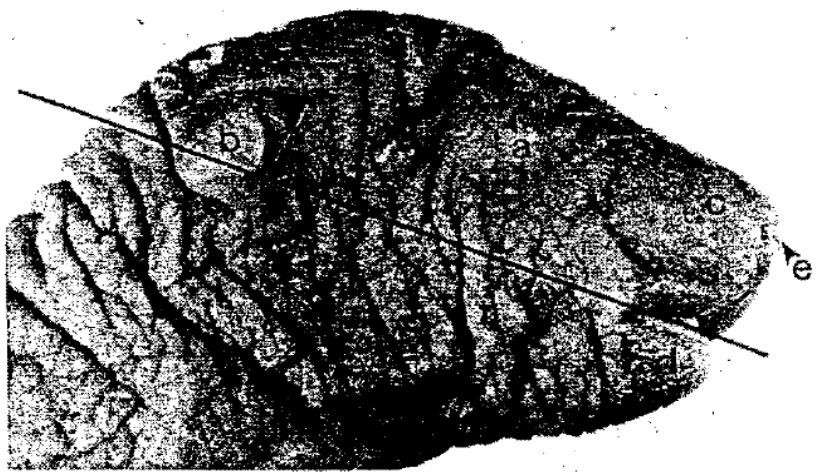
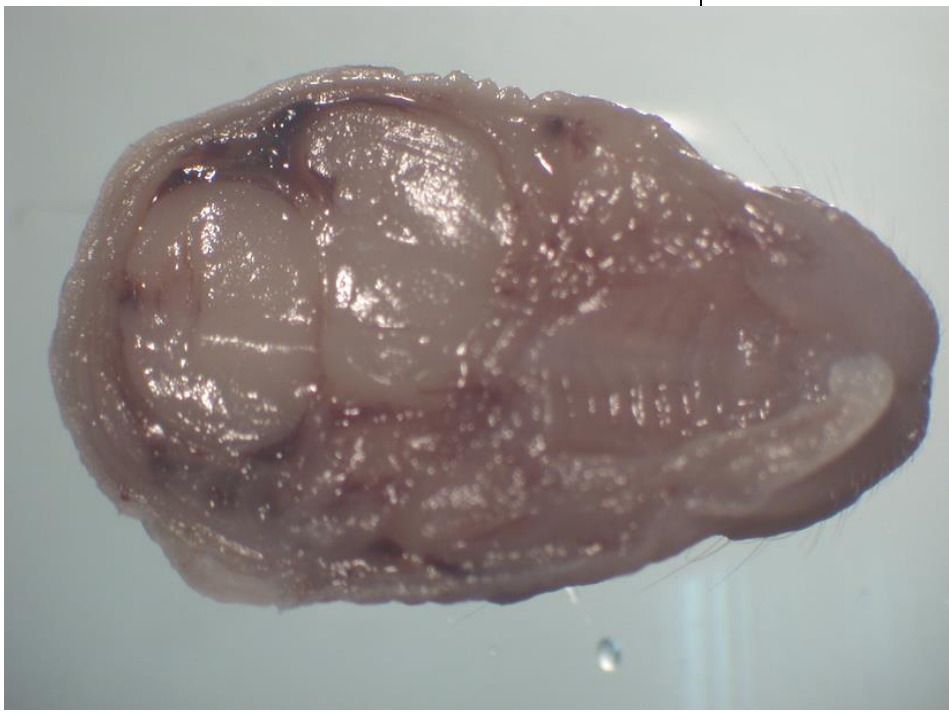


FIG. A-50. Normal head of rat fetus: *(a)* eyelids; *(b)* ear (pinna); *(c)* nares; *(d)* lower jaw; *(e)* nostrils. The line shows where section is made to examine the palatine shelf.



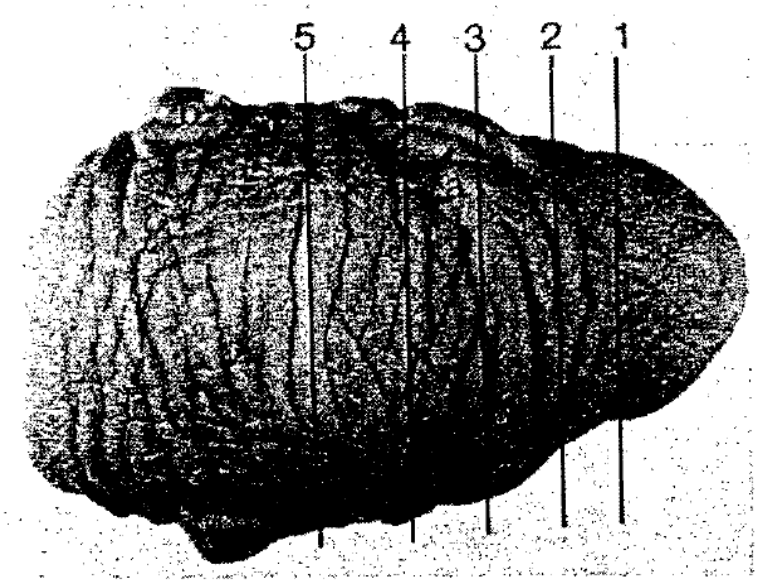
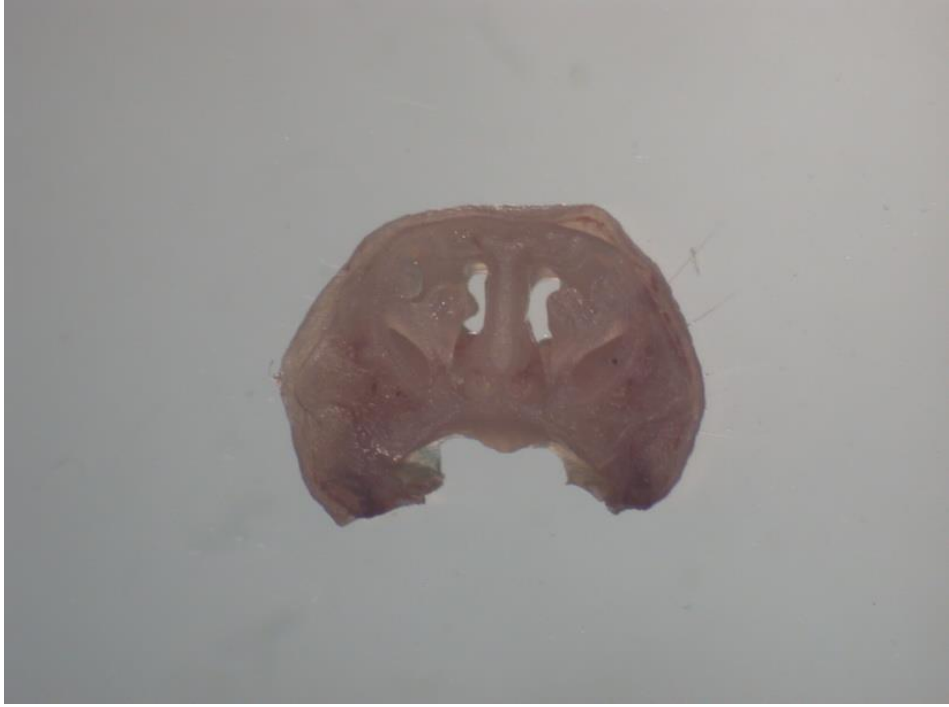
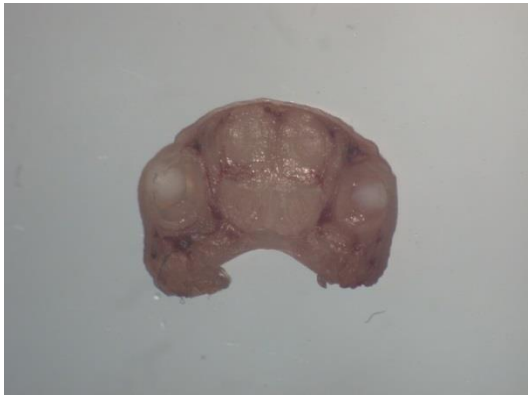
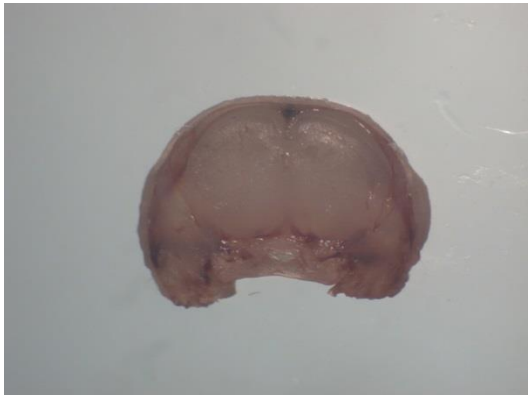


FIG. A-53. Normal head of rat fetus, coronal view: *(a)* eyelids; *(b)* ear (pinna). Lines 1–5 show where sections are made.







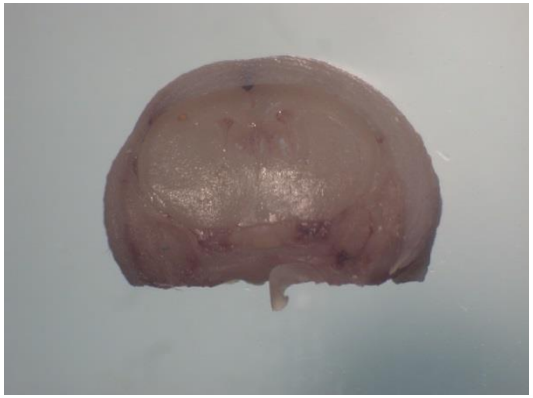


Table 2. Visceral anomalies in fetuses of mice on GD 20 following maternal treatment with substance

Group	Control
No. fetuses/litters examined	111 (21)
No. litters with abnormalities (%)[1]	0 (0.0)
No. fetuses with anomalies, N (mean %)	0 (0.0)
Type of anomalies, N (mean %)[2]	0 (0.0)

[1] %= Number of litters affected/total litters examined x 100.
[2] %= Number of fetuses affected/total fetuses examined x 100.

Skeletal abnormalities in fetuses of mice

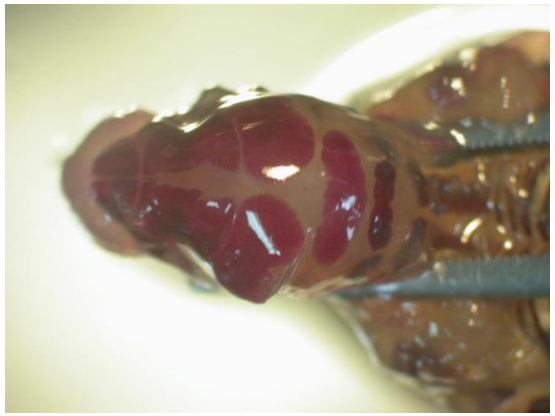
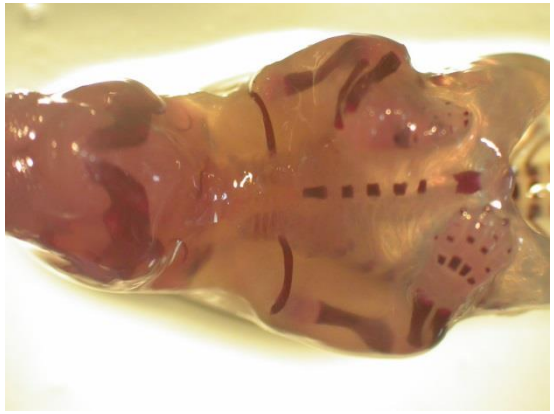
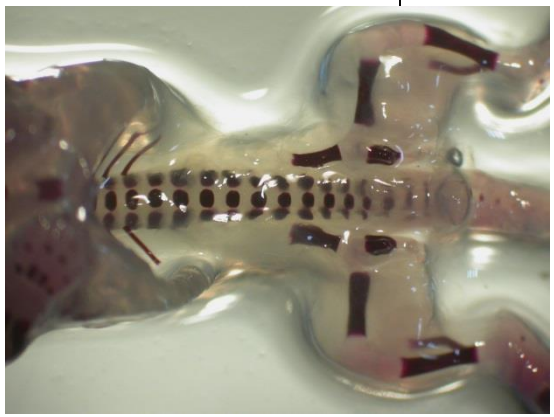
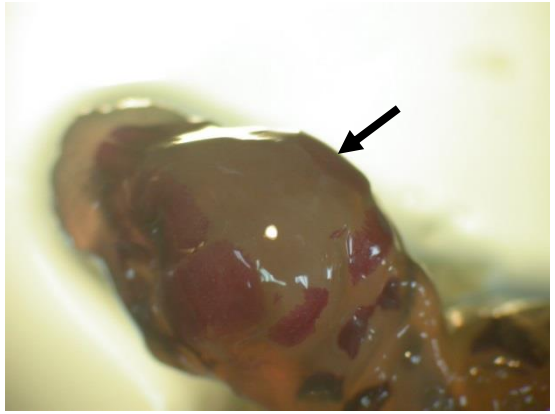
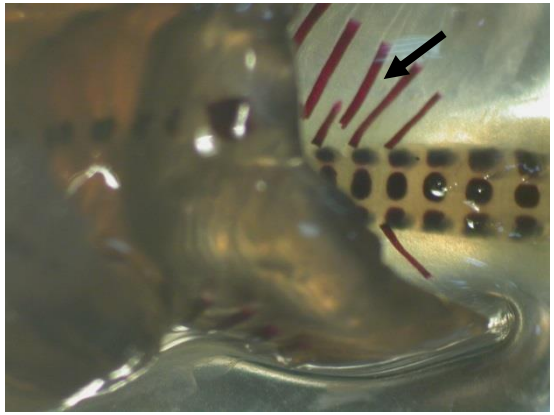
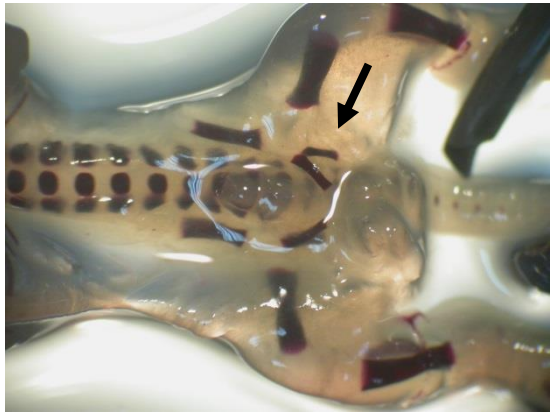







Table 3. Skeletal abnormalities in fetuses of mice on GD 20 following maternal treatment

Group	Control	Reference data3
No. fetuses/litters examined	121(20)	5,500
No. litters with abnormalities[1]	9 (20)	
No. fetuses with abnormalities, N (mean %)[2]	15 (12.4)	
Type of anomalies, N (mean %)		
Skull		
Braincase		
Missing	1 (0.8)	0.6-5.5
Break	1 (0.8)	0.4-4.0
Axial skeleton		
Sternal		0.5-5.0
Missing	0 (0)	
Vertebrae		0.5-5.0
Missing	2 (1.6)	
Break	1 (0.8)	
Winding	1 (0.8)	
Tail		0.5-5.0
Winding	1 (0.8)	
Ribs		0.4-4.3
Missing	6 (4.9)	
Break	1 (0.8)	

藥物致畸胎性之分類標準
(Classification of chemical teratogens)

致畸胎等級	分類標準
A 級	在良好控制下，在人類孕婦的實驗研究，顯示並無致畸胎的危險性。
B級	只有動物實驗而無適當的人類孕婦實驗(懷孕前三個月的安全性尚未確立，懷孕中期、末期則可以使用)。
C級	**動物實驗顯示有致畸胎性，但對人類尚無臨床實驗研究(孕婦使用必須非常小心)。**
D級	臨床使用資料顯示有致畸胎性危險性(非到萬不得已是不使用)。
X級	無論在動物或人體研究均證實會造成胎兒異常(任何情況均不建議使用)。

美國食品藥物管理局1980年

Skeletal abnormalities in fetuses of mice

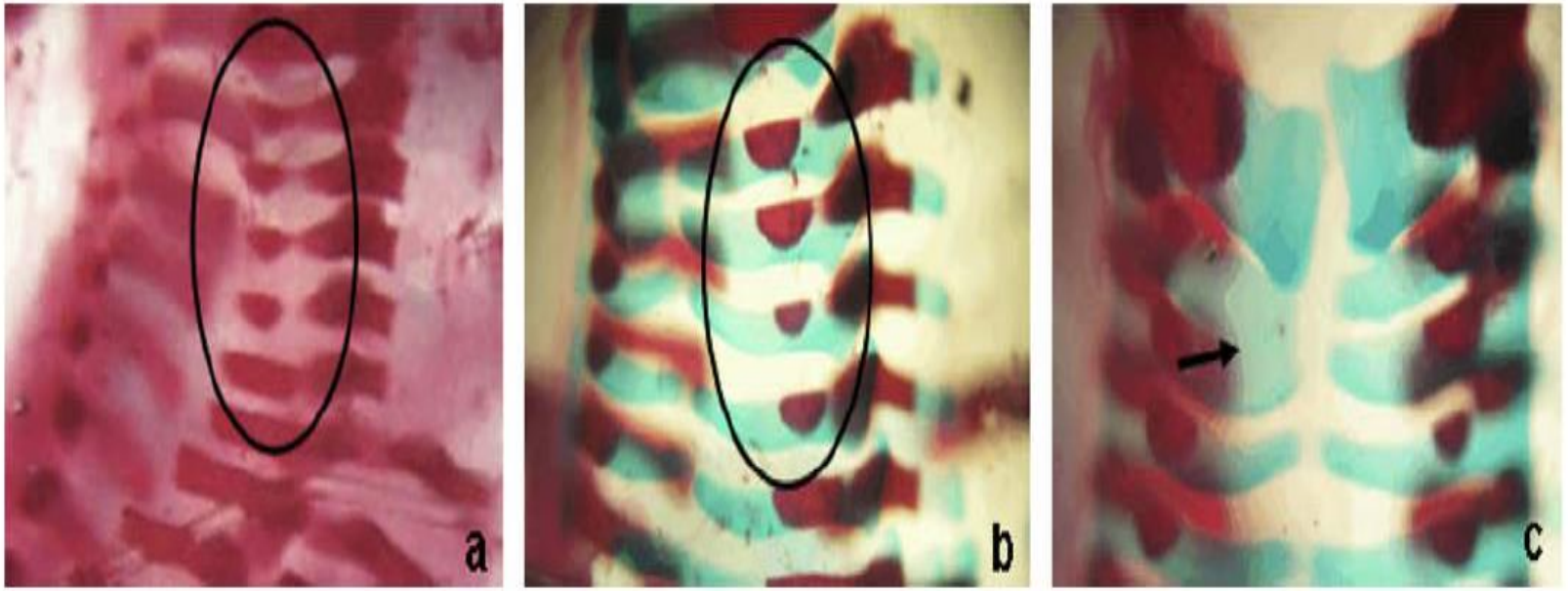


Fig. 1. Typical skeletal abnormalities in cervical arch in **acetylsalicylic acid-exposed** fetuses. Pregnant rats were treated with ASA at 250 mg/kg once on GD 10 and fetuses evaluated on GD 20. **Incomplete ossification of cervical arch** seen with single-staining (a) and double-staining (b), and fused cervical arch with double-staining (c). Note: Incomplete ossification of cervical arch was observed as a common finding in fetuses at 250 mg/kg with both single- and double-staining. **Fused cervical arch** was observed as a cartilage-specific finding.

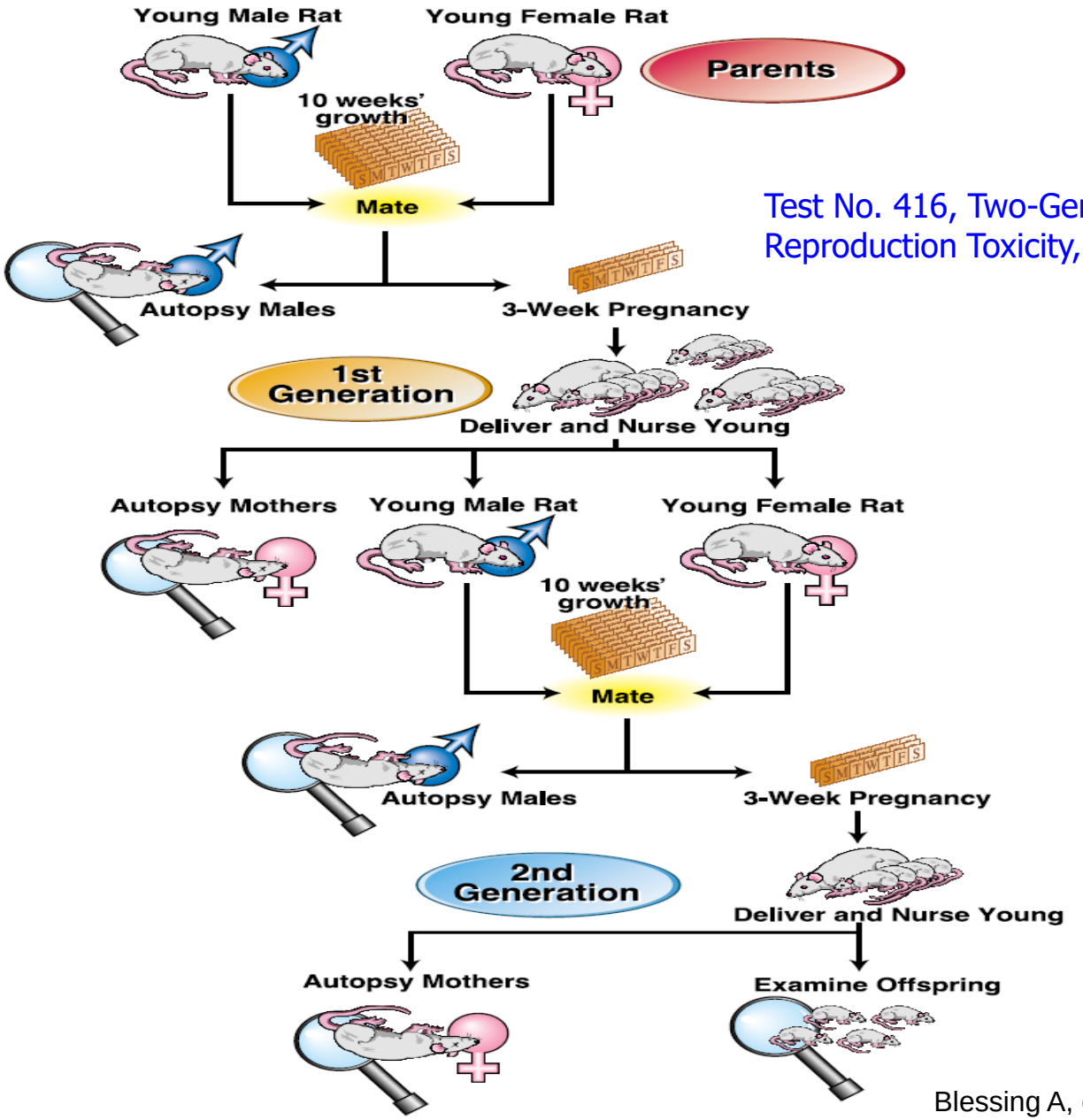
T. Dodo et al. / Regulatory Toxicology and Pharmacology 54 (2009) 308–313

Skeletal abnormalities in fetuses of mice

Fig. 3. Typical rib abnormalities in acetylsalicylic acid-exposed fetuses. Pregnant rats were treated with **ASA at 250 mg/kg** once on GD 10 and fetuses evaluated on GD 20. Fused rib (arrows) was observed as a common finding in single staining (a) and/or double staining. **Fused rib cartilage (b) and discontinuous rib cartilage (c)** were observed as cartilage specific findings.

T. Dodo et al. / Regulatory Toxicology and Pharmacology 54 (2009) 308–313

Two Generation Reproduction Study
With Daily Dosing of Adults and Offspring



Test No. 416, Two-Generation Reproduction Toxicity, 2001

Blessing A, et al., 2002

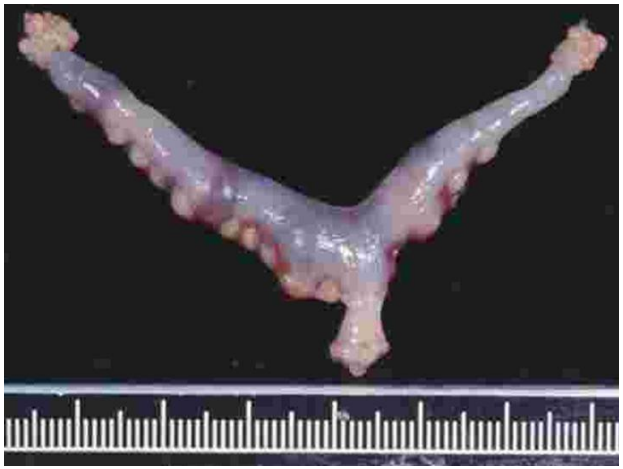
Reproductive toxicity *(in vivo)*

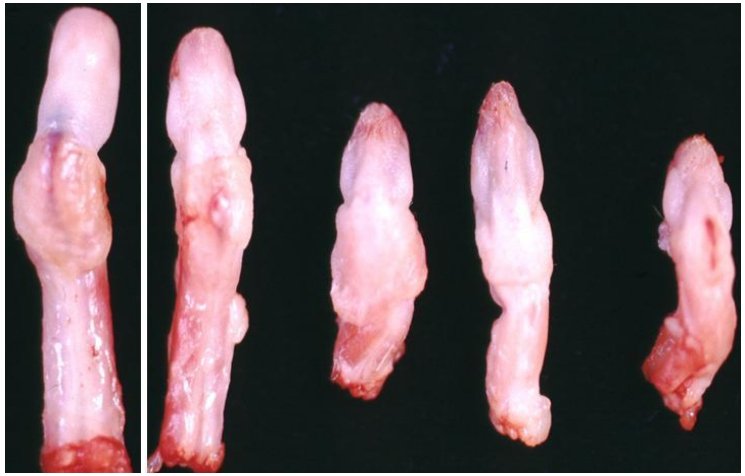
In utero

1. 黃體數(ovary)
2. 著床數(uterus)
3. 仔鼠數
4. 著床後胚死亡數
5. 畸形種類與數目
6. 性別比率

6-week old puberty

1.陰道開啓時所需時間
2.包皮與陰莖分離時間





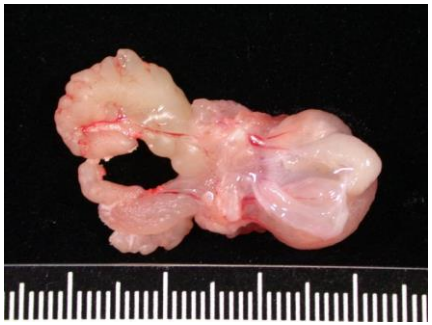
呂水淵, 2003

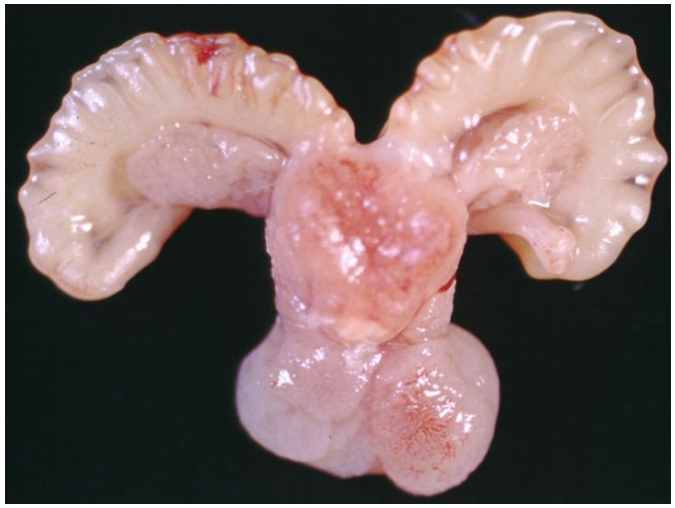
Endocrine disrupting activity

(in vivo assay)

Hershberger assay (male)

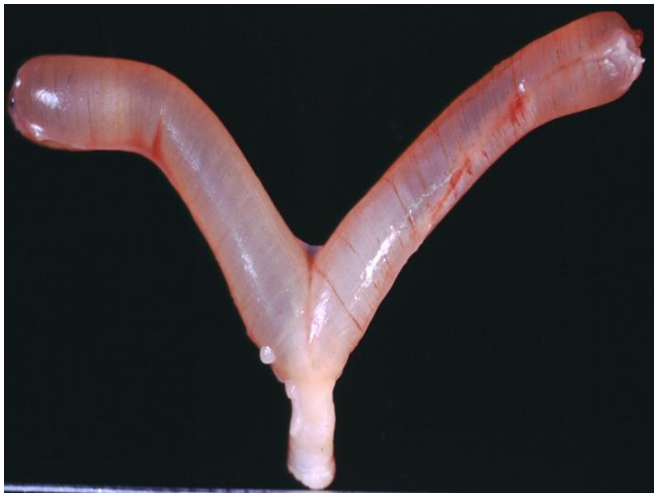
1.前列腺重
2.貯精囊重
3.球海綿體肌與提睪肌重





Uterotrophic assay (female)

1.子宮重



呂水淵, 2003

Table 2
Estrous cyclicity, natural delivery and litter observations; P generation and F1 generation.

Generation	P1					F1				
Group	Carrier control	Comparative control	NAA			Carrier control	Comparative control	NAA		
Dose	0	500	100	250	500	0	500	100	250	500
Estrous cycling observations										
Rats evaluated	25	25	25	25	24[a]	25	25	25	25	24[b]
Estrous stages/14 days (mean ± SD)	2.7 ± 1.1	3.1 ± 0.8	2.8 ± 1.1	2.9 ± 0.9	2.8 ± 1.2	3.2 ± 0.5	2.7 ± 0.9	3.0 ± 0.8	2.8 ± 1	3.0 ± 1
Rats with 6 or more consecutive days of dioestrus	0	1	2	1	2	0	1	2	5	3
Rats with 6 or more consecutive days of estrus	0	0	1	0	0	0	0	0	0	0
Natural delivery observations										
Rats assigned to natural delivery	25	25	25	25	25	25	25	25	25	24
Pregnant	20	20	20	23	21	24	22	22	22	22
Delivered a litter	20	20	20	22	21	24	22	21	21	22
Duration of gestation (mean ± SD)	22.8 ± 0.6	22.8 ± 0.6	22.8 ± 0.8	22.6 ± 0.6	22.7 ± 0.6	22.5 ± 0.5	22.4 ± 0.5	22.8 ± 0.5[*,#]	22.5 ± 0.5	22.4 ± 0.5
Implantation sites	292	278	293	320	307	378	337	333	320	328
Per delivered litter (mean ± SD)	14.6 ± 2.3	13.9 ± 3.9	14.6 ± 1.6	14.5 ± 1.9	14.6 ± 2.6	15.8 ± 2	15.3 ± 1.8	15.8 ± 2.4	15.2 ± 2.2	14.9 ± 1.9
Dams with stillborn pups	1	1	0	0	0	3	1	2	0	2
Dams with no liveborn pups	0	0	0	0	0	0	0	0	0	0
Gestation index[c]	100	100	100	100	100	100	100	100	100	100
Dams with all pups dying days 1–4 postpartum	0	1	0	0	0	0	0	0	0	0
Dams with all pups dying days 5–22 postpartum	0	0	0	0	0	0	0	0	1	0
Litter observations										
Delivered litters with one or more liveborn pups	20	20	20	22	21	24	22	21	21	22
Pups delivered (total)	275	259	278	311	289	361	308	317	295	310
Per dam (mean ± SD)	13.8 ± 2.4	13.0 ± 3.8	13.9 ± 1.9	14.1 ± 2.0	13.8 ± 3.0	15.0 ± 1.7	14.0 ± 2.0	15.1 ± 2.7	14.0 ± 2.3	14.1 ± 1.9
Liveborn (mean ± SD)	13.6 ± 2.3	12.8 ± 3.7	13.9 ± 1.9	14.0 ± 2.1	13.7 ± 3.0	14.7 ± 1.8	13.9 ± 1.9	14.8 ± 2.8	14.0 ± 2.4	14.0 ± 2.0
Stillborn (mean ± SD)	0.1 ± 0.4	0.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 1.2	0.1 ± 0.4	0.2 ± 0.7	0.0 ± 0.0	0.1 ± 0.5
Unknown vital status (mean ± SD)	2	0	0	2	1	1	1	2	2	0
Viability index (%)[d]	99.3	98.4	99.6	98.7	99.3	96.3	97.7	97.1	95.6	99.7
Lactation index (%)[e]	98.9	98.8	97.1	96.1	97.9	98.5	99	99.7	97.1	99.3
Surviving pups/litter at weaning[f]	13.3 ± 2.5	12.5 ± 3.8	13.4 ± 2.1	13.3 ± 3.3	13.3 ± 2.8	13.9 ± 1.8	13.4 ± 1.8	14.3 ± 2.6	13.0 ± 3.6	13.8 ± 1.8
Live litter size at weaning	13.3 ± 2.5	13.2 ± 2.4[g]	13.4 ± 2.1	13.3 ± 3.3	13.3 ± 2.8	13.9 ± 1.8	13.4 ± 1.8	14.3 ± 2.6	13.6 ± 2.2[h]	13.8 ± 1.8
Pup weight at weaning (grams)	39.5 ± 8.6	39.7 ± 7.8	37.0 ± 6.2	37.9 ± 6.1	39.2 ± 9.4	39.5 ± 4.2	41.2 ± 5.6	41.0 ± 6.6	39.9 ± 6.0[h]	38.9 ± 4.7

保養品、洗髮精 年底禁含雌激素

中時電子報 chinatimes.com

作者： 林宜慧／台北報導 | 中時電子報 – 2015年5月24日 上午5:50

中國時報【林宜慧／台北報導】**女性荷爾蒙「雌激素」為1級致癌物**，歐盟等國已禁用於化妝品，我國今年底擬跟進公告全面禁止，據食品藥物管理署統計，影響多達241件市售產品，包括萌髮566洗髮精、依必朗養髮洗髮精，聖卡禔亞睛亮雙效眼霜等知名廠牌，未來都不能添加雌激素。

台北榮總臨床毒物與職業醫學科主任楊振昌表示，雌激素被世界衛生組織列為1類致癌物，對人體的卵巢癌、子宮內膜癌有明確致癌性，長期過量暴露也可能增加乳癌風險，並導致年輕女性不正常出血，對嬰幼兒及孩童則會影響性徵及生殖功能。

TOXICOLOGICAL SCIENCES **103(1)**, 137–148 (2008)
doi:10.1093/toxsci/kfn020
Advance Access publication January 30, 2008

Motorcycle Exhaust Induces Reproductive Toxicity and Testicular Interleukin-6 in Male Rats

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Received December 13, 2007; accepted January 15, 2008

Fig 1. Gross finding of testes, epididymis of male rats in the ME119-treated rats. No significant gross change of the testes, epididymis (left), however, remarkable testis and epididymis atrophy were found in the ME119-treated rats (right). Tissues location ranged as the control (left), and M-treated (right) groups (A-D).

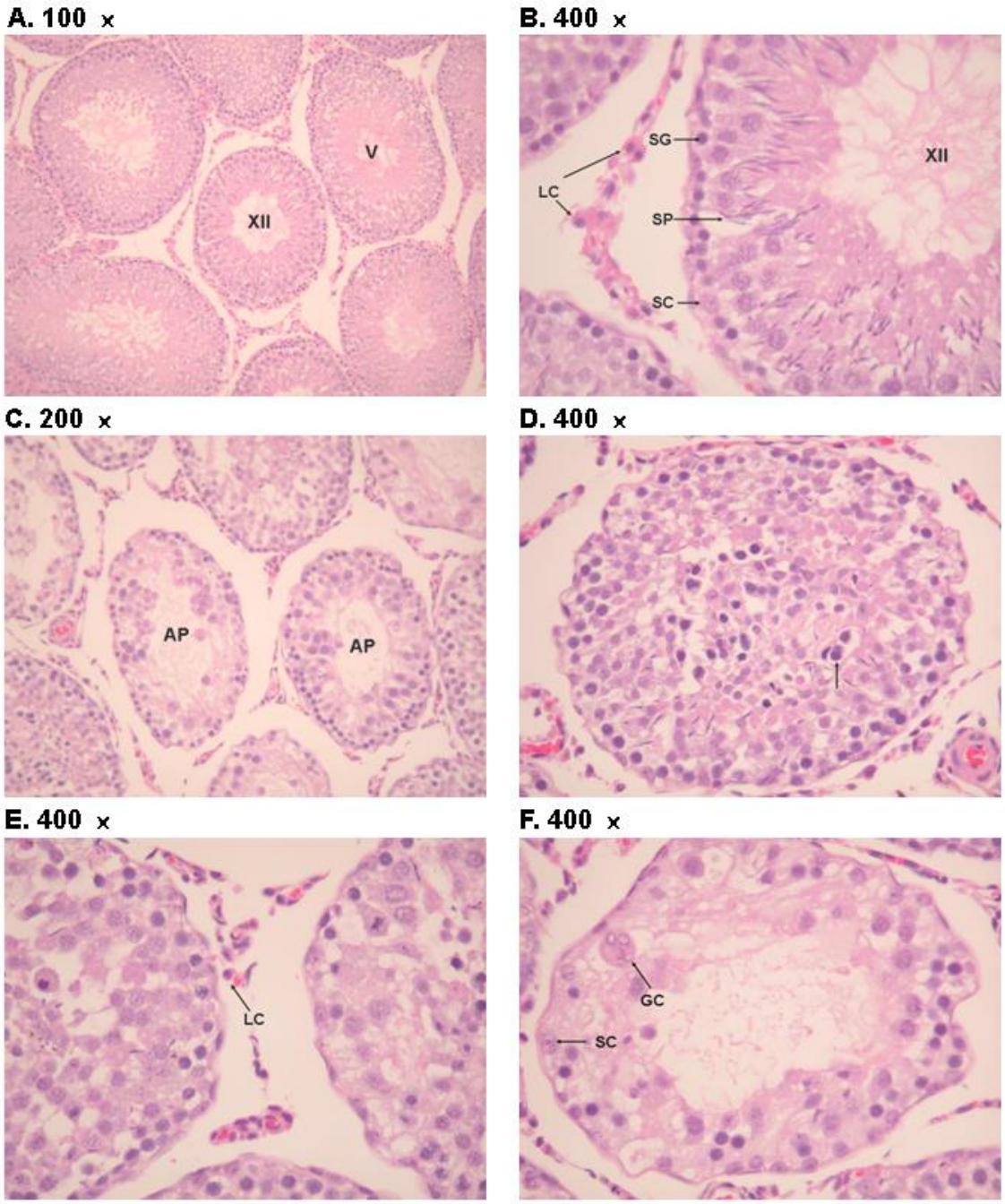

Figure 1. Morphology of testes from controls and male rats exposed to ME by inhalation. Male Wistar rats were exposed to 1:10 diluted ME by inhalation 2 h daily for 4 weeks. Control rats were exposed to clean air. Testis from a control rat showed normal seminiferous tubule morphology and sperm at varying stages (stage V and stage XII) of maturation (A, magnification x 100) and normal spermatognia (SG) and spermatid (SP) and the presence of Sertolic cells (SC) and Leydig cells (LC) (B, x 400). Testis from a ME-exposed rat showed seminiferous tubular atrophy (AP) (C, x 200), moderate to severe necrosis of spermatocytes (arrow) (D, x 400), dissociation of Leydig cells (E, x 400), and the absence of elongated spermatid and spermatozoa, decrease of spermatocytes, formation of multinuclear giant cells (GC), and the presence of Sertoli cells (F, x 400). Hematoxylin and Eosin stain

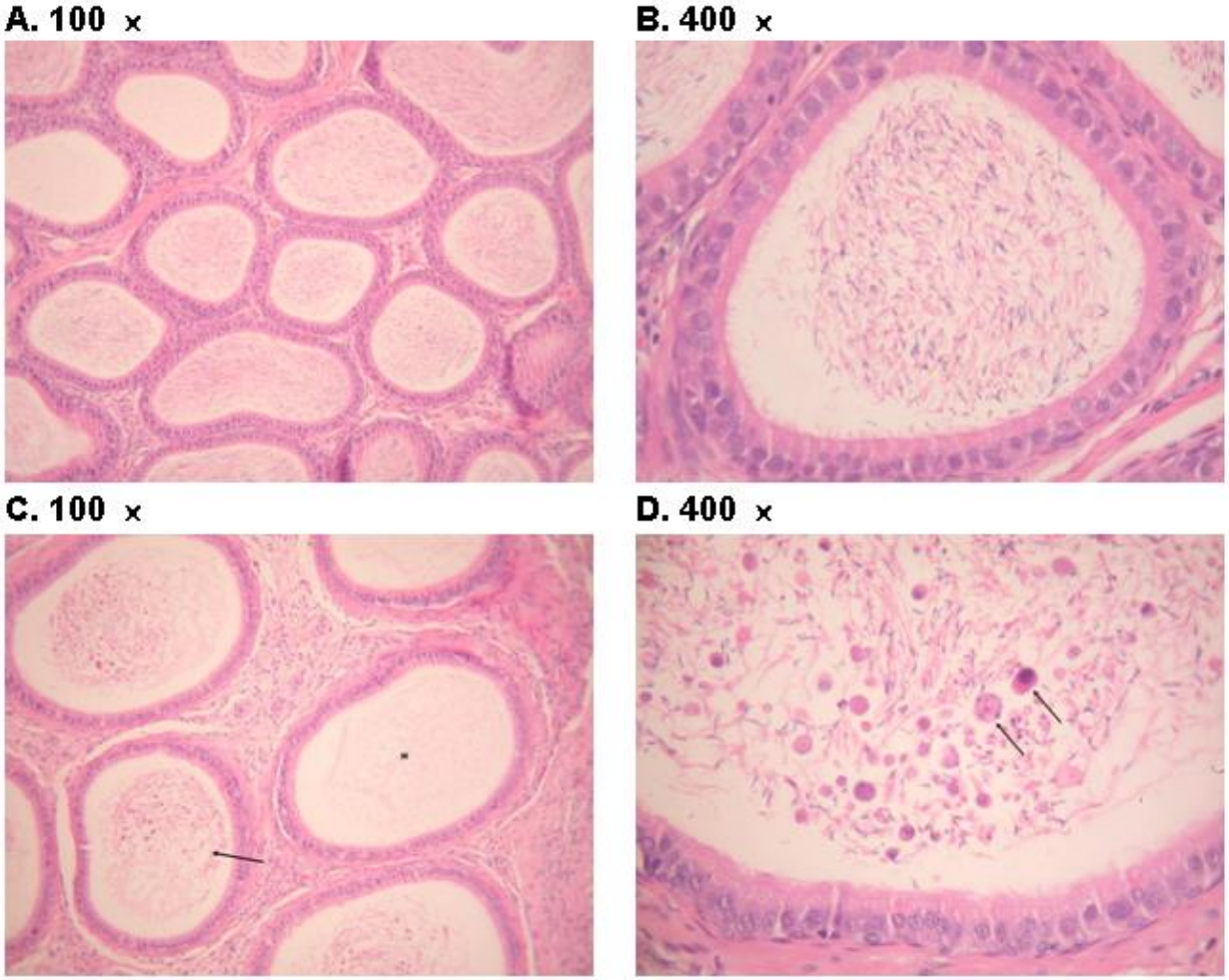

Figure 2. Morphology of cauda epididymides from control and male rats exposed to ME by inhalation. Male Wistar rats were exposed to 1:10 diluted ME by inhalation 2 h daily for 4 weeks. Control rats were exposed to clean air. Cauda epididymis from a control rat showed normal efferent ductules morphology (A, magnification x 100) and the presence of numerous normal spermatocytes (B, x 400). Cauda epididymis from a ME-exposed rat showed an empty of sperms (∗) or a mass cluster of pyknotic and necrotic sperm cells (arrow) in the lumen of efferent tubules (C, x 100) and cellular debris and necrotic sperm cells (arrow) (D, x 400). Hematoxylin and Eosin stain.

Chronic and Carcinogenicity Toxicity Test

- Rat and mouse (oral, dermal, or inhalation)
- Control, vehicle control, and treated groups
- Number: 20 males and 20 females per group (total no.: 400)
- Dosing:
  3 dose levels (minimal, intermediate, maximal doses)
  Interval sacrificed groups (at 6, 12, 18-mos for rats)
  Repeated doses treatment and a 2-year observation period
- Observations:
  Clinical signs, Mortality, Body weight change, Hematology, Gross and micro pathology, Urinary, Tumor incidence…
- Data analysis: NOAEL, mg/kg/bw/day

Test No. 453, Combined Chronic Toxicity/ Carcinogenicity Studies, 2018

OPPTS Harmonized Test Guidelines, Series 870.4100, EPA 712-C-98-210

Aflatoxin B1 and 2-Acetylaminofluorene Induced Hepatic Carcinogenicity and Gamma-Glutamyltranspeptidase Expression via Chronic Feeding in Rats

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[2]Department of Veterinary Medicine, National Taiwan University, Taipei, Taiwan, ROC

Plant Prot. Bull. 44：37 - 50, 2002

MATERIALS AND METHODS

1. Sixty male and female adult Wistar rats (*Rattus norvegicus*), 5 week-old, were bought from the National Laboratory Animal Production and Research Center in Taipei.

2. 2-AAF and AFB1 of 95% purity were purchased from Sigma Co., (Louis, MO, USA).

   2.1. The stock feed of 2% 2-AAF powder and 0.02% AFB1 (0.0526 g diluted with 250 ml acetone) were prepared as a stock feed.

   2.2. Weighing 100 g of stock feed was directly added into 4.9 kg of powder feed (Rodent Laboratory Chow 5001, Purina, MO, USA) and blended with stainless mixing machine (V-type, No. 40-564, RKJ, Japan) for 30 min.

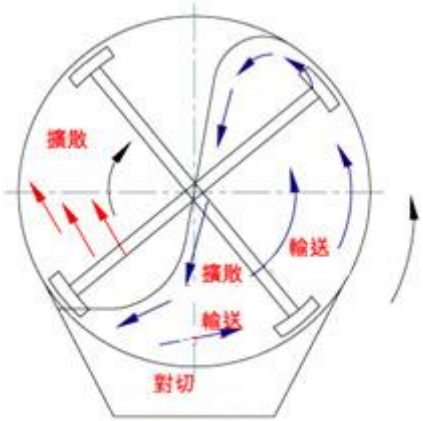
   2.3. Five kilogram feed containing test chemical was mixed weekly.

1. Stability of test article in diet feed
2. Homogeneity of test article in diet feed

二重圓錐式混合機

桌上型粉碎機

凌廣工業股份有限公司



混合示意圖

MATERIALS AND METHODS

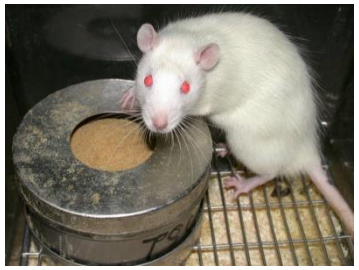
1. Rats were randomly assigned to the 2-AAF, AFB1 and control groups with 10 males and 10 females in each.

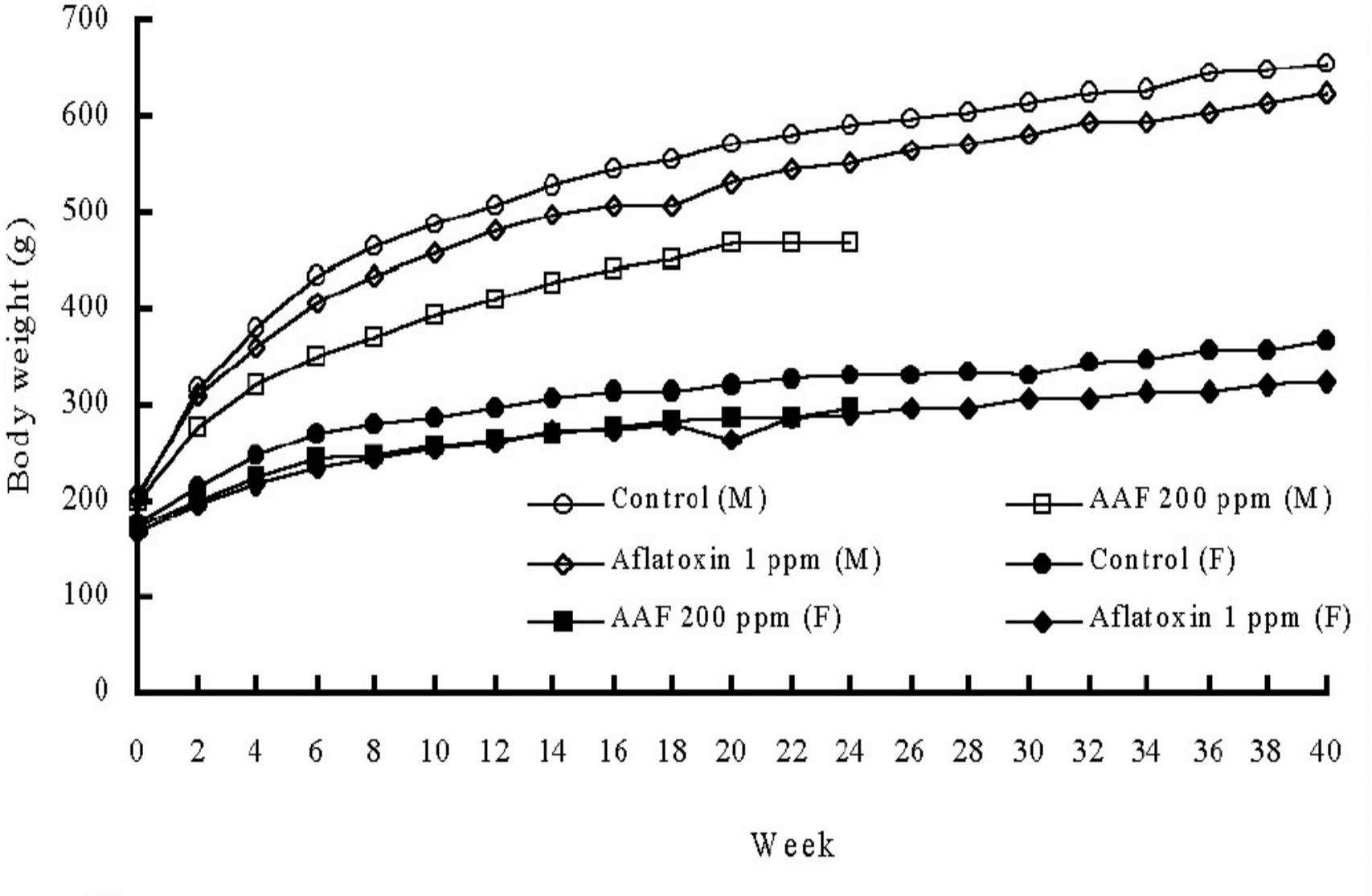
2. They were fed continuously on a diet containing **200 ppm 2-AAF for 24 weeks (2-AAF group),** 1 ppm AFB1 for 40 weeks (AFB1 group) or an equal amount of acetone (control group) for 40 weeks.

3. Health condition and behavior abnormalities were observed daily.

4. Feed consumption and body weight gains were measured weekly.

5. All animals received humane care in accordance with the guideline by A Guidebook for the Care and Use of Laboratory Animals.



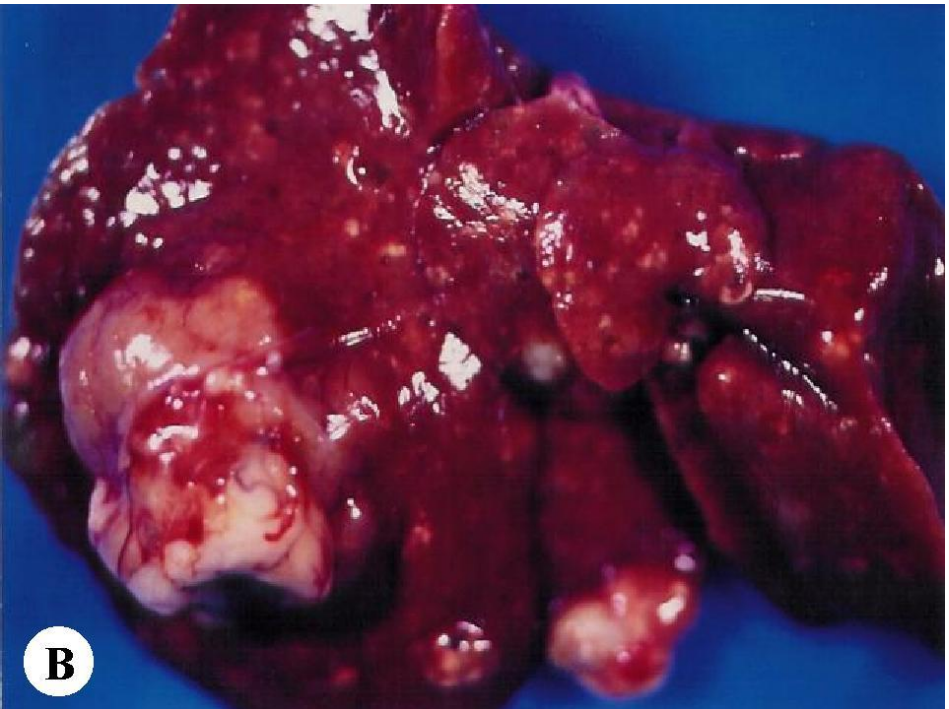


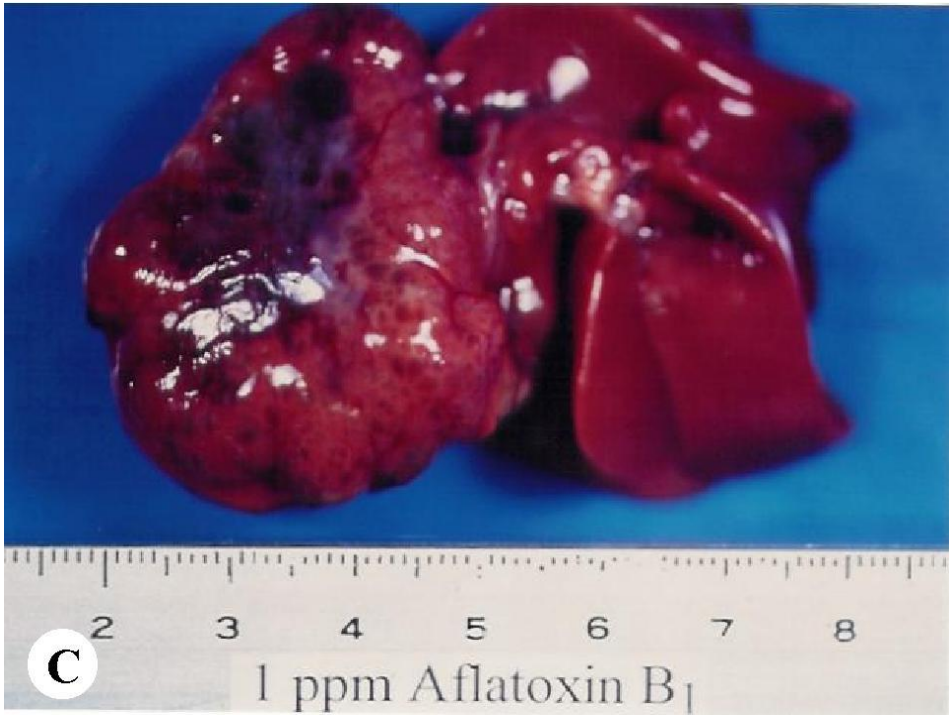
A

Fig. 1. Changes of body weight and feed consumption in rats fed on a diet contaning 200 ppm 2-acetylaminofluorene for 24 weeks and 1 ppm aflatoxin B1 for 40 weeks. (A) The body weight gain presented marked decline in 2-acetylaminofluorene treated male rats than that of aflatoxin B1 group. (B) The mean of feed consumption when compared with control group. M: male rats; F: female rats.

Aflatoxin B1 and 2-acetylaminofluorene induced hepatic carcinogenicity and gamma-glutamyltranspeptidase expression via chronic feeding in rats

Liao et al., 2002, Bull. Plant Protect. 44: 37 - 50, 2002





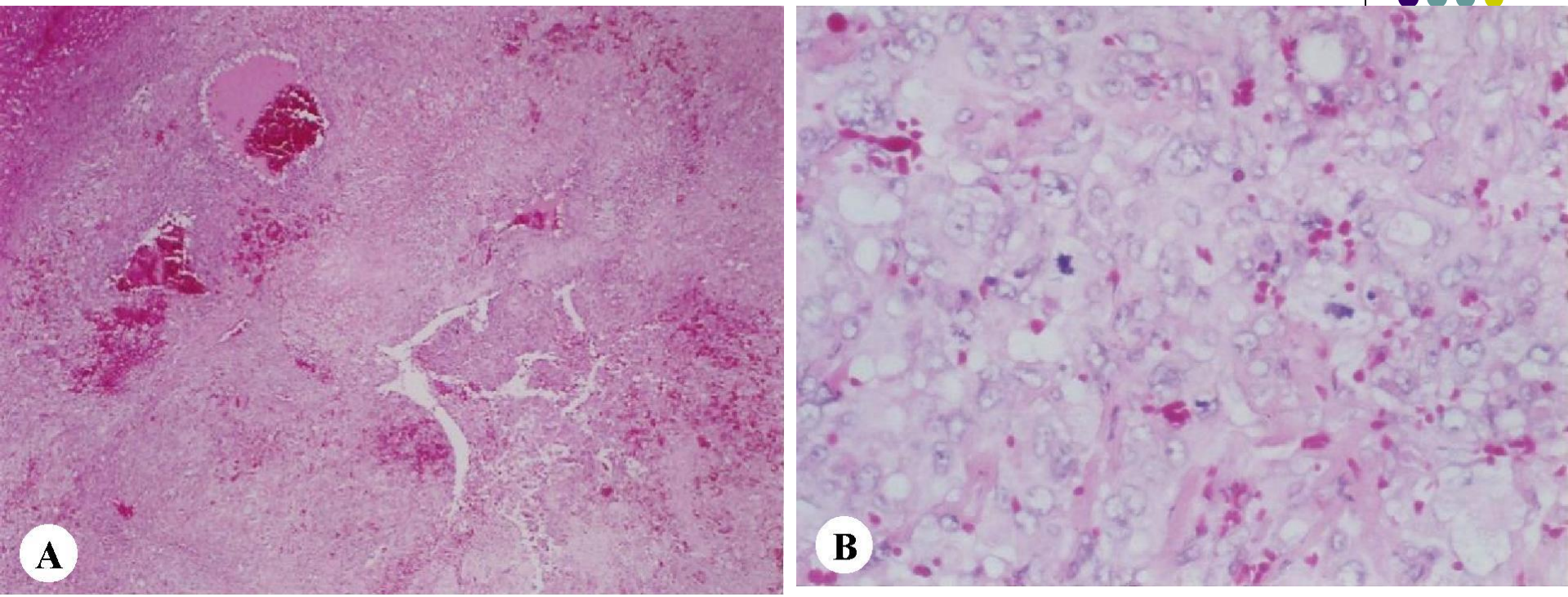


Fig. 3. Photomicrograph from Fig. 2. B. (A) Note the massive occupying and pressing to the normal hepatic cells by neoplastic cells (arrow) and massive hemorrhage (arrow head) in a 2-acetylaminofluorene treated rat. (H&E stain, 40×). (B) Higher magnification from A. Note the highly cellular mitotic figures (arrow) in the tumor masses (H&E stain, 400×).

Table 3. Histopathological incidence of rats fed continuously on a diet containing 200 ppm 2-acetylaminofluorene for 24 weeks or 1 ppm aflatoxin B_1 for 40 weeks

Organ/Lesion	Male			Female		
	Control	2-AAF	AFB_1	Control	2-AAF	AFB_1
Liver						
Preneoplastic foci	0/10[1)]	10/10	6/10	0/10	7/10	10/10
Bile duct proliferation	0/10	7/10	3/10	0/10	7/10	4/10
Cyst formation	0/10	7/10	1/10	0/10	7/10	0/10
Hepatocellular carcinoma	0/10	10/10	10/10	0/10	0/10	5/10
Lung						
Metastatic carcinoma	0/0	2/10	1/10	0/0	0/0	0/10
Skin						
Squamous cell carcinoma	0/10	1/10	0/10	0/10	0/10	0/10
Mammary gland						
Adenoma	0/10	0/10	0/10	0/10	2/10	0/10

[1)] Data presented as number of effect animals (included dead rats)/total number of examine animals.

Classification of chemical (drug) carcinogens

- **International Agency for Research on Cancer (IARC, USA)**

IARC Classification of the Evaluation of Carcinogenicity for the Human

GROUP	EVIDENCE	EXAMPLES
1. Agent is carcinogenic	Sufficient (human)	Arsenic, aflatoxin, benzene, estrogens, vinyl chloride
2A. Agent is probably carcinogenic	Limited (human) Sufficient (animal)	Benz(*a*)anthracene, diethylnitrosamine (DEN), polychlorinated biphenyls (PCB), styrene oxide
2B. Agent is possibly carcinogenic	Limited (human) Inadequate (human) Sufficient (animal)	TCDD, styrene, urethane
3. Agent is not classifiable as to carcinogenicity		5-azacytidine, diazepam
4. Agent is probably not carcinogenic	Inadequate (human) Inadequate (animal)	Caprolactam

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Tests for carcinogenic potential of chemicals

National Cancer Institute (NCI)/National Toxicology Program (NTP)

- **In vivo chronic rodent carcinogenicity studies**

1. **Rodent bioassay (The 2-year bioassay)** :

   ⇒ Exposure high doses and fractions of test chemical over 2-year in male and female rats and mice

   ⇒ The route of administration chemicals is **mimic natural route of human exposure**

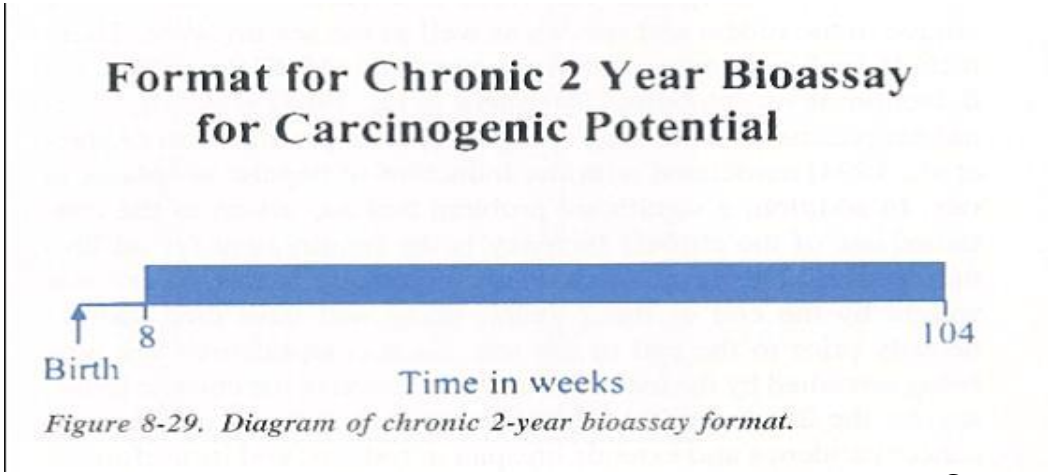

Figure 8-29. Diagram of chronic 2-year bioassay format.

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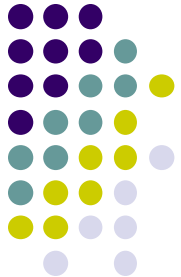
• In vivo chronic rodent carcinogenicity studies



2. Advantages

1) To be **the single best system** for identifying potential carcinogens
2) Sufficiently **sensitive** to known human carcinogens,
   e.g., Aflatoxin B_1

3. Disadvantages

1) High cost
2) Long term interval
3) Large number of animals
4) High dose has been criticized as unrealistic
5) How to interpret carcinogenic or not carcinogenic?
   $\Rightarrow$ Maximum tolerated dose (MTD)

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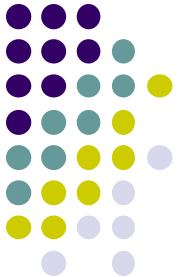
• Additional in vivo tests for carcinogenicity



• *In vivo* Short-term bioassay

1) Preneoplastic or neoplastic observed in a few weeks
2) Useful to define the nature of carcinogens
3) Help to elucidate the mechanism of carcinogenesis
4) Used as part of battery or tier approach to carcinogen testing

1. Strain A mouse pulmonary tumor test

⇒ Strain A mice lung tumor is sensitive for some classes of chemicals (bleomycin) but insensitive for others

⇒ Weaning animals were ip 3 times a week for 8 weeks and sacrificed at 24-wk

⇒ Positive control: urethane

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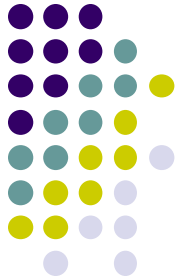
• Additional in vivo tests for carcinogenicity



2. Rat mammary neoplasm

⇒ Useful to assess the influence of hormone enhancement and dietary fat in promoting of mammary gland carcinogenesis

⇒ Single or multiple doses of agents are given virgin female Sprague-Dawley rats

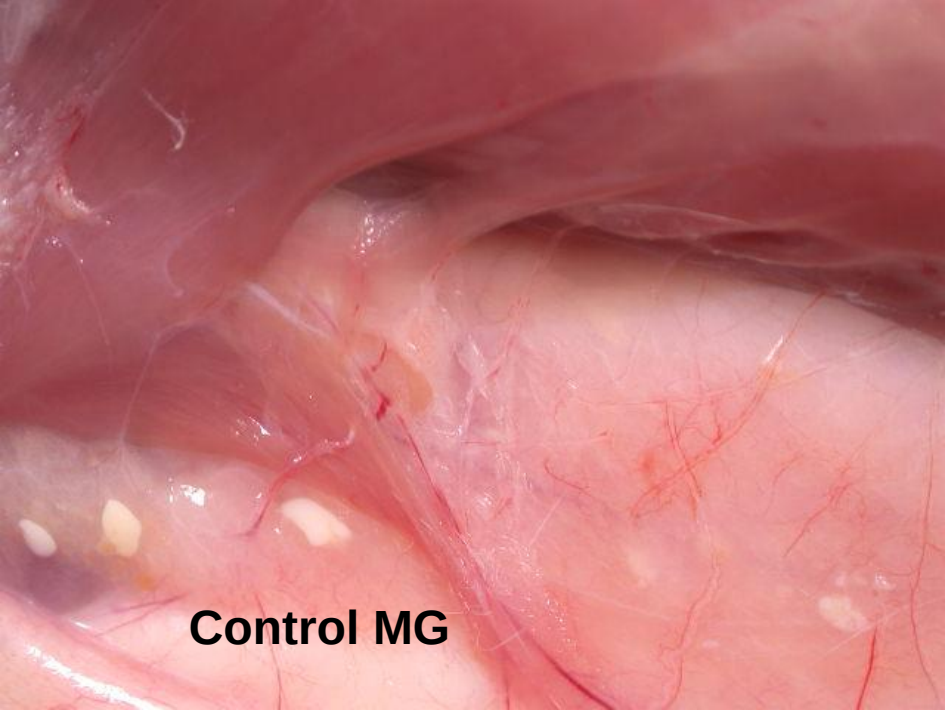
⇒ DMBA as positive control, detectable at 2 months, and 100% after 9 months

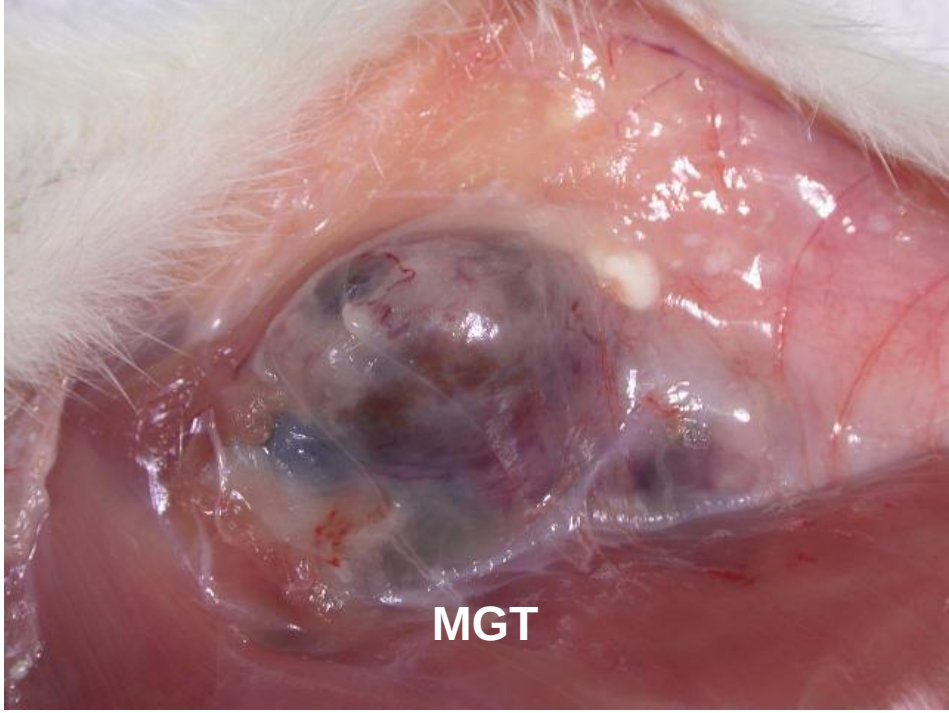
3. Subcutaneous injection test

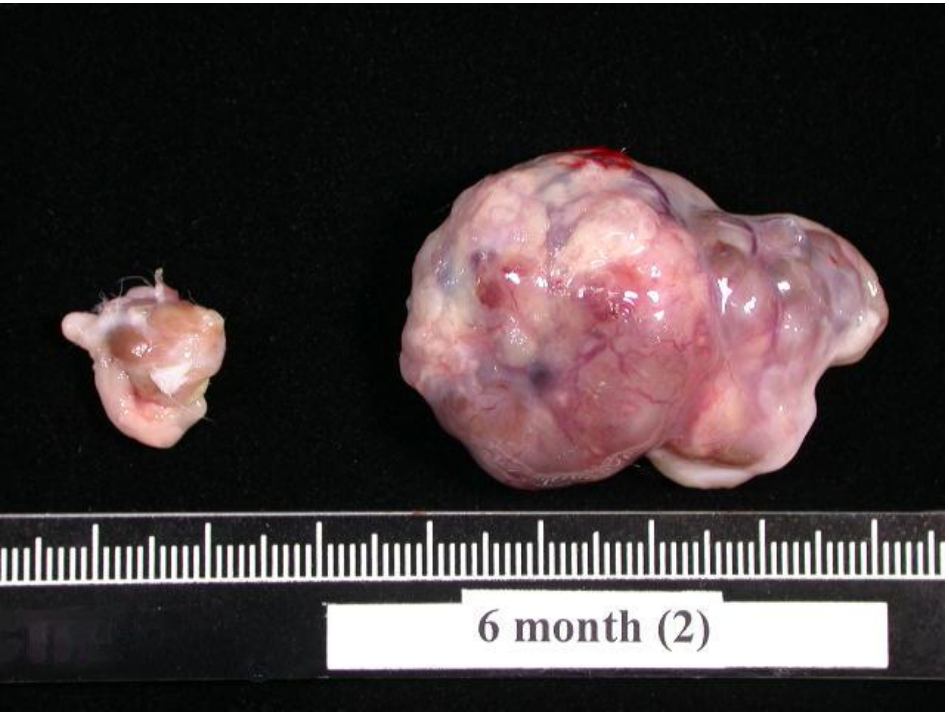
⇒ Single or multiple doses of agents are given to rats or mice

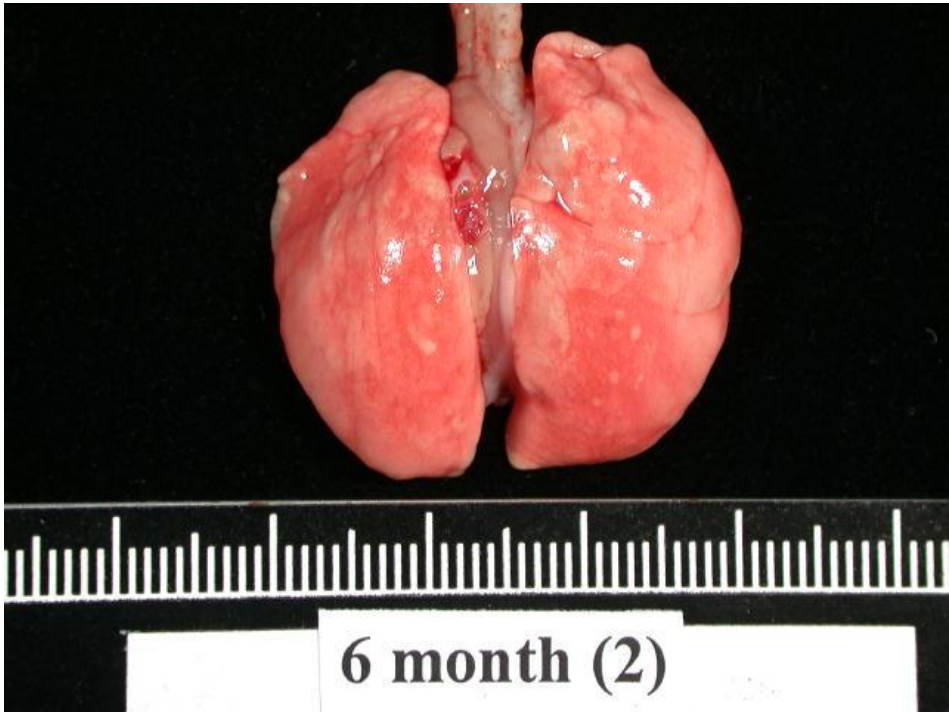
⇒ Some solid chemicals produce persistent foreign body reaction and cause localized sarcomas in rodents

⇒ Cadmium induced sarcoma in rats


Control MG


MGT


6 month (2)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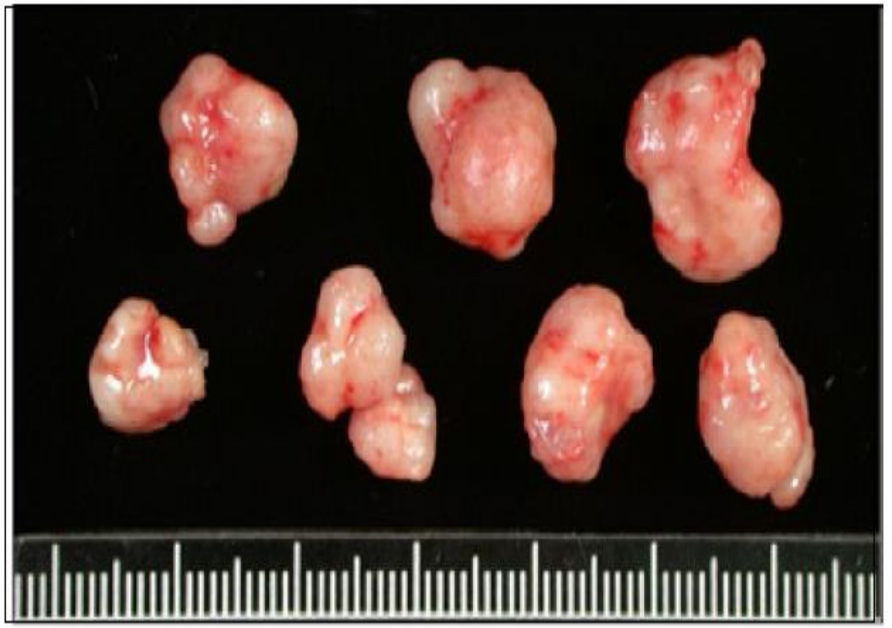
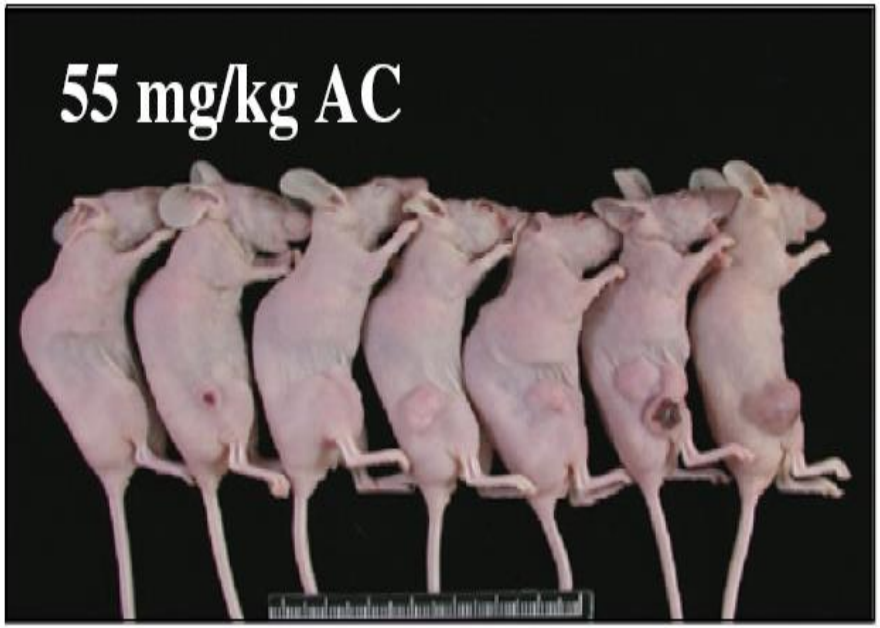
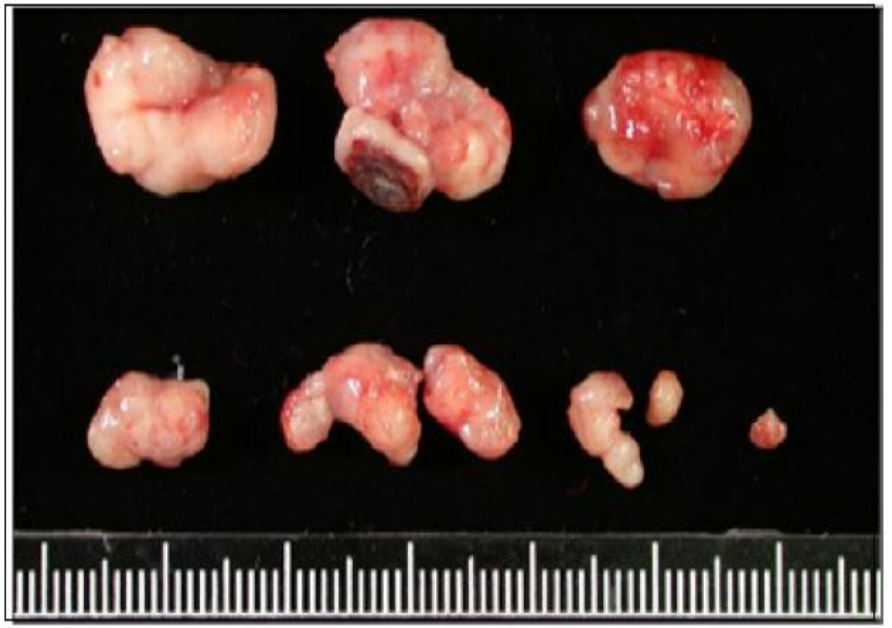
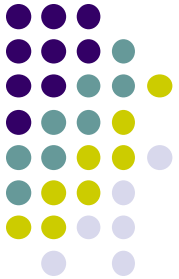






Y.-C. Hseu et al. / Food and Chemical Toxicology 46 (2008) 2680–2688

• Additional in vivo tests for carcinogenicity



4. Mouse skin-painting model

⇒ Single or multiple doses (no skin irritation) of agents are given to the shaved skin of the back throughout all or most of the life span of the mouse

⇒ Papillomas and carcinomas in the treated area

⇒ Benzo[a]pyrene as positive control

5. In vivo rat liver neoplasm model

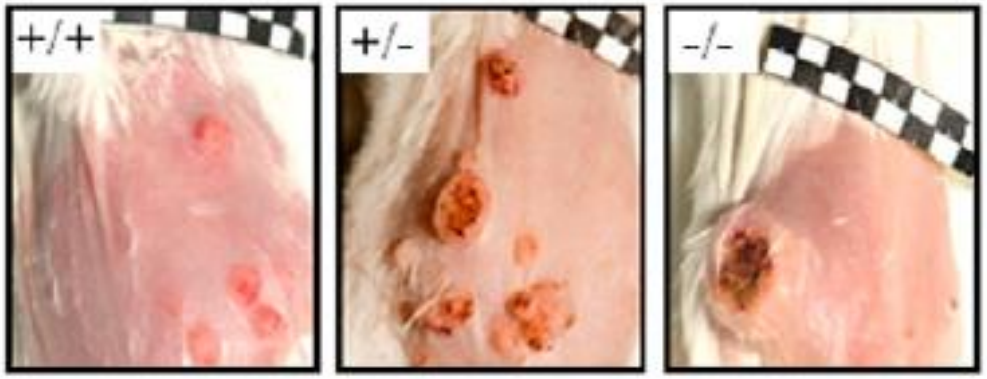
⇒ Useful in assessing carcinogen potency based on a quantitative endpoint

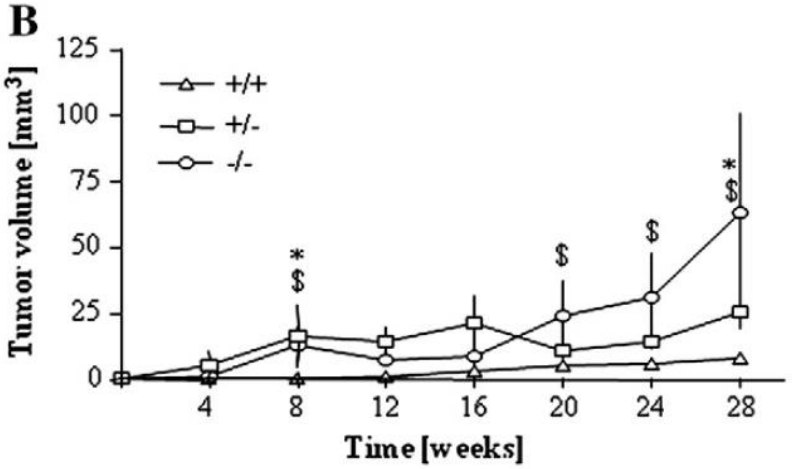
⇒ Single fed or treated with a necrogenic dose of carcinogen followed by a proliferative or natural stimulation (neonatal)

⇒ DEN→ Partial hepatectomy (CCl_4)→ 2-AAF → liver neoplasm

⇒ Histochemically identifiable foci with GST, GGT…

Casarett & Doull's Toxicology. 2002

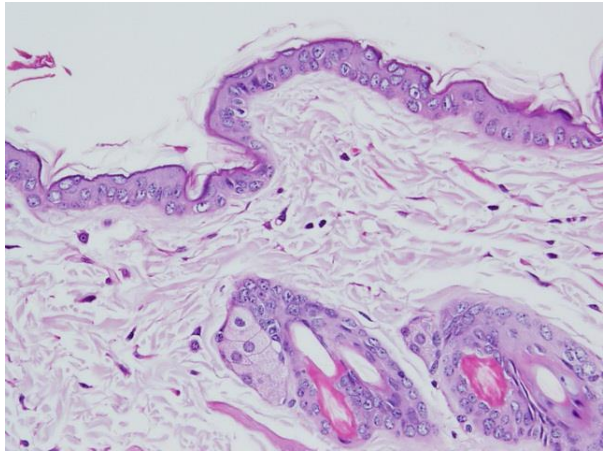


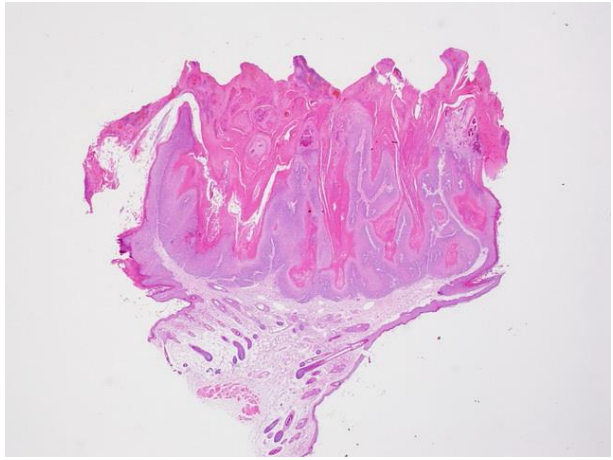


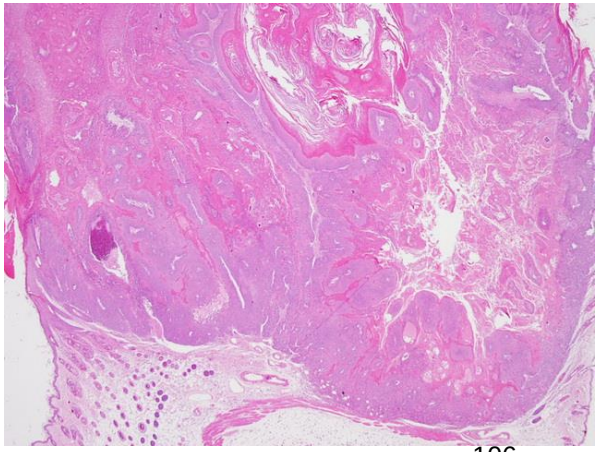
Heme oxygenase-1 (HO-1)

Fig. 1. Single dose of DMBA at wk 1 + repeated doses of TPA from wk 2 for 28 wks induced skin papillomas and squamous cell carcinoma in mice

H. Was et al. / Free Radical Biology & Medicine xxx (2011) xxx–xxx







Multistep process of carcinogenicity

(Barrett, 1993)

1. Initiation: 誘發期

1) the induction of a genetic alteration
2) as the mutational activation of a *ras* proto-oncogene by a mutagen
3) Irreversible
4) Starts the process toward cancer

2. Promotion:促進期

1) cellular proliferation in an initiated cell population
2) lead to the development of benign tumor, such as papillomas
3) agents called promoters
4) may be a mutagen or not a mutagen

3. Progression:腫瘤形成期

1) the continuation of cell proliferation and the accumulation of additional irreversible genetic changes
2) genetic instability
3) malignancy
4) The role of mutations is critical, and analyzing mutations and mutagenic effects is essential for understanding and predicting chemical carcinogenesis

Casarett & Doull's Toxicology. 2002

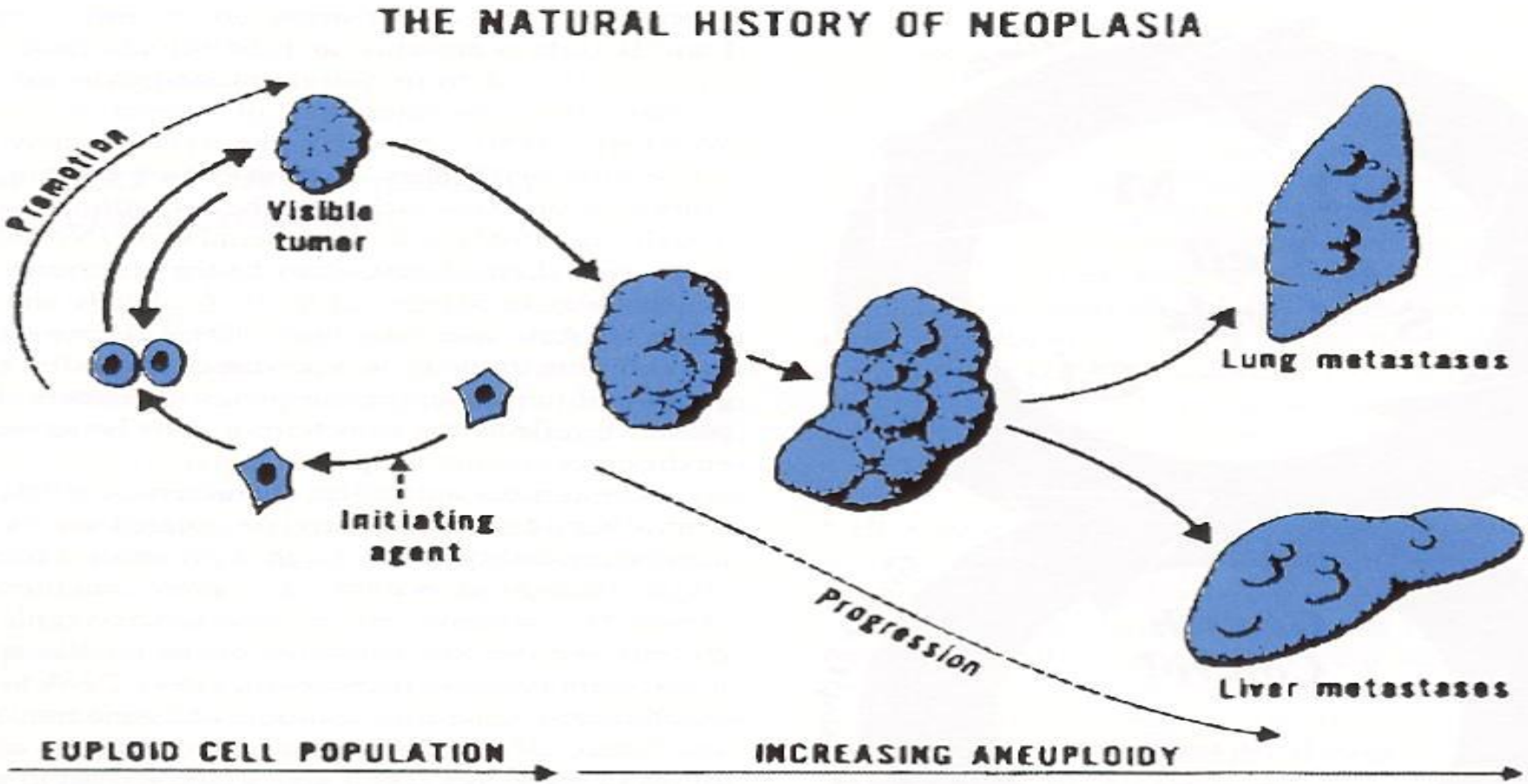


Figure 8-23. The natural history of neoplasia, beginning with the initiated cell after application of an initiating agent (carcinogen), followed by the potentially reversible stage of promotion to a visible tumor, with subsequent progression of this tumor to malignancy.

Casarett & Doull's Toxicology. 2002

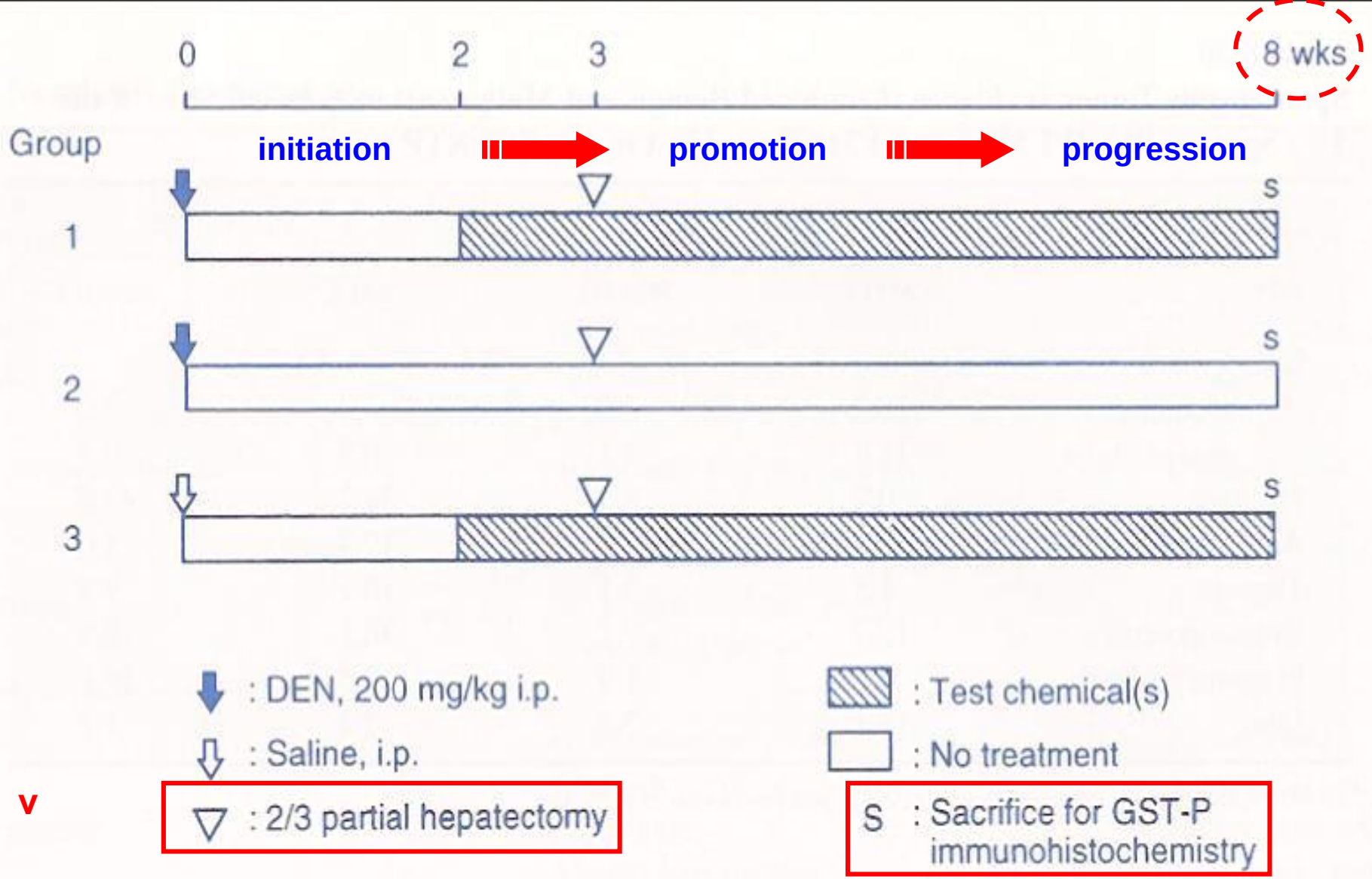


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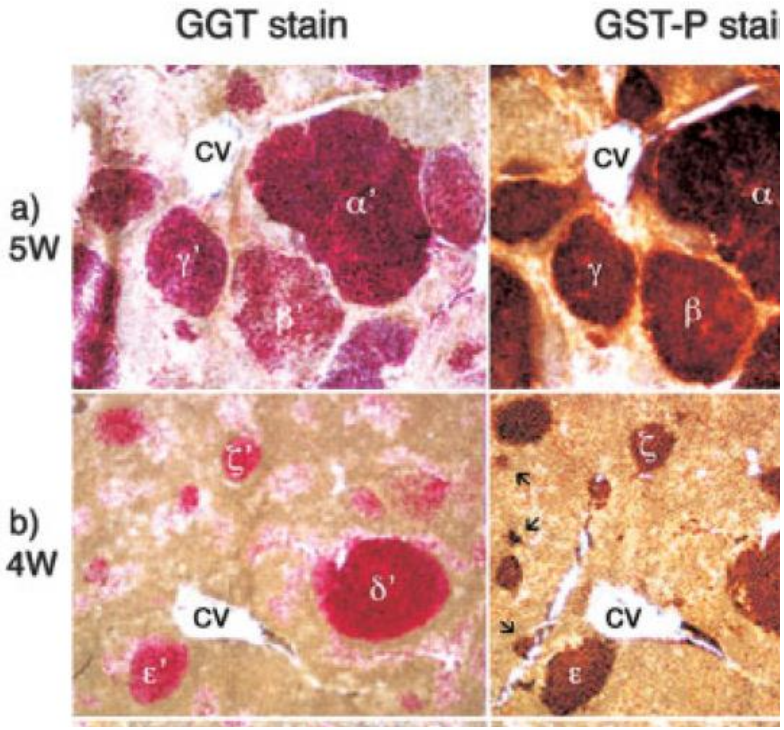
Figure 8-30. The medium-term liver bioassay protocol for identification of potentially carcinogenic agents.

DEN, diethylnitrosamine; GST-P, glutathione *S*-transferase–π. [Reproduced from Shirai (1997), with permission of author and publisher.]

Casarett & Doull's Toxicology. 2002

Enzymatic detection of precursor cell populations of preneoplastic foci positive for c-Glutamyltranspeptidase in rat liver



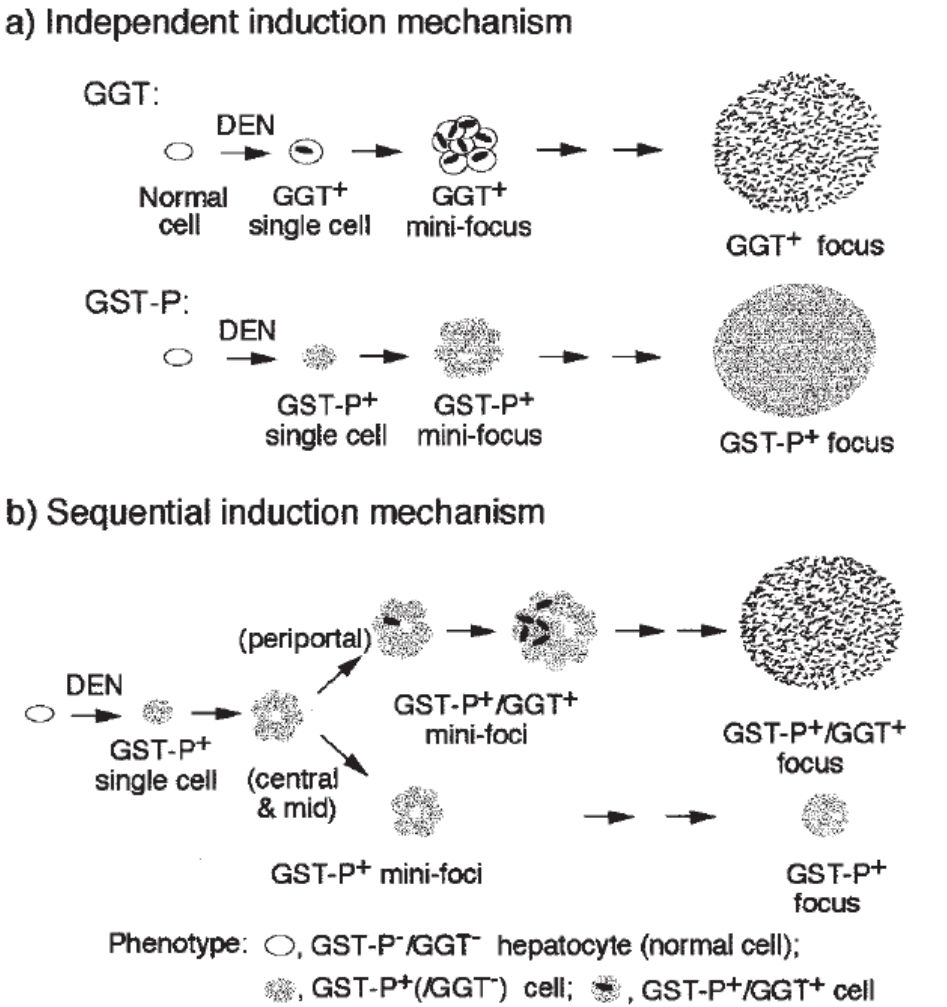
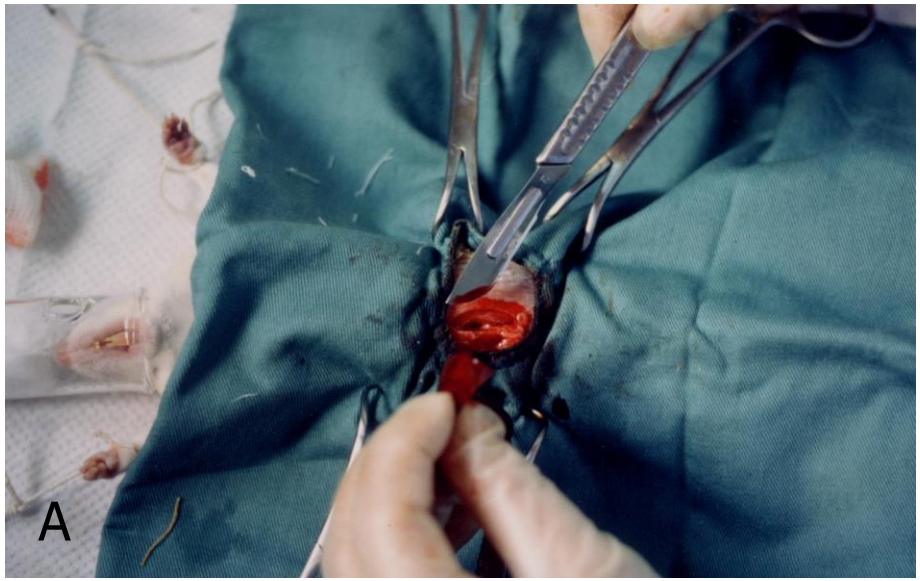
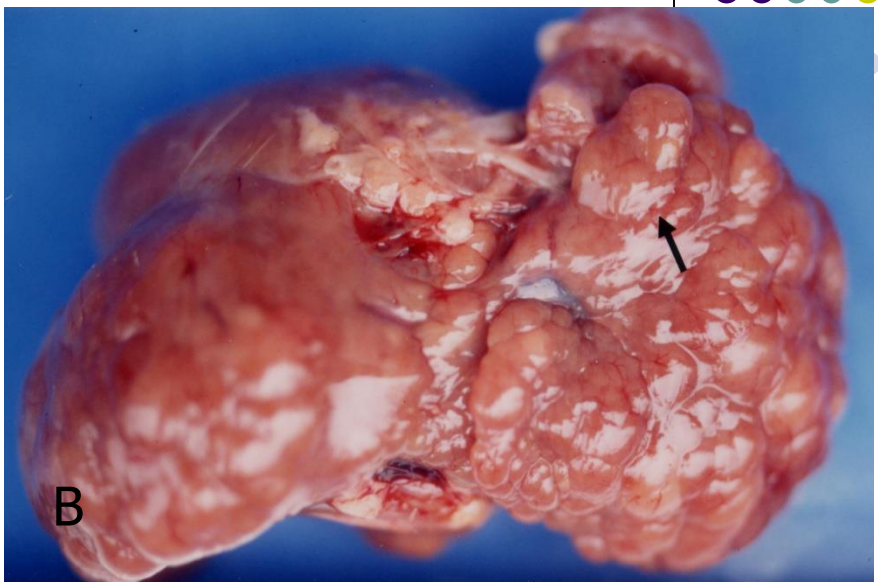


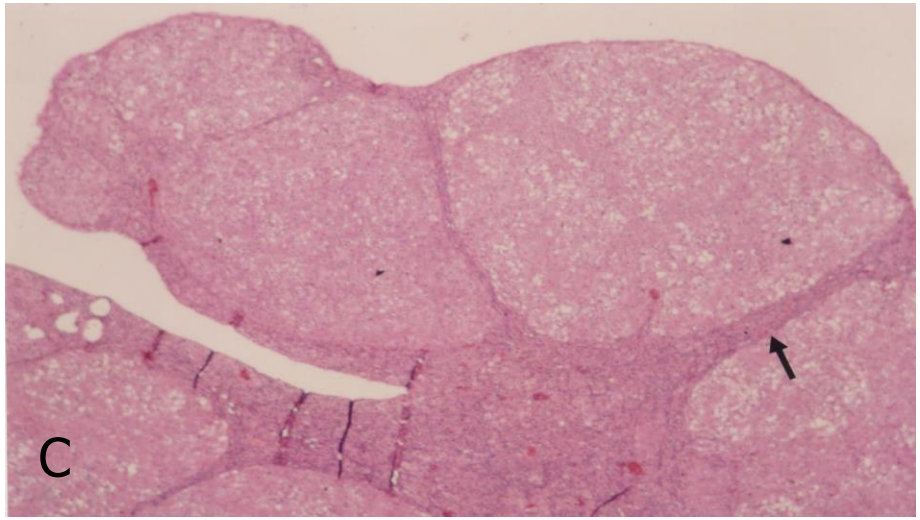
FIGURE 6 – Alternative mechanisms for induction of preneoplastic foci positive for GGT and GST-P in rat liver by DEN.

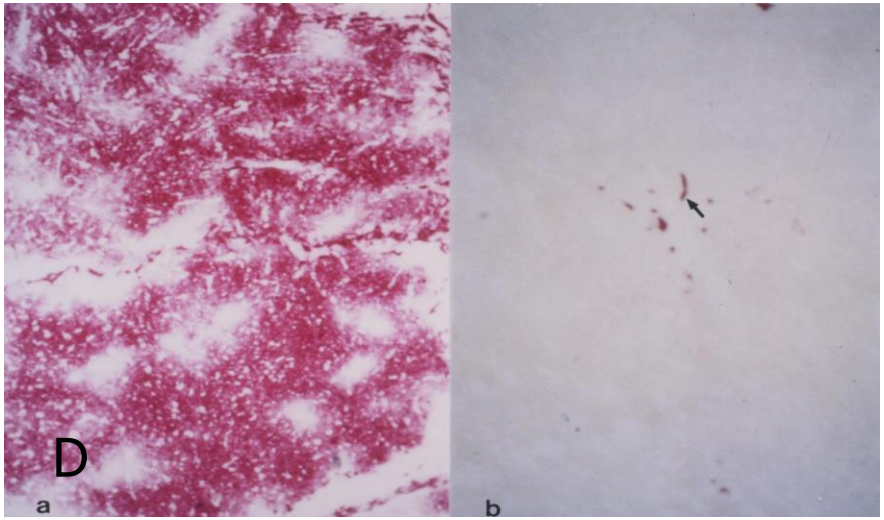
Satoh et al., Int. J. Cancer: 115, 711–716 (2005)

Rapid Bioassay for Hepatocarcinogenesis in Rats









(A) Partial hepatotectomy; (B) hepatoma was induced by DEN + 2-AAF in grossly and (C) histopathological examination at 12-week termination; (D) remarked GGT staining (a) in the DEN + 2-AAF-induced hepatoma and negative (b) control rat liver.

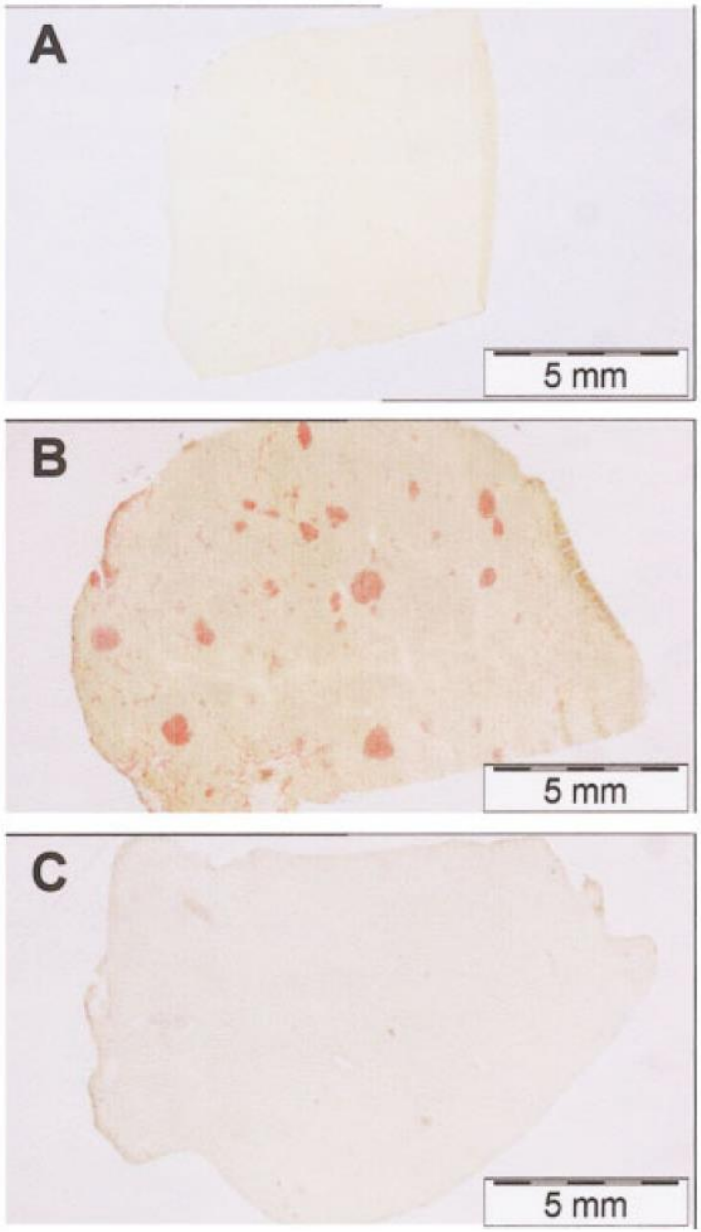


FIGURE 2 – Histochemically stained sections showing the effect of CAPE on the induction of GGT-positive AHF. Liver sections representative of each treatment. (*a*) NT, (*b*) CCT, (*c*) CCT plus CAPE during promotion.

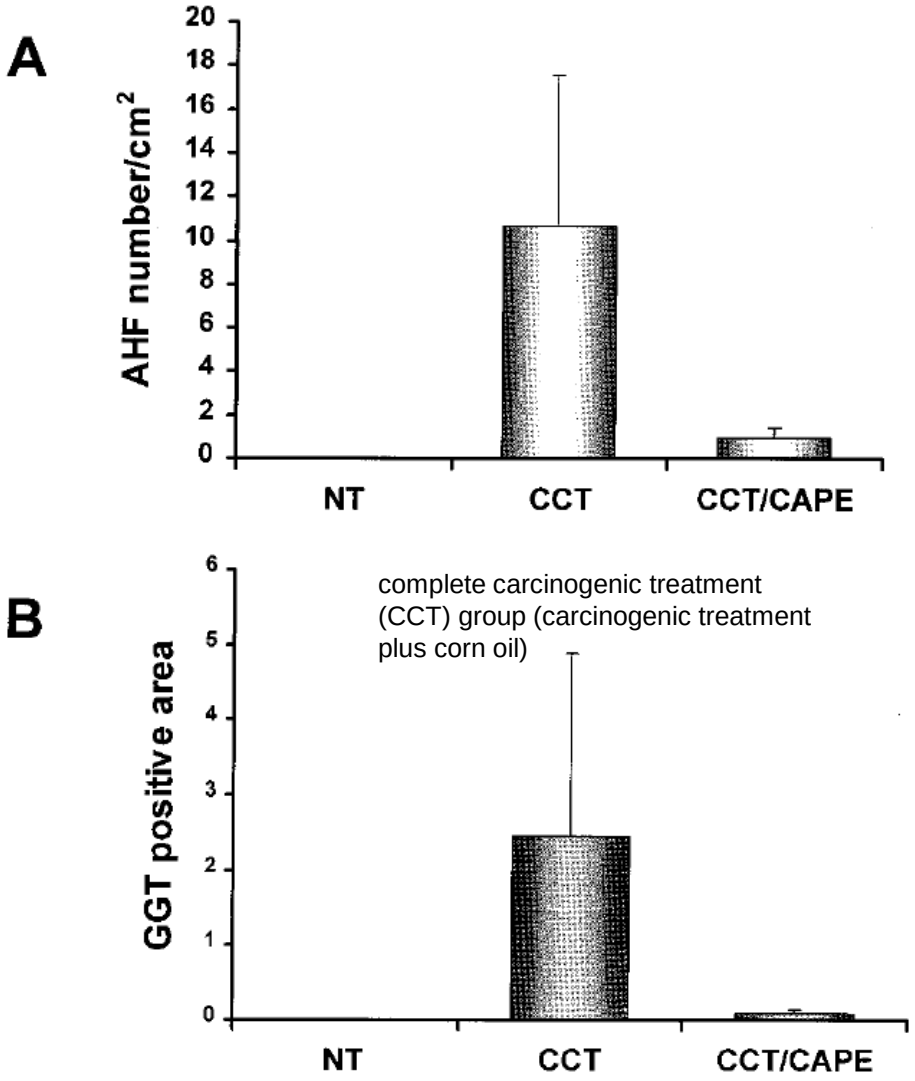


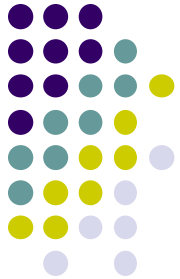
FIGURE 3 – Quantification of the CAPE effect upon number/cm^2 and % area of GGT-positive AHF. (*a*) AHF quantity number/cm^2. (*b*) The percent of GGT-positive area/tissue area. Twelve histological sections of the liver per rat from each treatment were randomly chosen and analyzed. NT ($n = 3$), CCT ($n = 3$), CCT plus CAPE ($n = 5$). Statistically different from CCT group, $p < 0.05$.

『健康食品』安全性毒理評估

- 第一類：(免提毒性測試資料)
  - 產品之原料為傳統食用且以通常加工食品形式供食者
  - 產品具有完整之毒理學安全性學術文獻報告及曾供食用之記錄
- 第二類：(產品之原料為傳統食用而非以通常加工食品形式供食者)
  - 基因毒性 (Ames with 5 strains, CA, MN assays)
  - 28天餵食毒性試驗
- 第三類：(產品之原料非屬傳統食用者)
  - 基因毒性
  - 90天餵食毒性試驗
  - 致畸胎毒性試驗
- 第四類：(產品之原料非屬傳統食用且成份含有致癌物之類似物者)
  - 基因毒性
  - 致畸胎毒性試驗
  - 致癌性試驗
  - 90天餵食毒性試驗
  - 後代繁殖試驗

(TFDA, 2002)

Objectives of toxicological tests



• **For general endpoint:**

1) To define the intrinsic toxicity, predict hazard to target species or organs
2) For the design and selection of doses for long-term studies
3) For clinician predict, diagnose and prescribe

•Academic endpoint:

1) To provide important clues on the mechanism of toxicity
2) The structure-activity relationship for a particular chemicals

•For regulatory endpoint

1) For the industrial formulate worker's safety
2) Risk assessment of acute exposure
3) For governmental regulator to set classification, labeling and transportation

Casarett & Doull's Toxicology. 2002

A comparison of spontaneous malignant tumors in humans, rats, mice and dogs

Table 1 : Mortality from cancer of various organs in humans, rodents and dogs.

	Male			Female			Male + Female
	Human	Rat	Mouse	Human	Rat	Mouse	Dog
Number of dead humans and animals by cancer or having cancer	139674	105	120	92243	117	100	5845
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Esophagus	4.7	0	0	1.4	0	0	0.3
Stomach	21.8	1.0	0	19.0	0.9	1.0	0.3
Rectum and related organs	4.4	0	0.8	4.3	0	0	1.0
Liver	14.0	0	52.5	8.1	1.7	24.0	0.7
Pancreas	5.6	0	0	6.9	0.9	2.0	0.5
Lung-trachea-bronchus	20.9	2.9	5.0	11.9	0	1.0	0.6
Mammary gland	<0.1	0	0	7.0	2.6	8.0	9.1
Uterus	–	–	–	5.1	10.3	11.0	0.3
Leukemia	2.4	53.3	20.8	2.6	59.8	31.0	4.3
Other	26.1	42.9	20.8	33.9	23.9	22.0	82.9

Kobayashi, et *al* ., *Journal of Environmental Biology*, 20 (3), 189 193 (1999)

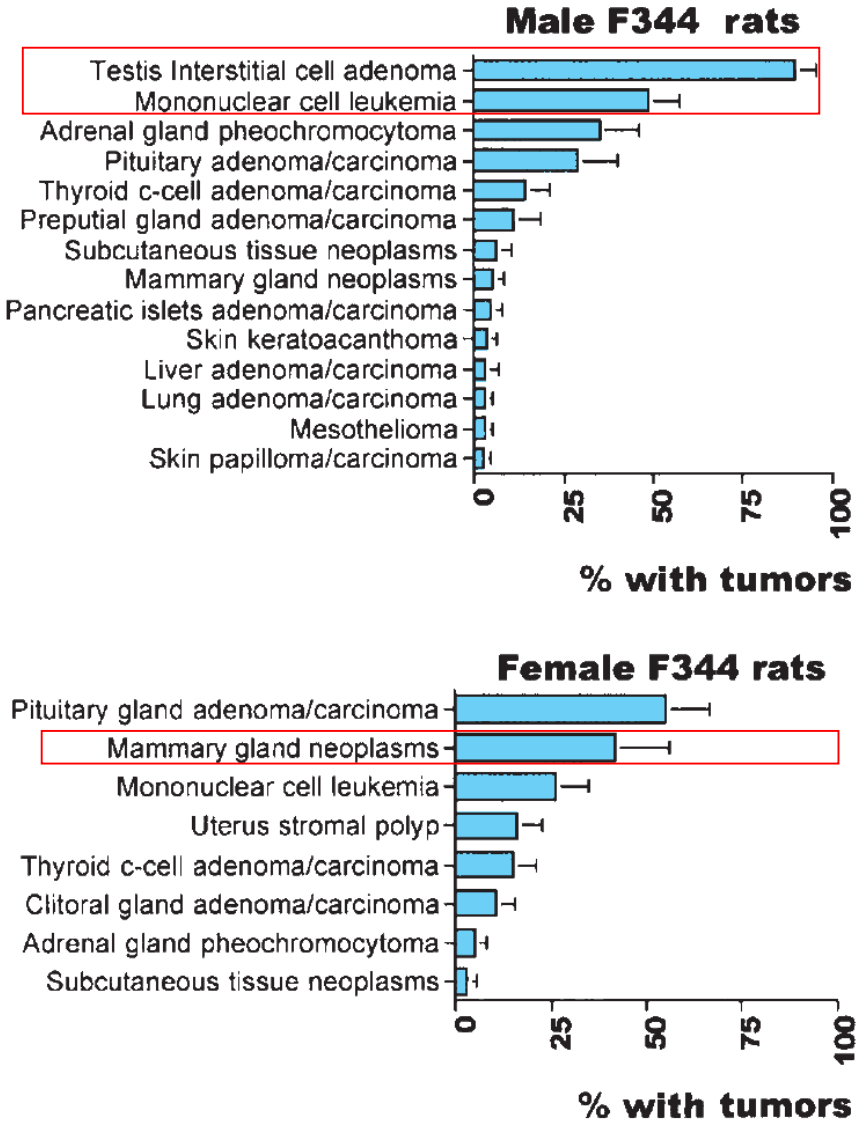


Figure 2-13. Most frequently occurring tumors in untreated control rats from recent NTP 2-year rodent carcinogenicity studies.

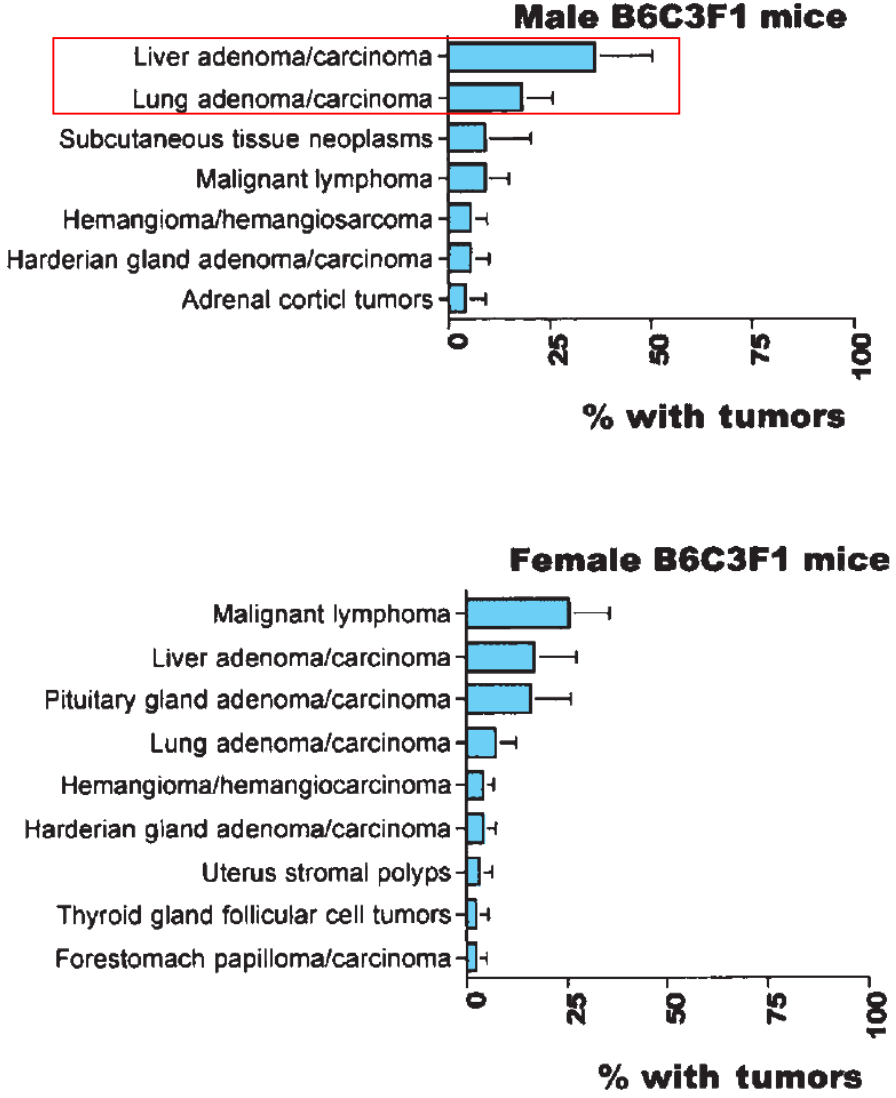


Figure 2-14. Most frequently occurring tumors in untreated control mice from recent NTP 2-year rodent carcinogenicity studies

Table 3 : Some rat-human differences important in chemical carcinogeneis.

	Rats	Human
Life-span (years)	2.5	70
Food consumption (g/kg/day)	50	10
Basal metabolic rate (k cal/kg/day)	109	26
Forestomach, zymbal's gland, harderian gland, preputial gland, clitoral gland	Present	Absent
Reproductive cycle	Estrus	Menstrual
Parity	High	Low
Stomach pH	4-5	1-2
Bacterial flora	Numerous	Few
$\alpha - 2\mu$ – globulin	Present (esp. in male)	Virtually absent
DNA excision repair	Low	High
Hepatic O_6-alkylguanine transferase	1	10

Adapted from Monro, *et al.*, 1995 in the reference.

Kobayashi, et *al* ., *Journal of Environmental Biology*, 20 (3), 189 193 (1999)

Table 1: Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area

Species	To Convert Animal Dose in mg/kg to Dose in mg/m², Multiply by k_m	To Convert Animal Dose in mg/kg to HED[a] in mg/kg, Either:	
		Divide Animal Dose By	Multiply Animal Dose By
Human	37	---	---
Child (20 kg)[b]	25	---	---
Mouse	3	12.3	0.08
Hamster	5	7.4	0.13
Rat	6	6.2	0.16
Ferret	7	5.3	0.19
Monkeys	12	3.1	0.32
Micro-pig	27	1.4	0.73
Mini-pig	35	1.1	0.95

Health food: 500 mg/cap; one cap. per day

⇒ Dose for human: 500 mg/cap x 1 cap /day ÷60 kg = 8.33 mg/kg

⇒ Animal dose: 8.33 mg/kg x 6.2 (rat to human HEDs) = 51.6 mg/kg

⇒ 28-day NOAEL (rat) > 2000 mg/kg day

⇒ > 2000 mg/kg day (rat) ÷ 8.3 mg/kg/day (human) = > 240 folds

05).

a Assumes 60 kg human. For species not listed or for weights outside the standard ranges, HED can be calculated from the following formula:
HED = animal dose in mg/kg x (animal weight in kg/human weight in kg) 0.33.

b This km value is provided for reference only since healthy children will rarely be volunteers for phase 1 trials.

c For example, cynomolgus, rhesus, and stumptail.

NOAEL

Table 1
Selected definitions of NOAEL

Source	Definition
Hayes (2001)	The highest dose that is without observed effects in properly designed and executed toxicology studies
ECETOC T R 85 (2002), EPA (1995), Faustman and Omenn (2001), Beck et al. (1993)	The highest exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effect between exposed and control groups. Some effects may be produced but they are not considered adverse or precursors to adverse effects
Leisenring and Ryan (1992)	Experimental dose level immediately below the lowest dose that produces a statistically or biologically significant increase in rate of adverse effects over control
Calabrese and Baldwin (1994)	Highest dose not statistically different from control yet significantly different from lowest-observed-adverse-effect-level (LOAEL)
FDA Guidance (2002)	The highest dose level that does not produce a significant increase in adverse effects… adverse effects that are statistically significant and adverse effects that may be clinically significant (even if they are not statistically significant) should be considered. The definition of the NOAEL in contrast to that of the NOEL reflects the view that some effects observed in the animal may be acceptable pharmacodynamic actions of the therapeutic and may not raise a safety concern
IPCS (1999)	Simple estimate of the highest dose in which the incidence of toxic effect was not significantly different from untreated group (statistically and biologically)

M.A. Dorato, J.A. Engelhardt / Regulatory Toxicology and Pharmacology 42 (2005) 265–274

Dietary risk

1. **An acceptable daily intake (ADI)** for human is based on the no observed adverse effect level **(NOAEL) in chronic toxicity in rodent tests**

2. **ADI** = NOAEL/UF x MF (mg/kg/day)
   ⇒ Species difference (10) x Individual viability (10) x Magnified factor (target organ, 10)

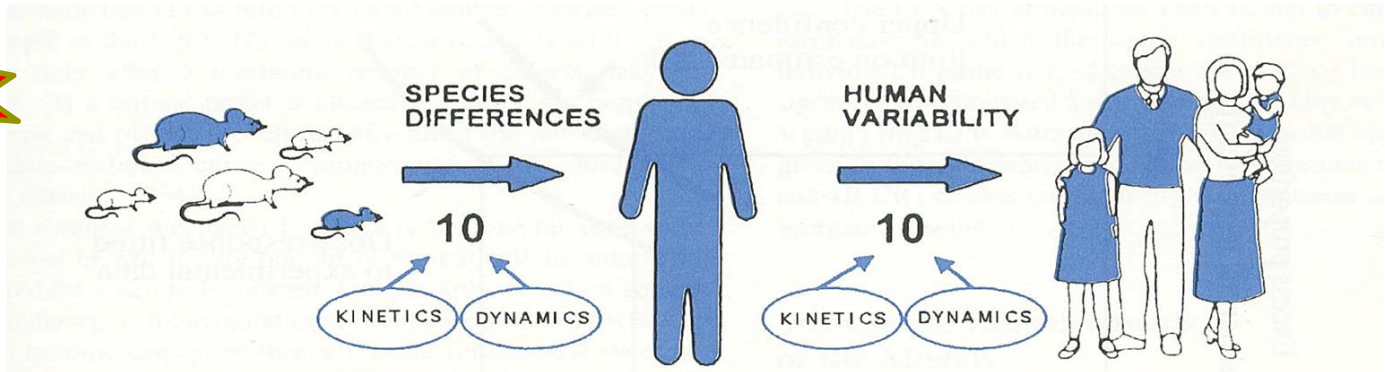


Figure 4-4. Toxicokinetic (TK) and toxicodynamic (TD) considerations inherent in interspecies and inter-individual extrapolations.

Toxicokinetics refers to the processes of absorption, distribution, elimination, and metabolism of a toxicant. *Toxicodynamics* refers to the actions and interactions of the toxicant within the organism and describes processes at organ, tissue, cellular, and molecular levels. This figure shows how uncertainty in extrapolation both across and within species can be considered as being due to two key factors: a kinetic component and a dynamic component. Refer to the text for detailed explanations. (Adapted from Renwick, 1999, 1998.)

Safety factors - in general

1. If only subchronic studies are available (14-day or 30 day studies), the SF = 2000 is likely
2. If chronic studies are available (90 day or in two species), the SF = 1000 is likely
3. If long-term chronic studies are available (1 year or 2-year), the SF = 100 is usually assigned
4. If NOEL is based on a concern about teratogenicity, a SF = 1000 is usually assigned

5. If human data are available, a SF = 10 is possible (or 50)
6. If there is same other uncertainly in the data, the SF may be increase from 100 to 200
7. If the NOEL was determined based on an effect known to have minimal relevance to humans (i.e. rat specific or dog specific effect, e.q. forestomach tumor), the SF may be lowered

EMEA, 2001; Gottschall, 2012

謝謝您 ！