ANIMAL MODELS FOR THE NTP RODENT CANCER BIOASSAY:
STRAINS & STOCKS - SHOULD WE SWITCH?

JUNE 16 - 17, 2005
NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES
RODBELL AUDITORIUM, RALL BUILDING
111 T.W. ALEXANDER DRIVE, RESEARCH TRIANGLE PARK, NC 27709

FINAL AGENDA

Thursday, June 16, 2005

8:30 AM	Welcome	Dr. John Bucher, NIH/NIEHS
	Introduction	Dr. James Popp, Stratoxon, LLC (Workshop Chair)
	Overview	Dr. Robert Maronpot, NIH/NIEHS
	Characteristics of Existing Models	Dr. Angela King-Herbert, NIH/NIEHS
	Selecting Stocks and Strains: Contract Research Organization Perspective	Dr. William Hooks, Huntingdon Life Sciences
	Selecting Stocks and Strains: Pharmaceutical Perspective	Dr. Daniel Morton, Pfizer
	Break	
	Use of Multiple Strains	Dr. Michael Festing, University of Leichester
	Statistics for Multiple Strains	Dr. Grace Kissling, NIH/NIEHS
	Public Comment [see public comment page]	
	Breakout Group Charges	Dr. James Popp, Stratoxon, LLC (Workshop Chair)
12:00 PM	Lunch	
1:00 PM	Breakout Group Meetings Mouse Models • Discussion topics: advantages and disadvantages of different mouse strains, isogenic versus outbred strains, multi-strain bioassays • Public comment	Dr. Norman Drinkwater, University of Wisconsin (Chair)
	Rat Models • Discussion topics: advantages and disadvantages of different rat strains, isogenic versus outbred strains, multi-strain bioassays • Public comment	Dr. Jerry Hardisty, Experimental Pathology Labs (Chair)

	Multiple Strain Approach • Discussion topics: evaluation, rare and common tumors, power, sample size requirements, reporting • Public comment	Dr. Julian Preston, US Environmental Protection Agency (Chair)
5:00 PM	Adjourn	

Friday, June 17, 2005

8:30 AM	Mouse Models Breakout Group Report • Discussion • Public comment	Dr. Norman Drinkwater, University of Wisconsin (Chair)
	Rat Models Breakout Group Report • Discussion • Public comment	Dr. Jerry Hardisty, Experimental Pathology Labs (Chair)
	Multiple Strain Approach Comment by Dr. Michael Festing • Discussion • Public comment	Dr. Julian Preston, US Environmental Protection Agency (Chair)
	Closing Remarks	Dr. Christopher Portier, NIH/NIEHS
12:00 PM	Adjourn	

Animal Models for the NTP Rodent Cancer Bioassay: Strains and Stock- Should We Switch?

June 16-17, 2005

National Institute of Environmental Health Sciences

NTP
National Toxicology Program

Strains and Stock Workshop Organizing Committee

- Dr. Angela King-Herbert
- Dr. Bob Maronpot
- Dr. Grace Kissling
- Dr. Kristina Thayer
- Dr. David Malarkey
- Dr. Rick Hailey

Strains and Stock Workshop
Immediate Context

- **Typical NTP Bioassay Design**
  - Animal numbers-- 50 to 100 per dose group
  - Number of doses-- 3 plus control
  - Study duration- 2 years
  - Life stage- young to late adult
  - Dose ranges- MTD, 1/2 to 1/3, 1/3 to 1/9 MTD
  - Pathology- “complete” approximately 40 tissues
  - Statistics- survival adjusted trend tests
  - Route- feed, gavage, drinking water, inhalation, dermal
  - Diet- NIH-07, NTP-2000
  - Species, strains- F344/N rat, B6C3F1 mouse

  - *Choices and Compromises*

Strains and Stock Workshop
Broad Context

- **Develop models to study environmental influences and impacts on human diseases**
  - **Rapid increase in understanding of rodent and especially mouse genetics- 15 mouse strain resequencing project**
  - **Appreciation of common mechanisms involved in many disease processes- NTP Vision**
  - **Paradoxical movement of toxicology away from the mouse**

Challenge is to appropriately incorporate expanding knowledge of genetics and phenotypic responses into our research and testing programs

Strains and Stock Workshop

- **Chair, Dr. James Popp, Stratoxon, LLC**

- **Breakout Chairs**
  - **Mouse models- Dr. Norman Drinkwater, University of Wisconsin**
  - **Rat models- Dr. Jerry Hardisty, Experimental Pathology Labs**
  - **Multiple Strain Approach- Dr. Julian Preston, US EPA**

Welcome to NIEHS and thanks for participating

Introduction & NTP Background

Bob Maronpot

NIEHS

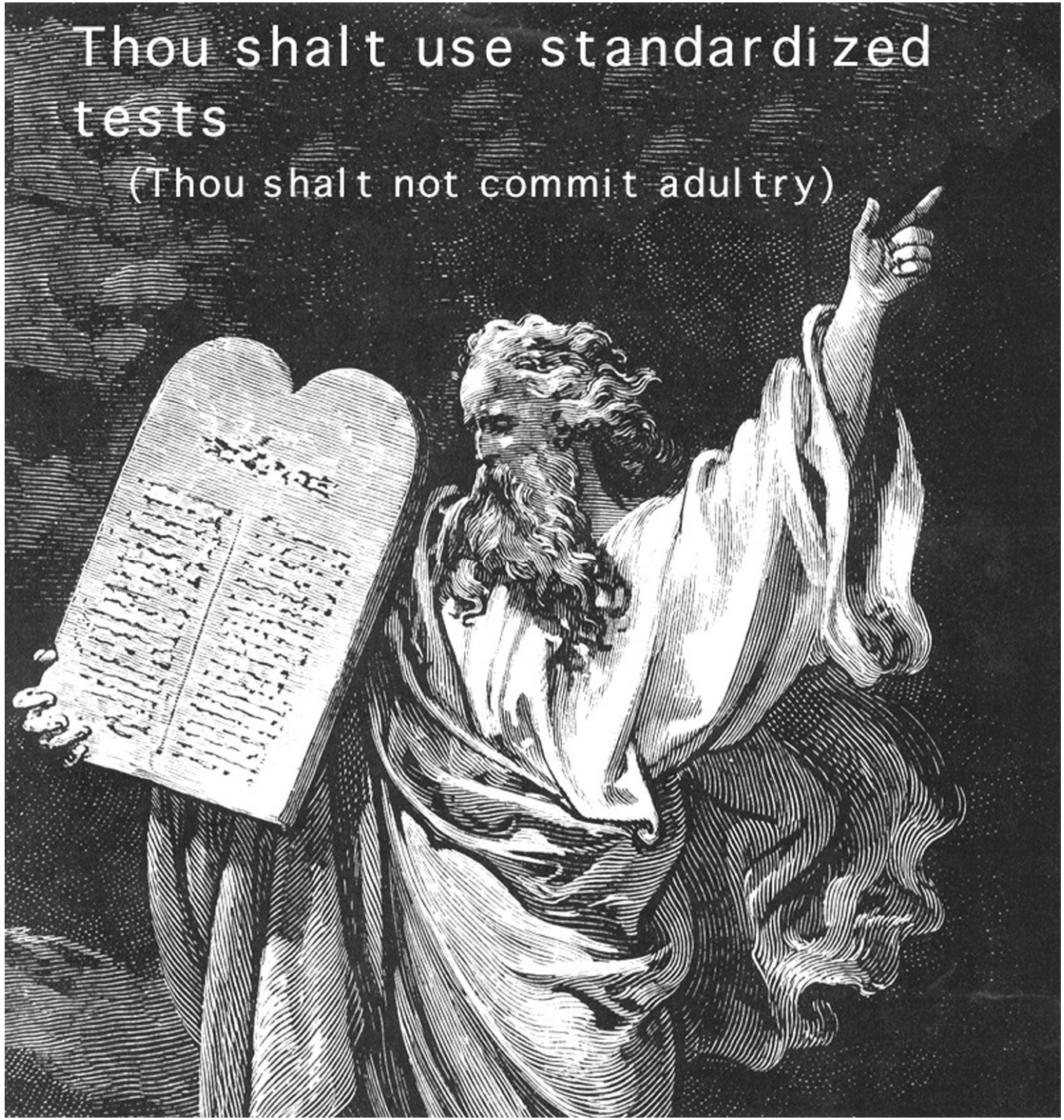
NTP
National Toxicology Program

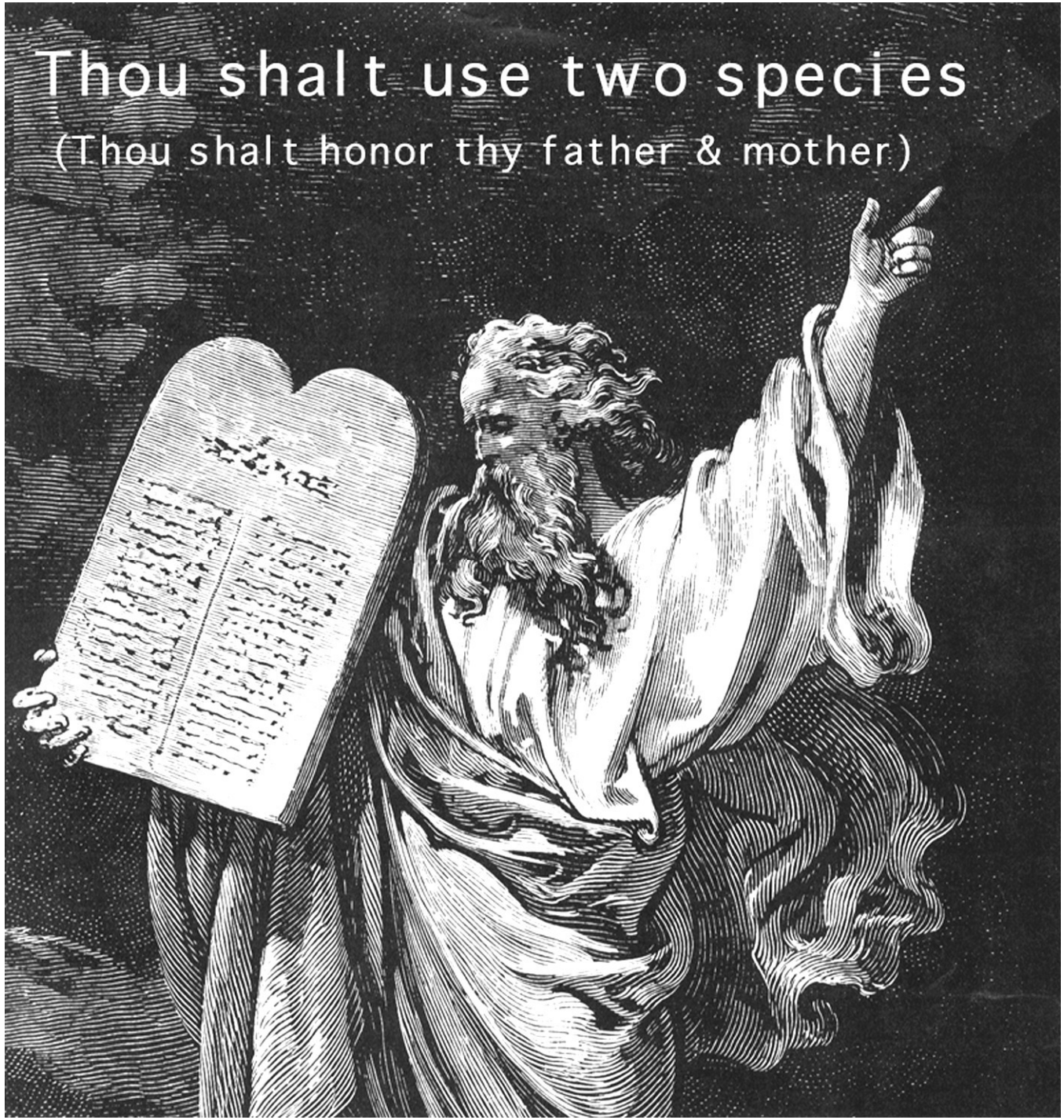
Early History of Animal Cancer Studies

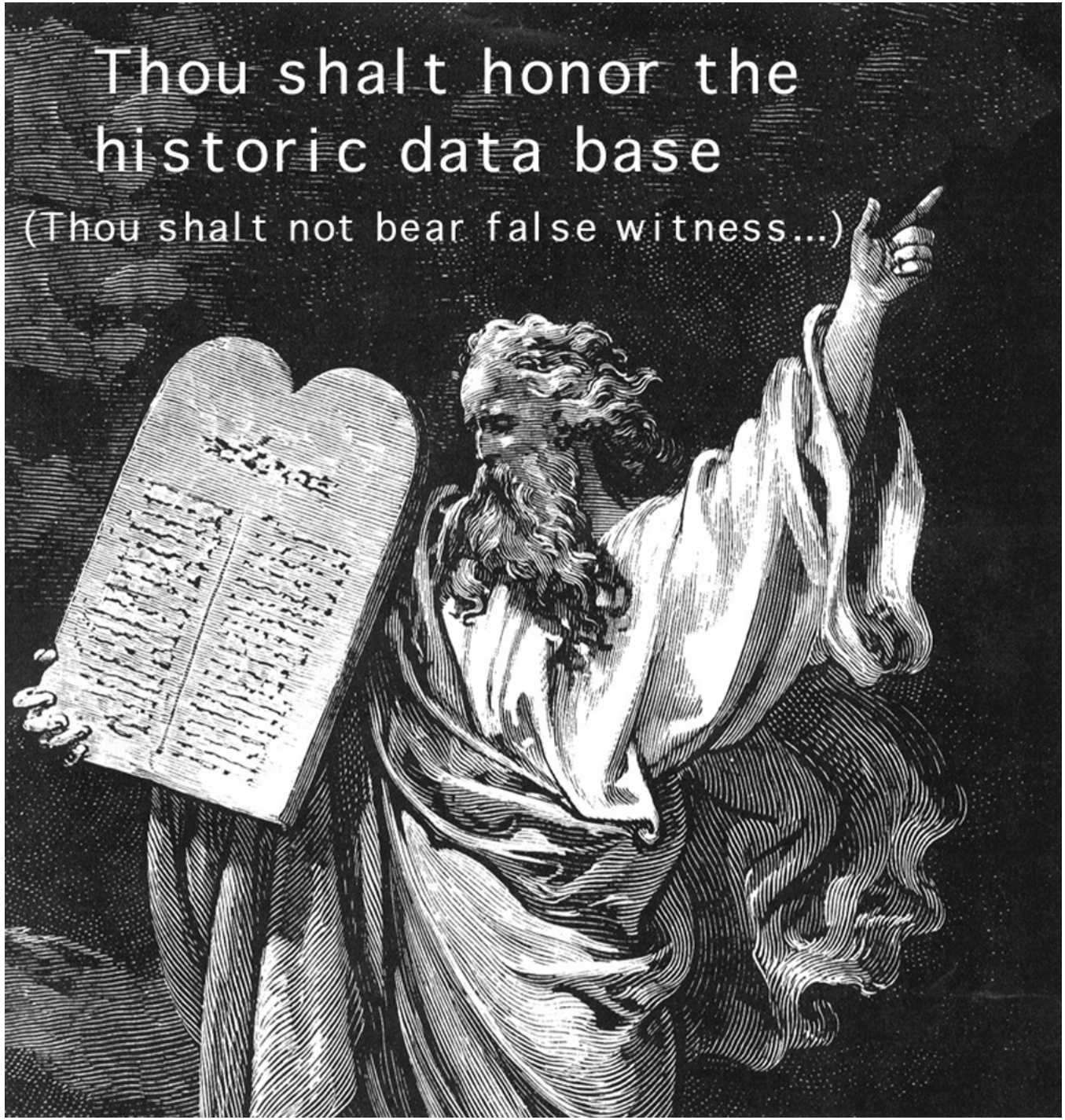
- Yamagiwa & Ichikawa - 1918
  - Coal tar & SCC of rabbit ears
- Murphy & Sturm - 1925
  - Coal tar skin exposure caused lung tumors in mice
- Cook et al. - 1932
  - PAHs caused skin cancer in mice
- Sasaki & Yoshida - 1935
  - o-Amidoazotoluene caused liver tumors in rats

NCI Bioassay History

- 1962 - First contracted bioassay
- 1969 - Innes et al., study published
  - Selection of B6C3F1 mouse
- 1971 - National Cancer Act
  - Decision made to standardize bioassay testing
- ~1975 - F344 rat selected
  - Small size, vigor & survival, disease resistance
  - Inbred


Thou shalt use standardized tests
(Thou shalt not commit adultry)

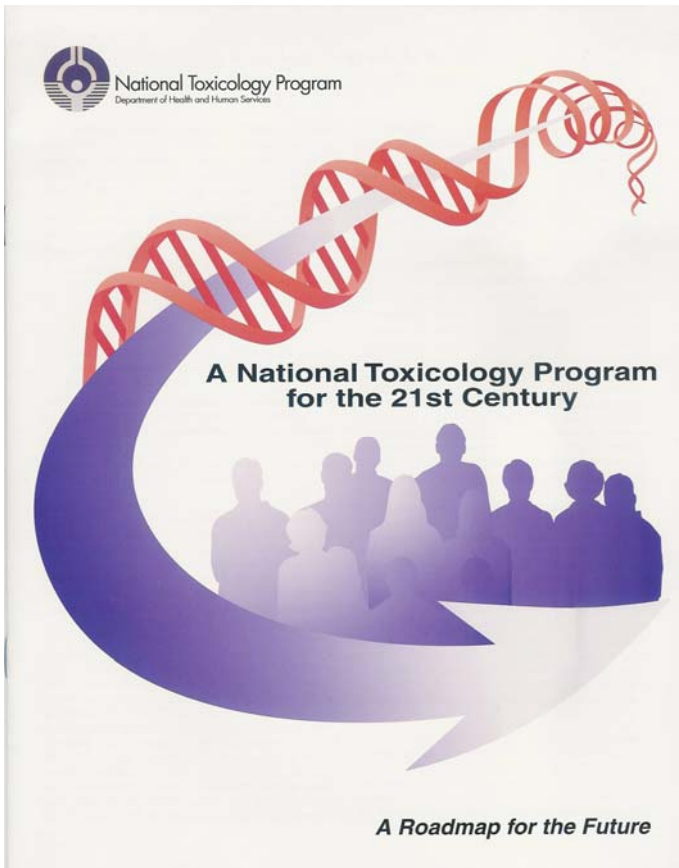

Thou shalt use two species
(Thou shalt honor thy father & mother)


Thou shalt honor the
historic data base
(Thou shalt not bear false witness...)

NTP Established in 1978

- Modified the rodent cancer bioassay
  - More doses
  - Incorporation of pharmacokinetics
  - Incorporation of mechanistic studies
  - Standardization of pathology evaluation
  - More emphasis on non-cancer effects
- Re-evaluate existing practices & research portfolio
  - “Doull” report - 1984
  - Mouse strain workshop - ~1985
  - Mechanism conference - 1995
  - NTP Roadmap - August 2003

NTP Roadmap



- From observational to predictive science
- High throughput screening (HTS)
- Diminished reliance on animal bioassays
  - Optimize utility of bioassays for scientific & regulatory decisions
- Incorporate relevant emerging technologies

Model! Model! Who's Got the Model?

- Two-year cancer bioassay
  - Societal & regulatory buy-in
  - Repeatable
  - Imperfect but the best we have
- Continued search for alternatives
  - Neonatal mouse liver model - Strain A
  - Ito medium term model -Transgenic mice
  - Medaka, zebra fish, guppies -SHE assay

Series of Workshops

- Selection of strains & stocks for bioassays
- Design of two-year bioassays
  - Life stages of exposure
  - Adequacy of current endpoints
  - Diet & dietary restriction
- Design of non-cancer studies
  - Developmental toxicity
  - Immunotoxicity
  - Neurotoxicity & developmental neurotoxicity

Why this specific workshop at this time?

Currently facing several problems with our F344 rat which, in the aggregate, may diminish the effectiveness of this bioassay model for identifying carcinogenic potential.

Characteristics of Existing Strains

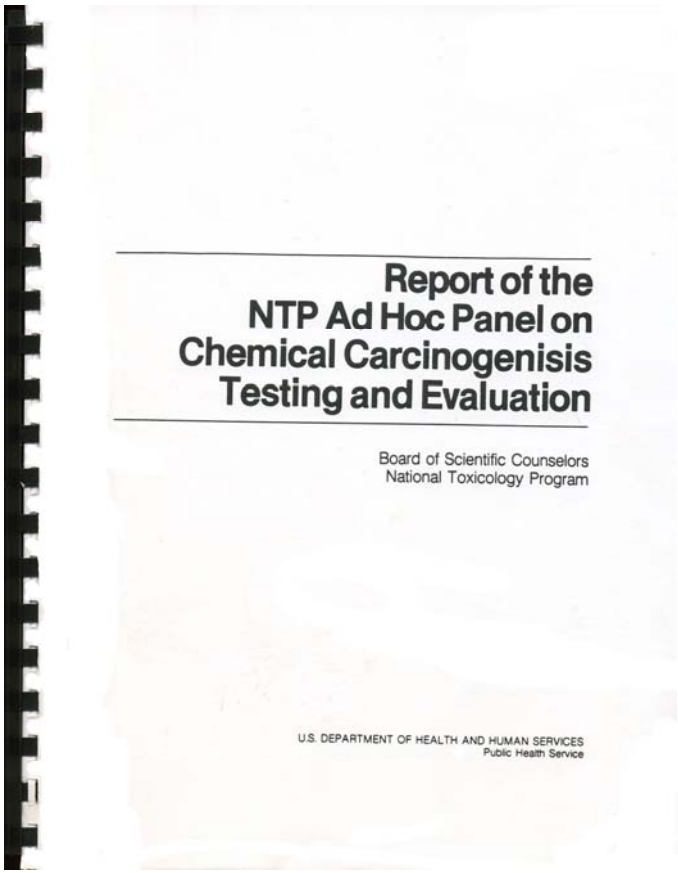
Animal Models of the NTP Rodent Cancer Bioassay: Strains & Stocks - Should We Switch?

Angela King-Herbert
National Institute of Environmental Health Sciences

NTP National Toxicology Program

NIEHS

Criteria for Selection of Animal Models



- Availability
- Cost
- Sensitivity to carcinogens
- Stable response
- Similar metabolism to man
- Similar pathology to man

Additional Criteria

- Survival
- Spontaneous tumor rate
- Experience with the model
- Sensitivity to other endpoints
  - Immunotoxicology
  - Neurotoxicology
  - Reproductive Toxicology

Current NTP Animal Models

- F344/N@Tac
  - Inbred rat
- B6C3F1/N@Tac
  - Isogenic hybrid mouse
  - F1 generation of C57BL/6- E84 female X C3H/HeN-MTV <-> male

Strains with Relevant Data

Rats

- F344/N
- F344/NCTR
- Wistar Han
- Wistar
- Sprague Dawley

Mice

- B6C3F1/N
- B6C3F1/NCTR
- CD-1
- C57BL/10J

F344

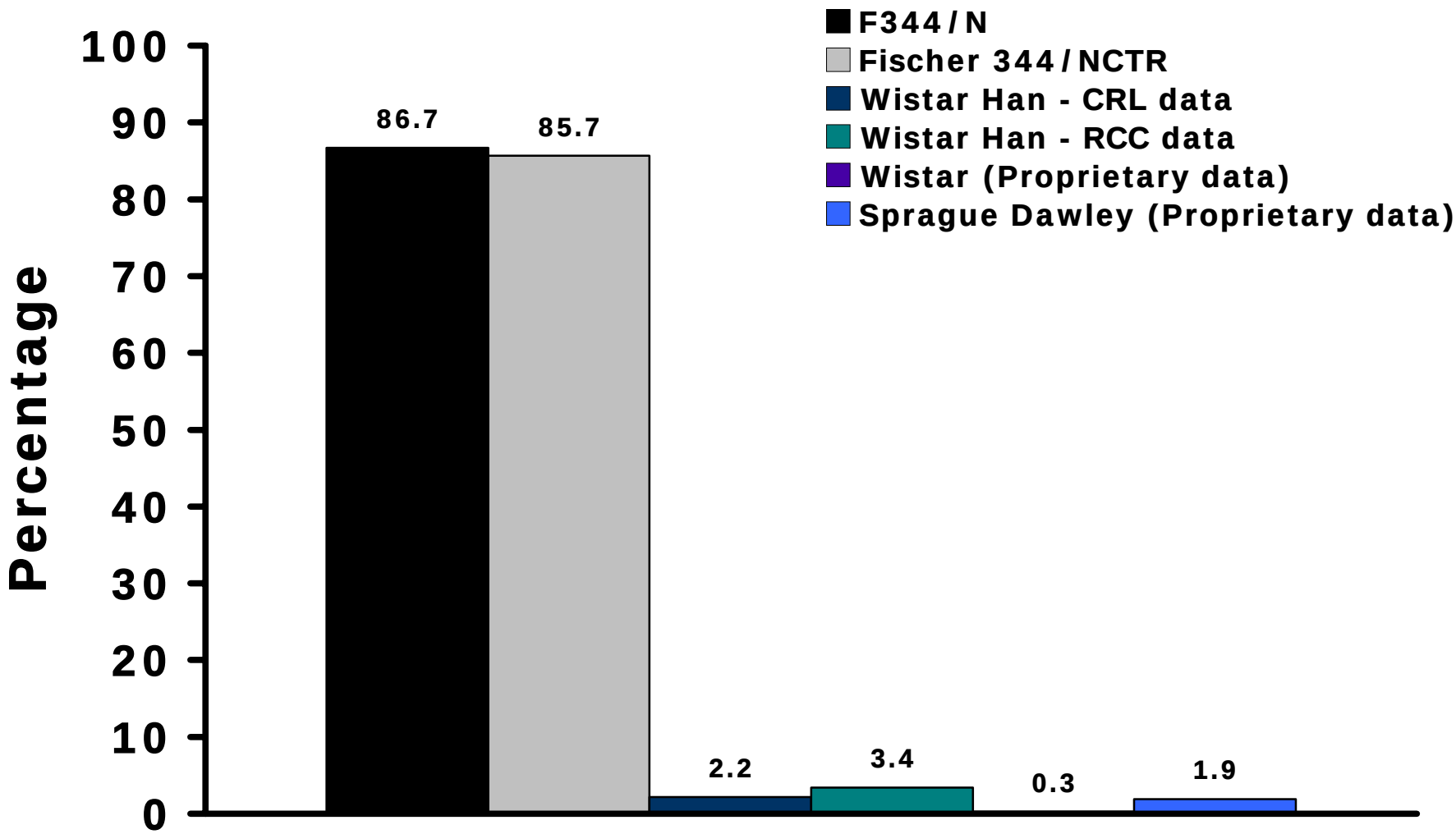
General characteristics

- Large historical database
- Small size
- Good survival
- Litter size 6-8/litter

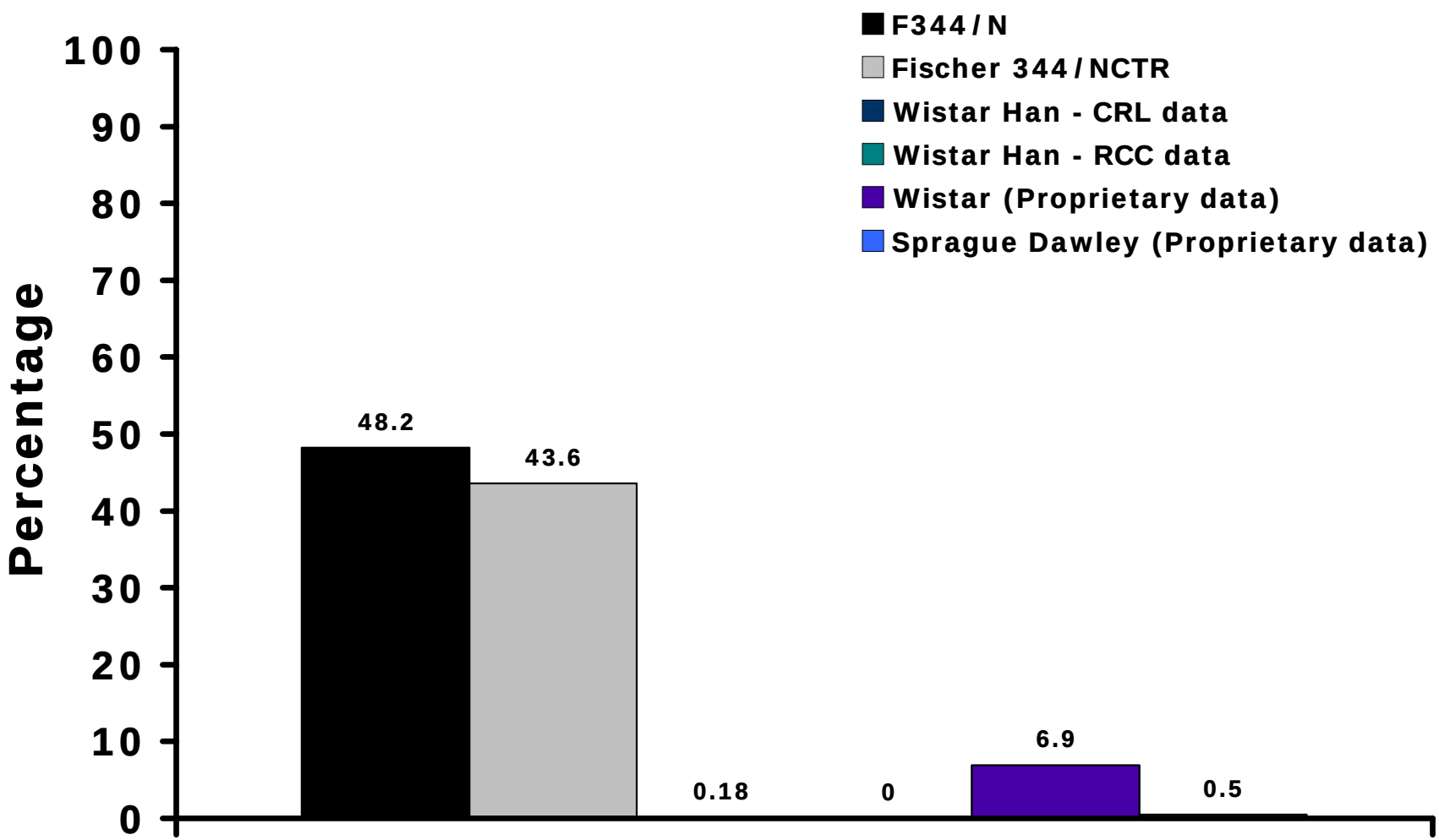
F344 General Concerns

- Testicular tumors (interstitial cell tumor)
- Mononuclear Cell Leukemia

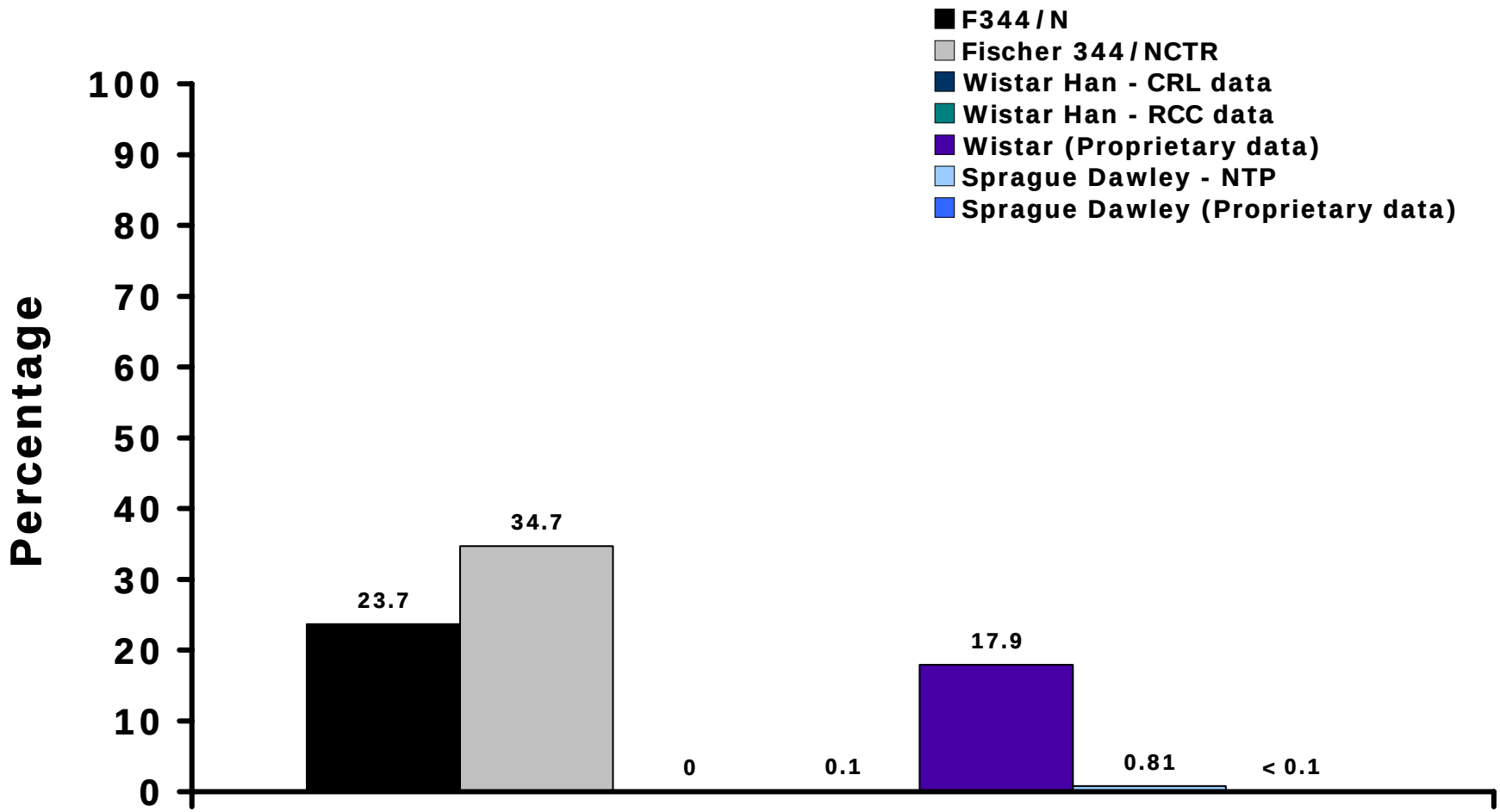
Male Rat Testes Interstitial Cell Tumors



Male Rat Mononuclear Cell Leukemia



Female Rat Mononuclear Cell Leukemia



F344 NTP Concerns

- Reproduction problems
- Seizures
- Chylothorax

Other Rats

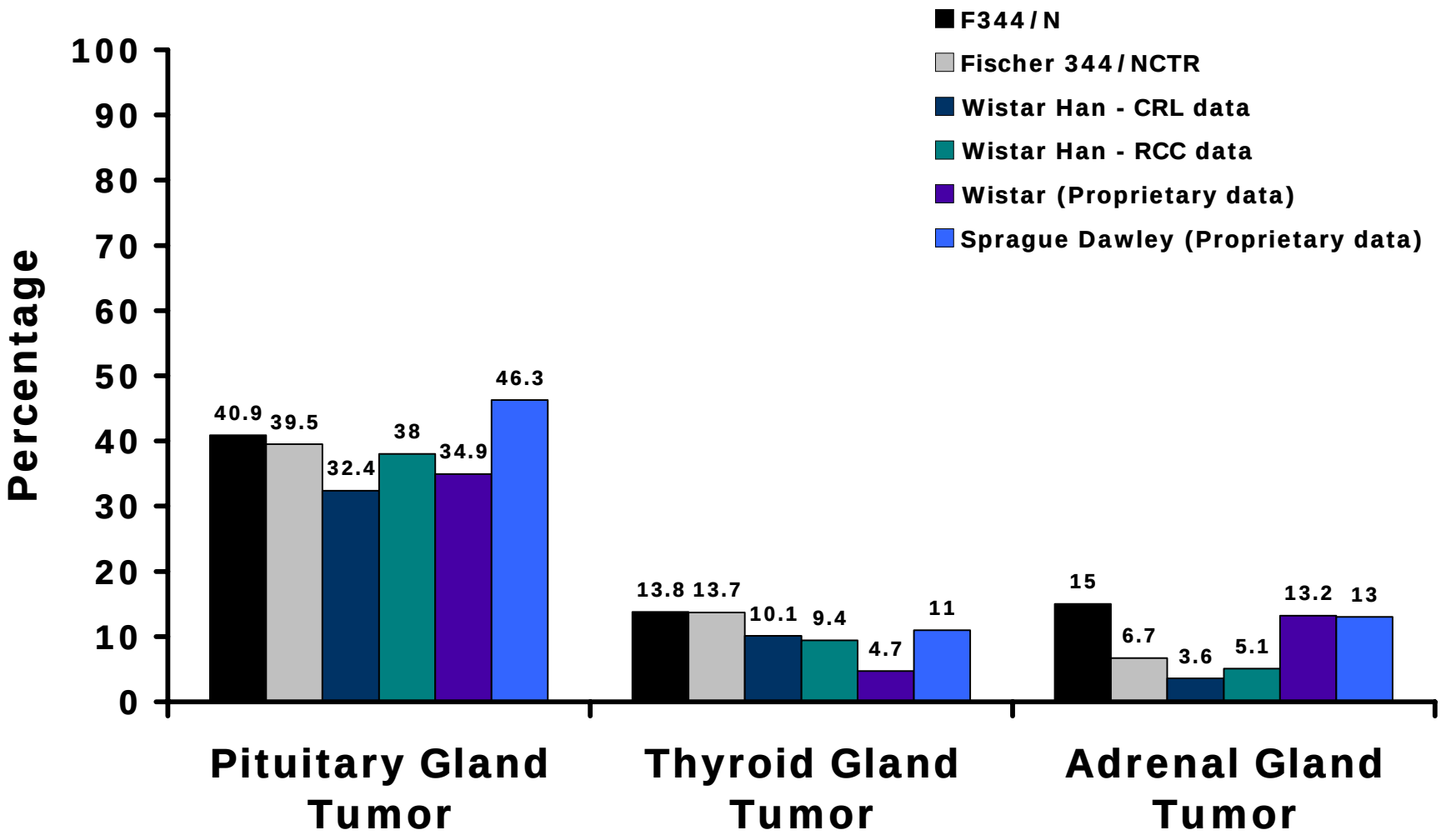
Wistar Han

- Outbred
- Small size
- Long survival
- Low tumor burden

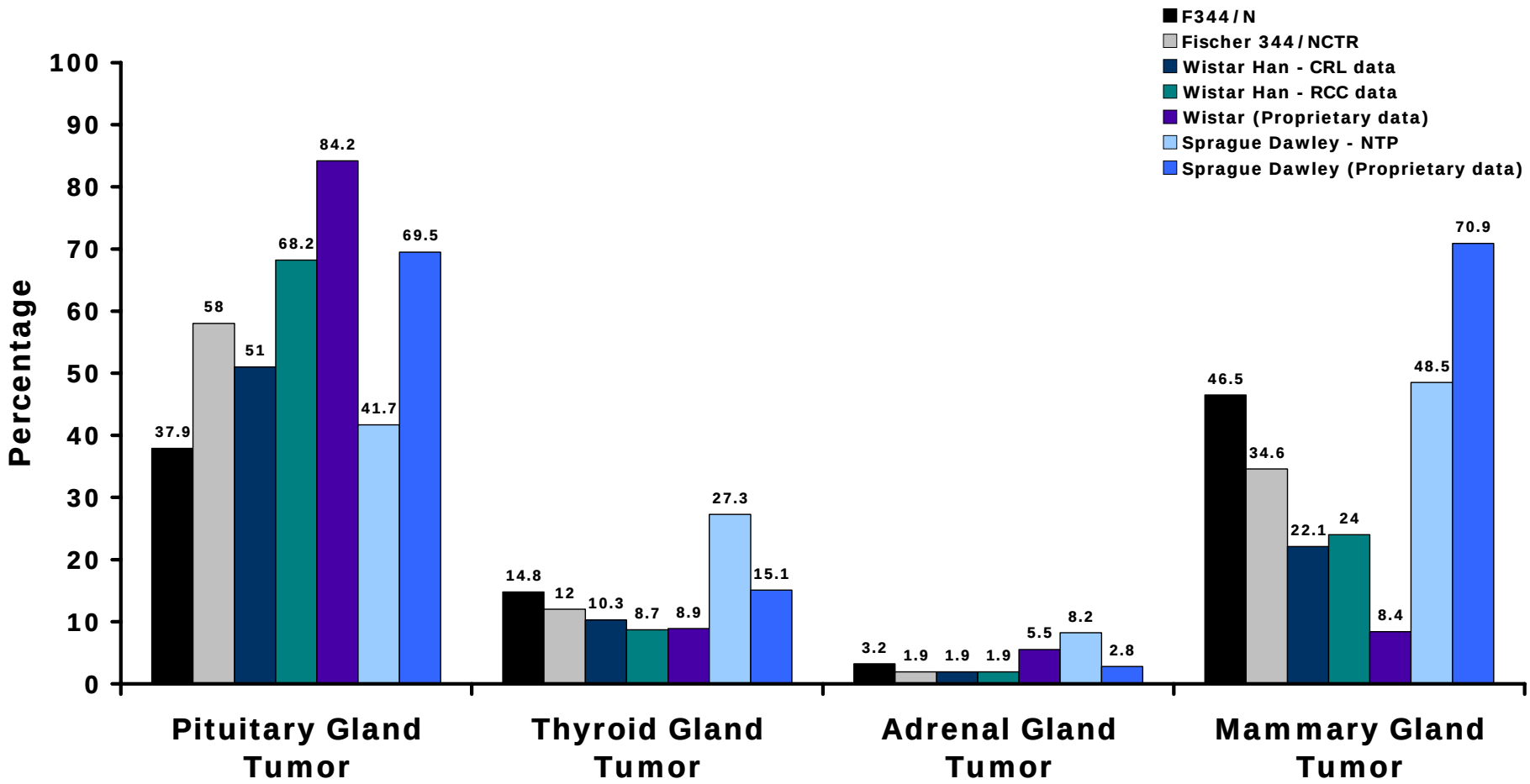
Sprague Dawley

- Outbred
- Widely used in toxicology studies
- Good reproductive characteristics
- Docile
- Hardy

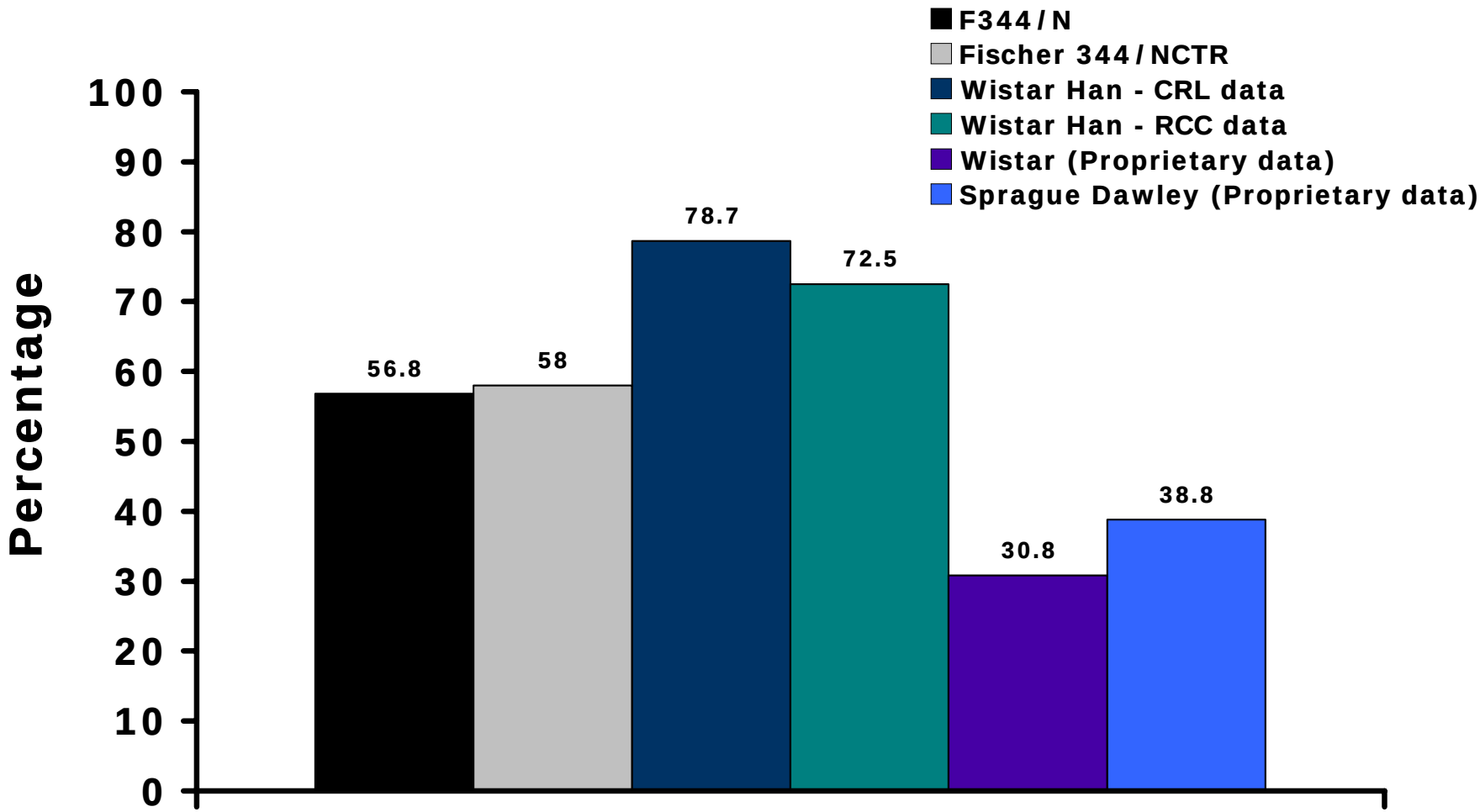
Male Rat Tumor Rates



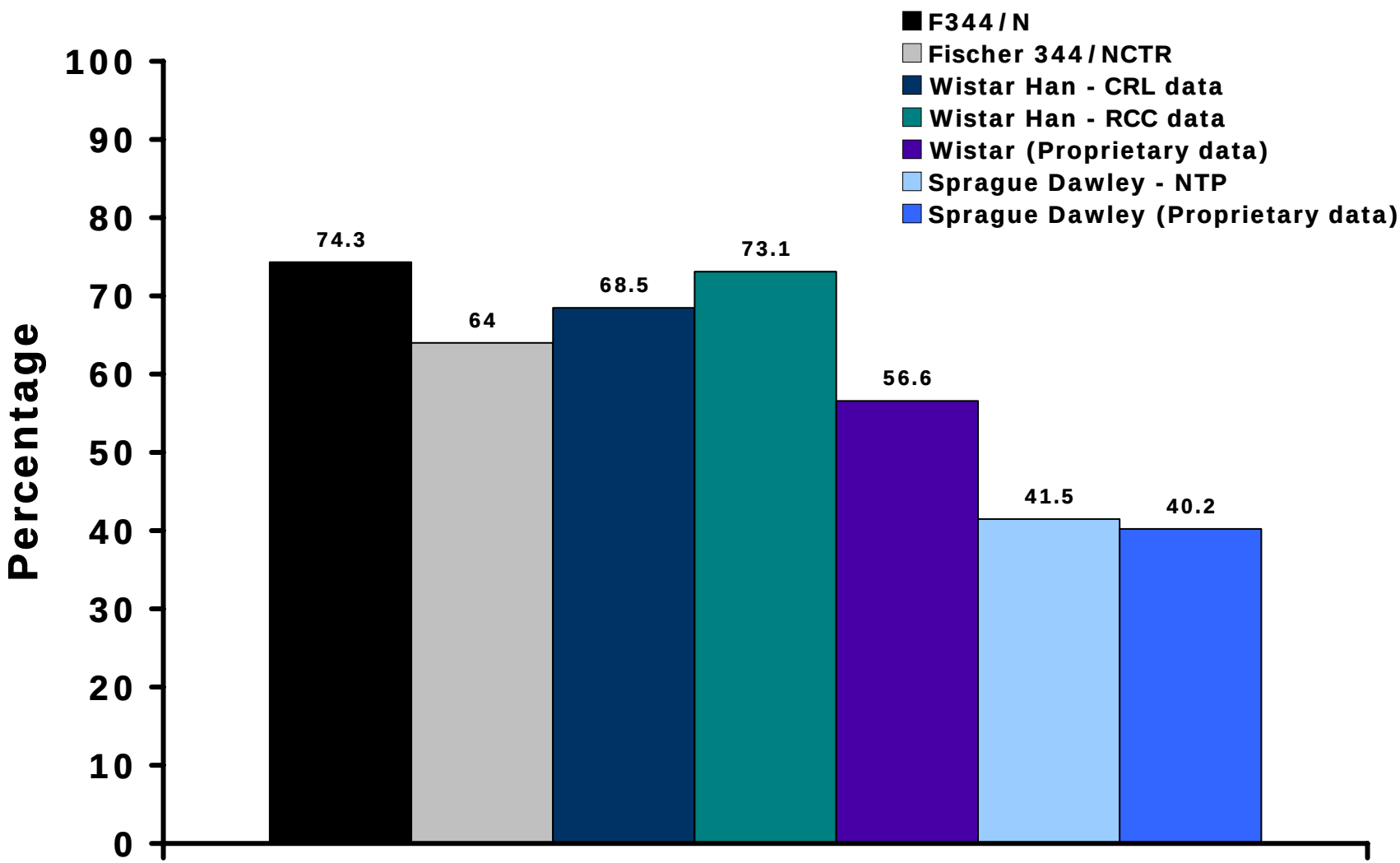
Female Rat Tumor Rates



Male Rat 2 Year Survival Rate



Female Rat 2 Year Survival Rate



B6C3F1

General characteristics

- Hardy
- Used often in toxicology studies
- Long survival

B6C3F1 Concerns

- Liver tumors
  - Increased background rate
  - Most common target organ
- Body weight gain
  - Increased body weight increases liver tumors

Other Mice

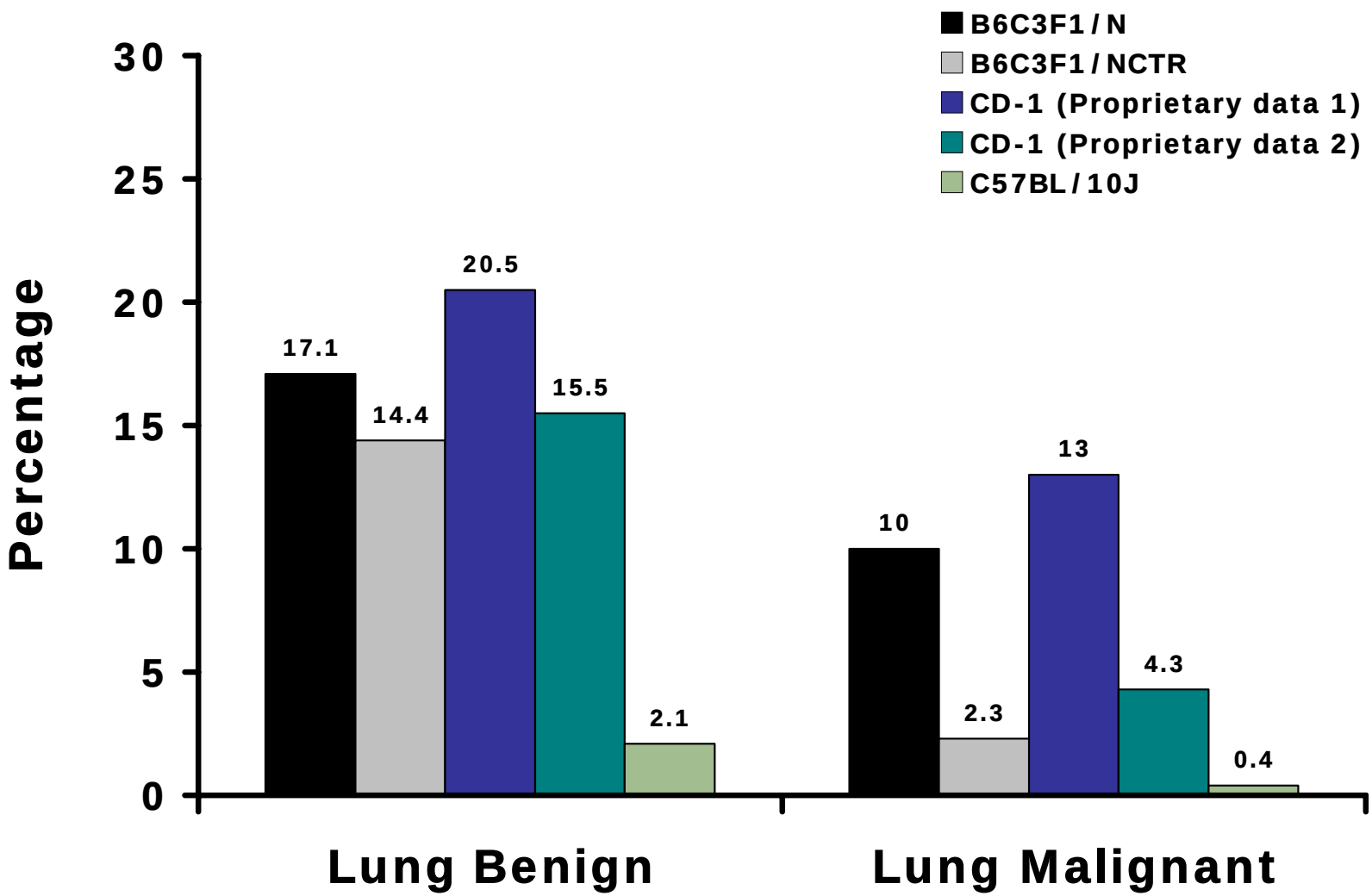
CD-1

- Outbred
- Widely used
- Good reproductive characteristics
- Docile

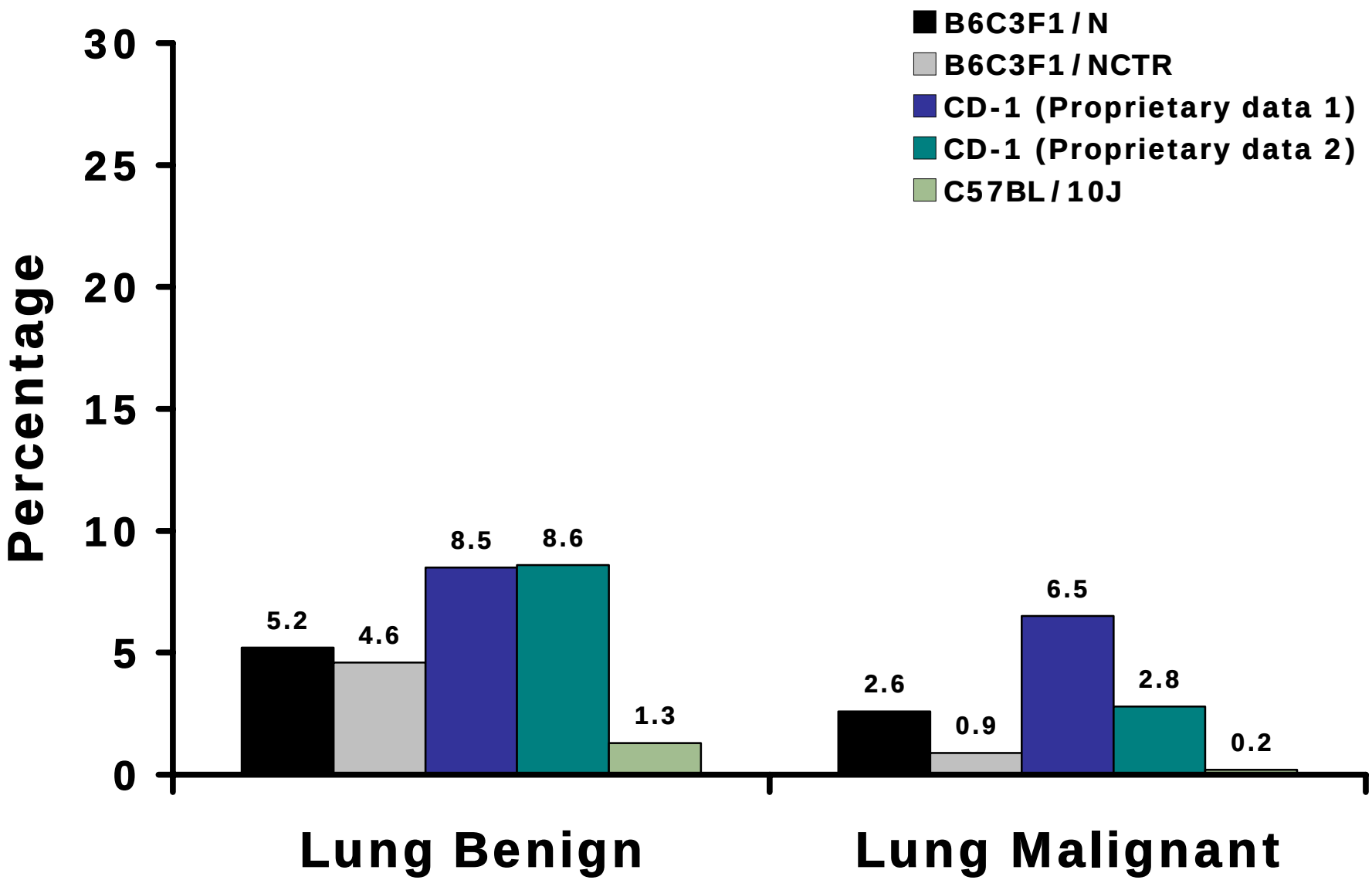
C57BL/10J

- Inbred
- Very similar to the C57BL/6J

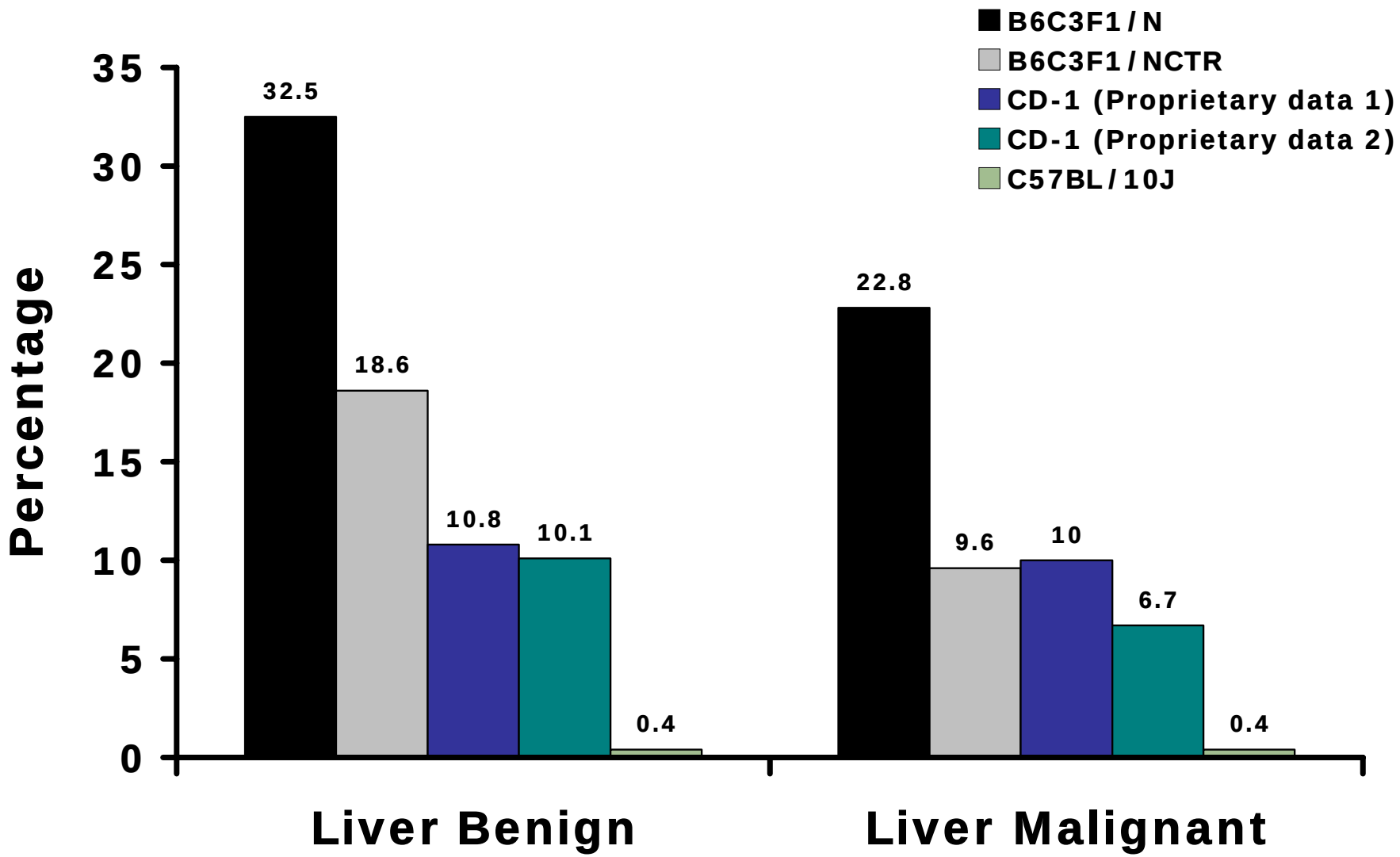
Male Mice Lung Tumors



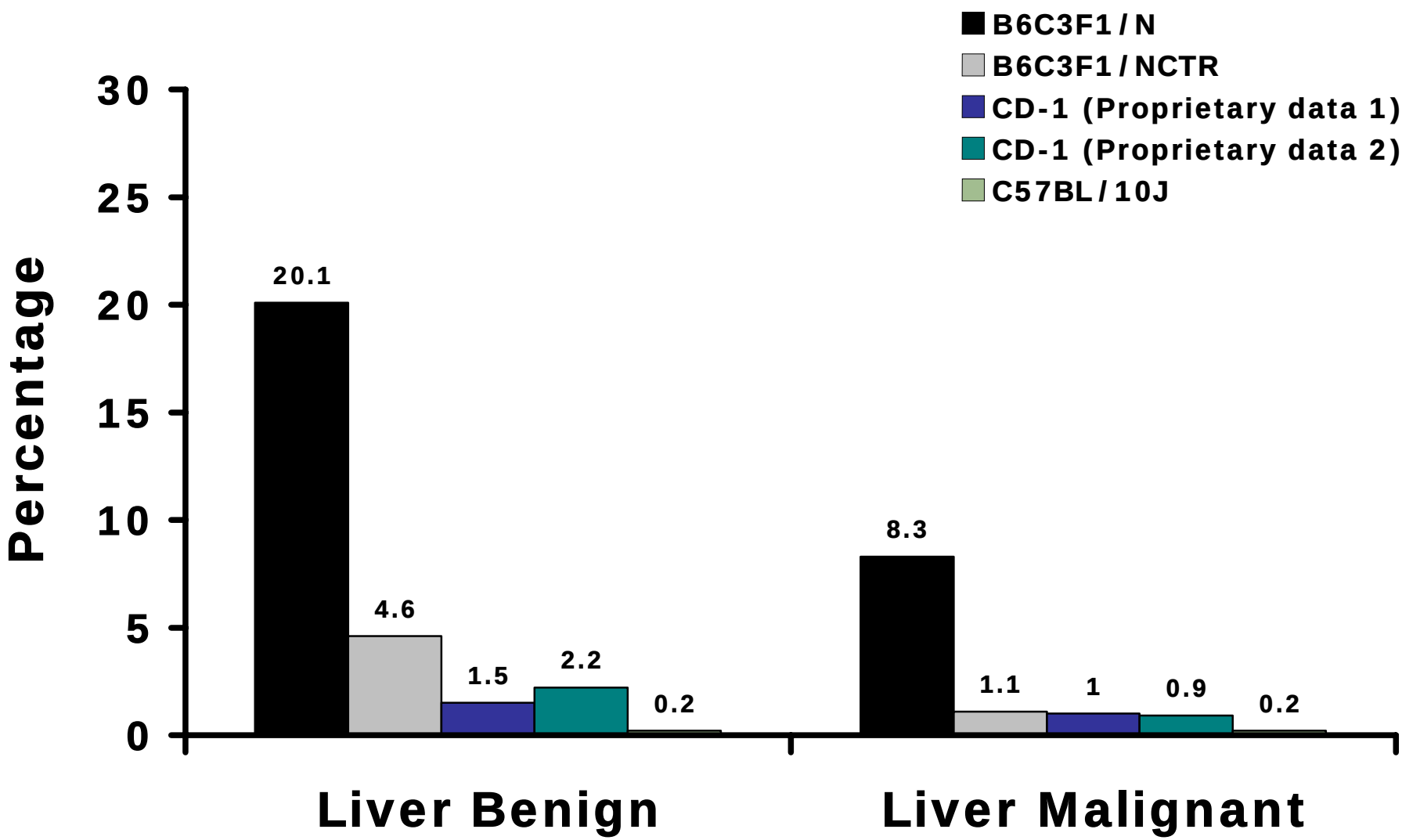
Female Mice Lung Tumors



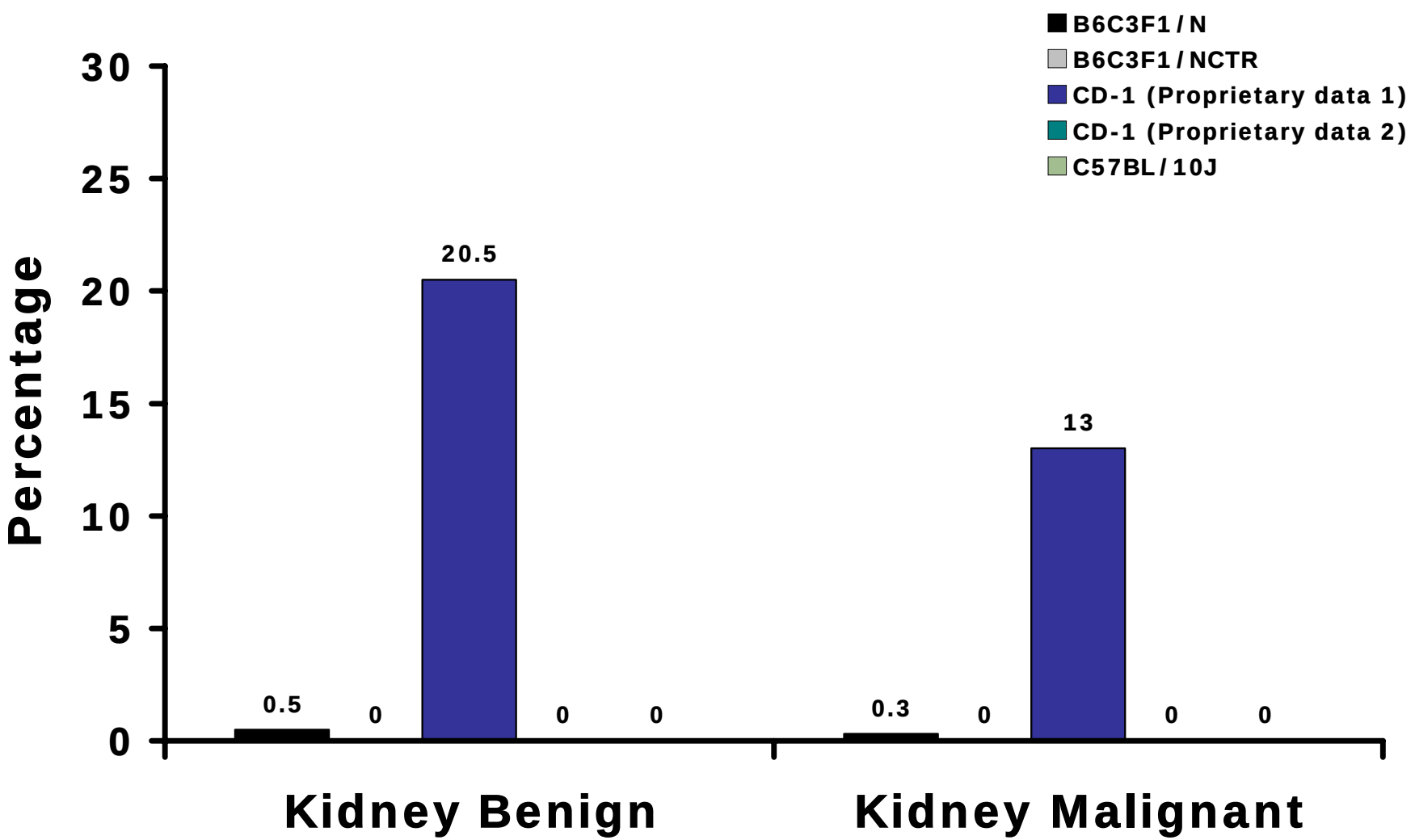
Male Mice Liver Tumors



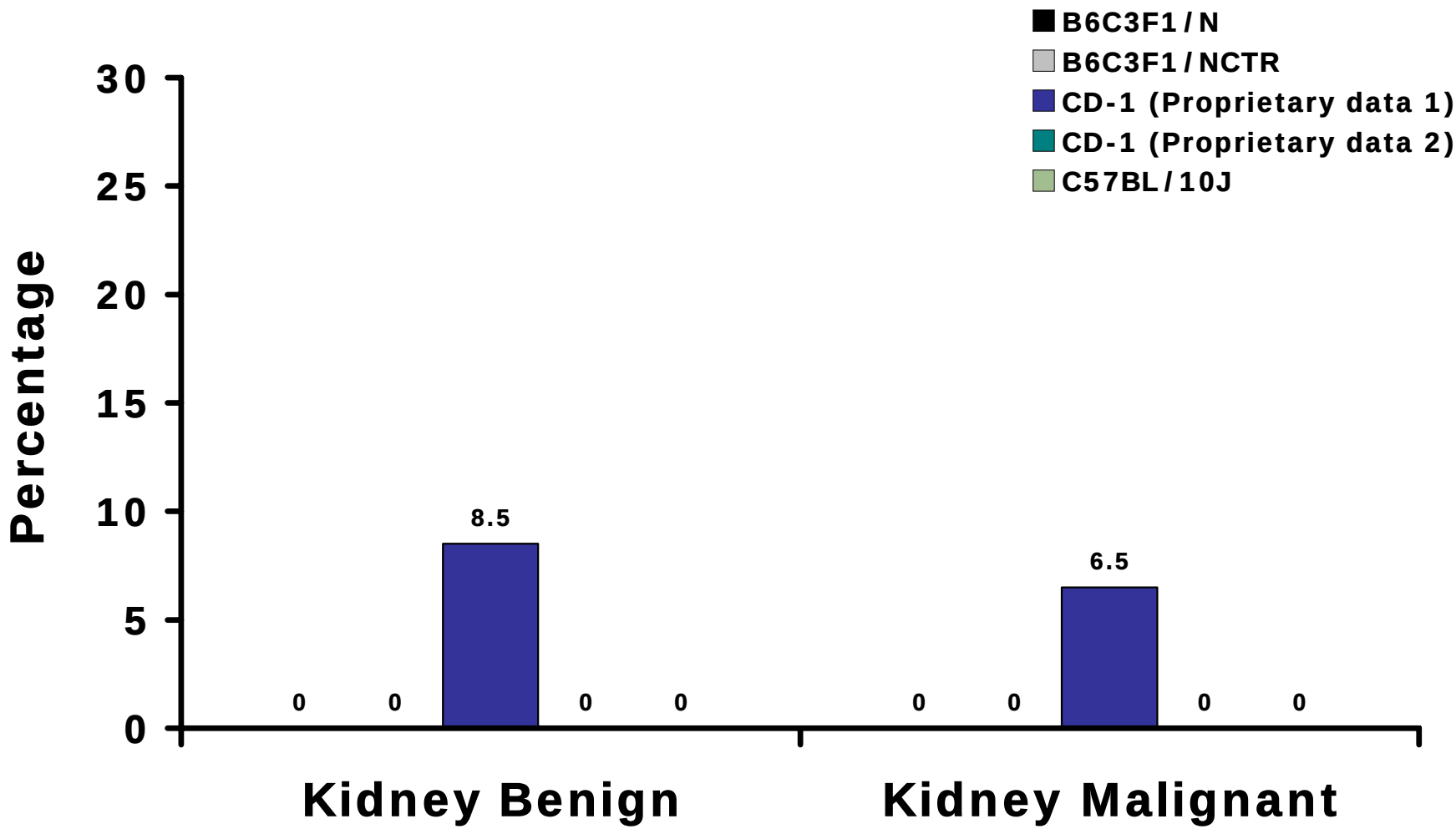
Female Mice Liver Tumors



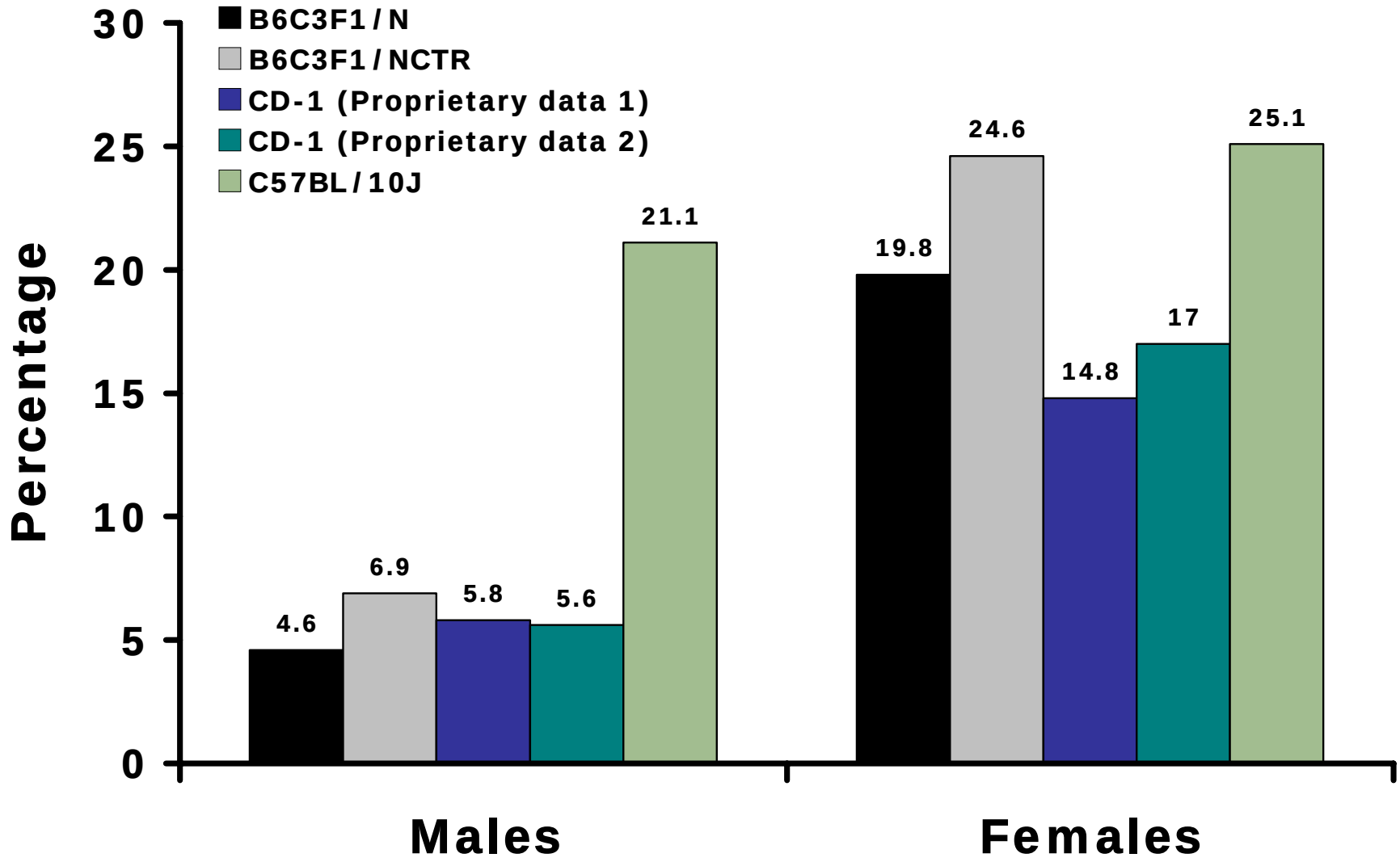
Male Mice Kidney Tumors



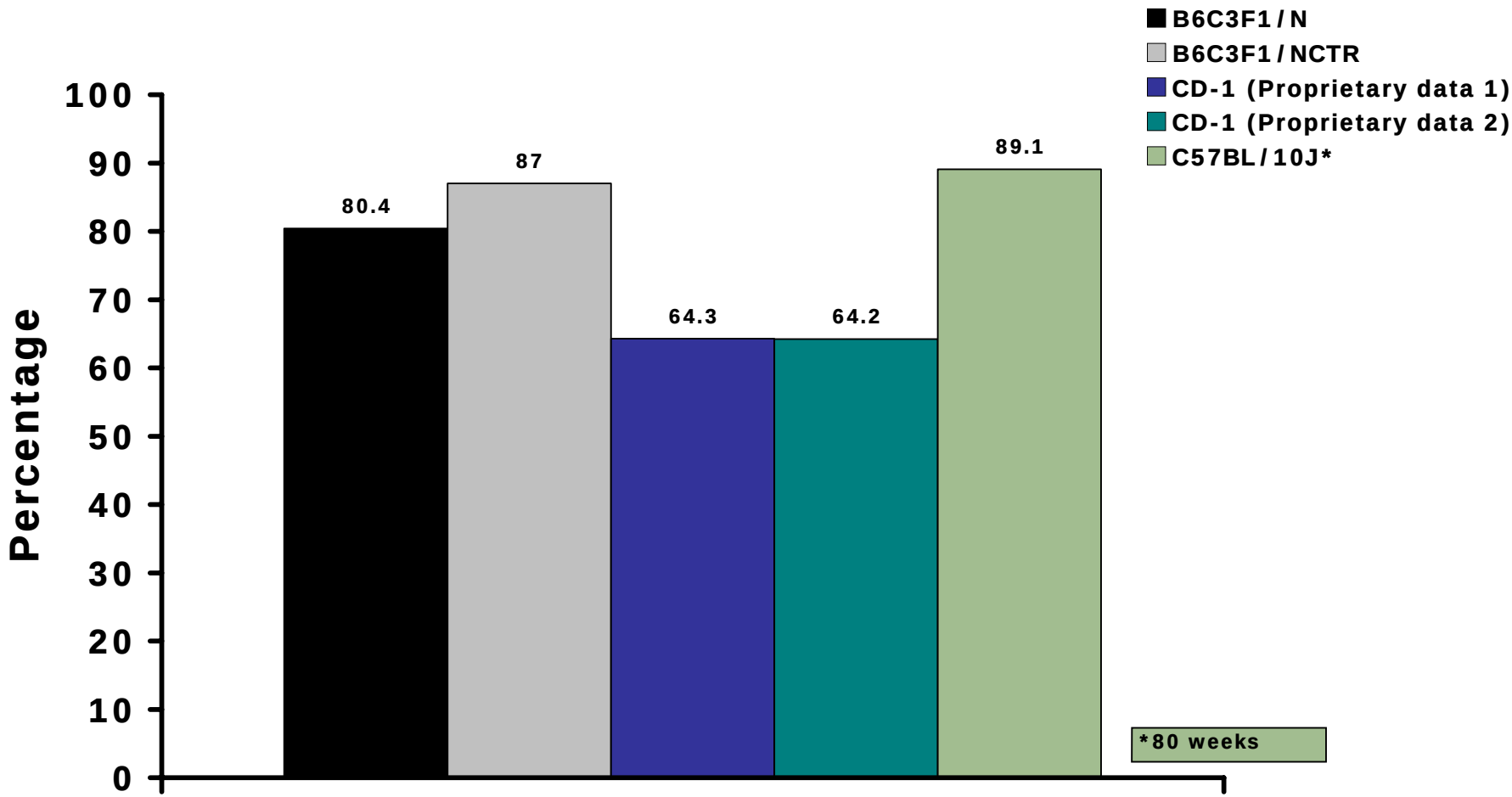
Female Mice Kidney Tumors



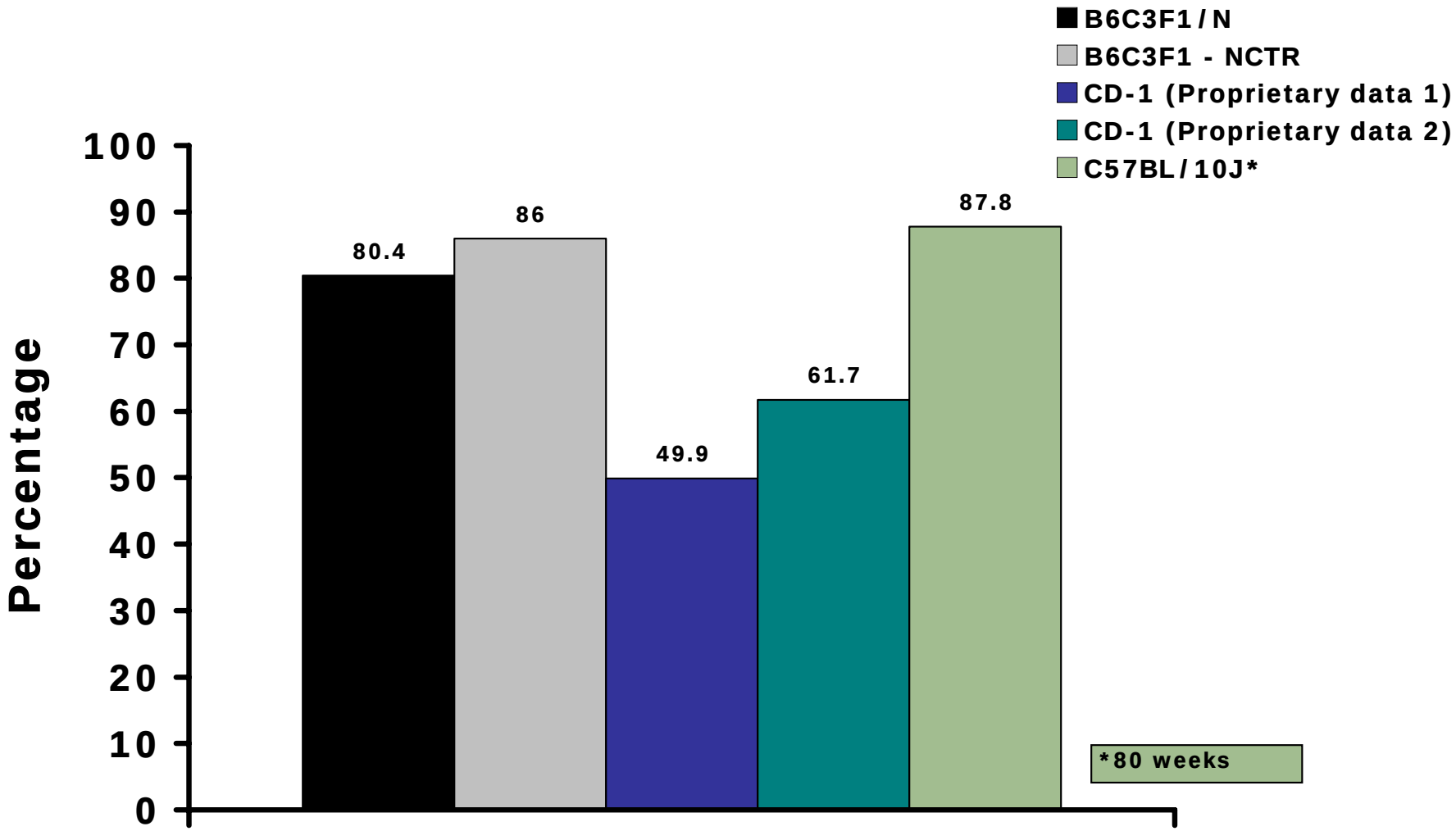
Mouse Lymphoma



Male Mice Survival Rates

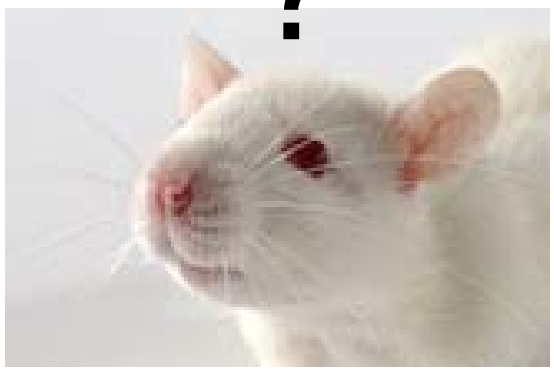


Female Mice Survival Rates



Should We Switch?

?



TUMORIGENICITY STUDIES: SELECTION OF RAT STOCKS AND STRAINS - a Contract Research Organisation perspective

William N Hooks
Huntingdon Life Sciences

June 2005

Introduction

In order to answer the NTP brief (*i.e.* why we conducted a review of rat strain, what we learned and what advice we could offer) the following points will be considered:

- Regulatory considerations
- Historical perspective
- The rat strain choice
- Advantages and disadvantages of each rat strain
- Summary and Conclusions

Regulatory considerations

- The 2 year endpoint for tumorigenicity studies is still required
- Definitive rat strains are not specified in the regulatory guidelines
- Laboratories should use a strain of rat that has sufficient background data
- In October 2000, the UK Pesticides Safety Directive suggested that if survival in tumorigenicity studies is to be compliant (with current UK and EC guidelines for the acceptability of a negative result from studies), test laboratories may wish to consider using alternative strains to the Charles River Sprague-Dawley rat

Regulatory considerations - continued

- Regulators (particularly the US FDA) have suggested that a diet optimisation method or increase in group size strategy should be employed if poor survival is expected at 2 years.
- Our standard rat strain, the Sprague-Dawley, was showing poor survival at 2 years.
- The regulatory acceptability of the rat strain used is of paramount importance. Therefore, we initiated a strategic review of the choice of rat strain used for tumorigenicity studies
- A short historical review is appropriate to place this in perspective

Historical perspective

The 1970's

- Consensus was that the duration of a rat tumorigenicity study should be a least 104 weeks
- Based on the survival of the major rat strains (*Sprague-Dawley (SD), Fischer-344 and Wistar rat*) at this time
- The SD rat was recognised as the shortest lived of the rat strains (*mortality was generally below 50%*)
- Regulatory authorities therefore adopted the tumorigenicity study endpoint as 104 weeks

Historical perspective - continued

The 1980's

- Charles River Sprague-Dawley (SD CR) rat studies started showing high terminal mortality values. At this time a high protein breeding diet was in use -

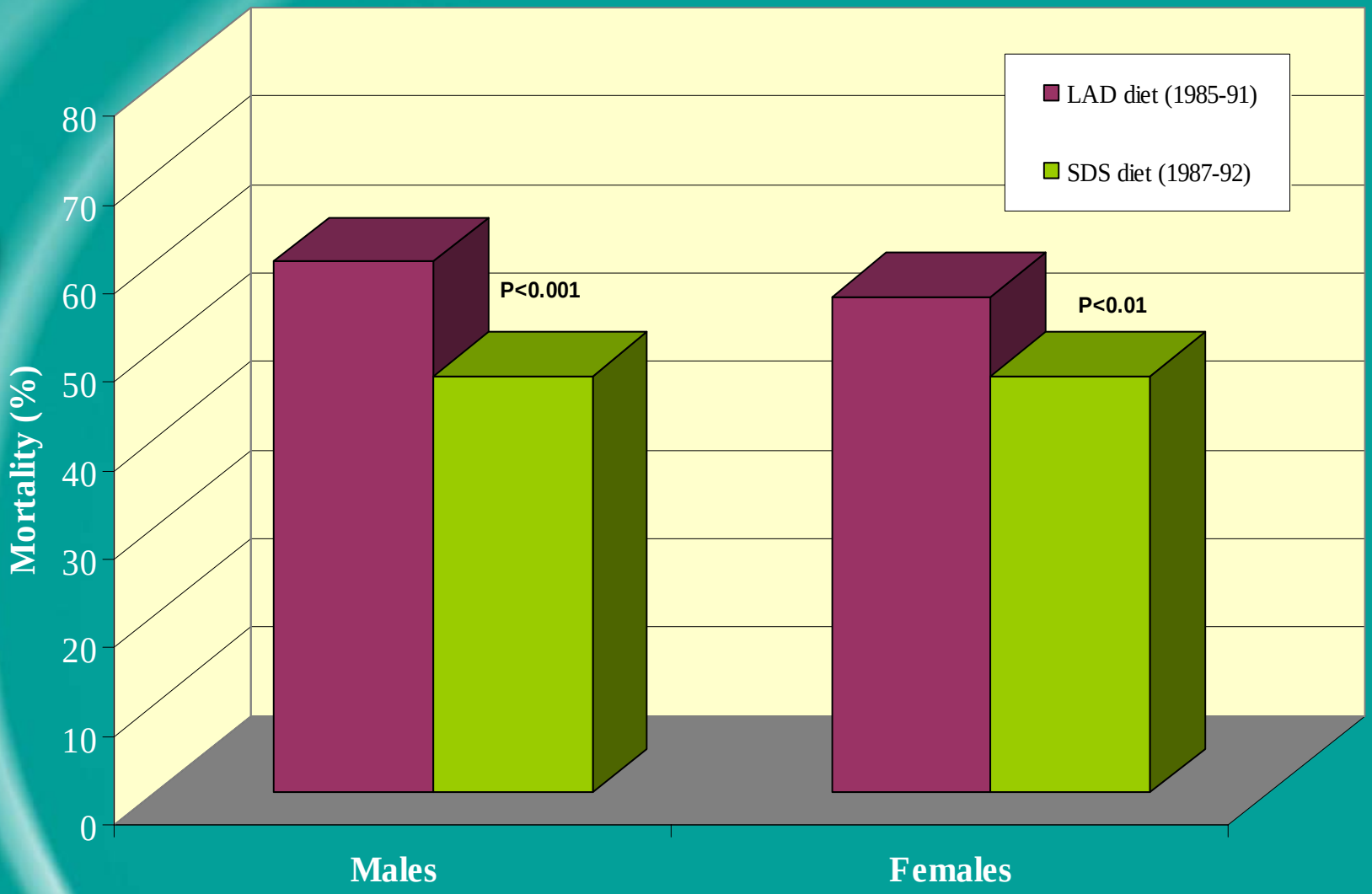
  Labsure Laboratory Animal Diet (LAD),
  typically 21.5% protein, 3.7% fat, 4% fibre

- The mortality rate was reduced (*Figure 1*) when a lower protein maintenance diet was used* -

  Special Diets Services (SDS),
  typically 14.5% protein, 3% fat, 4% fibre

(* mainly due to a reduced incidence of death due to renal progressive glomerulonephrosis)

Figure 1: Mortality at Week 104 in Sprague-Dawley rats - a comparison between diets

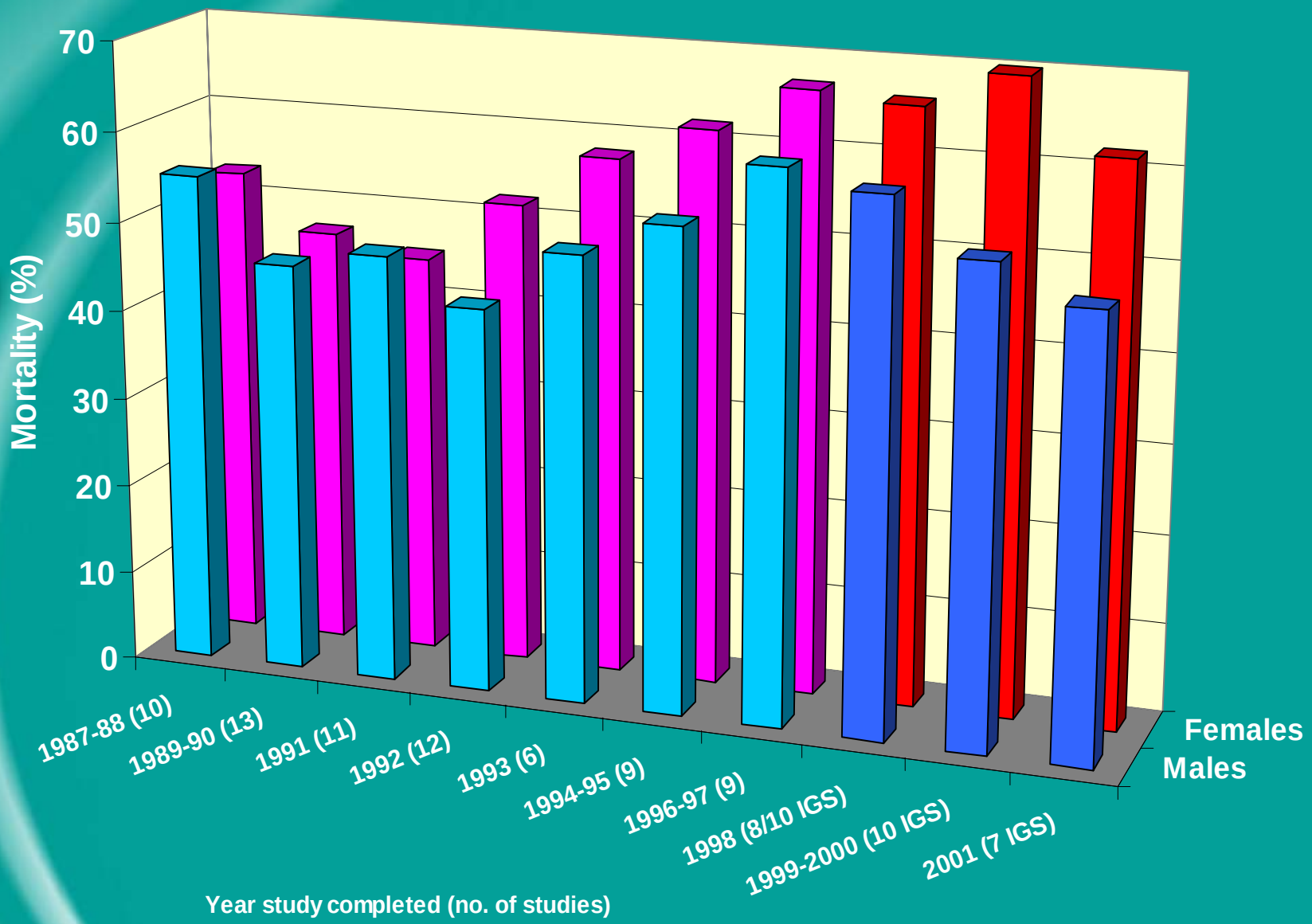


Historical perspective - continued

The 1990's

- Increasing trend towards higher terminal mortality values in the SD CR rat (*Figure 2*) - despite the use of lower protein diet
- Increasing use of alternative strains of rat
- In 1996, Charles River introduced the International Genetic Standard (IGS) rat (SD CR IGS)
- IGS Users Group formed in Japan (with world-wide contribution)
- In 1998, the first IGS rat studies completed two years

Figure 2: Mortality at Week 104 in SD CR rats - a comparison over time



The rat strain choice

The major strains of rat now in use at HLS include:

- Sprague-Dawley International Genetic Standard rat from Charles River, UK - SD CR IGS)
- Sprague-Dawley rat from Harlan UK - (SD HA)
- Fischer-344 rat from Charles River - (F-344)
- Wistar Han rat from Harlan UK or Bioresearch Laboratories, Switzerland - (WI Han)

The rat strain choice - continued

By the end of 2001, at the time of our review, HLS had completed up to the following number of tumorigenicity studies (*the terminal % mortality and survival values are also presented*):

	n	Mortality/Survival (%) *Males*	*Females*
SD CR IGS	27	*54 / 46*	*67 / 33*
SD HA	4	*44 / 56*	*54 / 46*
F-344	13	*51 / 49*	*36 / 64*
WI Han	22	*23 / 77*	*30 / 70*

Mortality data comparisons for these rat strains are given in Figures 3 - 5

Figure 3: Mortality at Week 104 in rat tumorigenicity studies

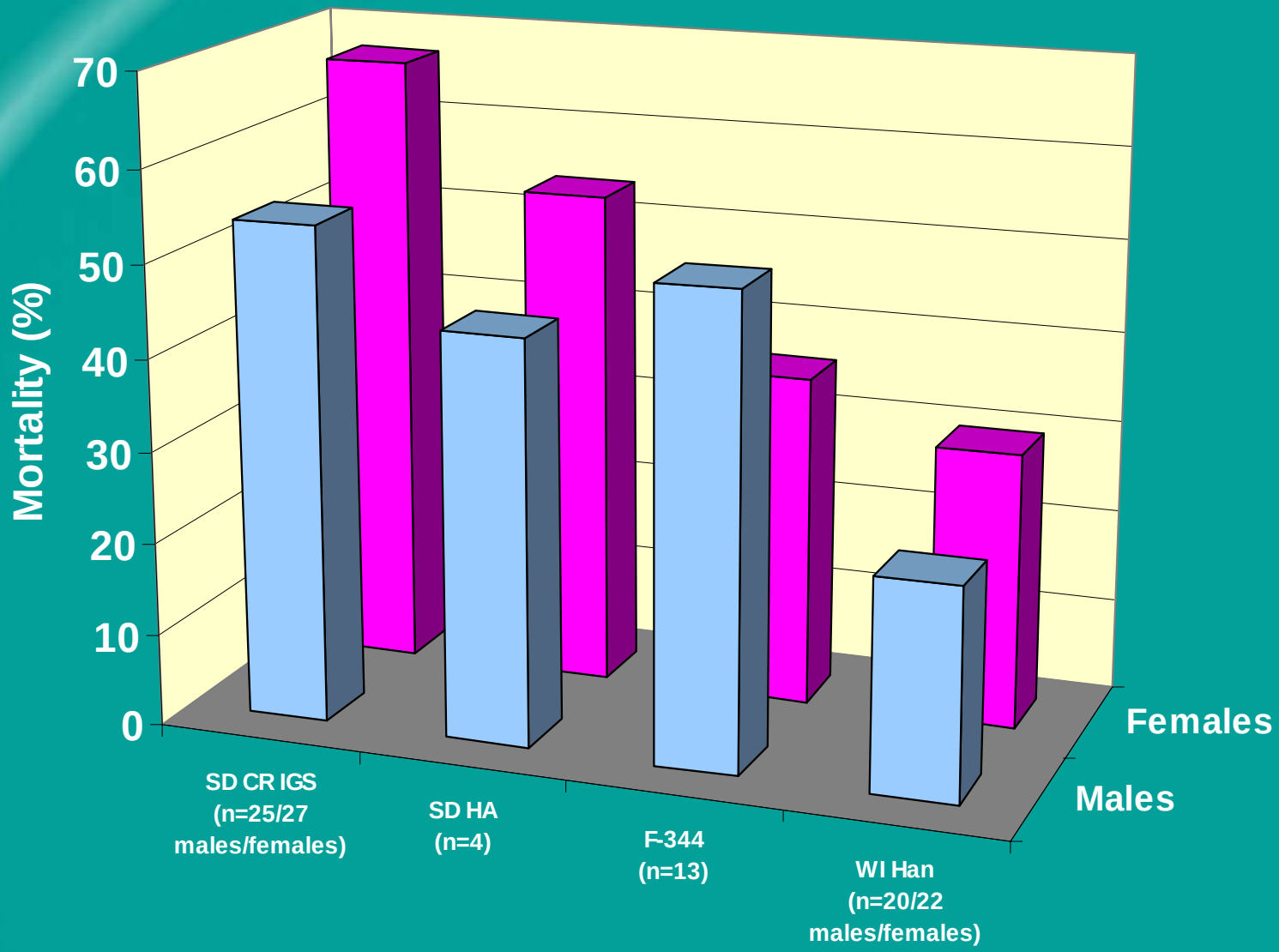


Figure 4: Mortality pattern in male rats

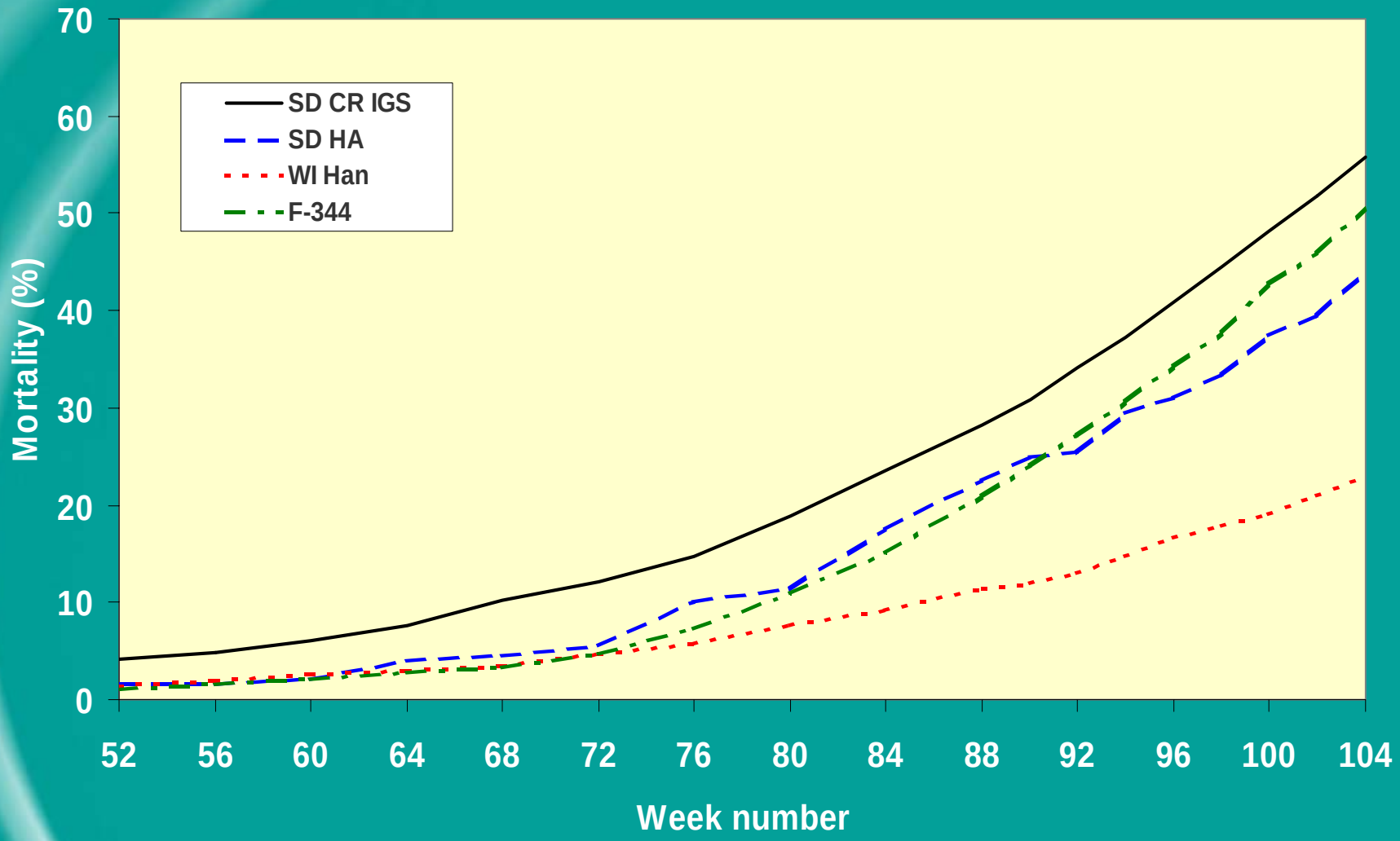
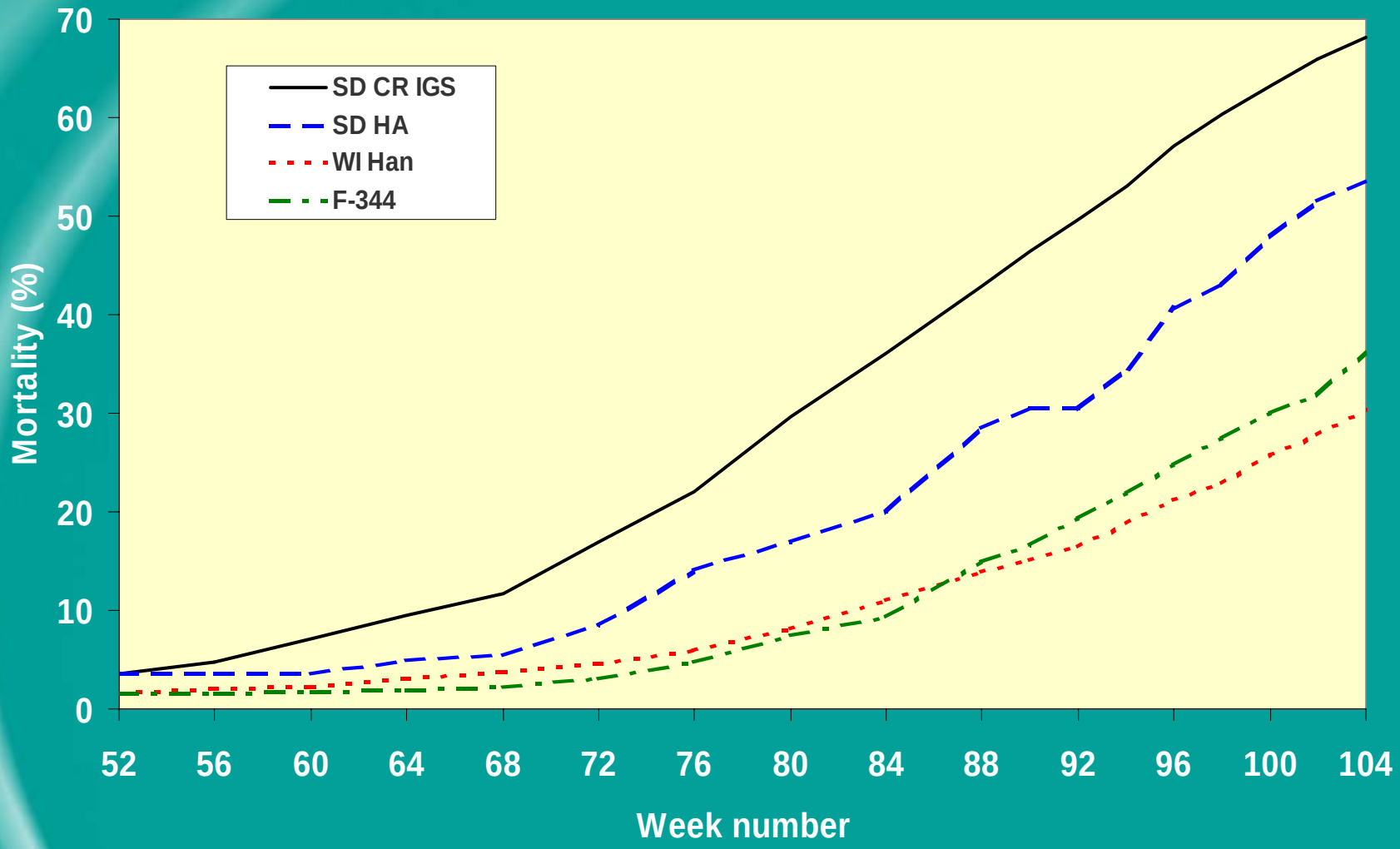


Figure 5: Mortality pattern in female rats



Advantages and disadvantages of rat strains

Sprague-Dawley rat (from Charles River, SD CR IGS):

Advantages -

- Vast amount of data available
- Internationally known and available strain

Disadvantages -

- Poor survival at 2 years, particularly in females
- Surviving animals are in a geriatric state
- High incidence of tumours (*e.g. pituitary adenoma and mammary adenoma/adenocarcinoma*)
- Strategies necessary to ensure sufficient number of surviving animals at 2 years

Advantages and disadvantages - continued

Sprague-Dawley rat (from Harlan UK, SD HA):

Advantages -

- Reasonable amount of data available
- Survival good in males, reasonable in females
- Increase in group size unlikely
- Test compound use and costs reduced (*a smaller rat in comparison to the SD CR IGS rat*)

Disadvantages -

- Variable survival at 2 years
- Data available relatively small

Advantages and disadvantages - continued

The Fischer 344 rat (from Charles River, F-344):

Advantages -

- Large amount of data available
- Chosen for the US National Toxicology Program
- Survival good in females, reasonable in males
- Increase in group size unlikely
- Test compound use and costs reduced
  (*a smaller rat in comparison to the other strains*)

Disadvantages -

- Various strain specific pathology
  (*e.g. high incidence of large granular lymphocytic lymphomas and testicular tumours*)

Advantages and disadvantages - continued

Wistar Han (from Harlan UK or Bioresearch Sw, Wl Han):

Advantages -

- Large amount of data available
- Survival excellent at 2 years (*always above 50%*)
- Increase in group size is not required (*minimises animal use and study costs*)
- Test compound use and costs reduced (*a smaller rat in comparison to the SD CR IGS rat*)

Disadvantages -

- Survival at 2 years is perhaps too good
- Strain less well known (*but data is available*)

Advantages and disadvantages - continued

WI Han rat compared with SD CR IGS rat:

Assessment of the tumour profile (*summarised in Figures 6 and 7*) has shown:

- Most prevalent tumours (*also the major FCTD*)
  - pituitary adenoma (males and females)
  - mammary tumours (females)
- Tumour incidences were lower in the WI Han rat
- Other differences
  - higher incidence of subcutaneous fibroma and adrenal benign phaeochromocytoma in SD CR IGS male rats and lymph node haemangioma in WI Han male rats

Figure 6: Tumour and FCTD incidence - male SD CR IGS and WI Han rats

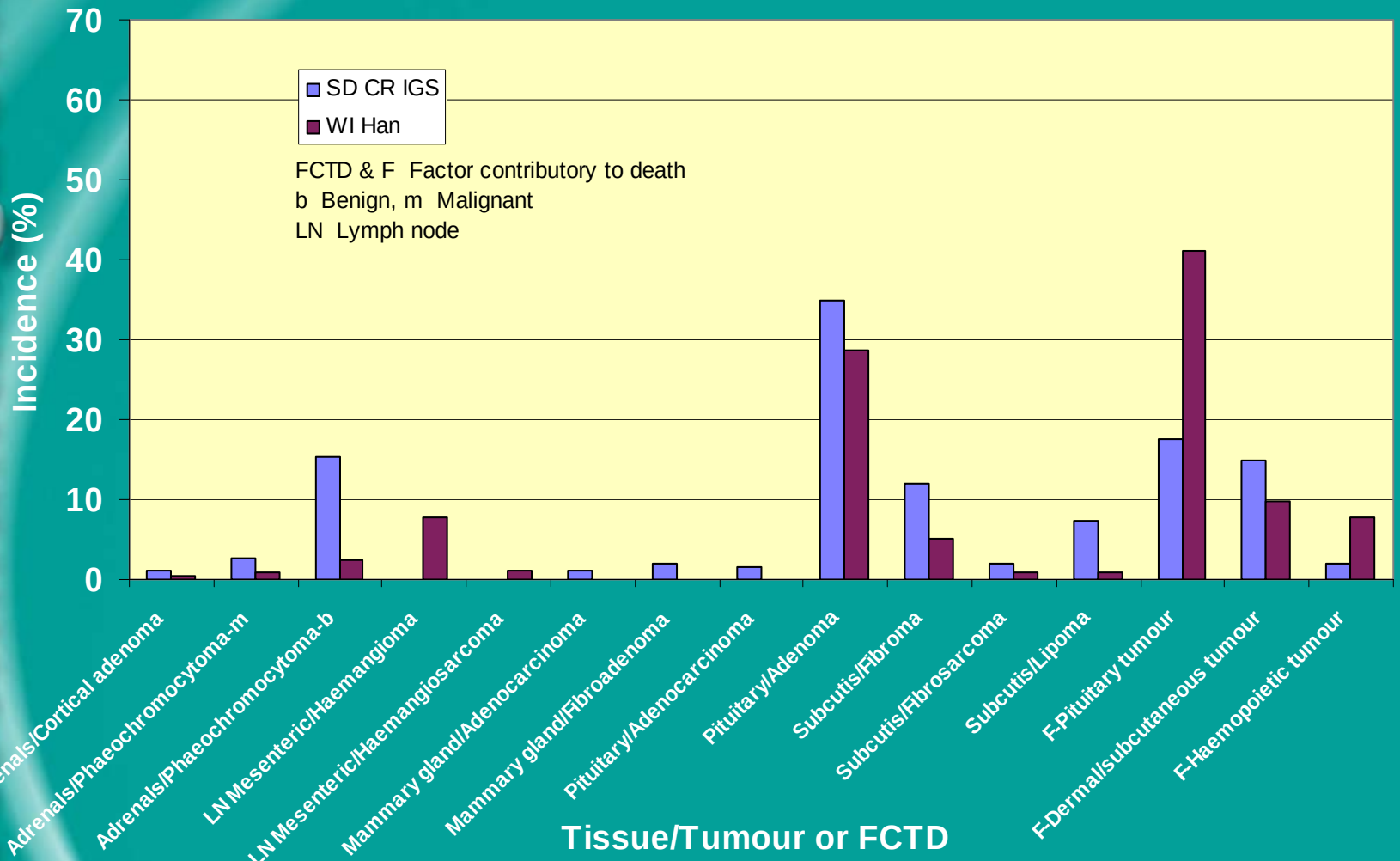
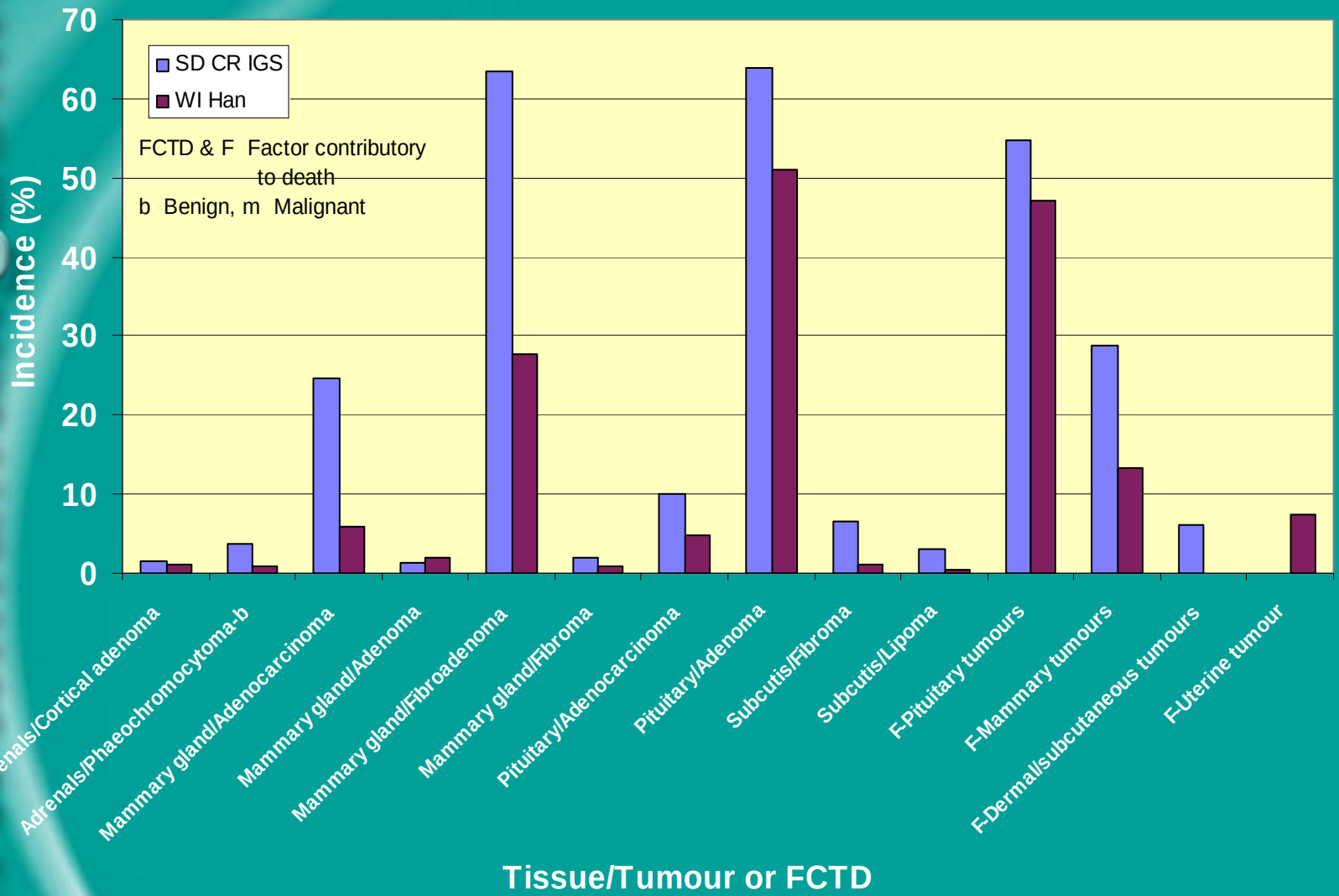


Figure 7: Tumour and FCTD incidence - female SD CR IGS and WI Han rats



Summary

- Regulatory authorities still consider 2 years as the endpoint for tumorigenicity studies
- The Sprague-Dawley (CR SD IGS) rat continues to show high terminal mortality
- The use of the F-344 or SD HA rat is viable (*but the mortality is higher than the WI Han rat and there are potential pathology problems in the F-344 rat*)
- The WI Han rat shows the lowest terminal mortality values (*always below 50%*)

Conclusions:

In consideration of the regulatory requirements to reach 2 years with an adequate number of survivors, our review of the data lead us to:

- Recommend the Wistar Han rat

Other solutions to ensure regulatory compliance regarding animal survival include:

- Use the Fischer 344 or the Harlan Sprague-Dawley rat, if required
- If the CR IGS Sprague-Dawley rat must be used, increase the group size or employ a diet optimisation (restriction) method
- and finally ……..

And finally ……..

In the final part of the NTP brief (what advice would we give the regarding the selection of a rat strain)

- Use a rat strain that will have an acceptable survival rate
- Ensure the rat strain chosen has an acceptable tumour profile
- Conduct regular retrospective reviews (to ensure that the profile of the chosen rat strain remains acceptable)

Selecting Strains and Stocks: A Pharmaceutical Company Perspective

Daniel Morton

June 16-17, 2005

Pharmacia's Problem

- Control CD rats fed ad lib had less than 20% survival at the end of 2-year carcinogenicity studies
- With mild toxicity, treated animals would weigh less and live longer than controls
- High incidences of pituitary neoplasia, mammary neoplasia, pododermatitis, and renal disease decreased survival
- Large rats with high incidence (~25%) of pododermatitis required special caging and care (AAALAC raised welfare concerns)

Possible Actions

- Caloric restriction in CD rats—labor intensive, uncertain regulatory acceptance, positive effects on survival and spontaneous lesions, which feeding regimen?
- Change stock/strain: Other SD rats,Wistar Han—few historical data, little regulatory risk
- Change to solid bottom cages with bedding

Effects of Diet Optimization
70-75% Ad Lib Consumption in CD Rats

	Body Wt	% 2 Year Survival	Pituitary Tumors	CPN
DO ♂	550	68%	46%	78%
Ad lib ♂	720	18%	62%	96%
DO ♀	300	56%	72%	23%
Ad lib ♀	606	18%	88%	78%

Concerns Over Diet Optimization

- Studies may not be readily accepted by regulatory agencies
- Diet optimization reduces ability to detect a carcinogenic treatment effect
- Historical control data will not be relevant
- ADME profiles may differ
- Diet optimization favors gavage studies, adding to cost

Pharmacia Tried Diet Restriction

- Pharmacia ran extra diet restricted high dose and control groups as part of a conventional carcinogenicity study
- Survival exceeded 50%
- Renal tumors in ad lib treated female rats, but not in diet restricted rats
- Renal neoplasia attributed to increased severity of CPN in ad lib females

We liked it!

Pharmacia Revisits "Should We Switch?"

- Following a merger, support for diet restriction was not universal
- Concerns included:
  - Cost of counting pellets or weighing feed daily
  - Preference for diet admix, and belief that diet admix studies would not be feasible or practical with diet restriction
  - Pressure in Europe to group house rats
  - European regulatory acceptance of diet restriction
- Preferred solution—change stock of rat

Pharmaceutical Preferences

- Outbred stocks traditionally preferred
- Availability of animals with the same general genetic history at multiple locations (a large vendor whose core breeding stock is distributed from a single colony)
- Caesarian-derived, barrier-maintained, pathogen-free breeding colonies
- Extensive distribution network

Strain Differences in Body Weights and Survival

		Crl:CD(SD)	Hsd:SD	F344	Wistar Han
Males	Mean maximal body weight (g)	824	578	480	640
	Mean % survival @ 104 weeks	29	49	65	82
Females	Mean maximal body weight (g)	547	344	310	360
	Mean % survival @ 104 weeks	44	63	64	82

CD and SD rats: Petterson, JC et al. A comparison of the Crl:CD®(SD)BR and HSD: Sprague-Dawley® SD rat. Ciba-Geigy Corp., Farmington, CT. F344 and Wistar rats: Harlan Sprague-Dawley Product Guide, July 1, 1998

Neoplastic Lesion Incidences

	CD	Wistar Han
Mammary tumors (F)	55 %	27 %
Pituitary	46/70 %	32/51 %
Thyroid C-cell	11/15 %	10/10 %
Adrenal medulla	13/3 %	4/2 %

Alternatives Considered

- Harlan SD Rat
- Longer lifespan
- High incidence of pituitary, mammary neoplasia, and renal disease
- Lower adult body weight that CD rat

- Wistar Han Rat
- CRL breeding program ensured consistency across different continents
- Low incidence of pituitary and mammary neoplasia
- Longer lifespan and lower body weight than CD rats fed ad lib

Both would benefit from diet restriction

Summary

- Wistar rat was not thoroughly tested before Pharmacia was purchased by Pfizer, but others have reported improved survival and low spontaneous tumor incidences.
- Pfizer uses CD rats fed ad lib in cages with wire mesh floors
- FDA has accepted all Pfizer studies in which survival has been a problem
- There are several approaches to improving survival
- Future decisions should be based on sound science and animal welfare, not cost
- A combination of strain/stock selection, diet manipulation, and optimal housing likely will provide the best scientific data.

Backup Slides

Diet Optimization for Rats

- Increased lifespan
- Decreased adult body weight
- Delayed tumor onset
- More uniform spontaneous tumor incidences
- Fewer degenerative lesions
- Only 50 rats/sex/group required for CA studies
- Less chemical required
- Better animal welfare (better health, fewer early deaths)

Diet Optimization Cost Considerations

- Survival allows using 50/sex/group vs. 65 with ad lib feeding
- Less in-life care
- Less pathology input
- More efficient necropsy (most animals come to scheduled sacrifice)
- Easier histopathology assessment (2-4 weeks faster)
- Greater than 23% savings in resources
- Better carcinogenicity study data

Effect of Diet Optimization in Male CD Rats

	Ad lib	80% AL	75% AL	50% AL
Survival	20%	46%	68%	80%
Neoplasms	78%	88%	78%	58%
Malignant Neoplasms	26%	54%	22%	16%
Benign Neoplasms	70%	78%	66%	46%
End stage CPN	24%	--	--	--

Effect of Diet Restriction on Causes of Deaths (Females)

	Ad lib	80%	75%	50%
Survival (weeks)	83	88	93	98
Survival	20%	42%	56%	82%
Pituitary Neoplasms	52%	26%	18%	6%
Mammary Neoplasms	8%	4%	4%	--
CPN	4%	--	--	--

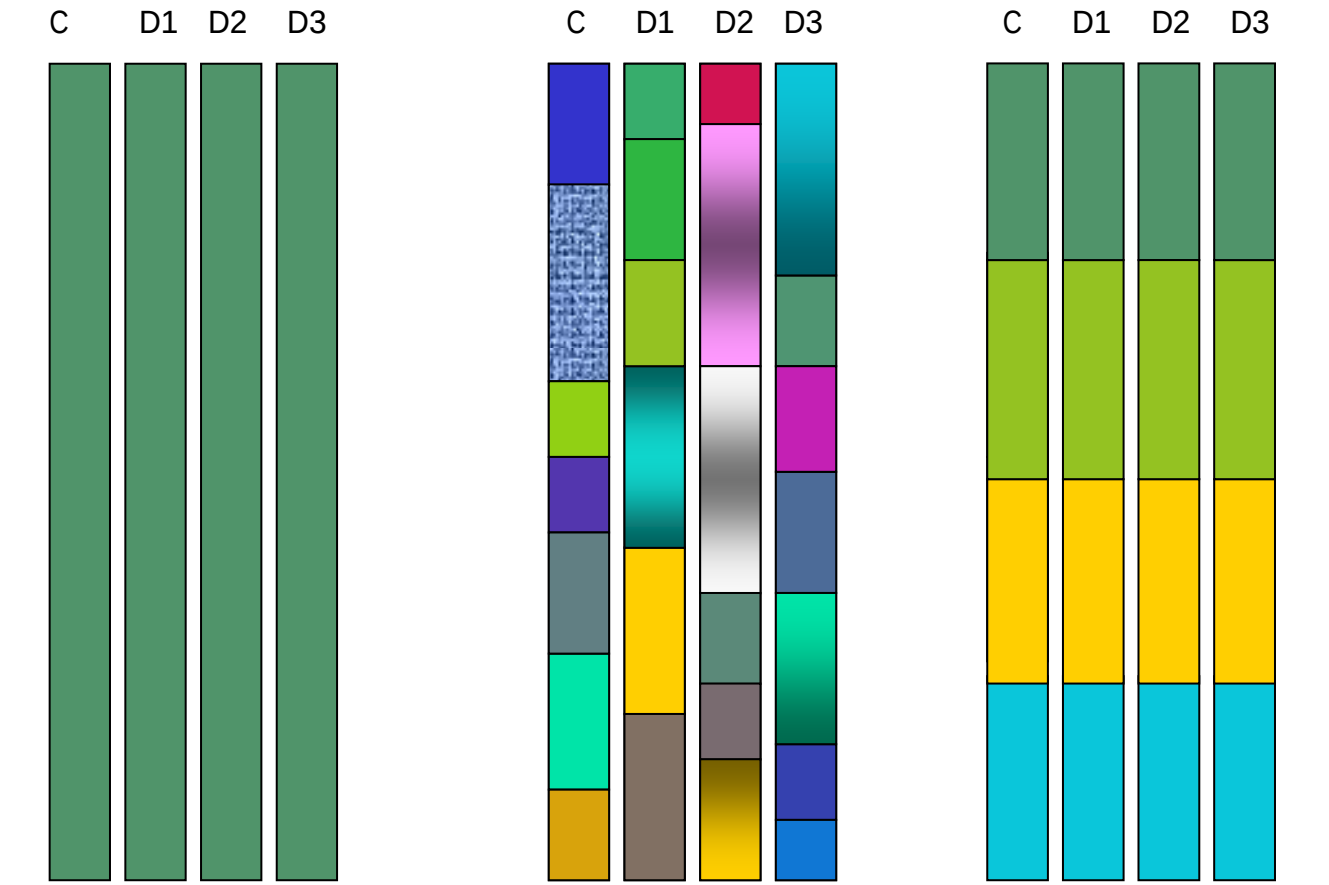
The Multi-Strain Assay

Michael FW Festing

c/o MRC Toxicology Unit, University of Leicester, UK

michaelfesting@aol.com

The multi-strain design

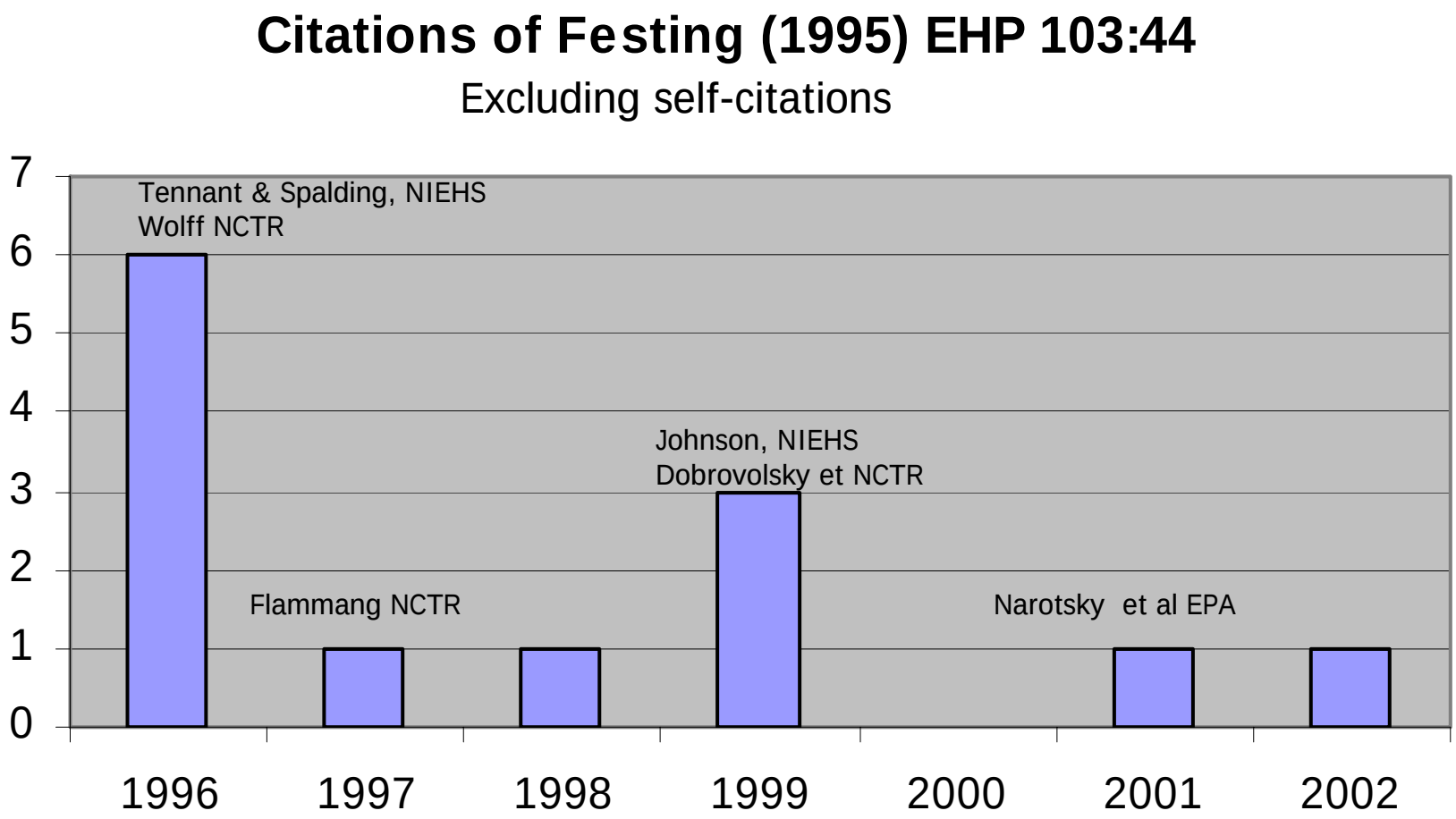


Single isogenic strain

Single outbred stock

Multi-strain design

Not exactly a citation classic!



Possible advantages of the multi-strain assay over use of a single strain

- Statistically more powerful*
  - Fewer carcinogens passed as safe
  - Better agreement between mouse and rat
  - Better validation of in-vitro/alternative assays
  - Less biased view of tumor type/organ sensitivity
- Biologically more powerful
  - Highlights importance of genetic variation in response
  - May lead to identification of resistance & susceptibility genes (human & animal)
  - Provides additional tool for studying mechanisms
  - Common response pattern may suggest common mechanism
- Psychologically important
  - Toxicologists should not ignore genetic variation in both animals and humans. Now is a good time to start their education.

* Felton RP, Gaylor DW. 1989. Multistrain experiments for screening toxi substances. Journal of Toxicology and Environmental Health 26:399-411.

Possible problems associated with the multi-strain assay

- Practical difficulties
  - How many & what type of strains
  - Supply of genetically authentic animals
  - Unfamiliar responses & pathology (lack of background data)
  - Setting dose levels (strains differ in MTD)
- Conceptual difficulties
  - Toxicologists will need to learn some genetics and experimental design
  - Statistical analysis & presentation of results may need some re-thinking

Most toxicologists still use outbred stocks

"..it is more correct to test on a random-bred stock on the grounds that it is more likely that at least a few individuals will respond to the administration of an active agent in a group which is genetically heterogeneous"

Arcos JC, Argus MF, Wolf G, eds. (1968) Chemical induction of cancer. 491pp, London, Academic Press.

The problem with genetic heterogeneity

Treated	Control
Beagle	Goat
Chicken	Pig
Mouse	Crow
Horse	Frog
Gerbil	Hamster
Guinea-pig	Quail
Lion	Beaver
Duck	Cat
Rabbit	Toad

Hexobarbital Sleeping time in mice: inbreds are more uniform and strains differ

Strain	n	Mean	SD	No needed*	Power**
A/N	25	48	4	23	86
BALB/c	63	41	2	7	>99
C57BL/HeN	29	33	3	13	98
C3HB/He	30	22	3	13	98
SWR/HeN	38	18	4	23	86
CFW	47	48	12	191	17
Swiss	47	43	15	297	13

* Power analysis: two-sample t-test to detect a 4 min. mean difference (2-sided) with α=0.05 and a power of 90% assuming response is the same

** power of an experiment to detect a 4 min. change in the mean if the sample size is fixed at 20 mice/group, with above assumptions

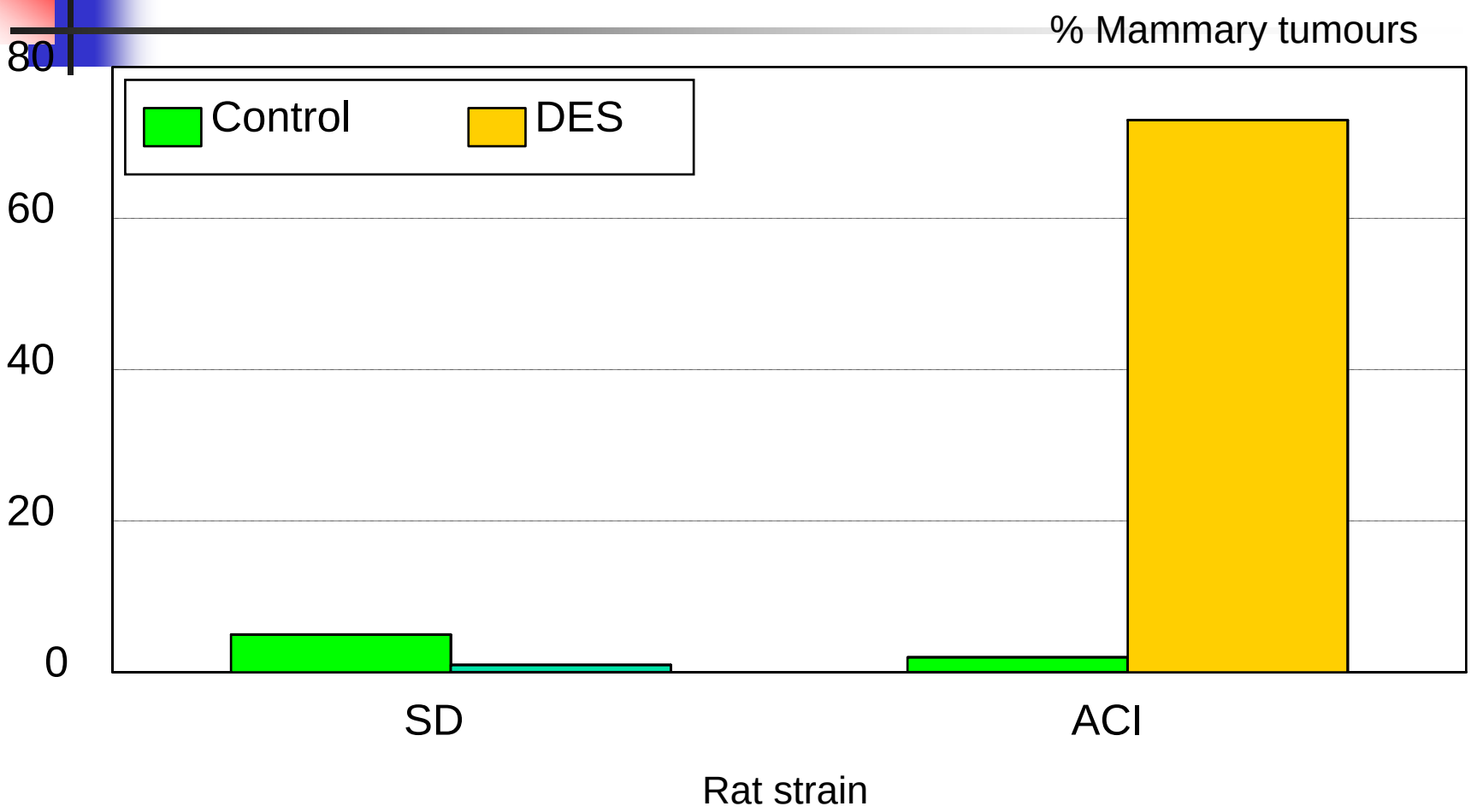
Data from Jay 1955 Proc Soc. Exp Biol Med 90:378

Most toxicologists still use outbred stocks

"..it is more correct to test on a random-bred stock on the grounds that it is more likely that at least a few individuals will respond to the administration of an active agent in a group which is genetically heterogeneous"

Arcos JC, Argus MF, Wolf G, eds. (1968) Chemical induction of cancer. 491pp, London, Academic Press.

Strain Differences in Response to DES



Shellabarger et al J.Nat.Can.Inst. 61:1505, 1978. Results averaged over 4 irradiation levels

Strain Differences

Response to TCDD

Strain	LD50 (microg./kg)
Han:Wistar	>3000
Long-Evans	10

Pohjanvirta et al 1988, Tox. Appl. Pharm. 29:131

A better design

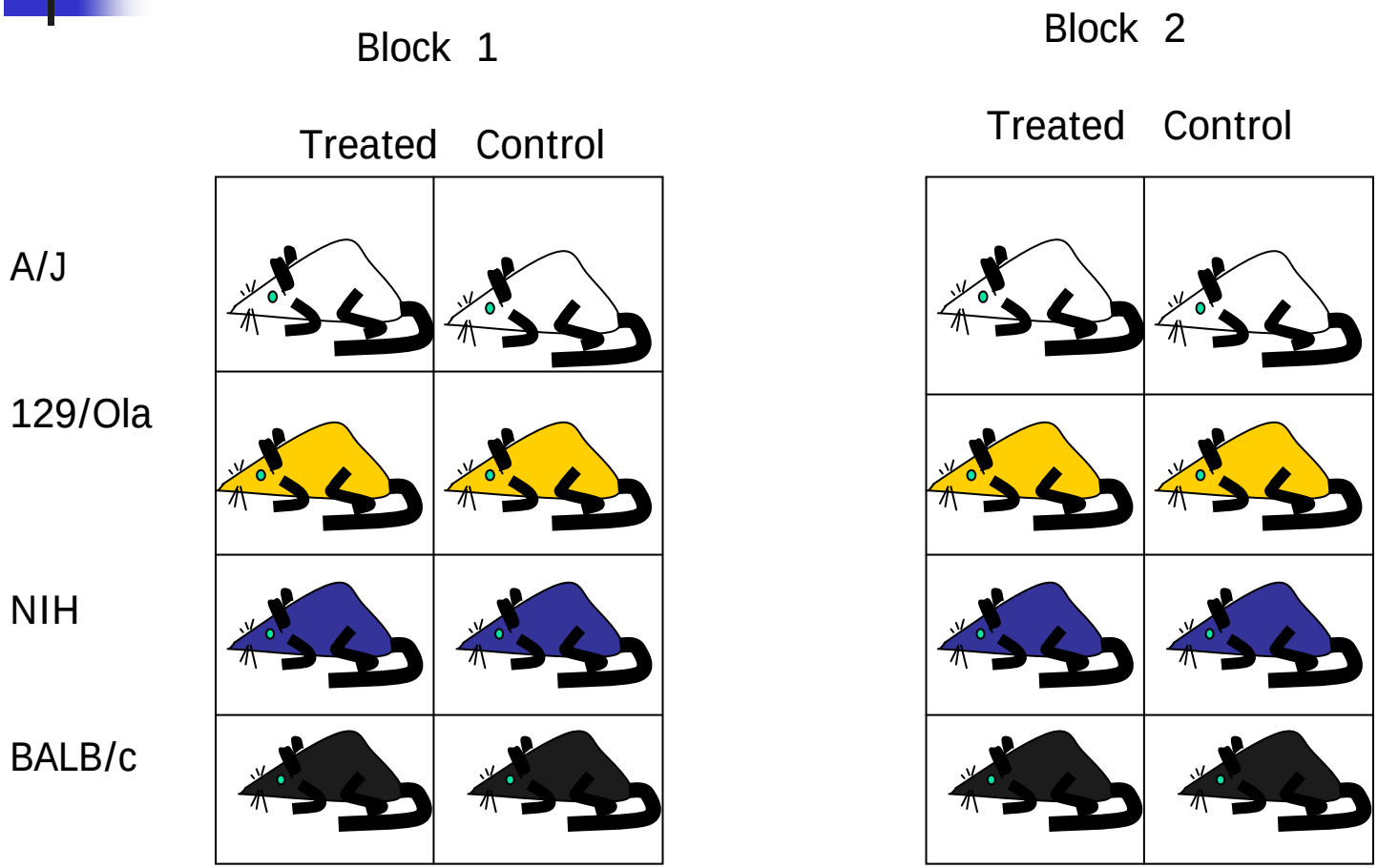
Treated	Control
Beagle	Beagle
Mouse	Mouse
Horse	Horse
Gerbil	Gerbil
Guinea-pig	Guinea-pig
Lion	Lion
Duck	Duck
Rabbit	Rabbit

A better design: a simple multi-strain assay

Group size 8
Sub-group size 1

Treated	Control
F344	F344
BN	BN
DA	DA
F344xBNF1	F344xBNF1
BDIX	BDIX
LEW	LEW
LEWxBDIXF1	LEWxBDIXF1
PVG/c	PVG/c

A real experiment to detect the effect of BHA on liver EROD activity



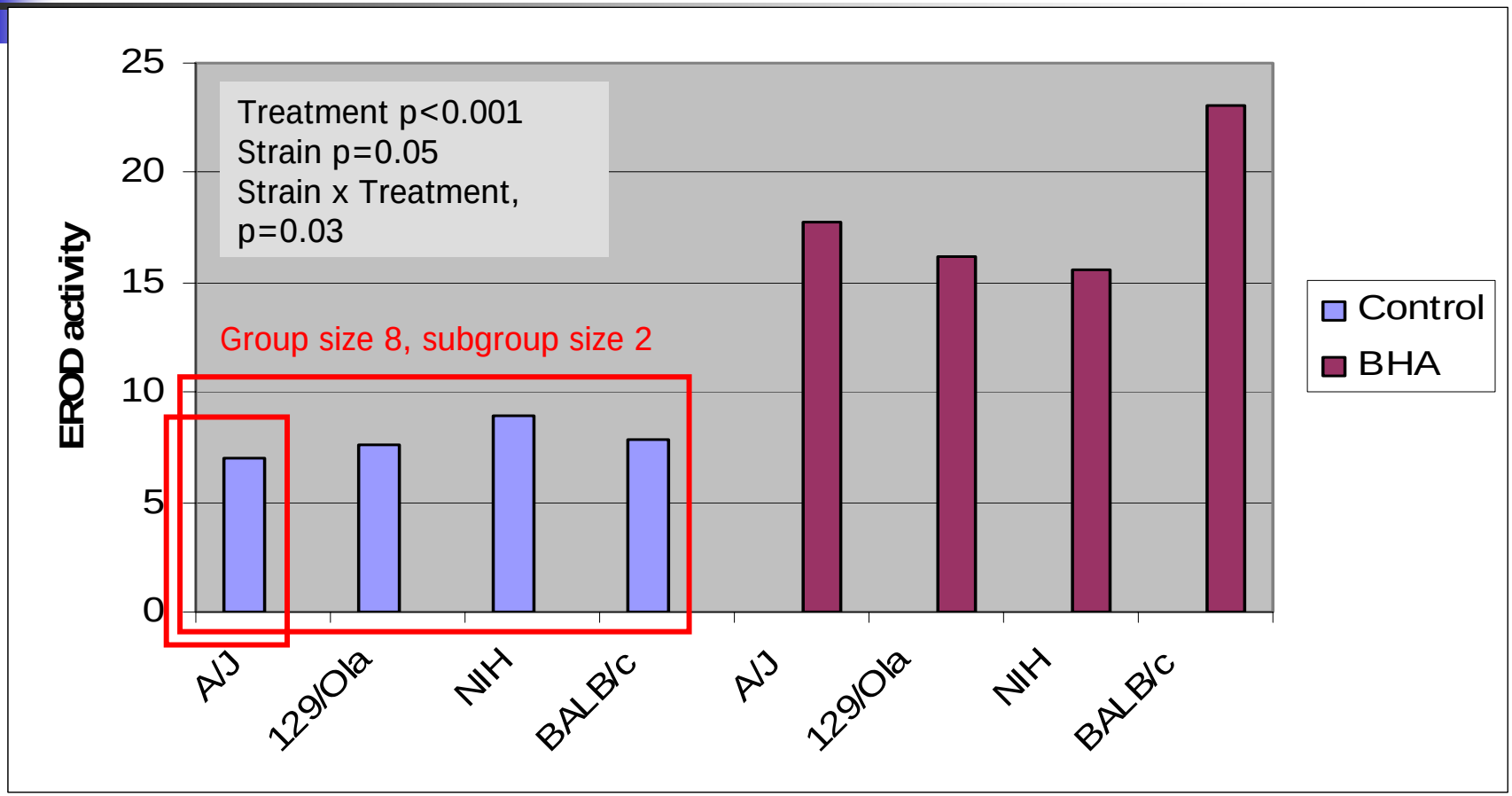
The two blocks were separated by approximately 3 months

A real experiment to detect the effect of BHA on liver EROD activity

	Block 1			Block 2		
	Treated	Control		Treated	Control	
A/J	18.7	7.7		16.7	6.4	
129/Ola	17.9	8.4		14.4	6.7	
NIH	19.2	9.8		12.0	8.1	
BALB/c	26.3	9.7	Mean 14.7	19.8	6.0	Mean 11.3 (diff 3.4)

The two blocks were separated by approximately 3 months

Effects of BHA on liver EROD activity in four mouse strains

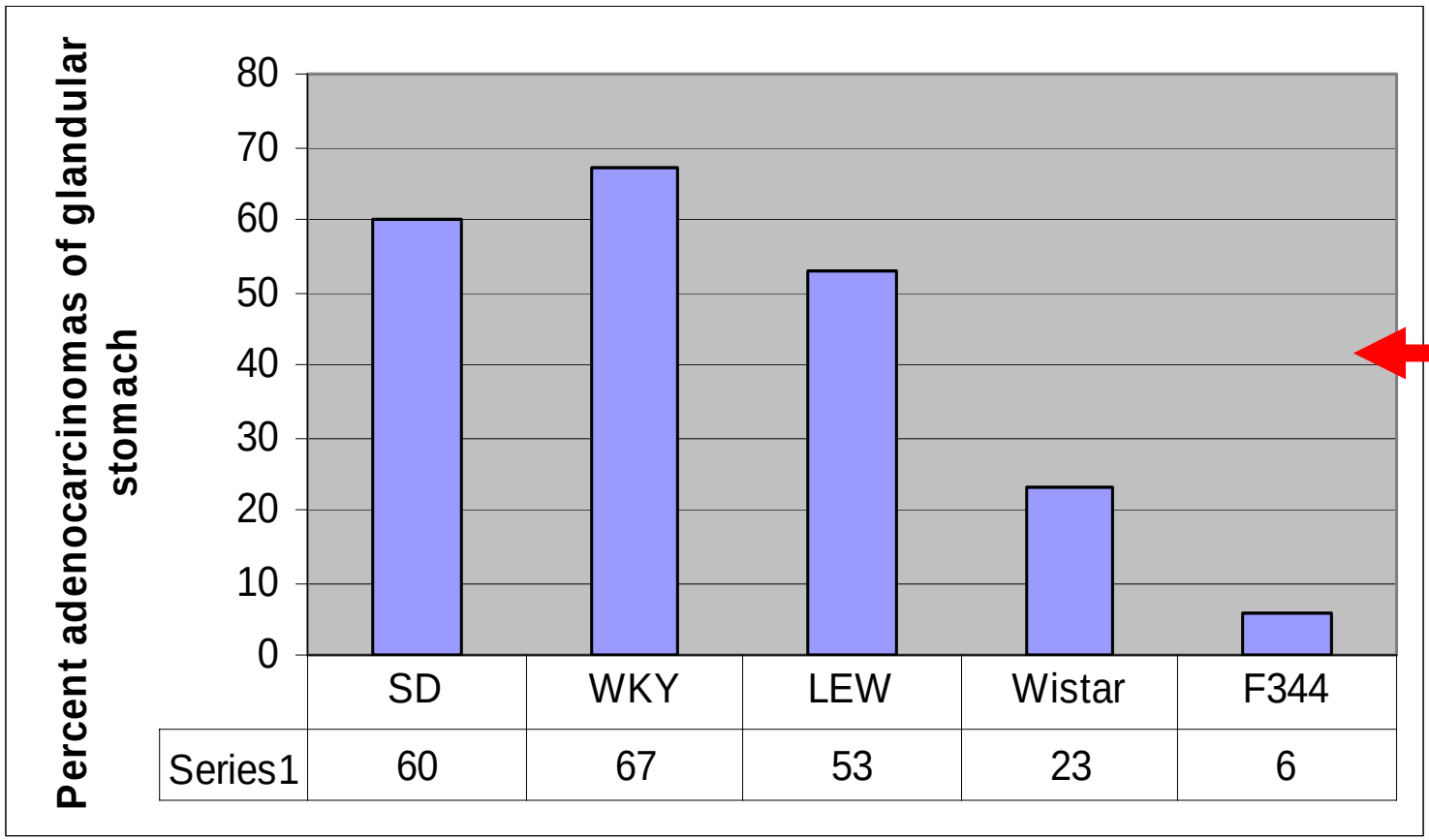


2 mice per mean (16 total), done as a randomised block design.

Festing MFW. (2003) Trends in Pharma. Sci. 24:341

Multi-strain assay statistically more powerful (response of rats to a carcinogen)

N-methyl-N'-nitro-N-nitrosoguanidine in drinking water



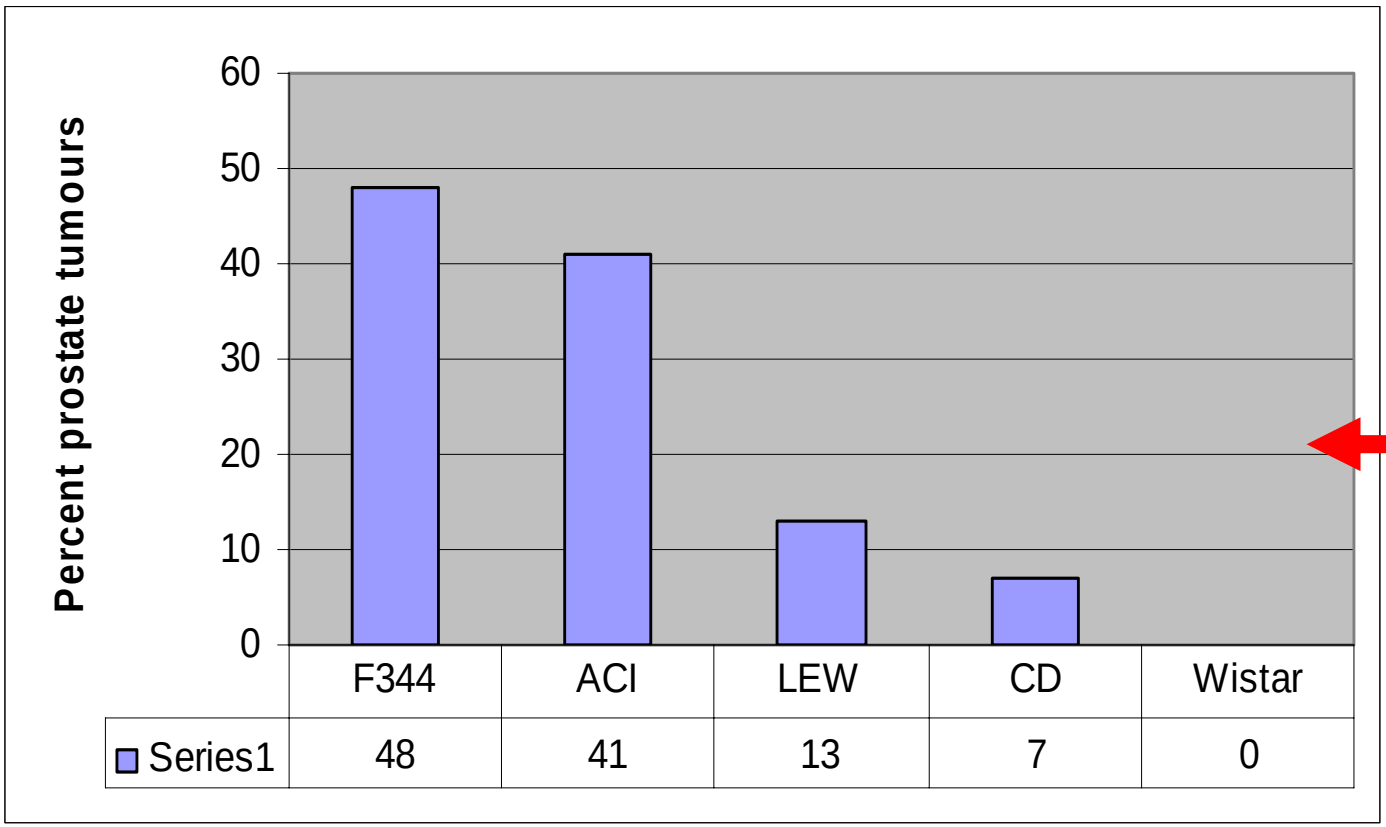
10 rats of each strain

41% (20 tums./50 rats)

Tatematsu et al (1988) Carcinogenesis 9:495

Multi-strain assay statistically more powerful

3,2'-dimethyl-4-aminobiphenyl



10 rats of each strain

21% (10 tums./50 rats)

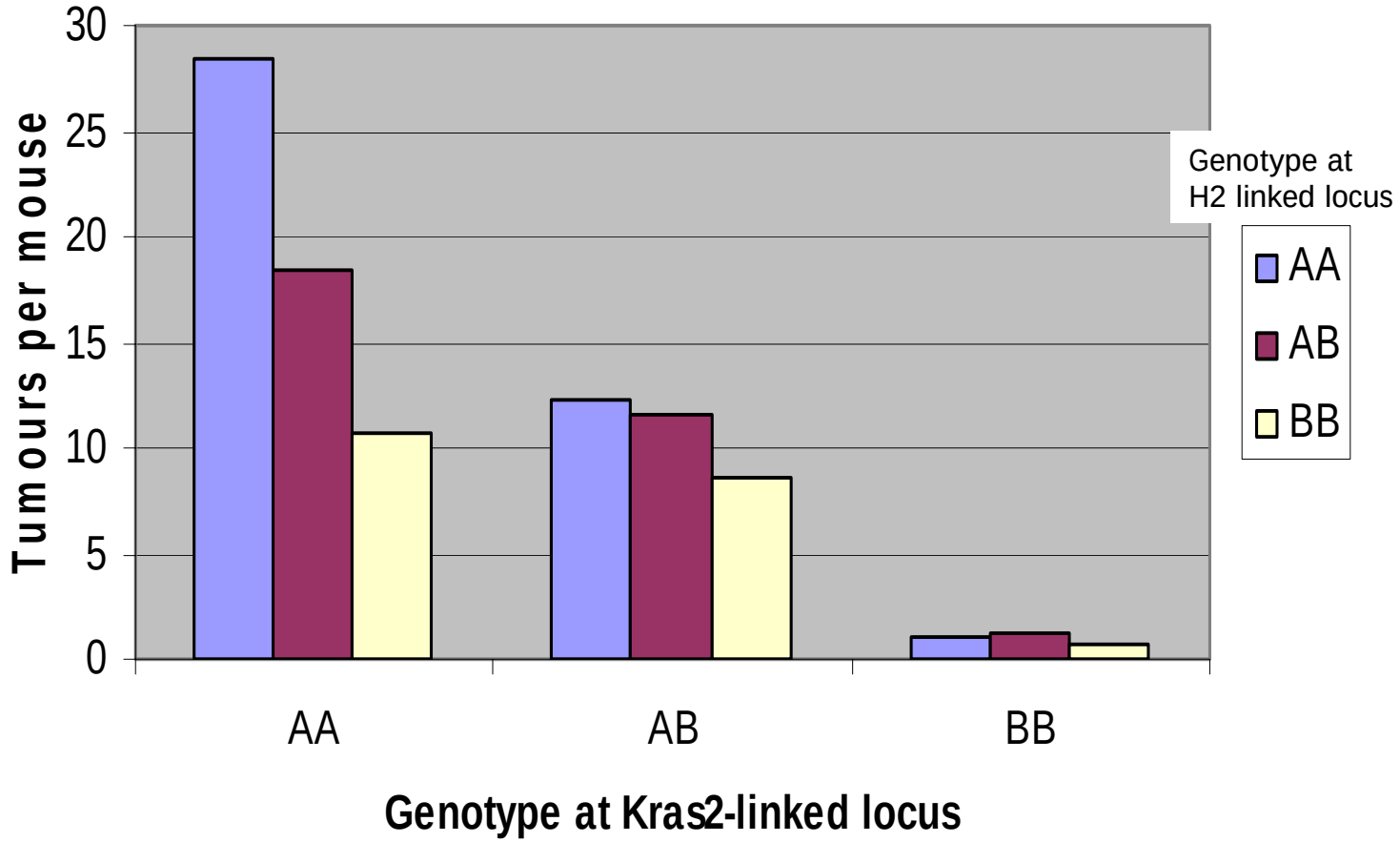
Shirai et al (1990) Carcinogenesis 11:793

Strain differences imply susceptibility genes

- New genetic techniques will enable susceptibility genes to be identified
  - Full DNA sequences of humans, rats, mice (and individual strains)
  - Microarrays
  - QTL analysis
  - Proteomics
  - Informatics

Urethane-induced lung tumours in mice: A/J susceptible C57BL/6 resistant

Loci linked to susceptibility to lung adenomas in B6xA/J F2 hybrids



Festing et al (1994) Genet. Res. 64:99

Microarray analysis of mouse lung mRNA

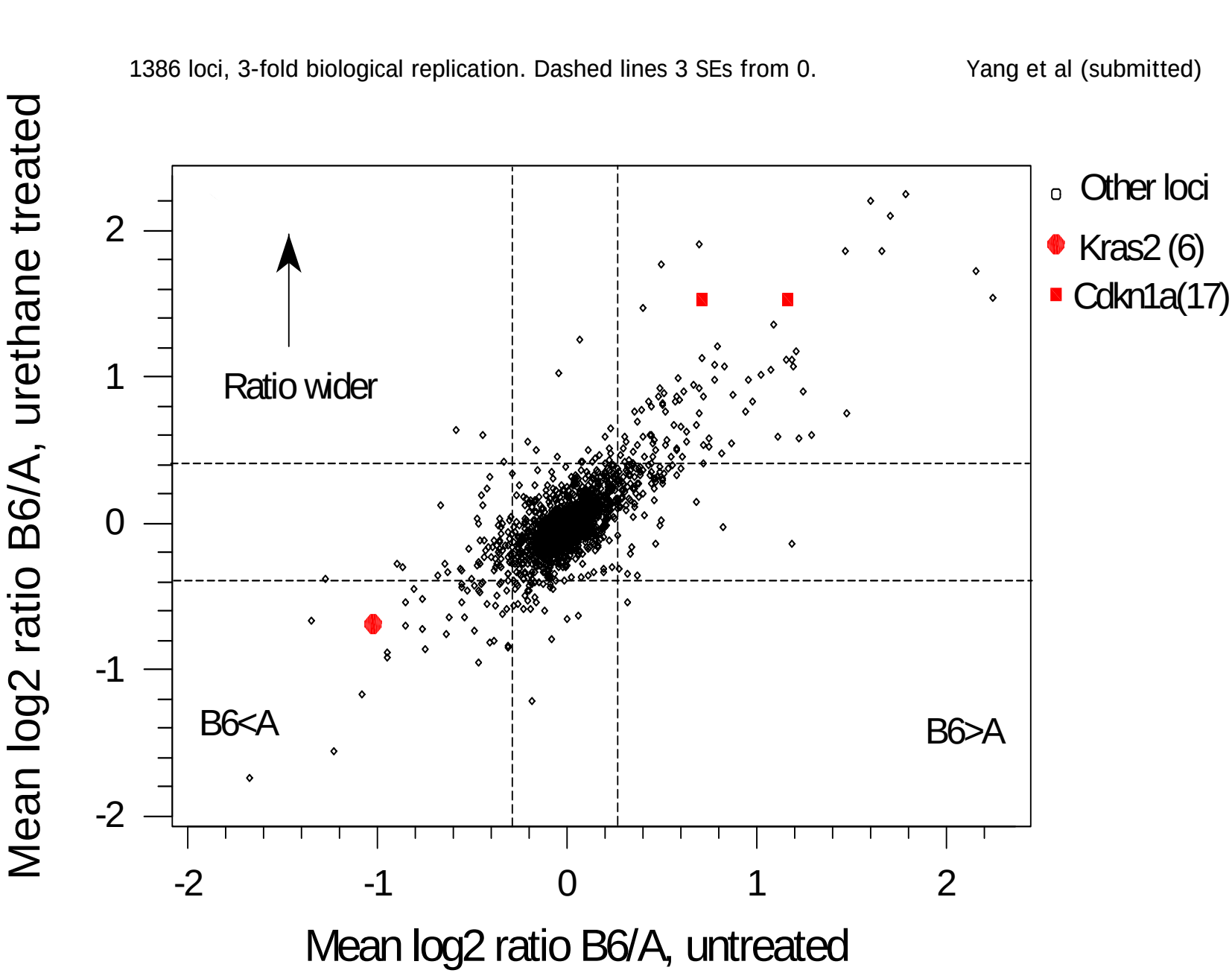
Comparative amount of mRNA for specific genes in two samples

Experiment 1

B6/A untreated, 3 replications averaged

B6/A urethane treated, 3 reps. averaged

cDNA from 1386 genes on each array


1386 loci, 3-fold biological replication. Dashed lines 3 SEs from 0.
Yang et al (submitted)
Other loci
Kras2 (6)
Cdkn1a(17)
Ratio wider
B6<A
B6>A
Mean log2 ratio B6/A, urethane treated
Mean log2 ratio B6/A, untreated
2
1
0
-1
-2
-2
-1
0
1
2

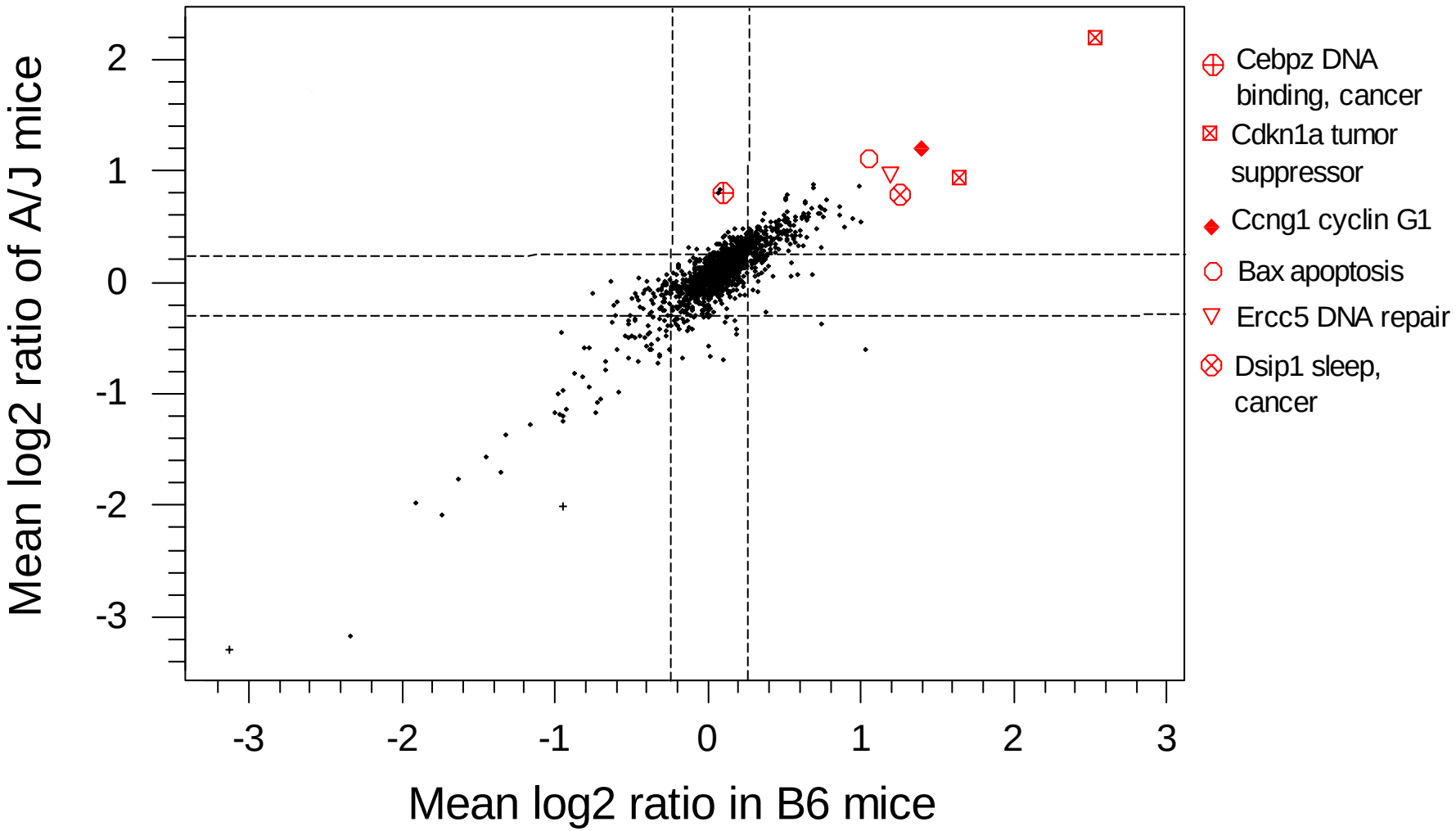
Microarray analysis of mouse lung mRNA

Experiment 2

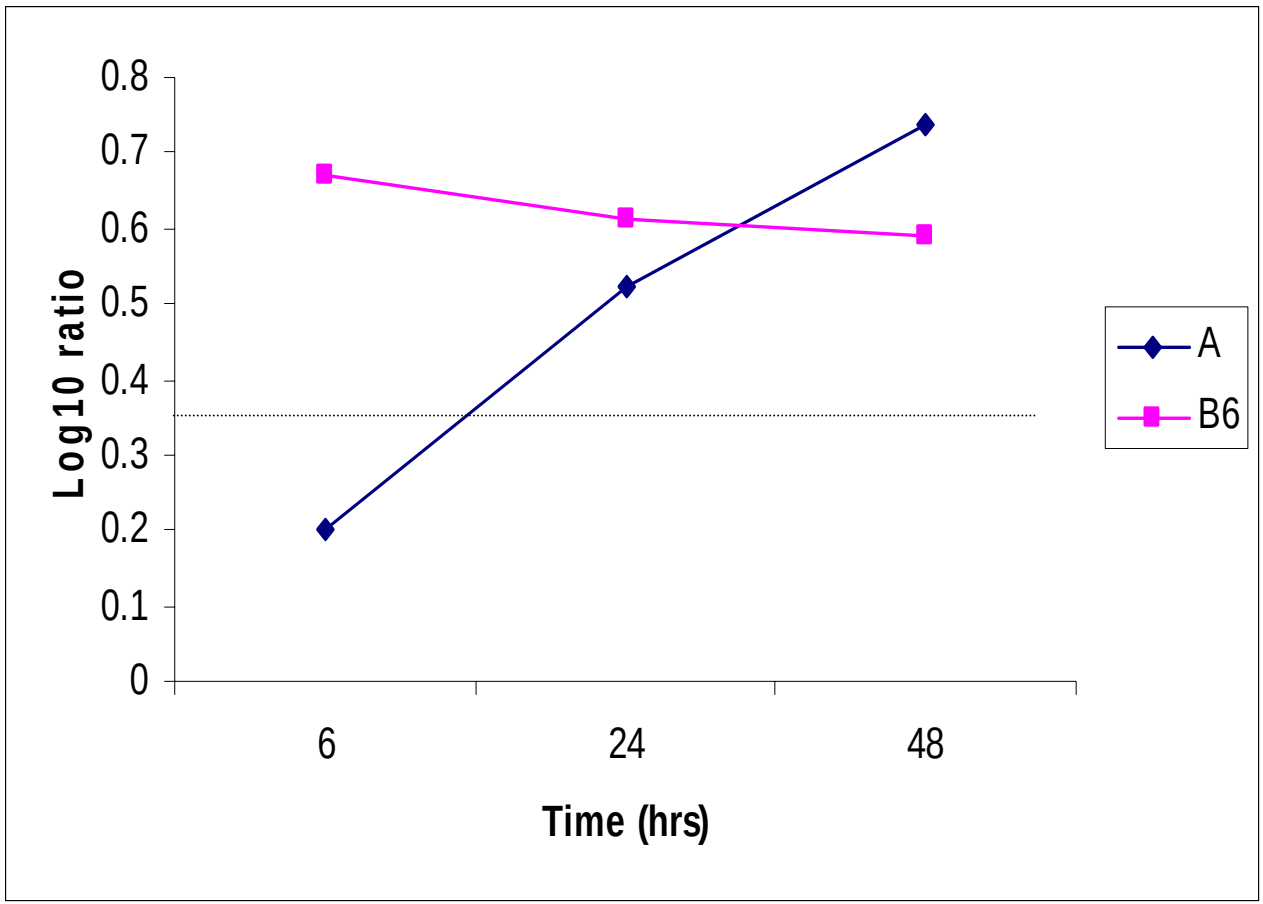
B6 Treated/untreated 4 arrays at each of 6, 24, 48 hrs

A/J Treated/untreated 4 arrays at each of 6, 24, 48 hrs

Up-regulation of cancer-related genes in lungs of B6 and A/J mice following urethane treatment



Up-regulation of Cdkn1a (p21) in response to urethane



Points above horizontal dotted line significantly different from zero @ p<0.05

A/J mice may be more susceptible to the development of lung tumours because in comparison with C57BL/6:

- In untreated mice they have
  - 2-fold elevated levels of lung Kras2
  - 2-fold lower levels of lung Cdkn1a
- Cdkn1a response is reduced and not evident at 6hrs

Comparative studies of multi-strain versus single strain experiments

- PhysioGenix studies of toxicity 8 compounds in SD, F344 & multi-strain studies
  - Gentamycin data summarised here
- Chloramphenicol study of CD-1 mice versus four inbred strains and C57BL/6
  - Available to breakout group if wanted

PhysioGenix's : Gentamycin toxicity

	Control	Treated*	Total
F344	15	31	46
SD	16	30	46
Seven strains**	25	21	46

*240mg/kg/day i.p. for 6 days

**Seven strains; six F1 hybrids and F344, 2-4 rats of each per treatment

Outcomes (characters measured)

- Body weight at 5 times
- L and R kidney weight
- Heart weight
- 26 biochemical characters

(34 characters per rat)

Response expressed in standard deviations

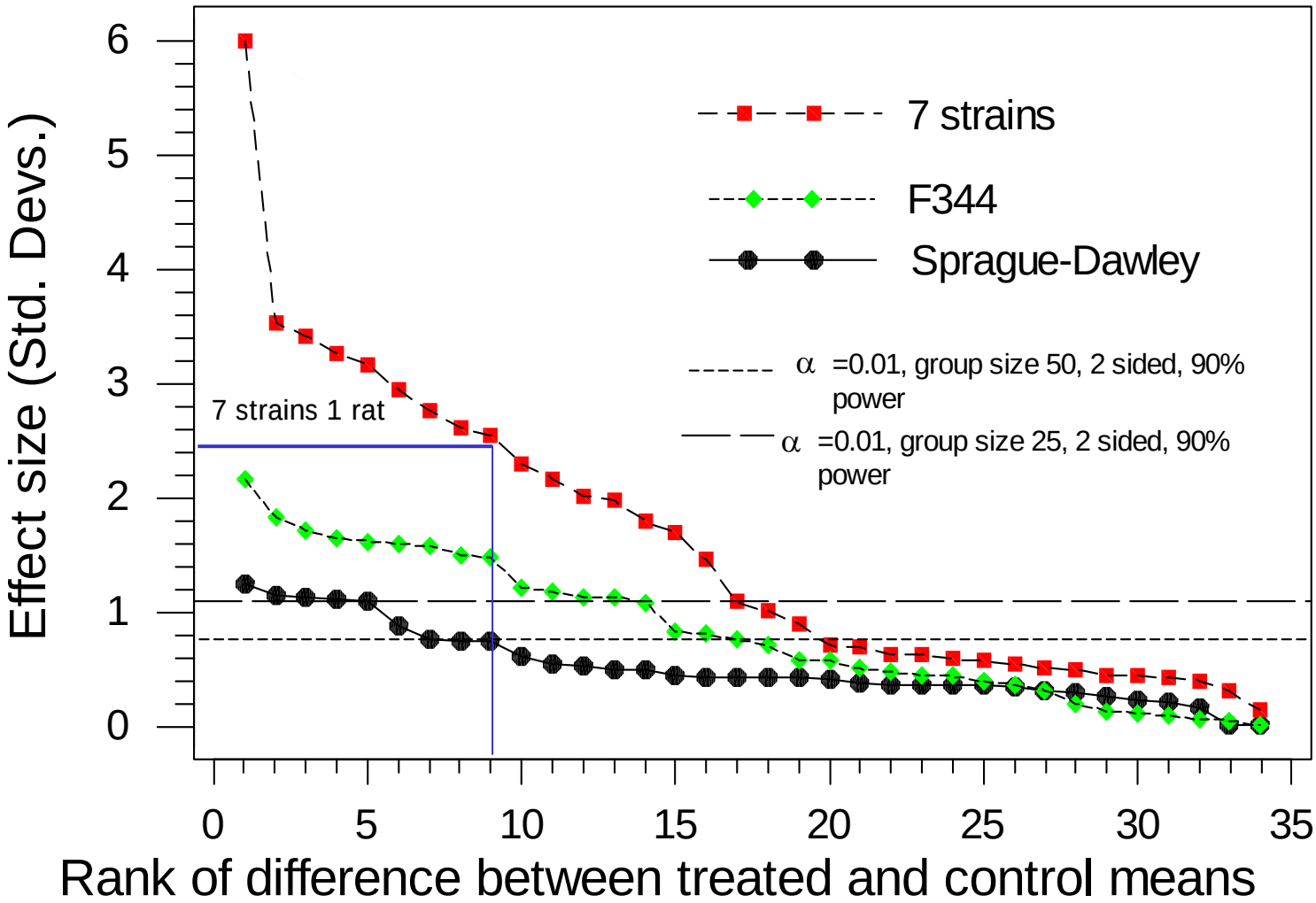
e.g. Results for right kidney

|Control mean-treated mean|/Std. Dev.

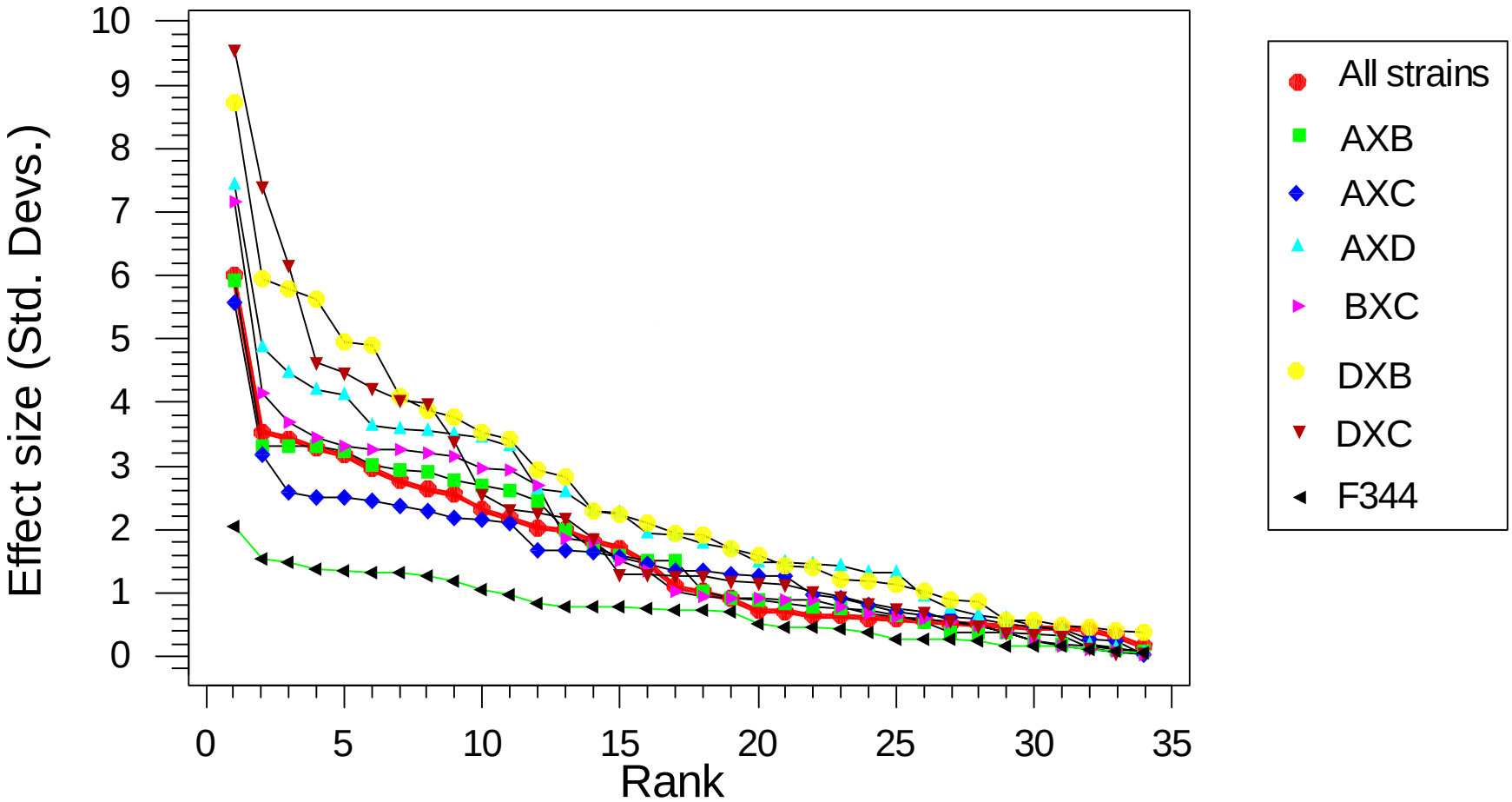
SD rats: |1.36-1.17|/0.49 = 0.19/0.49 = 0.38
F344: |0.94-0.88|/0.08 = 0.06/0.08 = 0.75
Multi-strain*: |1.18-0.98|/0.07 = 0.20/0.07 = 2.85

* main effect only, i.e. average across all strains

PhysioGenix Gentamycin toxicity study



Gentamycin study: responses of individual strains



Issues

- How many strains?
  - More is more powerful but less practical
  - Fewer provides background data more quickly
  - Same design for rats and mice?
  - Fixed battery or flexible
- Which type of strains?
  - Inbred; F1 hybrid; Sets of RI strains; sub-species (e.g. *Mus castaneous*)?
  - Life span data only available for about 8-10 rat, but many mouse strains
- Dose levels
  - Strains will differ in MTD

This is the era of informatics: use correct nomenclature

BALB/c	not Bagg albino
F344/N	not Fischer-344
	Certainly not CDF
DA/OlaHsd	not Dark Agouti
WF/NCrl	not Wistar Furth
PVG	not ??????

Electronic databases/informatics depend on correct nomenclature
Substrain symbols may be important
Common names add nothing except confusion

Rules for nomenclature of inbred strains are the same for mouse and rat and are given in www.informatics.jax.org

Conclusions. The multi-strain assay is:

- Statistically more powerful than use of a single strain
  - Fewer false negative results
  - Will lead to better agreement between mouse/rat/in-vitro (long-term financial implications)
- Biologically more powerful
  - Highlights genetic variation in response
  - Potential to identify susceptibility genes and mechanisms
- Psychologically important: Toxicologists will have to recognise genetic variation soon
- Use of a single outbred stock is the **worst possible strategy.** The FDA should now review its policy on strain use

We live in a hostile chemical environment and have evolved genetic mechanisms to allow us to survive. Toxicology is an important discipline within the science of genetics!

Ragwort and the Cinnebar moth

Power Calculations for Multiple-Strain Designs

Grace E. Kissling, PhD.
Biostatistics Branch
NIEHS

NTP
National Toxicology Program

DEPARTMENT OF HEALTH & HUMAN SERVICES • USA

Background

- NTP carcinogenicity and toxicity bioassays use single isogenic strains of mice and rats.
- Would a multiple-strain design be better?
- The statistical power of multi-strain designs is an important consideration.

Definition

- **Power is the probability of detecting an effect when it is present.**

Previous Studies

- Haseman and Hoel (1979) found that a multi-strain design could be more powerful than using a single outbred strain.

- Felton and Gaylor (1989) found that :
  - in low power situations, the one strain design is more powerful
  - otherwise, when strains respond differently, multi-strain designs are more powerful.

Testing strategies

I. Single strain design

II. Multi-strain design
   A. Pool results over strains
   B. Test each strain separately

General Approach

- Control group, Treated group

- 48 animals per group
  - 1 strain, 48 animals
  - 2 strains, 24 animals each
  - 3 strains, 16 animals each
  - 4 strains, 12 animals each

General Approach

- Uncommon tumors and common tumors (arbitrarily defined)
  - Uncommon: baseline tumor rate = 5%
  - Common: baseline tumor rate = 20%

- Different strains may have different strengths of tumor responses to the treatment.

Strength of Tumor Response

Strength	Uncommon		Common	
	Control	Treated	Control	Treated
Strong (S)	5%	35%	20%	50%
Moderate (M)	5%	25%	20%	40%
Weak (W)	5%	15%	20%	30%
No (N)	5%	5%	20%	20%

Combinations of Strengths of Tumor Responses

Individual strains may or may not respond similarly to a given carcinogen.

Mix of Response Strengths Among Strains

0%, 25%, 50%, 75% or 100% of the strains are S, M, W, N responders

Some examples …

- **Homogeneous:** 100% M
- **Heterogeneous:** 25% S, 25% M, 25% W, 25% N
- **Heterogeneous:** 75% S, 0% M, 25% W, 0% N

Assumptions

1) To keep it simple, all animals survive to the end of the study.

2) Strains are randomly sampled from a population of strains having a specified mixture of response strengths.

Statistical Tests

- **Pooled test, pooling over strains**
  - **Mantel-Haenszel χ^2 statistic, corrected for continuity**
- **Separate tests for each strain**
  - **Fisher's exact test for each strain**

Power Calculations

- **Mantel-Haenszel χ^2 test**
  - **Wittes & Wallenstein, *J. Am. Stat. Assoc.* 82: 1104-1109, 1987.**
- **Fisher's exact test**
  - **Bennett & Hsu, *Biometrika* 47: 393-398, 1960.**
- **$\alpha = 0.05$**

Sampling of Strains

Suppose that 2 strains are to be selected from a population composed of 75% S, 25% W strains.

Possible samples	Pr(sample)
S S	$\frac{2!}{2!0!}.75^{2}.25^{0} = 0.5625$
S W	$\frac{2!}{1!1!}.75^{1}.25^{1} = 0.3750$
W W	$\frac{2!}{0!2!}.75^{0}.25^{2} = 0.0625$

Power Calculation

For any given mixture of tumor responses, the probability of detecting an effect when it is present (Power) is

$$\text{Power} = \sum_{\substack{\text{all possible} \\ \text{samples}}} \Pr(\textit{sample}) \times \text{Power}_{\textit{sample}}$$

Example Calculation for 2 Strains From a 75%S, 25%W Population

Sample	Pr(*sample*)	Power$_{sample}$
S S	0.5625	0.9620
S W	0.3750	0.7989
W W	0.0625	0.3624

$$\text{Power} = \sum_{\substack{\text{all possible} \\ \text{samples}}} \Pr(\textit{sample}) \times \text{Power}_{\textit{sample}}$$

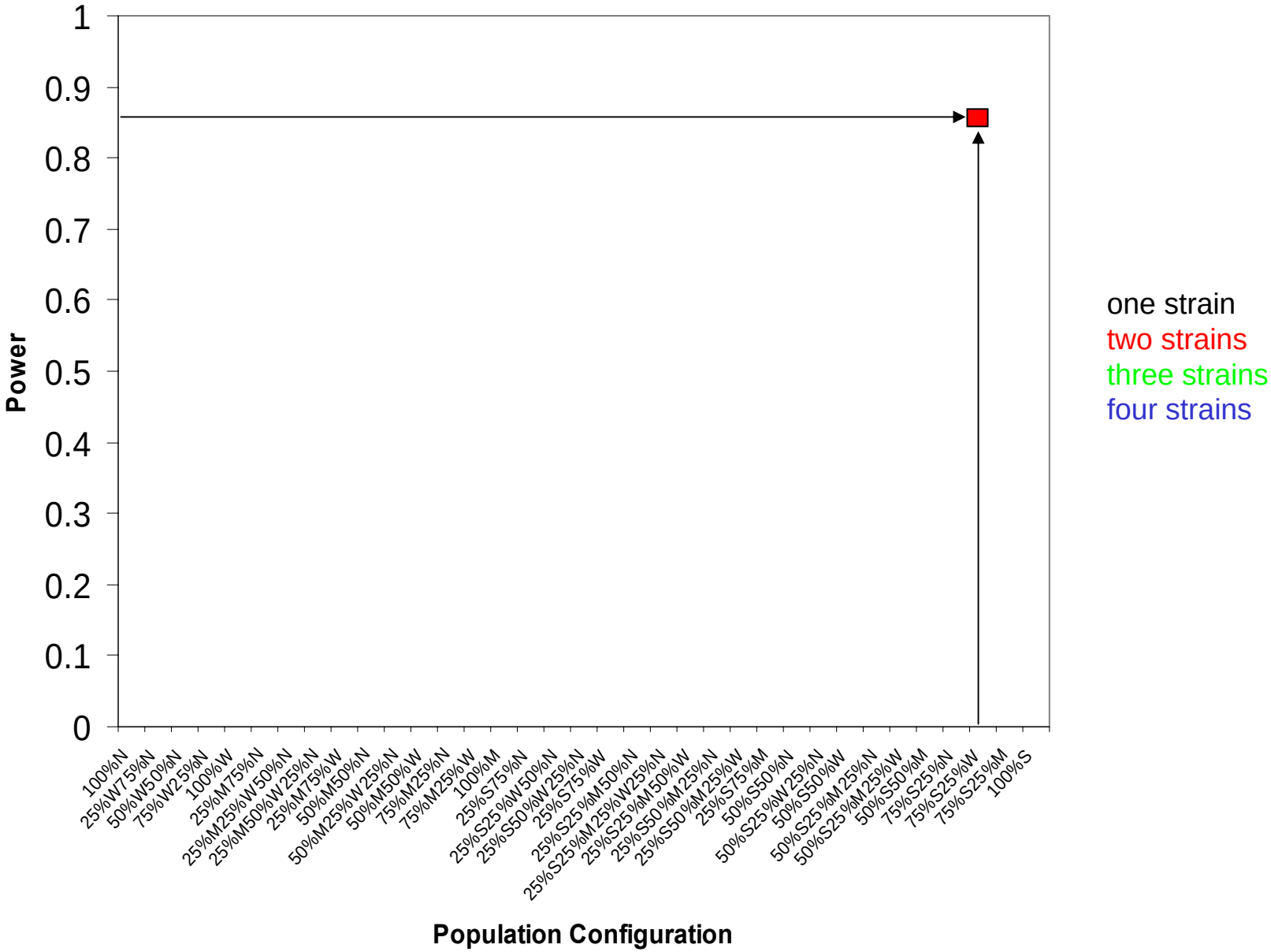
$$= 0.5625 \times 0.9620 + 0.3750 \times 0.7989 + 0.0625 \times 0.3624$$

$$= 0.86336$$

$$= 86.3\%$$

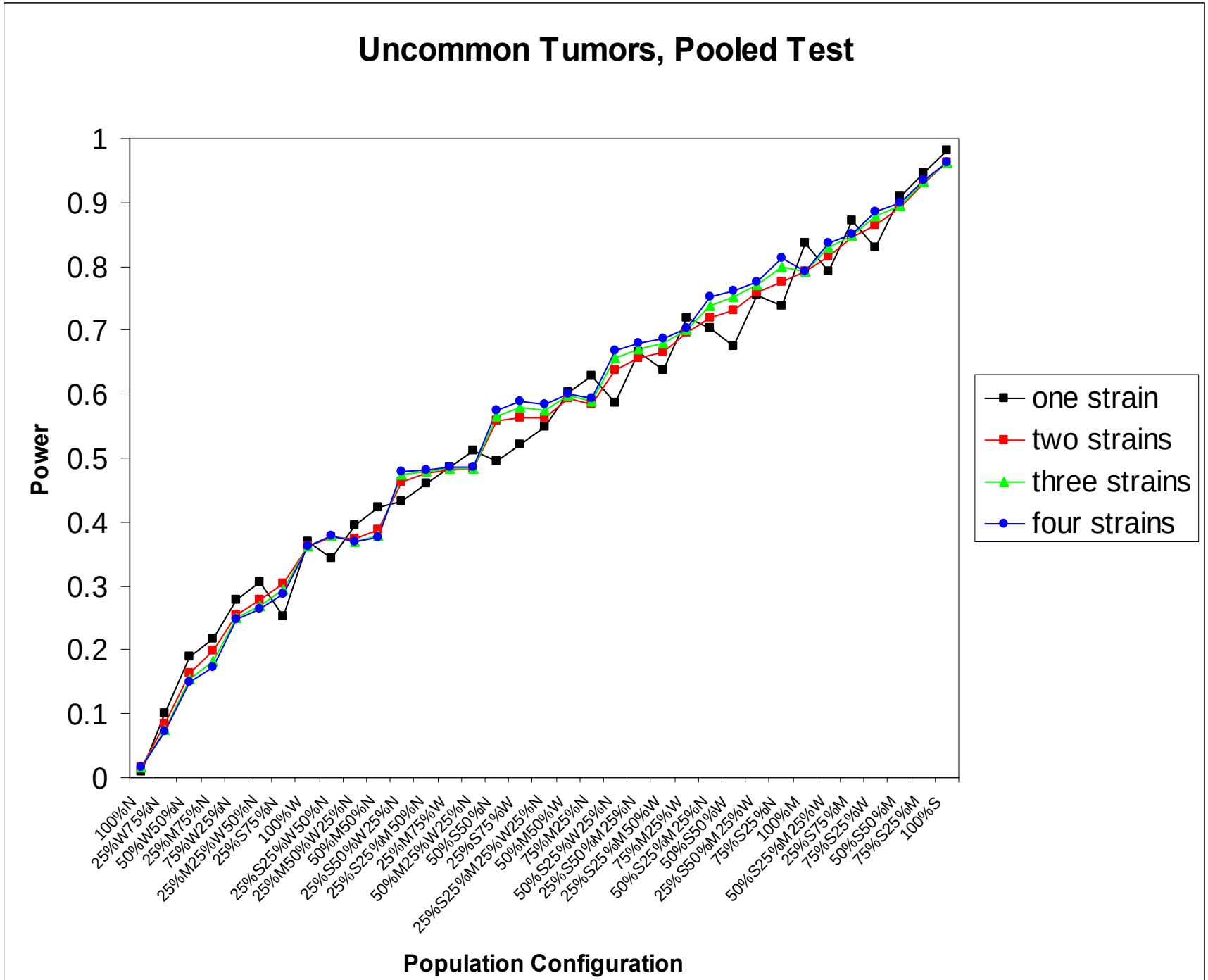
NTP National Toxicology Program

Sample Layout of Power Plots



Testing Strategy: Pooled Test

- One strain: Fisher's exact test
- Two or more strains: Mantel-Haenszel χ^2 test with continuity correction


Uncommon Tumors, Pooled Test
Power
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
100%N
25%W75%N
50%W50%N
25%M75%N
75%W25%N
25%M25%W50%N
25%S75%N
100%W
25%S25%W50%N
25%M50%W25%N
50%M50%N
25%S50%W25%N
25%S25%M50%N
25%M75%W
50%M25%W25%N
50%S50%N
25%S75%W
25%S25%M25%W25%N
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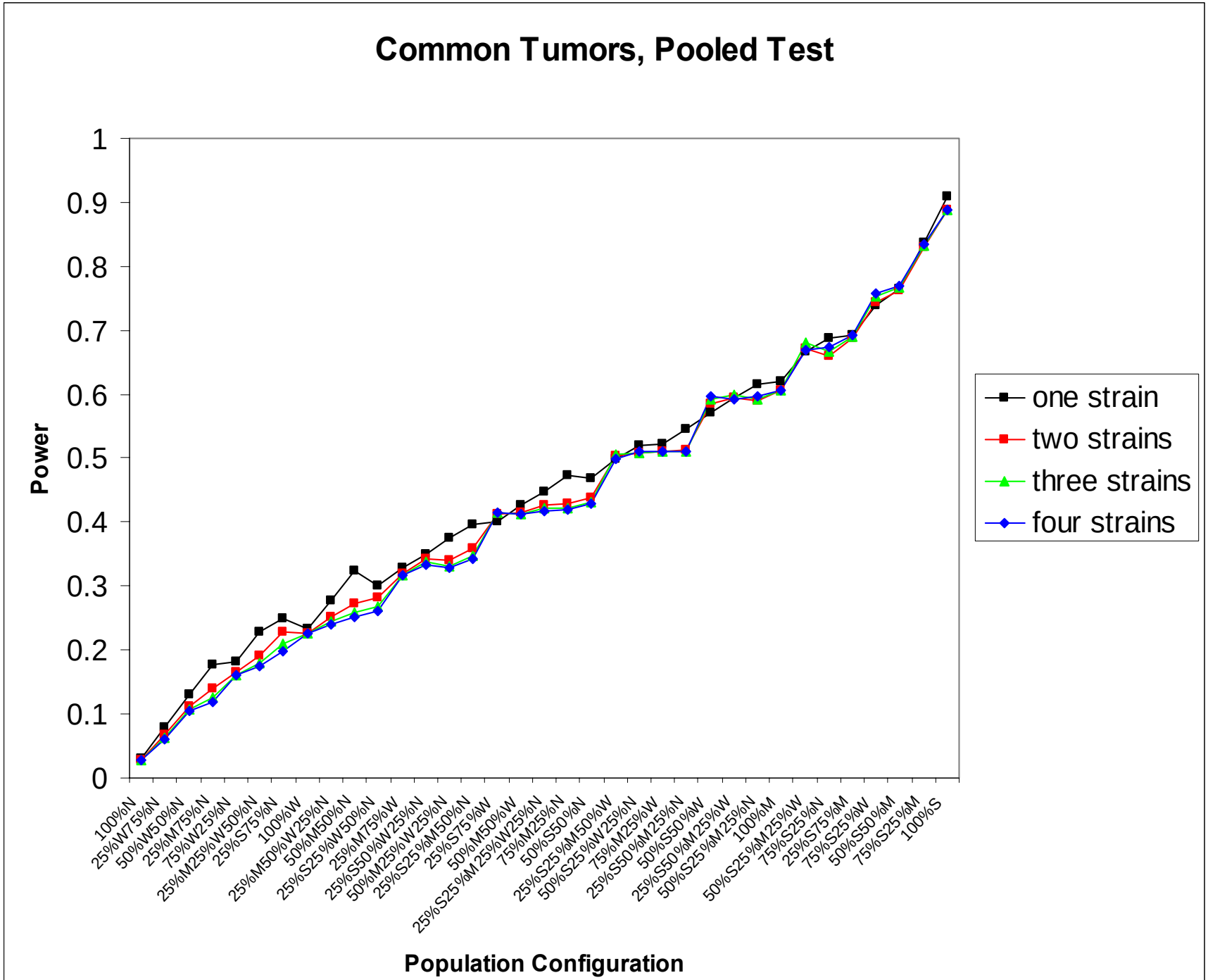
50%S25%M25%N
50%S50%W
25%S50%M25%W
75%S25%N
100%M
50%S25%M25%W
25%S75%M
75%S25%W
50%S50%M
75%S25%M
100%S
Population Configuration
one strain
two strains
three strains
four strains


Common Tumors, Pooled Test
Power
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
one strain
two strains
three strains
four strains
100%N
25%W75%N
50%W50%N
25%M75%N
75%W25%N
25%M25%W50%N
25%S75%N
100%W
25%M50%W25%N
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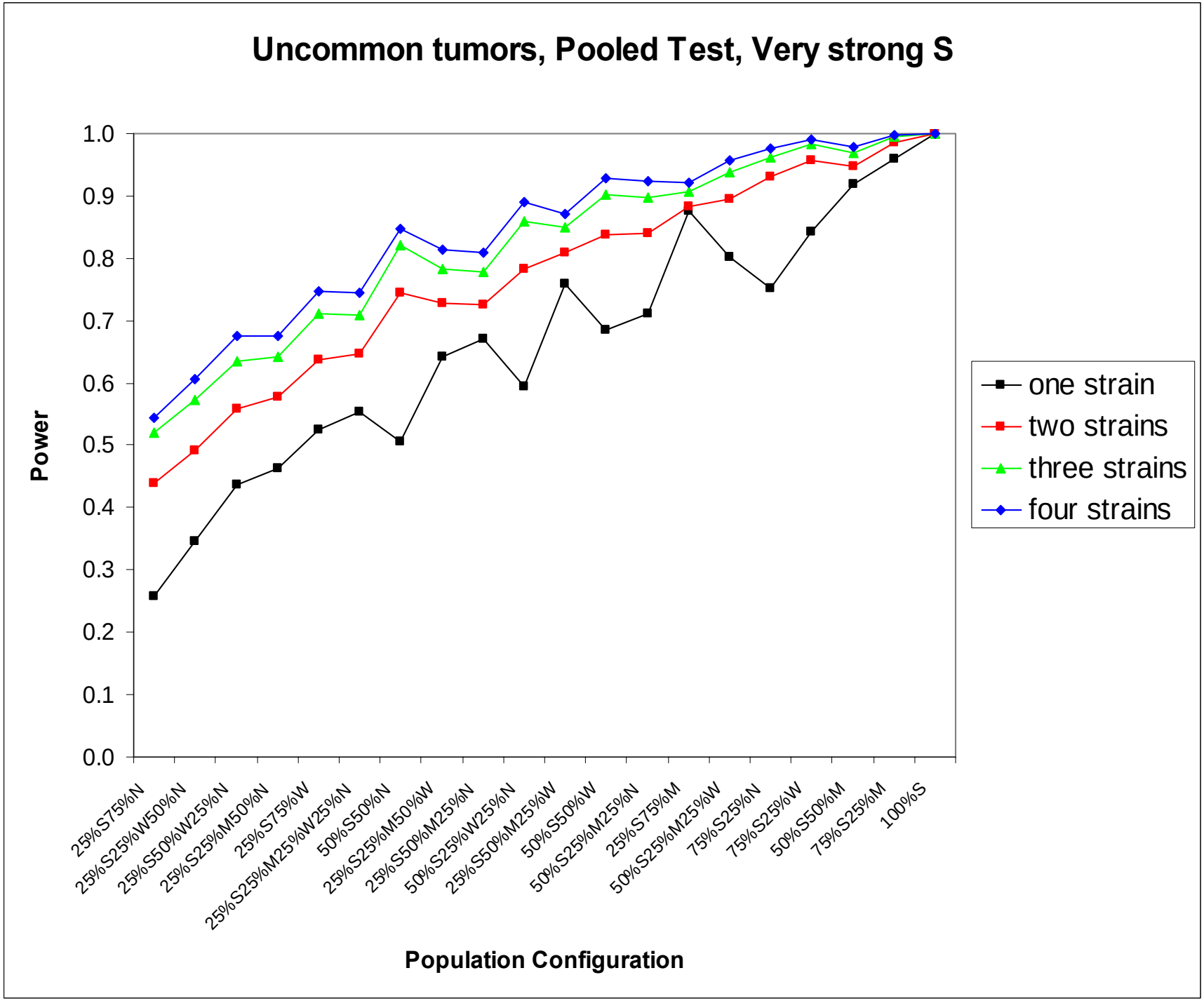
100%M
50%S25%M25%W
75%S25%N
25%S75%M
75%S25%W
50%S50%M
75%S25%M
100%S
Population Configuration

False Positive Rates for Pooled Test

	Uncommon Tumors	Common Tumors
1 strain	0.0104	0.0296
2 strains	0.0173	0.0287
3 strains	0.0173	0.0287
4 strains	0.0173	0.0287

Very Strong S

Strength	Uncommon		Common	
	Control	Treated	Control	Treated
Strong (S)	5%	**65%**	20%	**90%**
Moderate (M)	5%	25%	20%	40%
Weak (W)	5%	15%	20%	30%
No (N)	5%	5%	20%	20%


Uncommon tumors, Pooled Test, Very strong S
Power
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
25%S75%N
25%S25%W50%N
25%S50%W25%N
25%S25%M50%N
25%S75%W
25%S25%M25%W25%N
50%S50%N
25%S25%M50%W
25%S50%M25%N
50%S25%W25%N
25%S50%M25%W
50%S50%W
50%S25%M25%N
25%S75%M
50%S25%M25%W
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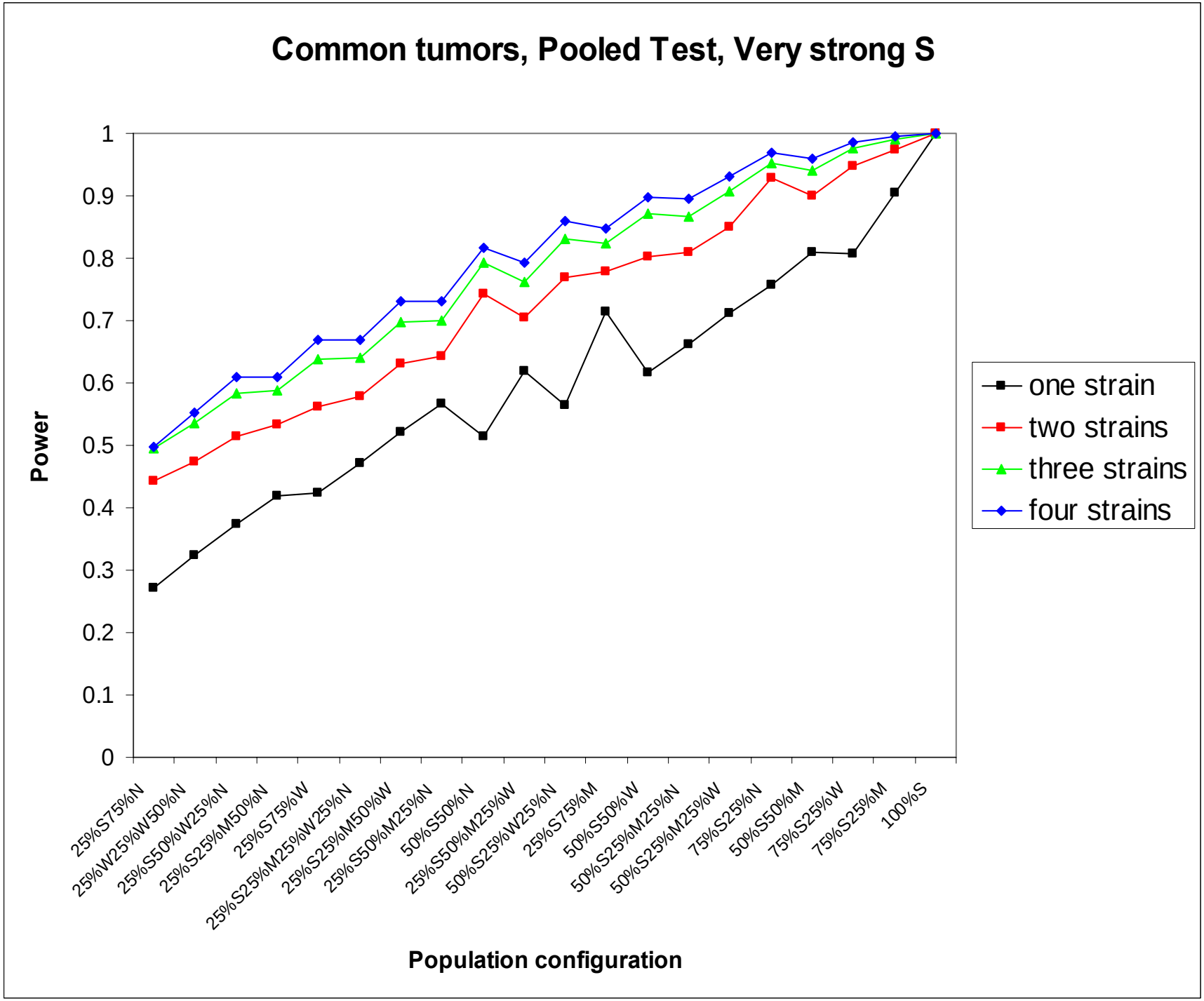
50%S50%M
75%S25%M
100%S
Population Configuration
one strain
two strains
three strains
four strains


Common tumors, Pooled Test, Very strong S
Power
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
25%S75%N
25%W25%W50%N
25%S50%W25%N
25%S25%M50%N
25%S75%W
25%S25%M25%W25%N
25%S25%M50%W
25%S50%M25%N
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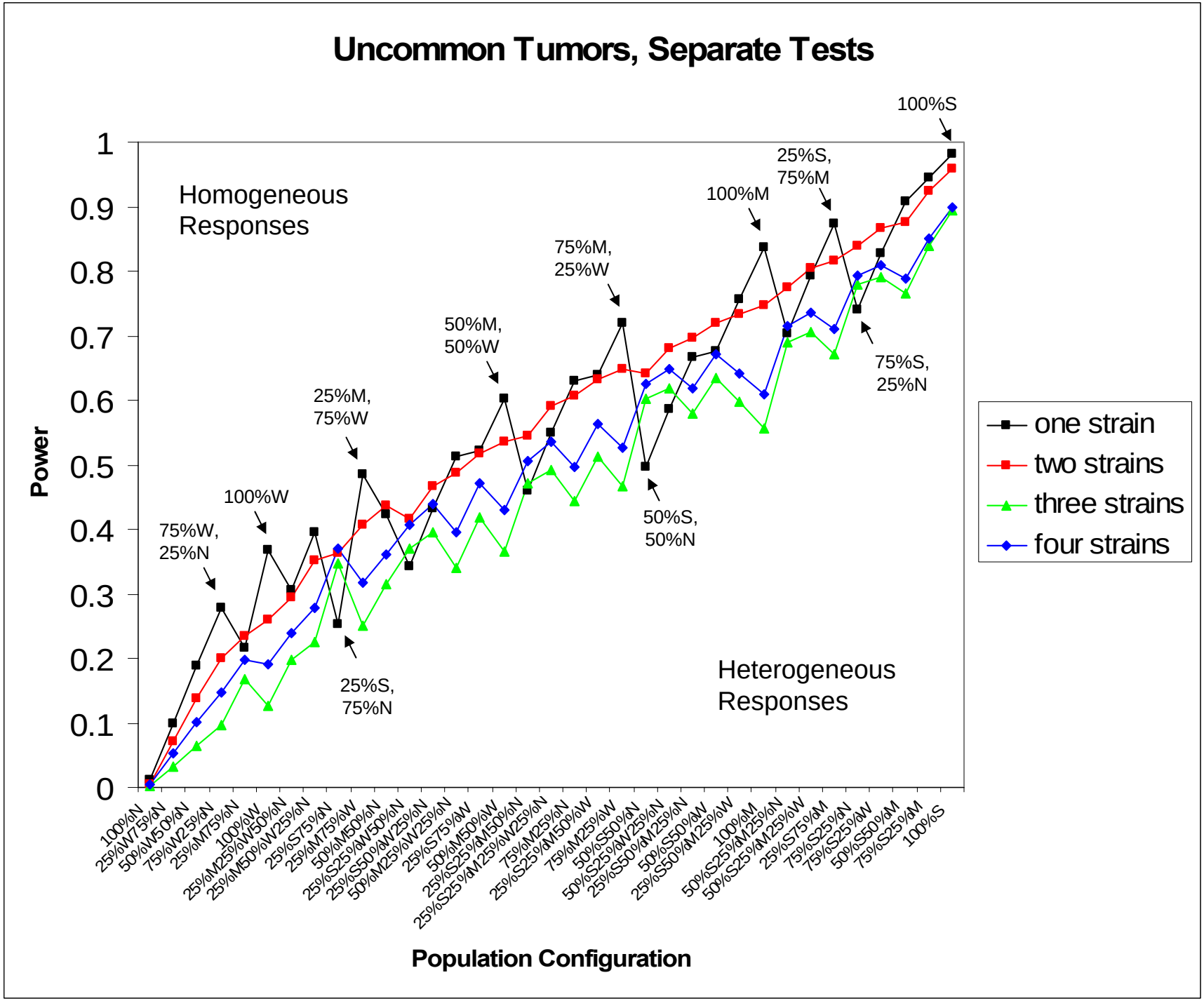
25%S50%M25%W
50%S25%W25%N
25%S75%M
50%S50%W
50%S25%M25%N
50%S25%M25%W
75%S25%N
50%S50%M
75%S25%W
75%S25%M
100%S
Population configuration
one strain
two strains
three strains
four strains

Testing Strategy: Separate Tests

- Fisher's exact test on each strain
- Treatment effect is considered significant if at least one strain's test is significant


Uncommon Tumors, Separate Tests
Power
Population Configuration
Homogeneous Responses
Heterogeneous Responses
100%S
25%S, 75%M
100%M
75%M, 25%W
50%M, 50%W
25%M, 75%W
100%W
75%W, 25%N
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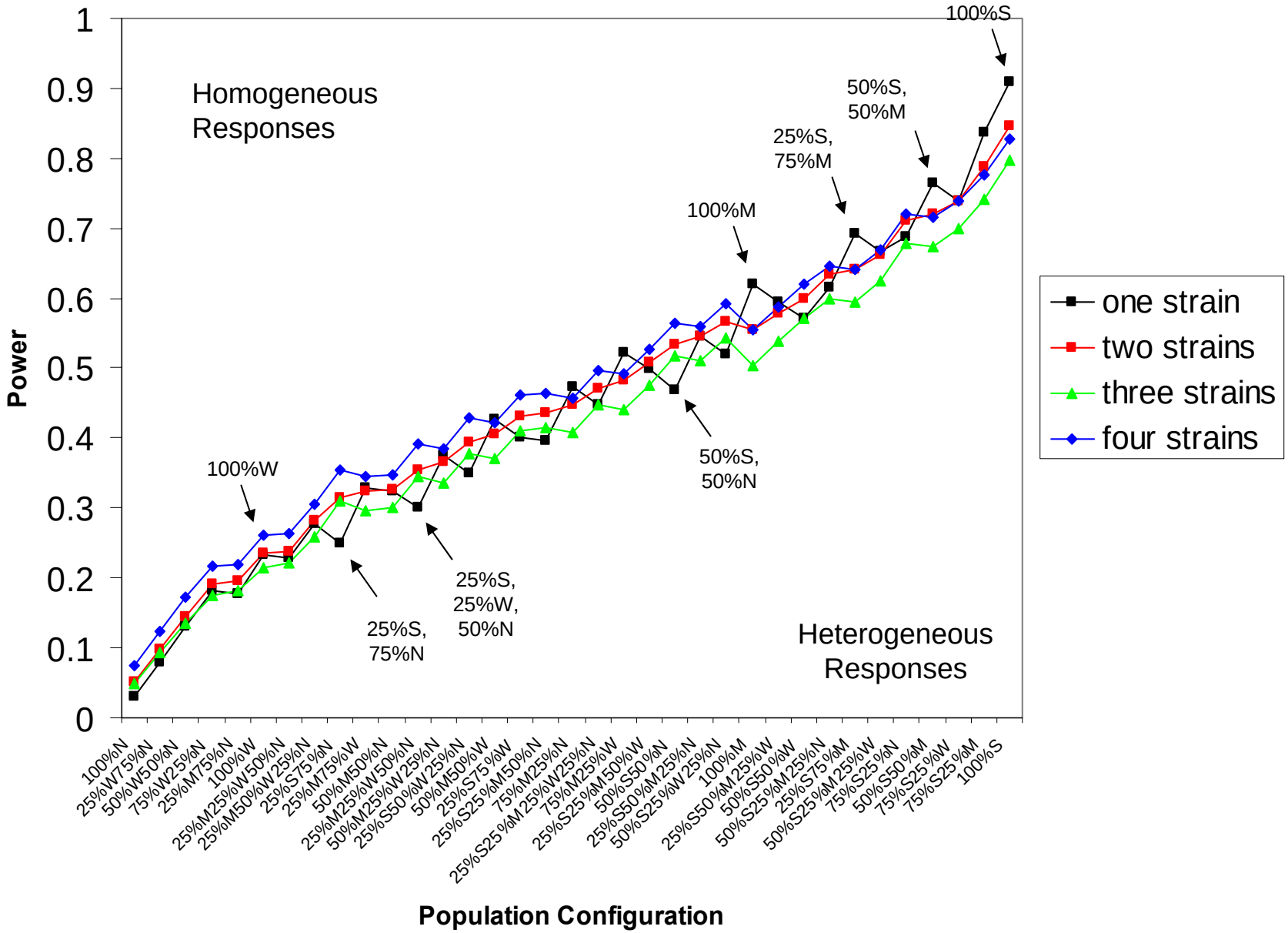
50%S, 50%N
75%S, 25%N
one strain
two strains
three strains
four strains

Common Tumors, Separate Tests

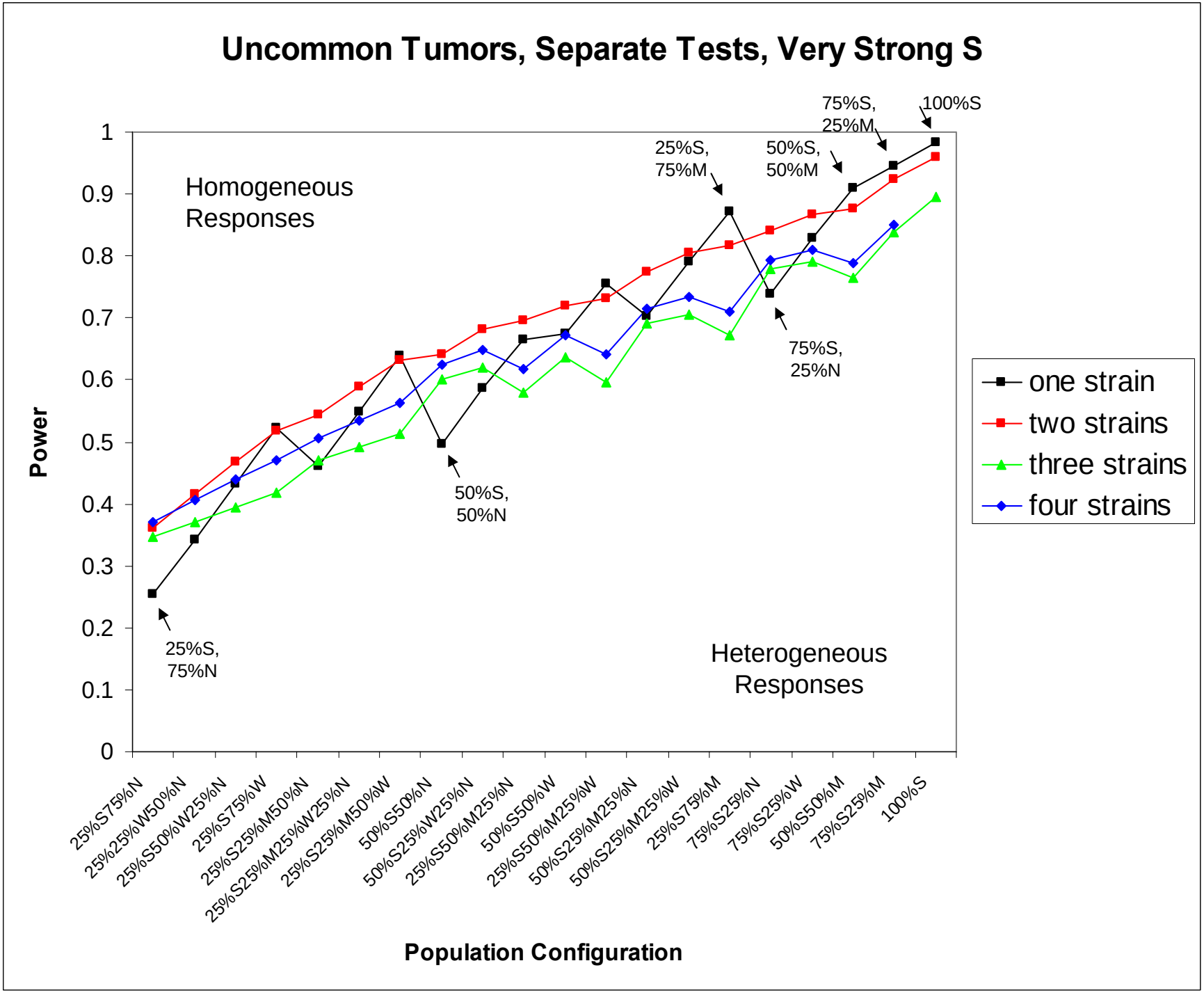


False Positive Rates for Separate Tests

	Uncommon Tumors	Common Tumors
1 strain	0.0104	0.0296
2 strains	0.0042	0.0511
3 strains	0.0012	0.0496
4 strains	0.0048	0.0754

Very Strong S

Strength	Uncommon		Common	
	Control	Treated	Control	Treated
Strong (S)	5%	**65%**	20%	**90%**
Moderate (M)	5%	25%	20%	40%
Weak (W)	5%	15%	20%	30%
No (N)	5%	5%	20%	20%


Uncommon Tumors, Separate Tests, Very Strong S
Power
Population Configuration
Homogeneous Responses
Heterogeneous Responses
25%S, 75%N
50%S, 50%N
25%S, 75%M
50%S, 50%M
75%S, 25%M
100%S
75%S, 25%N
one strain
two strains
three strains
four strains
25%S75%N
25%25%W50%N
25%S50%W25%N
25%S75%W
25%S25%M50%N
25%S25%M25%W25%N
25%S25%M50%W
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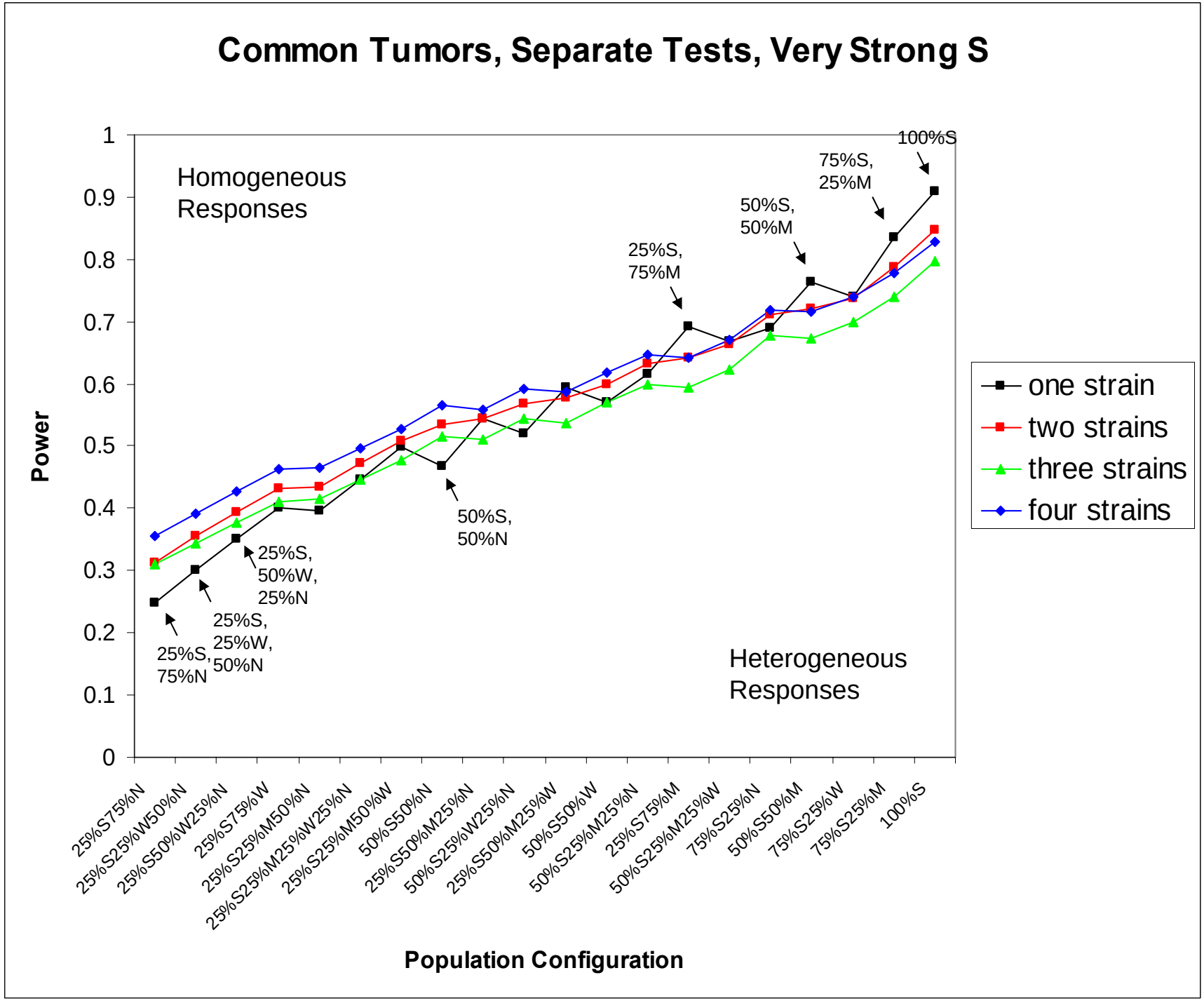
75%S25%M
100%S


Common Tumors, Separate Tests, Very Strong S
Power
Population Configuration
Homogeneous Responses
Heterogeneous Responses
25%S, 75%N
25%S, 25%W, 50%N
25%S, 50%W, 25%N
50%S, 50%N
25%S, 75%M
50%S, 50%M
75%S, 25%M
100%S
one strain
two strains
three strains
four strains
25%S75%N
25%S25%W50%N
25%S50%W25%N
25%S75%W
25%S25%M50%N
25%S25%M25%W25%N
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50%S50%W
50%S25%M25%N
25%S75%M
50%S25%M25%W
75%S25%N
50%S50%M
75%S25%W
75%S25%M
100%S

Conclusions

- **Pooled test:**
  - **Multi-strain designs are more powerful if the effects are very heterogeneous across strains and very strong in the sensitive strains.**
  - **Otherwise, single and multi-strain designs have similar powers.**

Conclusions

- **Multiple separate tests:**
  - **False positive error rates can be**
    - **unacceptably high for common tumors**
    - **unacceptably low for uncommon tumors.**
  - **Multi-strain designs have**
    - **lower power for homogeneous strains**
    - **higher power for heterogeneous strains.**

References

- Bennett & Hsu, *Biometrika* 47: 393-398, 1960.
- Felton & Gaylor, *J. Toxicology and Environmental Health* 26: 399-411, 1989.
- Haseman & Hoel, *J. Toxicology and Environmental Health* 5: 89-101, 1979.
- Wittes & Wallenstein, *J. Am. Stat. Assoc.* 82: 1104-1109, 1987.

Mouse Models

Chair: Dr. Norman Drinkwater

Rapporteur: Dr. Glenda Moser

NTP
National Toxicology Program

Is there a preference for isogenic or outbred strains?

- Isogenic strains should be used to insure reproducibility over time (requires management of drift), and to facilitate both genetic monitoring and subsequent mechanistic studies.
- F1 hybrids are preferable to inbreds
  - Many of the cancer modifiers identified to date are semidominant. F1 mice often many fold more susceptible than resistant parent and approx. half as responsive as sensitive parent.
- No good argument for use of outbred stocks.

Would any of the strains or stocks for which we have data be considered a better model than the NTP B6C3F1 mouse ?

- Very limited data on other F1 hybrids in two year assay (B6D2F1, others?).
- Desirable properties for any model to be considered include survival > 50% at two years, reproducible tumor profile, amenable to group housing, accessible to other investigators, parents part of resequencing effort.
- B6C3F1 (NCTR) does not (yet) suffer from concern related to increasing liver tumor incidence.

Are the liabilities associated with the with NTP B6C3F1 mouse significant enough to justify switching strains?

- Major liability for B6C3F1 (NTP) is increasing incidence of liver tumors in control male mice (60%+), likely associated with increasing body weight.
- Not critical yet but could become so.

Do you have any suggestions for ways the B6C3F1/N mouse currently used could be improved to address the issues raised?

- **Need to understand basis for lower liver tumor background for B6C3F1 mice in NCTR studies.**
  - **NCTR, NTP parental stocks may have diverged genetically (do reciprocal test bioassays). Could require return to frozen foundation stocks.**
  - **Dietary differences (fat content or other) could contribute to higher weight gain (and liver tumor incidence) in NTP studies.**
  - **Intestinal flora may influence nutritional status and give rise to differences in liver tumor response.**
  - **Other environmental factors?**

If a switch is made, how should it be implemented?

A. Just Switch? Absolutely not.

B. Use new strain(s) in addition to currently used NTP strains? Yes.

- Cost of parallel studies in B6C3F1 and another hybrid likely to be prohibitive.
- If use of alternative model is desirable, first implement as a 25x2 study, with equal numbers of B6C3F1 and the alternative hybrid.
- Above approach would provide continuity with existing database while experience is gained with new model.

If a multiple strain approach is utilized:

(We're not all persuaded.)

Fixed set of strains? Or should strains vary in relation to their genetics or unique susceptibilities with regard to study agent?

- Use of varying strains has two disadvantages: accumulation of useful historical data is slowed and act of choosing biases the results.
- Choose fixed set using rationale on next slide.

Should NTP utilize “highly sensitive” strains? If so, in what proportion?

- Any single inbred or hybrid may be highly sensitive at some tissue site (possible exception- recent wild-derived strains).
- To define a panel of suitable F1 hybrids, start with the set of inbreds being resequenced by NIEHS.
  - Eliminate inbreds with high spontaneous incidence of lymphoma or leukemia (likely to limit long term survival).
  - Choose parental pairs that are genetically distant from each other.
  - Each inbred only used for one hybrid.

We believe that continued use of the mouse in the bioassay is essential.

- Tumor response in multiple species should generate greater concern with implications for eventual risk assessment.
- Availability of data from both mouse and rat is useful in cases of equivocal response.
- Mechanistic understanding of tumor responses will be aided greatly by our increasing knowledge of genetic modifiers of cancer risk in mice and the availability of genomic sequence for multiple sensitive and resistant strains.

Rat Models

Chair: Dr. Jerry Hardisty

Rapporteur: Dr. David Allen

NTP
National Toxicology Program

Is there a preference for isogenic or outbred strains (stocks)?

- There is not a specific preference, although there are reasons for choosing one over the other as follows:
- Isogenic:
  - Can use a relatively small number of animals and still conduct an effective bioassay
  - Need a much larger number of animals if using outbred stocks due to their genetic variability (therefore less practical) – although this has not been substantiated; there are also survival issues
  - In general, isogenic strains are much better for obtaining mechanistic information
- Outbred:
  - Some isogenic strains are "flawed" (e.g., F344/N has very high incidences of testicular tumors and LGL leukemia)
  - Pharma favors outbred because:
    - They are not overly sensitive and are more representative of the human response
    - They avoid the sensitivity/resistance that may develop in inbred
    - Although there is genetic drift in all models, outbred may be less likely
    - May not be as outbred as believed due to current breeding and genetic monitoring practices

Would any of the strains or stocks for which we have data be considered a better model than the NTP F344 rat?

- Must define parameters upon which a better model is defined:
  - Survival
  - Background Tumor Incidence
  - Cross-species metabolism
- Better isogenic?
  - Different source of F344/N
  - Too little data on other inbred strains
- Preferred Outbred?
  - Wistar-Han, based on favorable survival and background tumor incidence, but metabolism must be evaluated (i.e., TCDD)
- Recommend the evaluation of historical data on other less commonly used strains/stocks prior to final selection

Are the liabilities associated with the NTP F344/N rat significant enough to justify switching strains?

- Liabilities in the current strain of F344/N that NTP is using mandate that it should not be used.
- Mutations in the current strain appear to be causing these liabilities.
- There are three options:
  - Re-establish the F344/N strain (which doesn't address some liabilities – e.g., spontaneous tumor incidence; inhalational route issues – e.g., leukemia)
  - Create an F1 Hybrid (which would lack historical data and experience in carcinogenicity bioassays)
  - Choose an appropriate alternative strain/stock (such as Wistar Han)

Do you have any suggestions for ways the F344/N rat currently used could be improved to address the issues raised?

- No.
- As stated previously, liabilities in the current strain of F344/N that NTP is using mandate that it should not be used.

If a switch is made, how should it be implemented?

- **Just Switch? No.**
  - **Complete current studies already initiated with F344/N**
- **Use new strain(s) in addition to currently used NTP strains?**
  - **For the purposes of the 2-year bioassay, only the new strain would be necessary (testing both strains would require excessive resources – i.e., not intending to evaluate the differences in carcinogenicity of a given substance between the two strains)**
  - **Any new strain/stock may be considered the "default" strain/stock unless metabolism data suggests otherwise.**

If a multiple strain approach is utilized:

Fixed set of strains? Or should strains vary in relation to their genetics or unique susceptibilities with regard to study agent?

- Must use the same principles discussed previously (i.e., survival, tumor incidence, cross-species metabolism).
- A multi-strain study would have to be scaled up appropriately to mimic a single strain study design, and therefore is not practical for a screening bioassay.

Should NTP utilize “highly sensitive” strains? If so, in what proportion?

- The predictive value of using “highly sensitive” strains is uncertain.
- Rather, the NTP should use “highly predictive” strains.

Multiple Strain Approach

Chair: Dr. Julian Preston

Rapporteur: Dr. Kris Thayer

NTP
National Toxicology Program

Multiple Strain Approach

Work Group:

Michael Festing

Joe Haseman

Howard Jacob

Ralph Kodell

Julian Preston

Kris Thayer

Hiroyoshi Toyshiba

Is a multiple strain assay a viable approach for cancer hazard identification?

YES

Advantages:

- Better captures range of rodent genetic variability
- Statistical power advantage for heterogeneous responses without increasing the number of animals used in 2-year bioassay
- Help identify mechanisms of cancer induction and susceptibility
  - relevance of rodent tumors to humans

Disadvantages:

- Added cost (multiple 90-day MTD dose finding studies)
- More opportunity for operational error (e.g., more doses)
- Increased logistical problems with use of multiple strains
- Need to collect background data for strains
- If regulatory acceptance is an issue

Data Analysis and Statistical Considerations

- **Pooled vs. separate analysis for each strain?**
  - **Pooled analysis recommended**
- **Possible statistical approach for hazard identification**
  - **poly3 test (survival adjusted), then pool**
- **Possible statistical approach for dose response**
  - **calculate point of departure for each strain, then choose most sensitive or average point of departure**
- **No reason to add additional doses beyond those currently used in the 2-year bioassay**

Study design factors: Dose Selection

- **Test up to each strain's MTD?**
  - **Yes, otherwise lose power benefits**
- **Use MTD from most sensitive strain?**
  - **No, would reduce power**

Should NTP vary the strains selected based upon our knowledge of the anticipated action or target tissue for the agent being tested?

- GOAL: Select strains based on preliminary data and known sensitivities of strains
- Few of these data are currently available

Should NTP utilize “highly sensitive” strains? If so, in what proportion?

- Sensitive refers to agent-induced tumors rather than high background tumor frequency
- Review data to select for use potentially sensitive strains
- Relevance of mechanism for humans important
- Would want relatively low background tumor frequency
  - would also be associated with better 2-year survival

Should background data be required in order to make decisions about new strains?

Would want at least a minimal amount of 2-year historical control data

- Including histopathology, body weight, 2-year survival, natural life span

Strains to consider

General characteristics

- genetic diversity, position in evolutionary tree
- sequenced strains (mouse)
  - focus on genes known to be important
- commercially viable (more of a rat issue)
- known biological data
- Always include:
  - B6C3F1/N
  - F344/N

How many strains should be used in a multiple strain bioassay?

- 12 strain fixed pool (at least for rats)
  - beyond this number there will be little additional benefit in terms of capturing the range of genetic variability
- From this fixed pool, select a subset of strains (e.g., 4) to test for a given agent
  - selection based in part on pilot data

If so, is there a preference for isogenic or outbred strains?

- **Isogenic (inbred and/or F1 hybrid)**

Should we attempt to “validate” a multiple strain model?

- “Characterization” is a better term than “validate”
- Yes, to the extent practical
  - Would want background data for strains at a minimum
  - Characterizing and generating pilot data would be sufficient
- Use known human carcinogens and compounds generally recognized as safe as part of characterization.
- Could study an agent previously tested in one strain to validate prediction for untested strains

If a switch is made, how should it be implemented?

A) Just Switch?

No

If a switch is made, how should it be implemented?

B)Use strain(s) in addition to currently used NTP strains?

- **Conduct pilot studies to collect adequate background information for strains to be used**
- **Characterize strains with known human carcinogens and agents generally recognized as safe**
- **Incrementally add strains to current 2-year bioassay**

Final Note

- **These are all recommendations and suggestions – they are not prescriptions**

Can it be valid to pool across genotypes?