The A/J Mouse lung tumor model: strengths and weaknesses

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Toxicology of Tobacco smoke

- TS is a complex mixture – but, in contrast to other complex mixtures, we know <u>exactly</u> what it does to man – cancer in multiple sites, COPD, cardiovascular disease etc.

- Why then is experimental toxicology as we know it so much behind – or: why do we not have (yet) a safe cigarette?

Highly recommended reading

- Richard Kluger: "ASHES TO ASHES, or: Americas Hundred-Year Cigarette War, the Public Health, and the Unabashed Triumph of Philip Morris"
- Published in 1996
- Describes in detail the NCI effort to develop a less hazardous cigarette ("Breeding a One-Fanged Rattler")

Tobacco smoke and lung tumors in mice

- When strain A mice are exposed to tobacco smoke, they develop multiple visible tumors on the lung surface. Carcinogenic potential is evaluated by tumor multiplicity rather than by the more customary tumor incidence.

- At present, this is, if not the first, but arguably the best model to show that cigarette smoke causes lung cancer in experimental animals.

- While the model has many advantages, it also has some weaknesses

Main objections

- Pathologists: Mouse lung tumors are not representative of human lung cancers caused by smoking, because it does not produce bronchogenic carcinoma.

- Toxicologists: the strain A/J mouse lung tumor model is not a good tumor model because it occurs in a particularly sensitive strain and might only reflect acceleration of tumorigenesis, not create new tumors.

A simple model made complex

- The simple model:
- Expose mice for 5 months smoke/4 months air
- Count surface nodules
- Average number of tumors per lung (multiplicity) is measure of carcinogenicity
- Do limited histology (it is monotonous)

- Questions
- Arise often because of unfamiliarity with the assay (which has a history on its own)
- Is it reproducible? (yes!)
- Why not incidence? (Multiplicity gives dose response even at 100% incidence)

Criteria for positive lung tumor assay in strain A mice (Shimkin and Stoner, 1975)

- Preferably more than 1 tumor per lung, although 0.7 to 0.9 are acceptable (?)

- Evidence of dose response

- Controls should be within historical range and not abnormally low

- Positive controls should be included to test sensitivity of strain used

Facts to remember

- High spontaneous tumor incidence; more then 50% in 1 year old mice, 100% in 2 year old ones.

- No good response to amines, metals and several hepatocarcinogens.

- Excellent dose - response to polycyclic aromatic hydrocarbons, nitrosamines and carbamates.

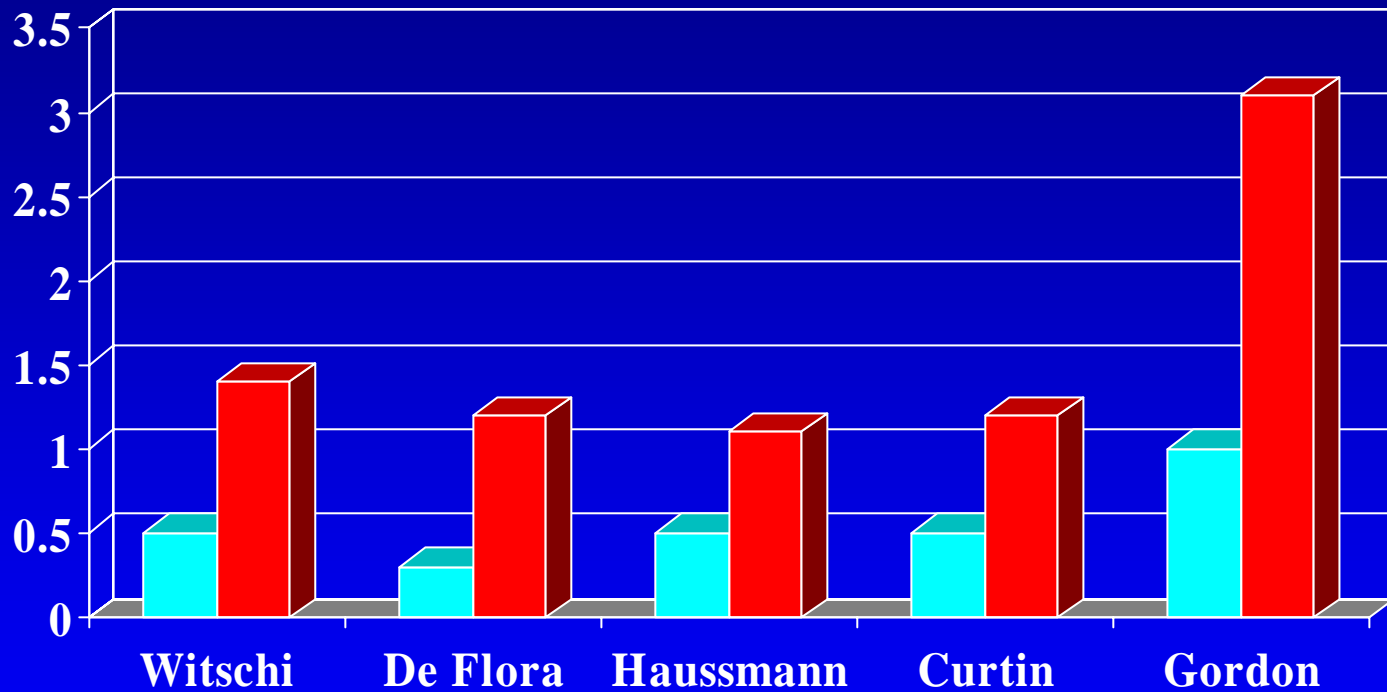# Carcinogenesis Assays with ETS 1995-2004 (Lung tumor multiplicity)
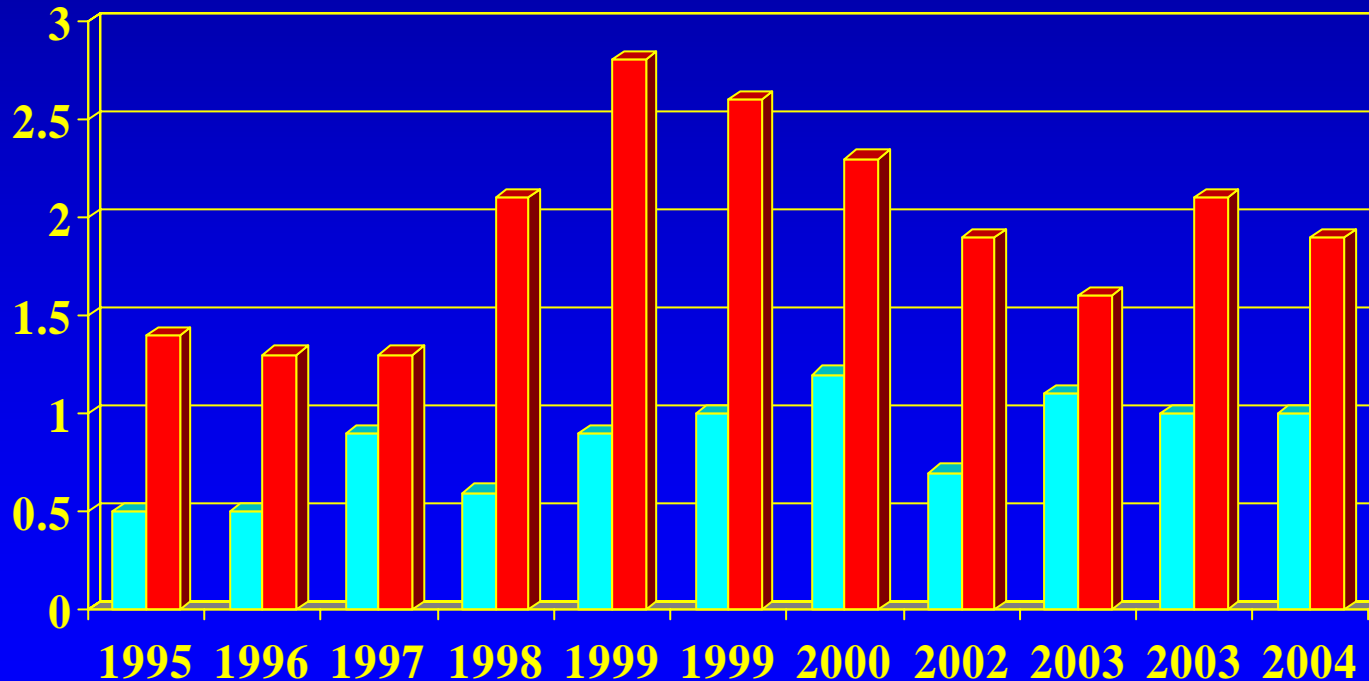
Data from different laboratories

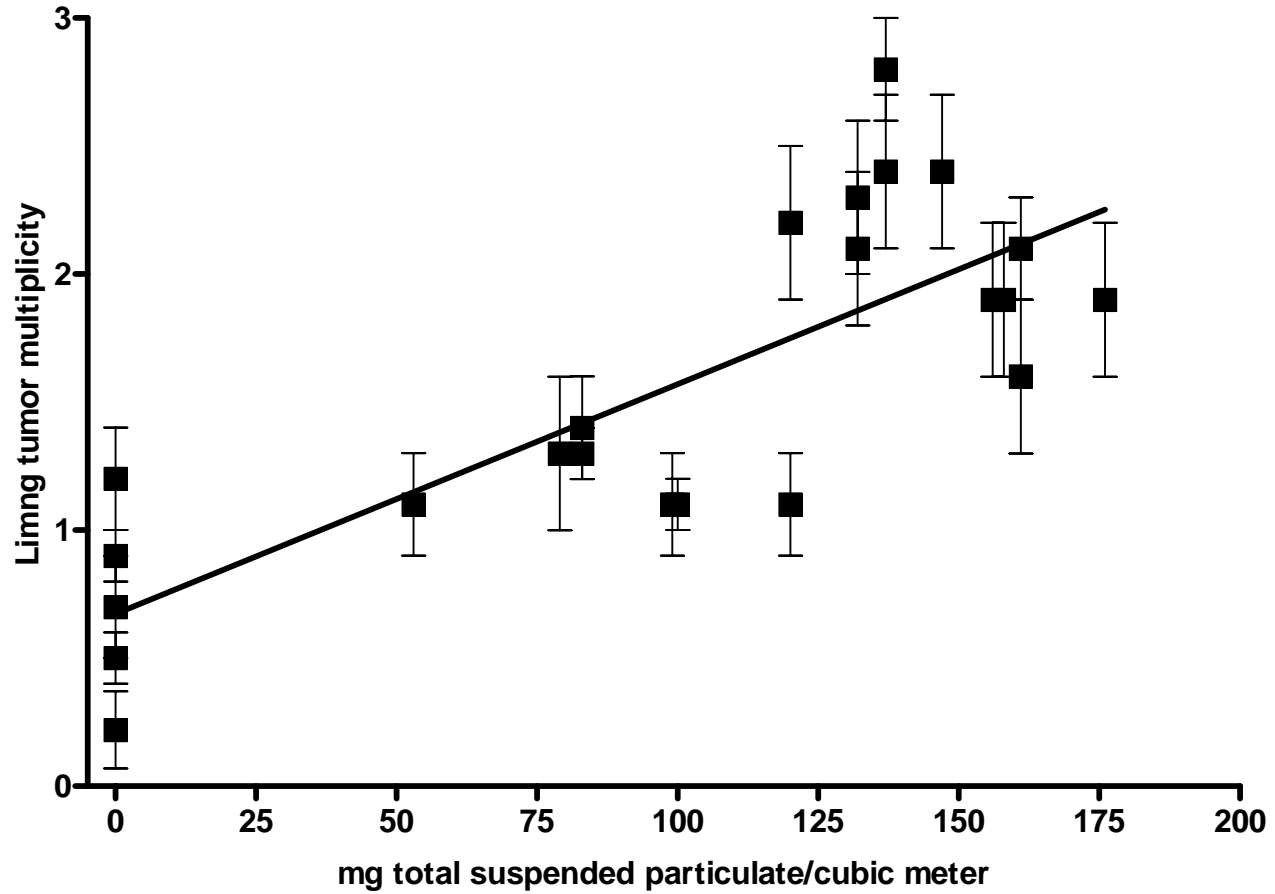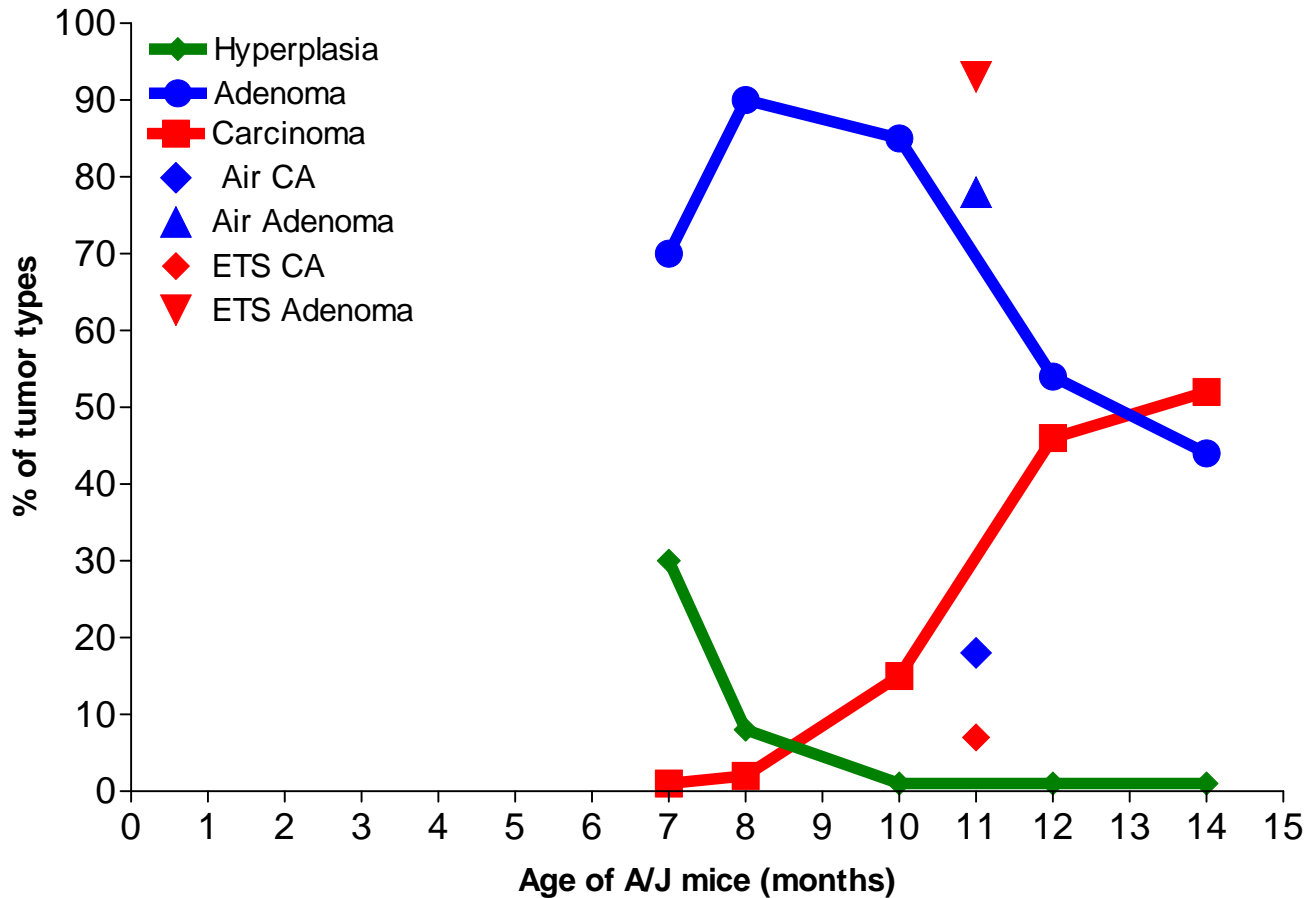**Dose-Response to Tobacco Smoke**

Distribution of tumor types after:
- single injection of Vinyl Carbamate (Foley et al., 1991)
- in ETS experiments

Legend:
- Hyperplasia
- Adenoma
- Carcinoma
- Air CA
- Air Adenoma
- ETS CA
- ETS Adenoma

Y-axis: % of tumor types

X-axis: Age of A/J mice (months)

"We contend that the majority of pulmonary tumors tumors in strain a mice treated with vinyl carbamate arise as hyperplasias, progress to adenomas, and finally become carcinomas".

Mouse (rodent) lung tumors differ from human lung cancer

- Progression from hyperplastic foci to adenomas to carcinomas.

- However, even after 2+ years, more than 50% of tumors show benign features.

- Even after 2+ years, metastasis to distant organs is an extremely rare event, although invasion of adjacent tissue is quite common.

- Not to be confused with human bronchioloalveolar carcinoma.

Response of different strains to tobacco smoke in on-off protocol
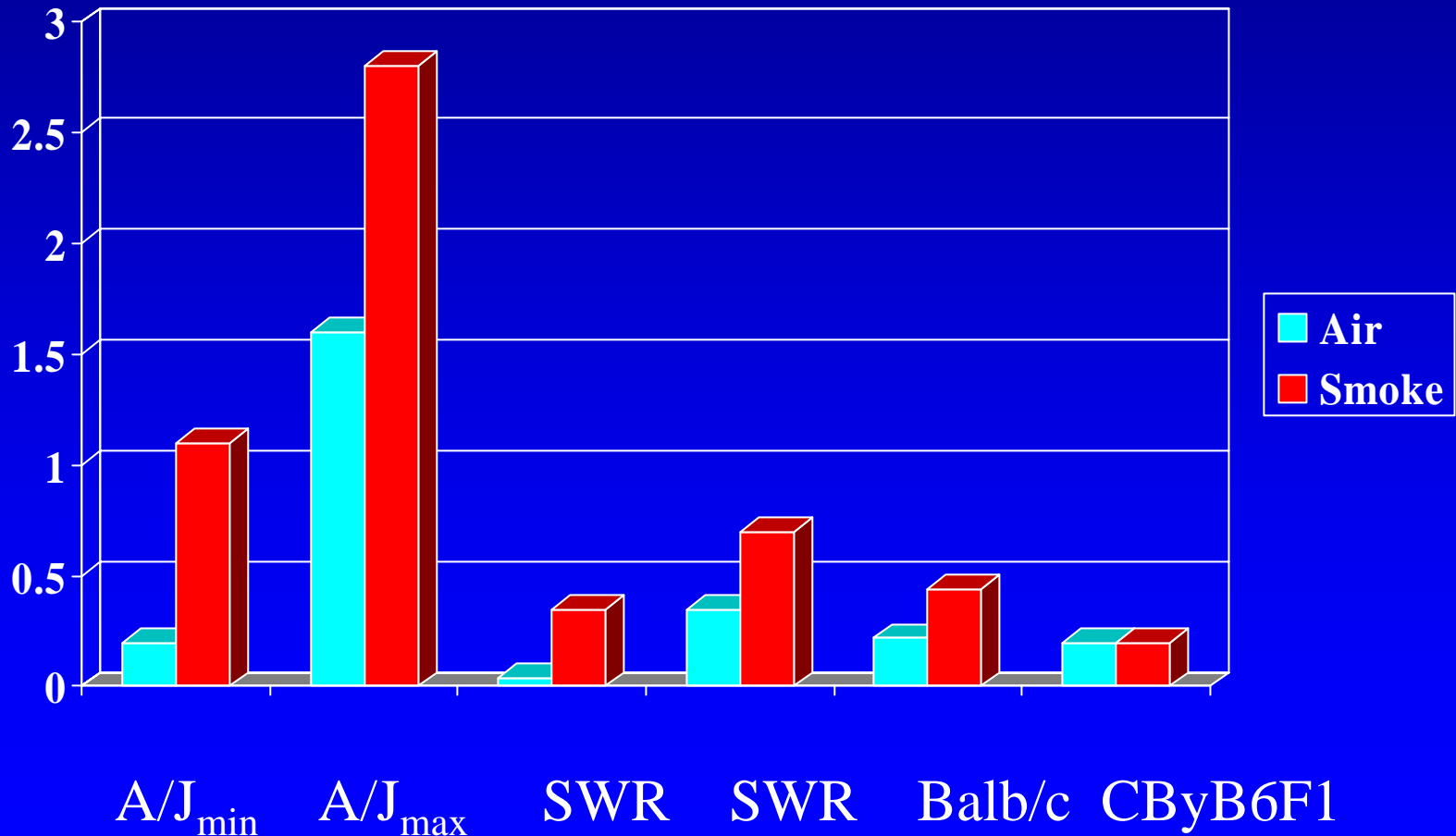
Transgenic mice exposed to TS

Transgene	Air	Tobacco
UL53-3X A/J p53 mutant	0.2	0.7
wild type	0.4	0.3
A/J mice rasH2 Tg	0.6	1.4
wild type	0.5	1.2
FVB/N Prostacyclin synthase overexpression		0.4
wild type		1.2

So far, no better response with transgenic models, but good for mechanistic studies on chemoprevention

"Acceleration" or de novo?

Age of mice
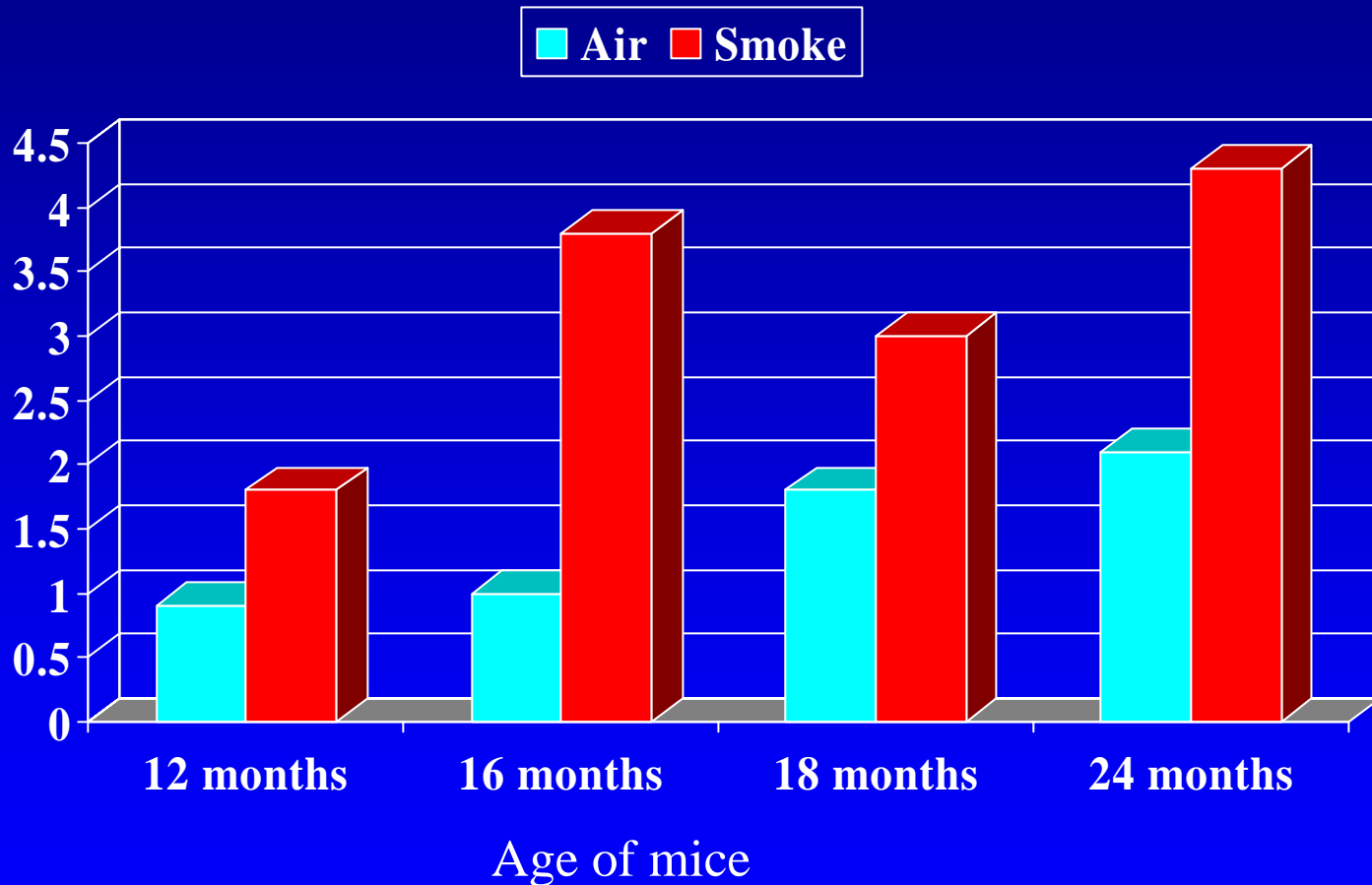
Tobacco smoke does not simply accelerate the growth of tumors that would have developed spontaneously, but it leads to the formation of additional (new) tumors.

Weight Gain

Body weight (g)

Months 1-5 Tobacco smoke; 6-9 Air

A/J Air
A/J TS
SWR Air
SWR TS
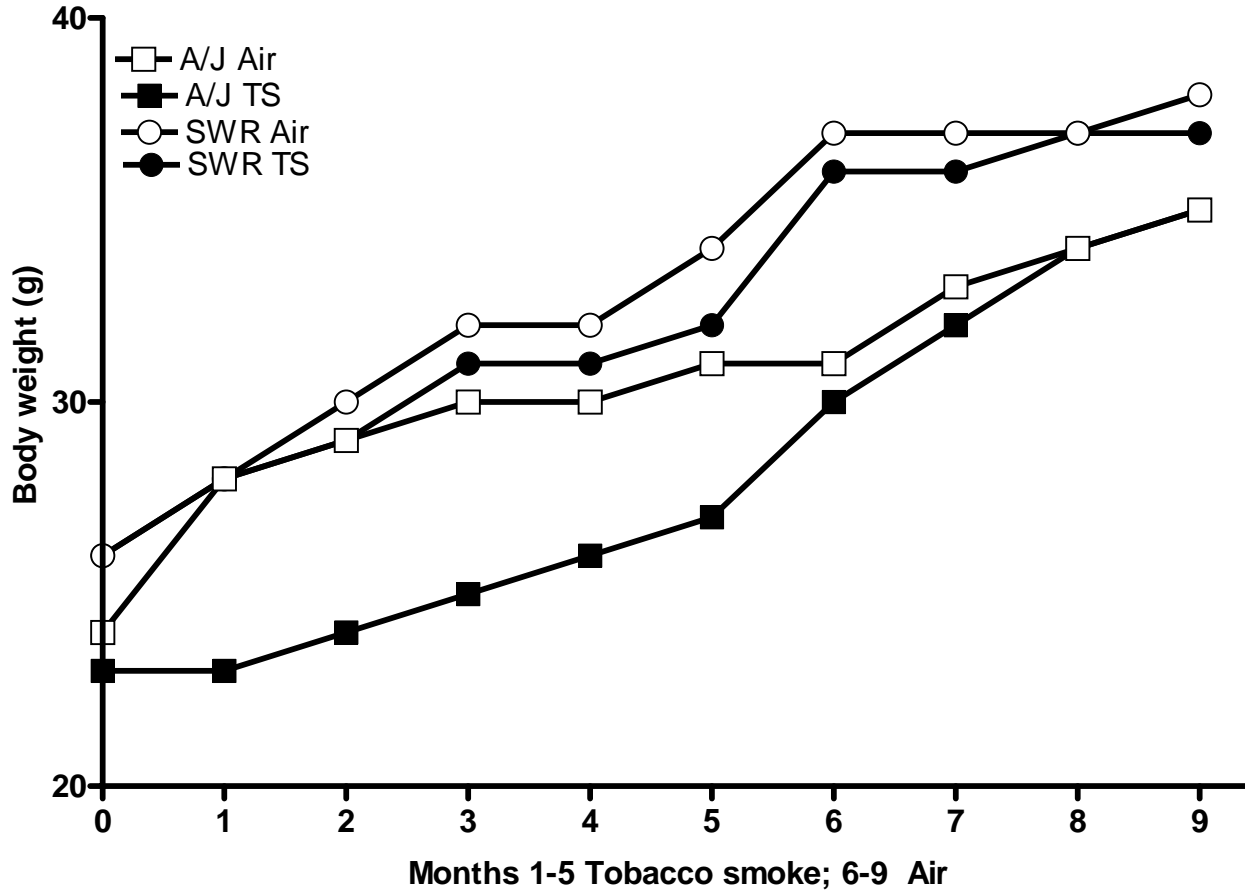
Reduced Weight Gain

- While A/J mice show reduced weight gain, SWR and FVB/N do not, yet still develop tumors
- Well controlled pair-feeding shows that initially reduced weight gain is unrelated to tumor development (Stinn, Haussmann et al.)
- Reduced weight gain in A mice explained by stress
- Other possibilities: food palatability, nicotine?
- If anything, reduced weight gain underestimates tumor rates

Effects of full and filtered smoke
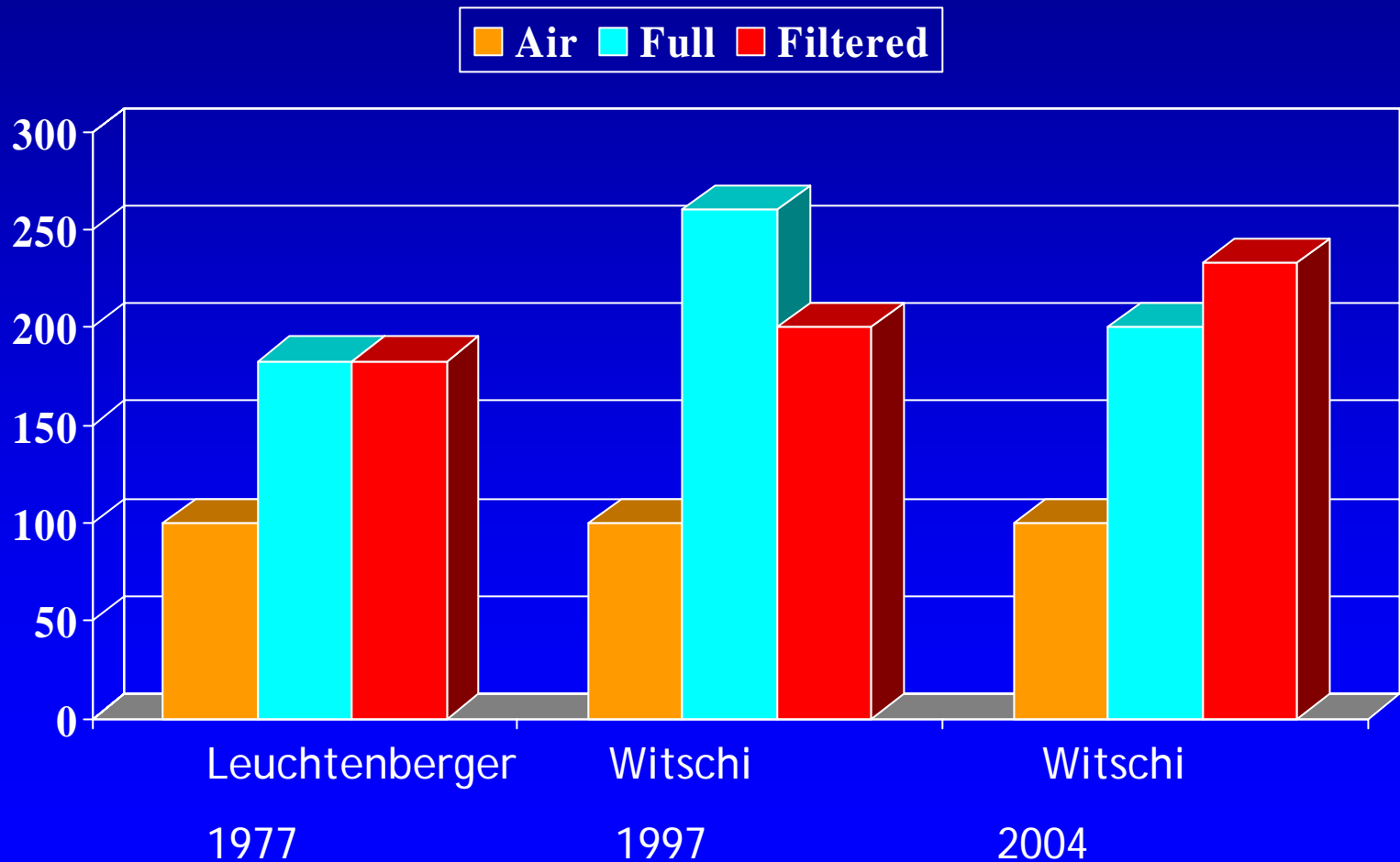
How important is gas phase?

- In mice, lung tumors develop even when PAH's and TSN's are absent.

- Analytical data and dosimetric considerations suggest only one agent to be a major carcinogen: 1,3-butadiene.

- How does this relate to humans? (Impact of filter, low tar cigarettes)

- What about skin painting studies for hazard assessment of modified products?

Mainstream smoke (MS) vs. sidestream smoke (SS) toxicity

- Experiments at INBIFO show short term toxicity of inhaled SS to be 2 to 4 times more pronounced than MS toxicity.

- SS condensate more potent skin carcinogen than MS condensate (Mohtashamipur et.al).

- Experiments from 3 different laboratories with MS in A/J mice were negative or effect was only discovered with serial sectioning of lungs.

- All SS experiments in A mice (4 laboratories) were positive with surface counting alone.

Chemoprevention

- Beta carotene and N-acetylcysteine (NAC) two agents known for low (if any) toxicity. Hope that they might prevent an otherwise incurable disease.

- No convincing preclinical data, except for some mechanistic information, often with in vitro approach.

- Clinical trials a big disappointment.

- Need for good preclinical model?

Chemoprevention against NNK or tobacco smoke (% of controls)
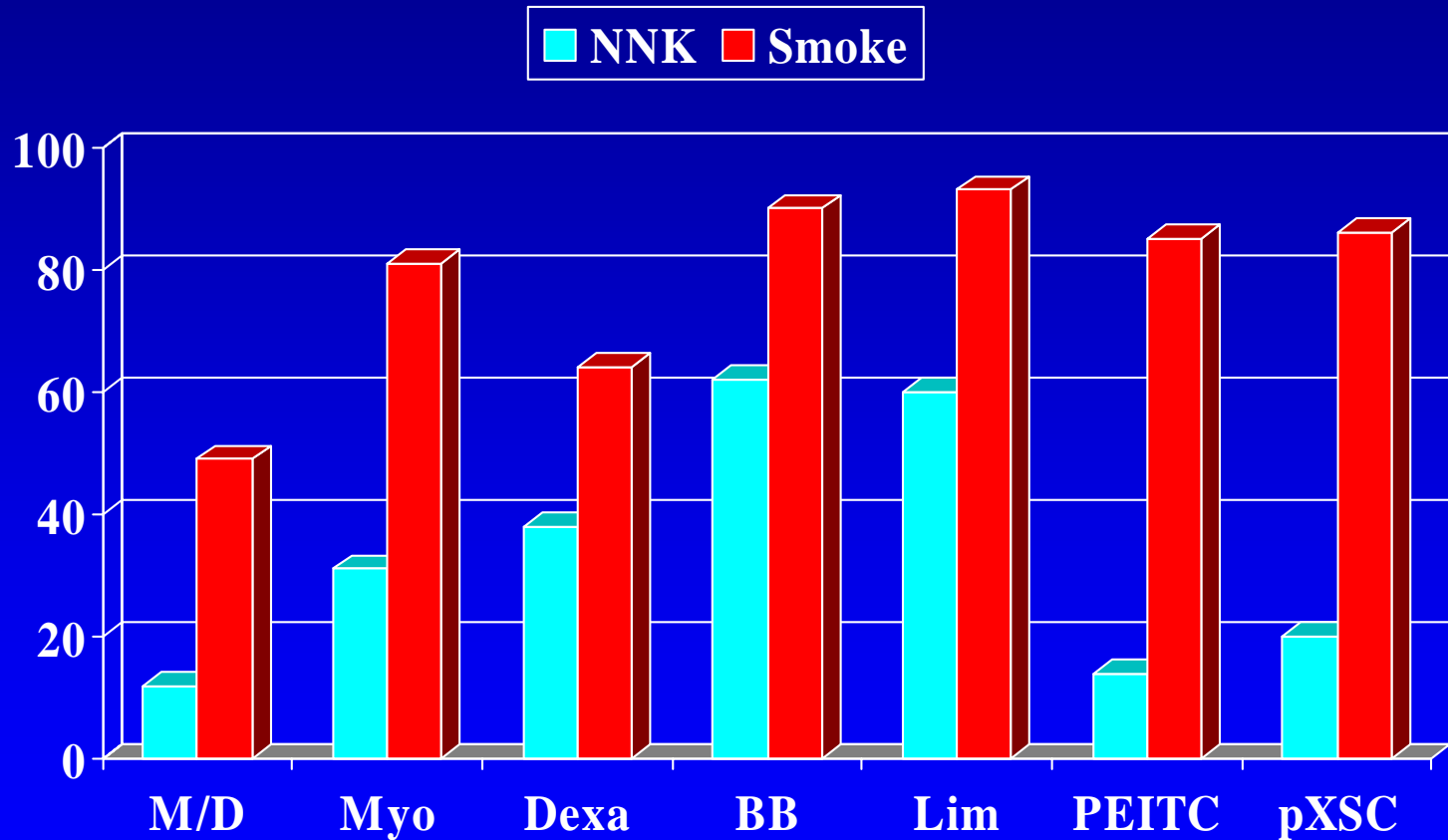
Significant effects against ETS found with one treatment only (M/D; Myoinositol+dexamethasone)

Tobacco smoke exposed A/J mice as a preclinical model for chemoprevention

- Would have correctly predicted the failure of NAC and beta carotene in clinical trials
- Many promising agents yield reduction in tumor multiplicity to about 80% of controls
- A 20% reduction would be great in humans
- Is system sensitive enough?
- Mutatis mutandis, also applies to product improvement

Statistical power: the weakest aspect of the lung tumor assay

- With single carcinogens, anywhere from 10 to 50 (or more) tumors per lung can be produced; this makes it easy to detect small differences

- With tobacco smoke, usually 1 to 2 tumors per lung (with SE of 0.2 to 0.3); this makes it next to impossible to detect small differences (20% to 30%) without using hundreds of animals

- *Unfortunately, similar considerations apply to other animal tobacco smoke models with small tumor rates*

B6C3F1 mice and tobacco smoke (656 mice, 30 months)

- Tumor incidence: 10% in controls, 45% in smoke exposed animals
- Percentage of mice with benign tumors: controls 7%, smoke exposed 28%
- Percentage of mice with malignant tumors: controls 3%, smoke exposed 20%
- Local invasion of neoplastic cells (pleura, heart, aorta) "not uncommon" (25-30%)
- Distant metastasis: controls 0.3%, smoke exposed 1.3%
- Final weight – 73% of controls. Live longer!

Are rats better?

- First positive rat study (Dalbey et al.) only significant if all respiratory tract tumors are counted (difference: 1 tumor).

  Controls 2.6%; tobacco smoke 10.3%

- Modern rat study (Mauderly): 753 rats, 30 months
- Significantly increased tumor rates in females

  Controls 0%, high dose 13.6%; low dose 5.7%

- 67% of tumors benign

Complex Mixture Toxicology, Tobacco Smoke and Lung Cancer

- The complex mixture of tobacco smoke is the most important human carcinogen (lung, bladder, pancreas, colon, prostate, others?). It is one of the few carcinogens that was detected in man <u>before</u> animal experiments suggested its carcinogenicity (others: radon, aniline dyes, asbestos).

- It is the only human carcinogen for which we could accomplish zero exposure.

Preclinical TS toxicology

Species	Expt.	Animals	Comment
Mouse (not A/J)	7	> 10,000	Only 3 studies positive – the best one involving one sex, one dose, 600+ animals
Rat	3	> 1500	One study positive because of 1 tumor; other: incidence max. 13%
Hamster	3	> 4000	Larynx tumors only

Was it for inhalation studies, TS never would have been recognized to be a carcinogen (it was skin painting studies with cigarette tar that did the trick).

The "best" (definitive?) studies

Species	N	Comments
A/J mouse	768	Addresses weight gain problem, Ki ras mutations no differences between TS and controls; 12 months
B6C3F1	656	30 months, one sex, one dose, thorough pathology, no differences in molecular biology (perhaps "trends") TS/controls
F344 rat	753	Only conclusive rat study; top incidence in females 13% (significant because controls have 0%), males NS; thorough pathology, inc. noses; 30 months.

"To rely on larger and larger experiments involving more and more animals is a gesture of defeat" (W.N. Aldridge, 1973)

The issue at hand

- Will we continue to rely on the phenotype, i.e. in vivo development of lung tumors, for assessment of reduced harm?

- Given what we know how laboratory animals react to tobacco smoke, can we afford a new round of megamouse experiments from a financial standpoint? Let alone ethical considerations? (animal welfare)

- Or should we muster the courage to rely on biomarkers for decision making?

- *However – remember NAC and beta carotene*

The real challenge

- The real challenge will not be to develop more and more animal models.
- The real challenge will be to convince the powers there are that mechanistic research is justified, can be relied upon and is acceptable for decision making. Five decades of "mechanistic" research should pay off! (it already has in explaining that some animal carcinogenesis data are not relevant for human cancers)
- If we continue to rely on the phenotype for the validation of "omics" – why do invest in this kind of research at all?

Ten Reasons to Develop the Strain A/J Mouse Model

- The phenotype (tumor response to tobacco smoke) is well established, incl. dose-response

- Intra-and interlaboratory reproducibility

- 100% incidence allows to study mechanisms on tumor development with some confidence over time, which is manageable (6 to 12 months)

- Group sizes are reasonable

- A/J mice not worse than transgenic, which are much more expensive

Ten reasons (continued)

- Strain A mouse demonstrably not simply a tumor accelerator model

- Other rodents also show more benign tumors than malignant ones, even after 30+ months

- Cells of tumor origin known, can be isolated, and cell lines already available

- Much information available on molecular biology, signal transduction and genetics

- *Therefore: strain A mouse lung tumors are worst possible model except for all the other ones*