Animal testing in toxicology: Does it work?

Thomas Hartung

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Professor of pharmacology and toxicology, University of Konstanz, Germany

Johns Hopkins University
Center for Alternatives to Animal Testing

Promoting for 29 years alternatives to animal testing where they are not fit for purpose

CAAT – the information and communication hub

$

- **Global clearinghouse; 26 member project team**
- **5.000 individual visitors per month, 8.000 fans on facebook**
- **Workshops, info days, stakeholder networks**
- **Lecture and courses, open source**
- **ALTEX, CAATfeed, CAATwalk**
- **Funding program ($7.1 million, 350 grants),**

Creation of CAAT- Europe in 2010

- CAAT is the only transatlantic competence center for 3Rs
- EU excellence center
- US/EU dialogue

You too can be a toxicologist in two easy lessons, each of ten years.

Arnold F. Lehman, FDA

$3 trillion

$3 trillion

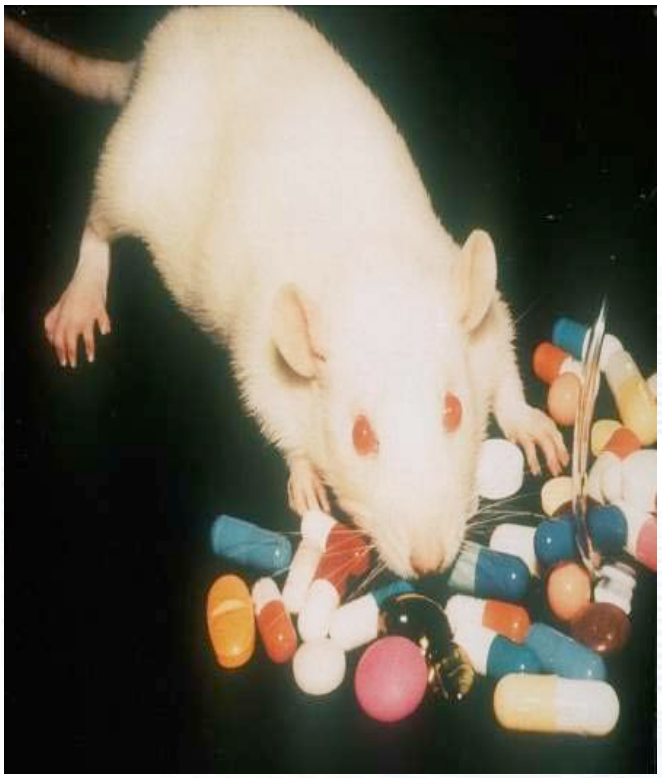
Animal testing at $3 billion per year

$200 billion

$3 trillion

Regulatory Toxicology desperately needs to renew its toolbox

Some limitations of toxicology



- Species differences
- Predictive capacity: false negatives and false positives (precautionary)
- Through-put
- Animal use
- High-dose to low-dose extrapolation
- Poor statistics
- Traditions - little adaptation to scientific progress, not knowledge- and hypothesis-driven
- Not applicable to new products
- Costs
- Lack of scientific control mechanisms

R22 harmful if swallowed

(LD_{50} = 150-200mg/kg in rats)

R 36 irritant to eyes

R 37 respiratory irritant

R 38 irritant to skin

Not carcinogenic,
but co-carcinogen (promotor)

Unclear mutagenicity

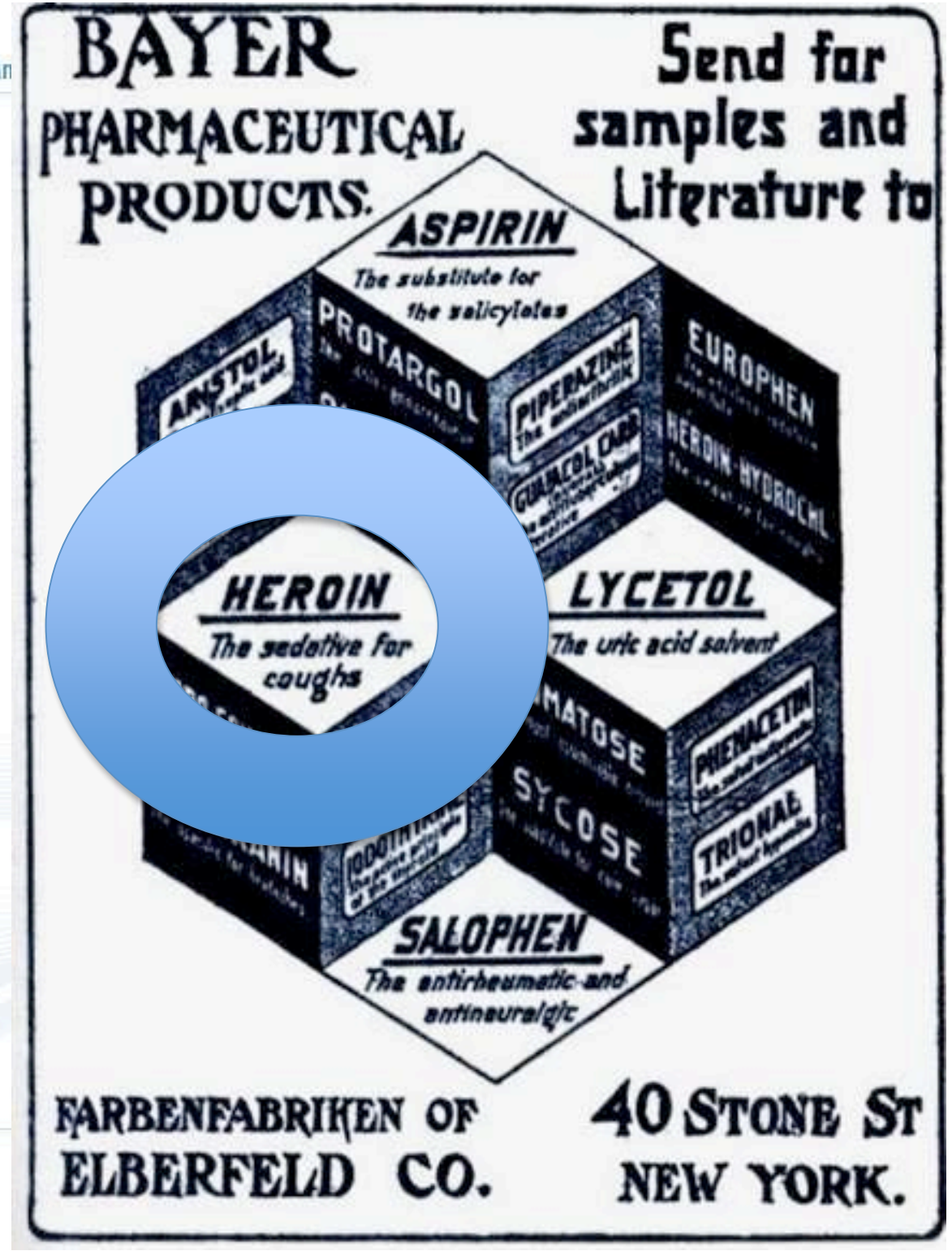
Embryonic malformations in cat, dog, rat, mice, rabbit, monkey

Unlikely to be brought to the market today

Actual use of aspirin

- > one million billion doses taken
- 50,000 tons produced and 35,000 tons consumed per year
- >23,000 scientific papers on aspirin
- 74 percent of the US population regards Aspirin as the eighth wonder of the world
- 840 million $ sales per year (35-40% in US)
- Britons: average 70 per person per year
- Even used for pre-eclampsia in pregnancy

There is some good reason for regulating new products….



Some chemicals produce unknown health effects

the problem:

- **70 million chemicals synthesized**
- **100.000 chemicals in consumer products and environment**
- **2-3.000 chemicals extensively tested**
- **mixtures?**
- **natural substances?**

Forerunner REACH

picture
© ChemSec

Originally expected:

- **180.000 pre-registrations by about 27.000 companies**
- **30.000 substances**

State of the play 12'08:

- **> 2,7 million pre-registrations by about 65.000 companies**
- **144.000 substances**

Hartung&Rovida, Nature 2009

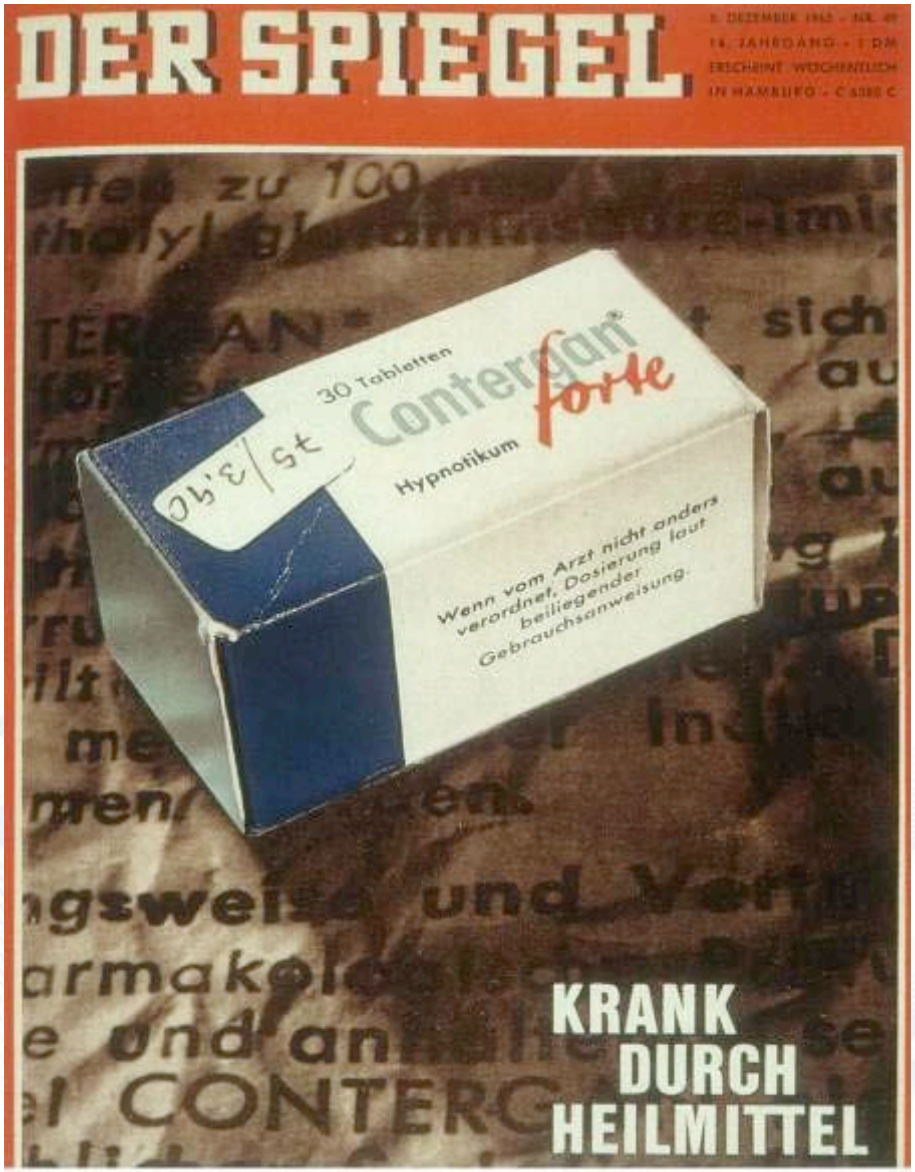
- **68.000+ chemicals**
- **> 54 mill. animals**
- **> 9 bill. €**

Toxic Substance Control Act

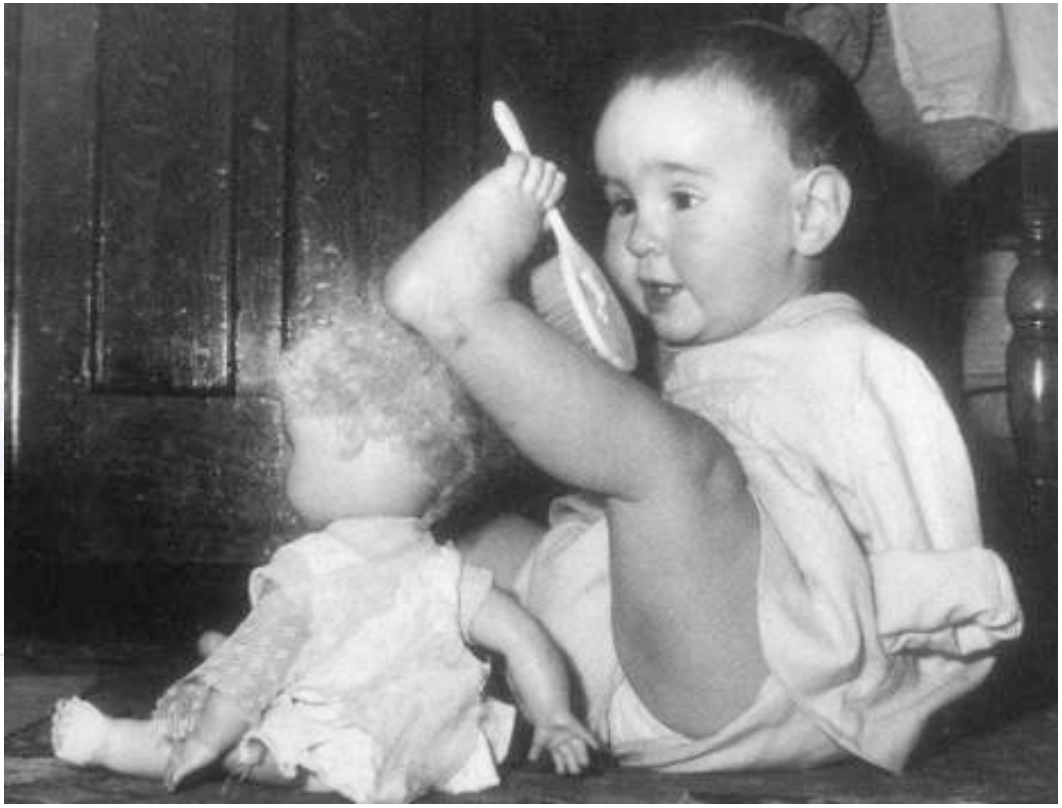
(1976, no major amendments; regulates manufacture of chemicals in commerce)

- **Screening of existing chemicals**
- **Burden of proof with EPA**
- **Original TSCA inventory**
  – 55,000 'old' chemicals
- **Today's TSCA inventory**
  – 88,000 chemicals

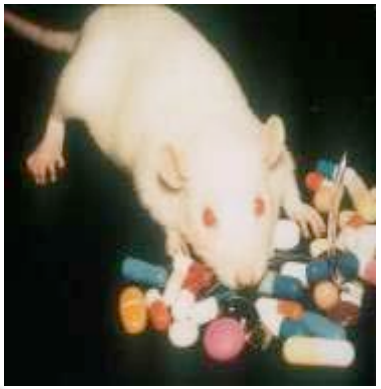
- **Pre-marketing notification for new chemicals**
  –Only 15% contain toxicology data
  –24,000 received only for 200 EPA required more testing



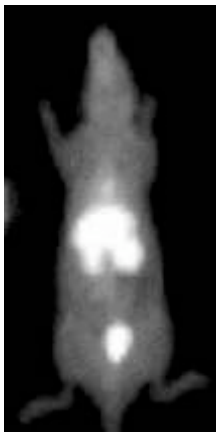
Titelbild des Nachrichtenmagazins »Der Spiegel« vom 5. Dezember 1962



Interspecies prediction of cancer



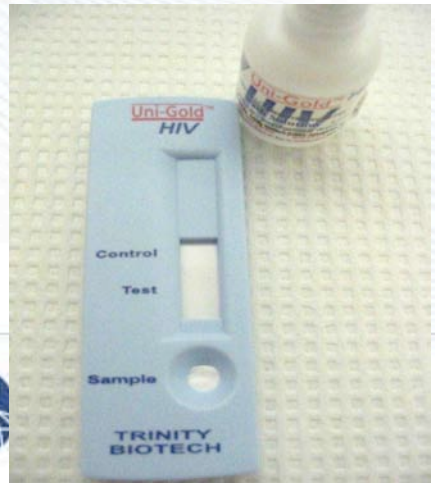
Correlation 57%



Animal test	**Cancer**
	18-24 months
	$1-1.5 million
	600 animals
	53% positive*
Estimate human	**5-20% positive**

*Ames&Gold Mut.Res 2000

Diagnosis: toxic! - Trying to apply approaches of clinical diagnostics and prevalence in toxicology considerations



Thought starter:

Healthy European without HIV risk factors: Prevalence of infection is 1:10.000

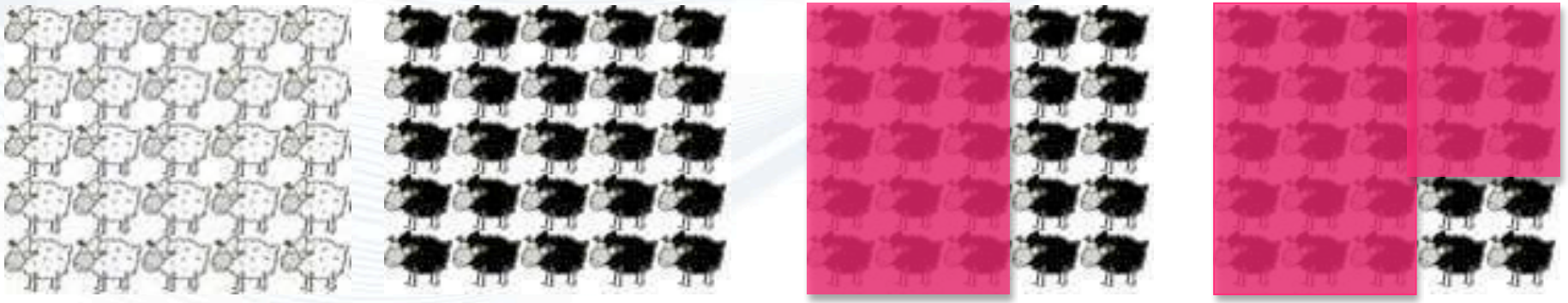
The result of 99.9% accurate test is positive

Testing 10.000 people with this test will result in 1 real-positive but 10 false-positive

Probability of HIV infection: 1/11 = 9%

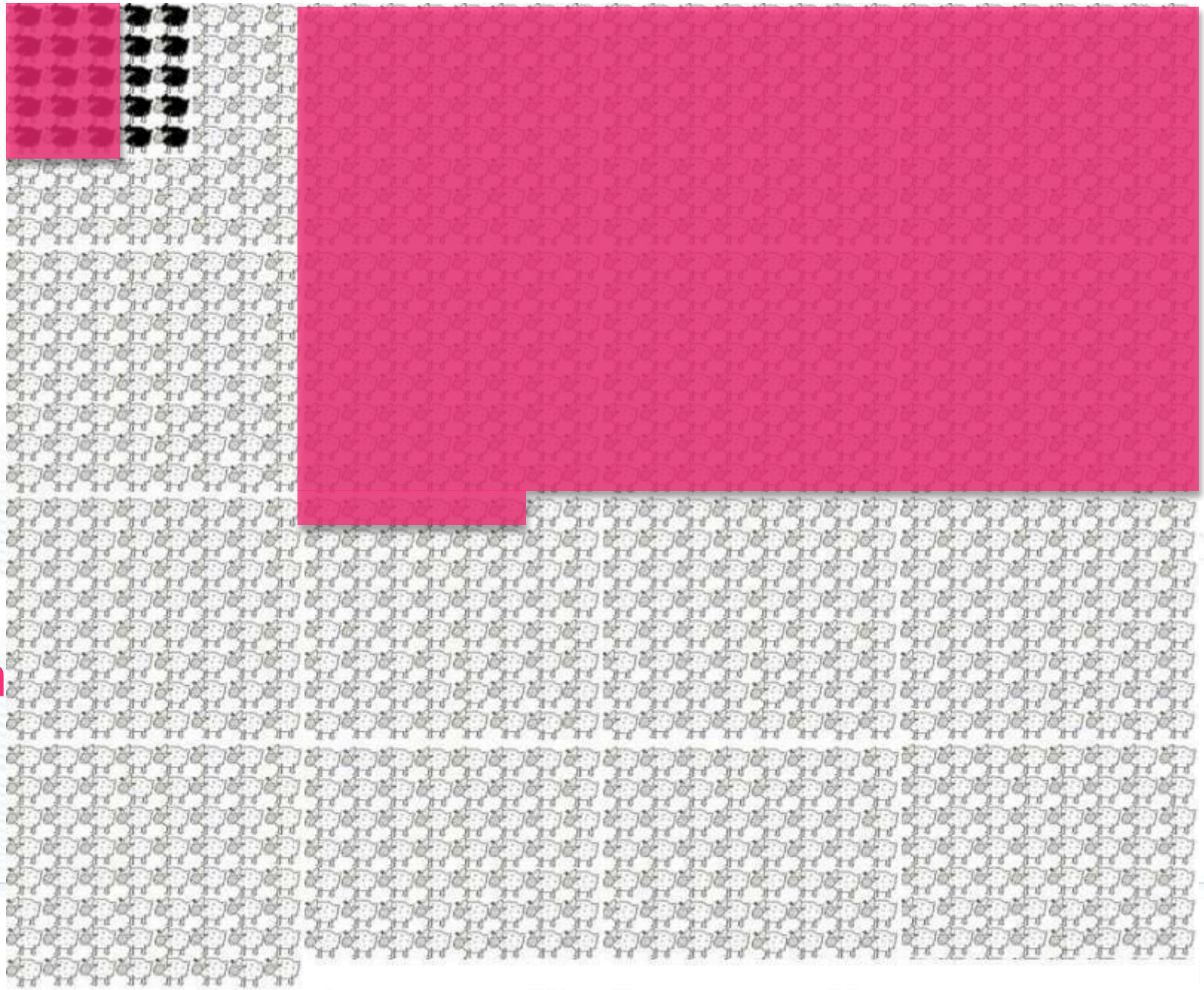
Example reprotox study in two species to find "black sheep" among chemicals

Test substances **60% 1st species** **60% 2nd species**



The real situation: black sheep are rare

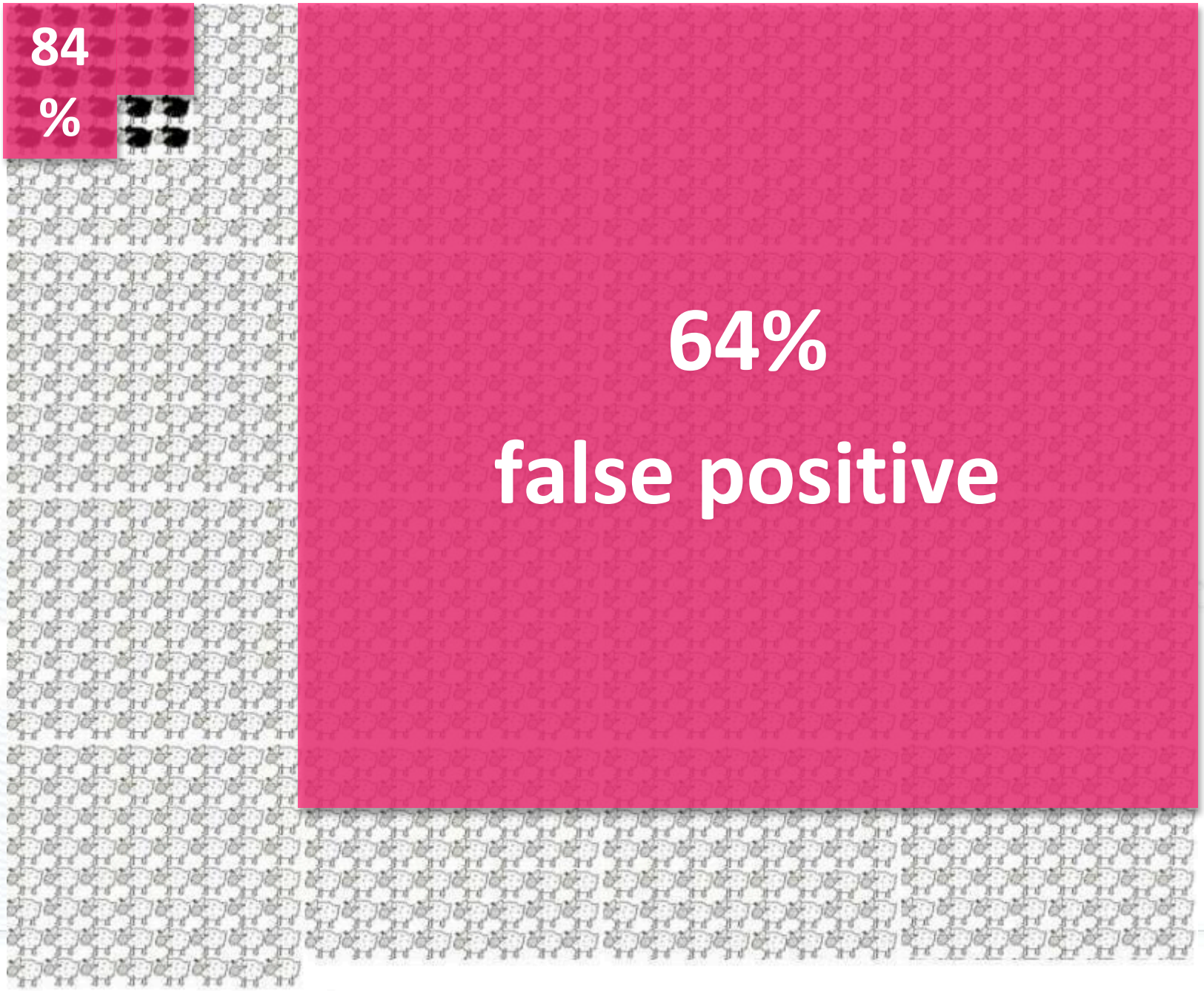
2-3% reproductive toxicants among chemicals



1st Species Two-gen positive results

Plus 2nd Species One gen

Pos. results



Hartung T. Nature 2009, 460:208-212.

We forget the compromises made when introducing tests over time

Learning from experience may be nothing more than learning to make the same mistakes with increasing confidence.

Petr Skrabanek, James McCormick

Follies and Fallacies in Medicine
Tarragon Press, Glasgow, 1989

From a presentation
by G. Daston, P&G, 2009

EVB
rct. EAI

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
FOOD AND DRUG ADMINISTRATION
WASHINGTON, D.C. 20204

March 1, 1966

Procter and Gamble Company
Ivorydale Technical Center
Cincinnati 17, Ohio

Attention: Dr. Fred H. Snyder

Gentlemen:

During the past several years following the thalidomide episode, we have been recommending a study designed to determine the potential of drugs for producing adverse effects on the reproductive process. The guidelines for this study reflected a modification of a test used for many years by the food industry to provide evidence of safety of food additives. The introduction of the two-litter test appeared to offer a reasonable approach to the over-all problem of assessing the safety of drugs on reproduction. It was anticipated that the two-litter test would prove an adequate screening procedure for the elucidation of adverse effects of a new drug on the reproductive process and that such effects could be subjected to a critical evaluation.

modifications be necessary, they can be instituted earlier. Of paramount importance, of course, is that studies designed along the lines of our new recommendations should yield more meaningful data upon which to base an evaluation of safety.

It must be realized that even these improved guidelines reflect merely the "state of the art" at the present time, and undoubtedly further modifications will be needed in the future as additional knowledge in this area is developed. We hope these suggestions will prove helpful.

Sincerely yours,

Edwin I. Goldenthal

Edwin I. Goldenthal, Ph.D.
Chief, Drug Review Branch
Division of Toxicological Evaluation
Bureau of Scientific Standards
and Evaluation

Enclosure

Outdated technologies when safety is at stake?

20ies: LD_{50} for acute toxicity

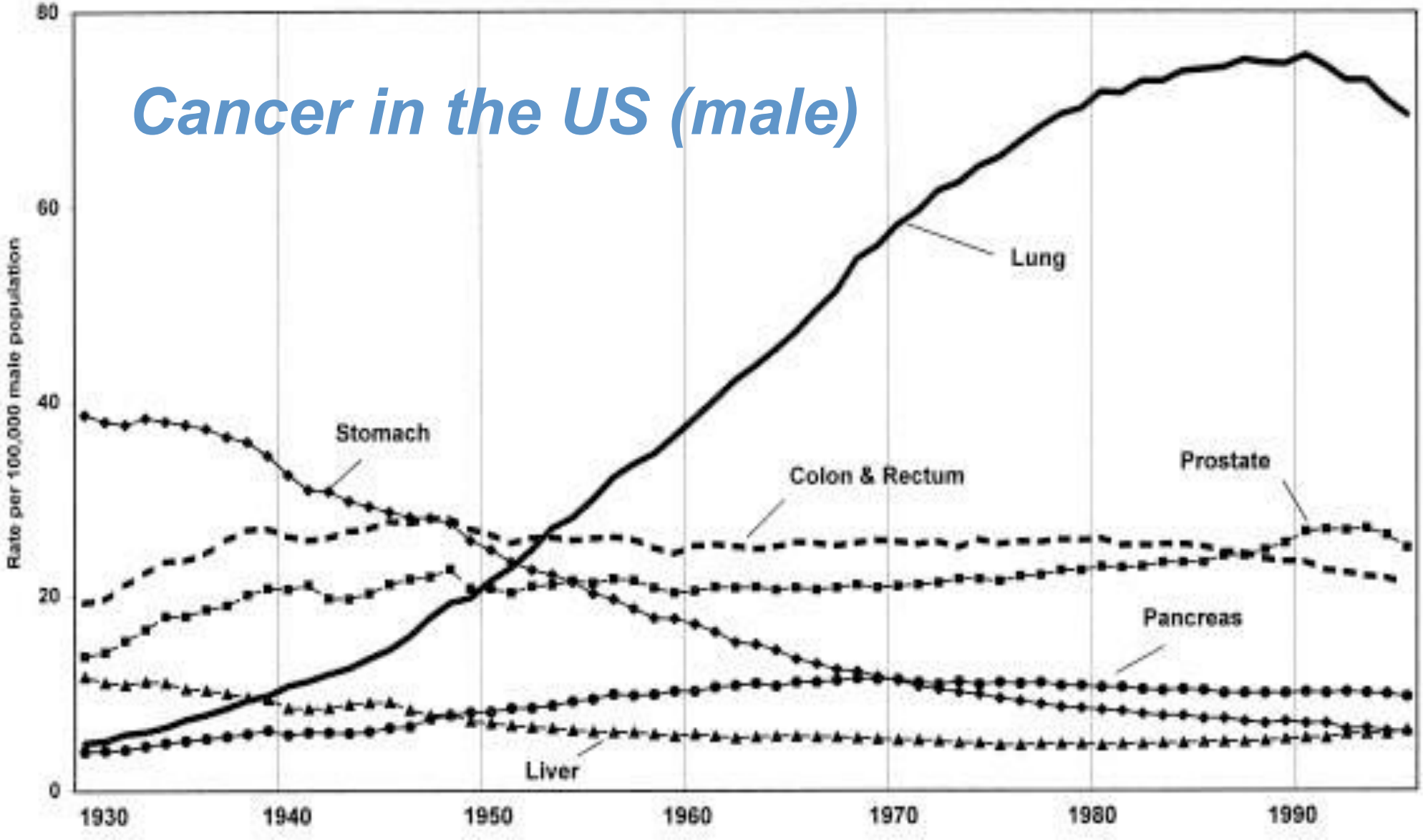
30ies: chronic toxicity

40ies: eye and skin irritation

60ies: reproductive tox.

70ies: cancer

Cancer in the US (male)

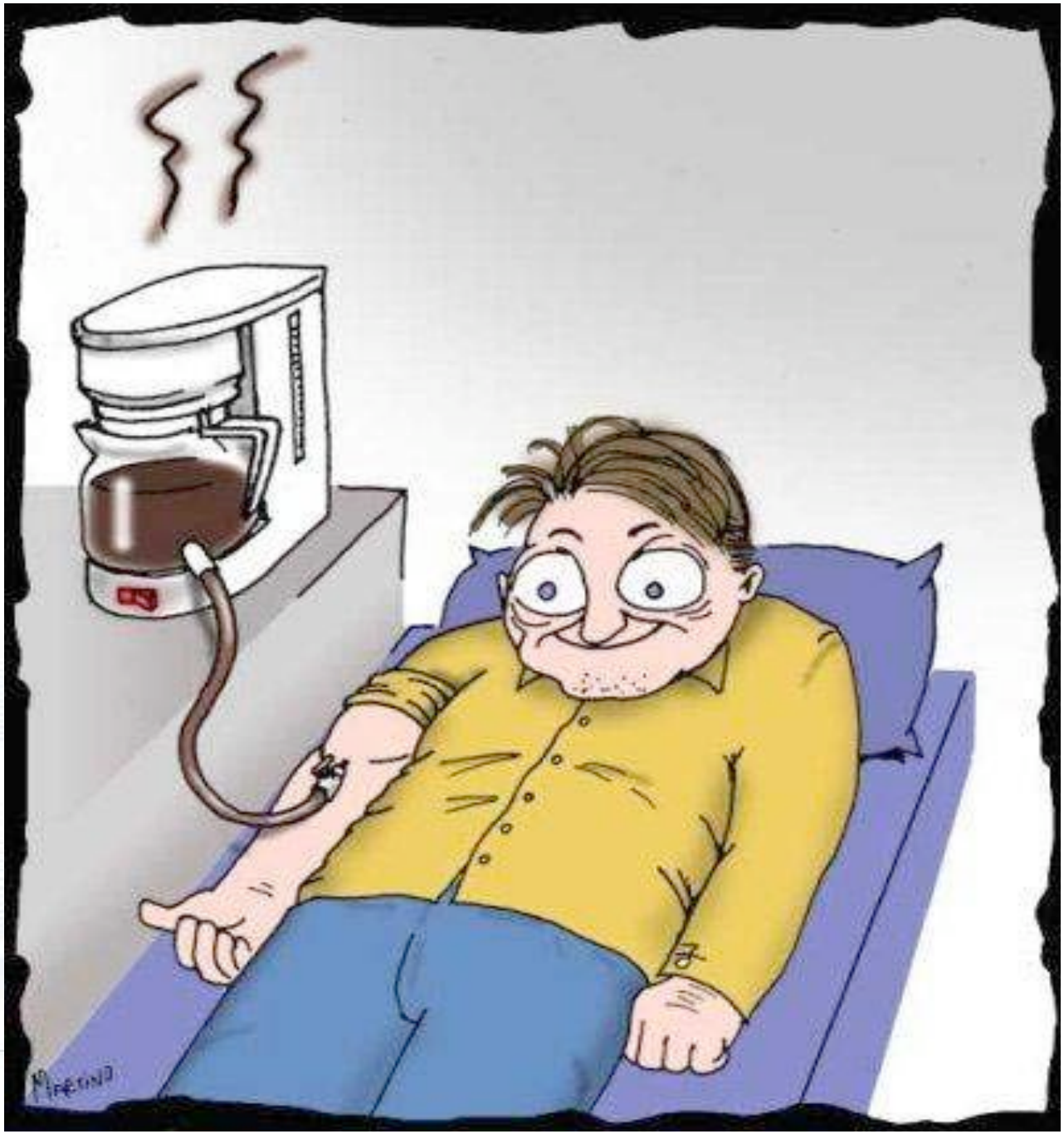


Cancer bioassay results

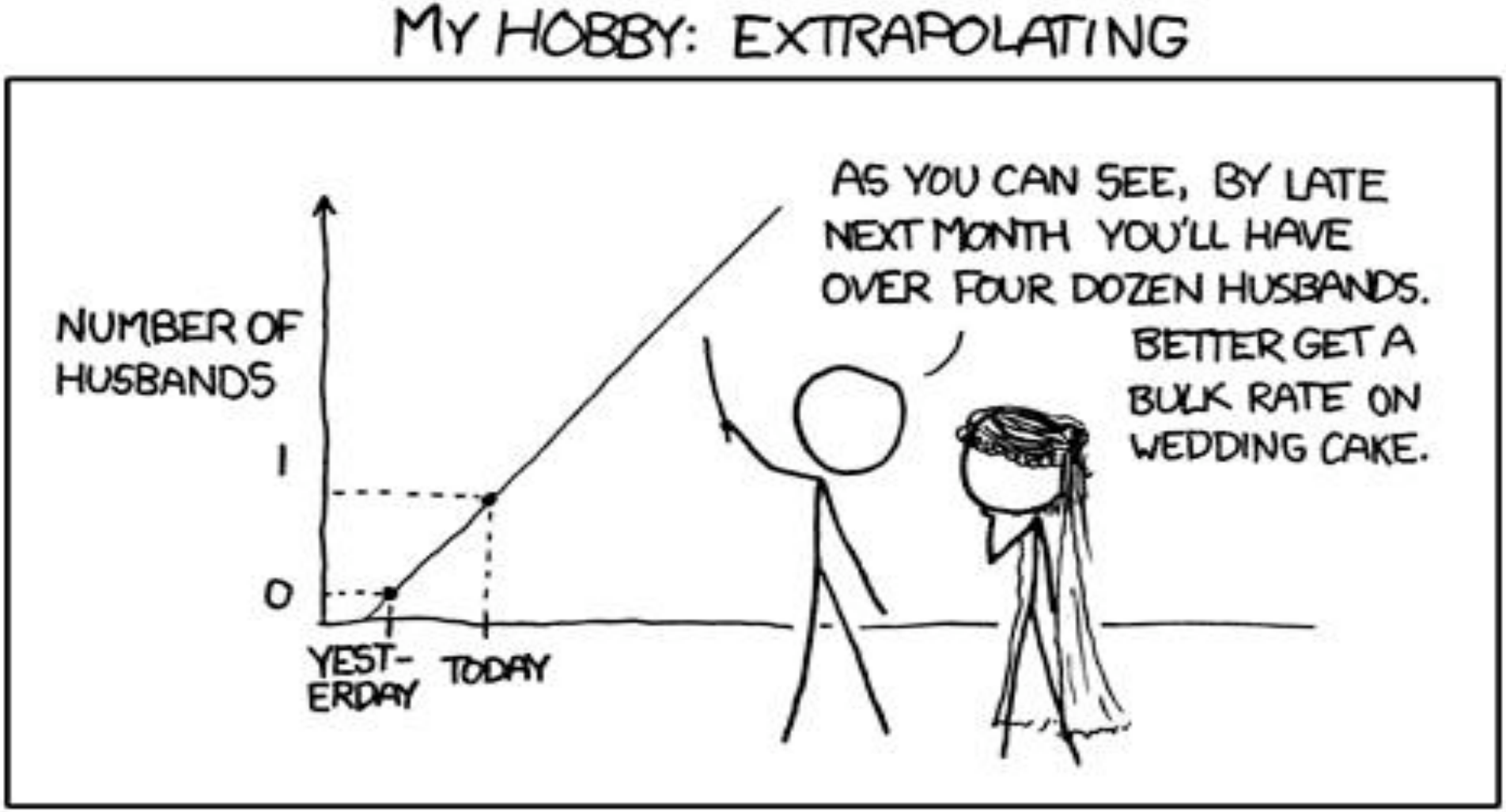
[Ames & Gold 2000]

	Proportion	% pos
Chem. tested in rats and mice	350 / 590	59 %
- natural	79 / 139	57 %
- synthetic	271 /451	60 %
Chem. tested in rat or mice	702 / 1348	52 %
- Natural pesticides	37 / 71	52 %
- Chem. in coffee	21 / 30	70 %
- Mold toxins	14 / 23	61 %
Drugs (PDR)	117 / 241	49 %
Drugs (FDA)	125 / 282	44 %

Despite various carcinogens, coffee drinking is actually reducing liver cancer



Human Exposure vs. Test Doses



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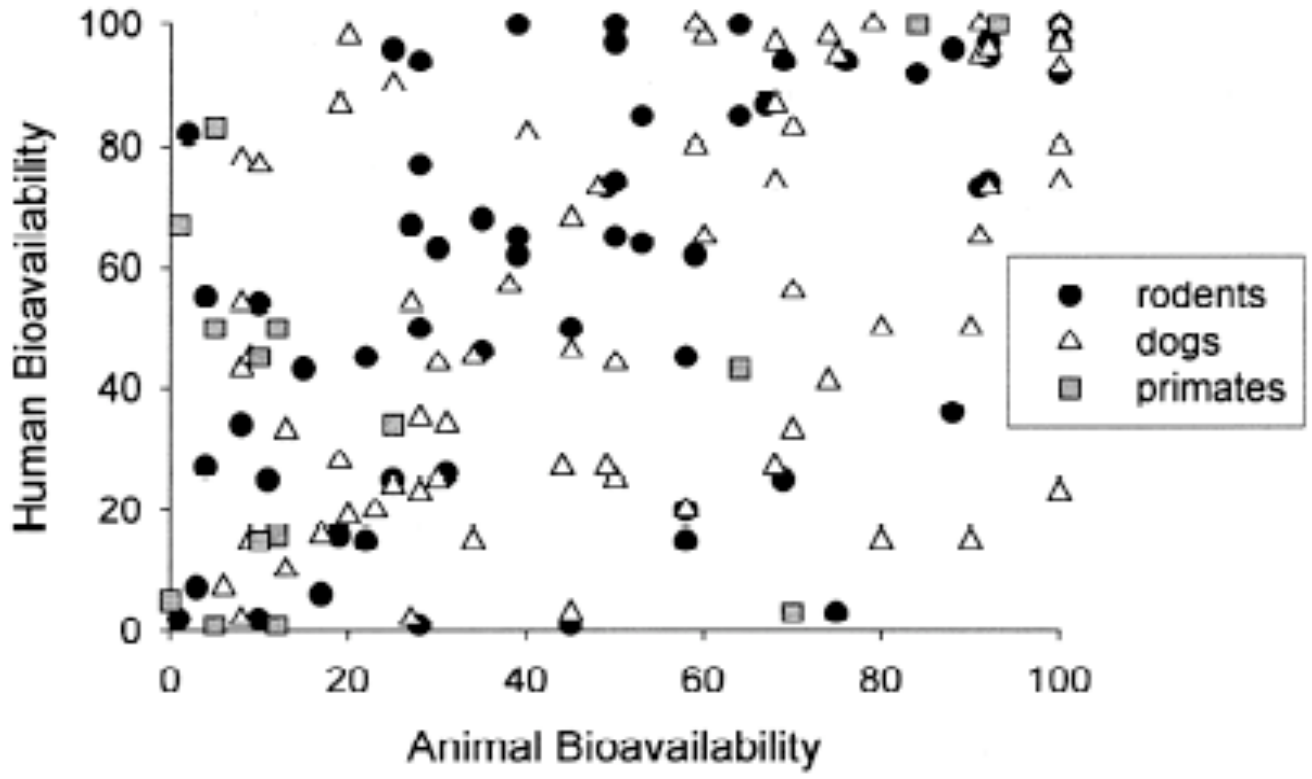
How can we do quantitative risk assessment, if already oral bioavailability differs dramatically?



Grass GM and Sinko PJ. Adv Drug Delivery Rev 2002, 43:433-451

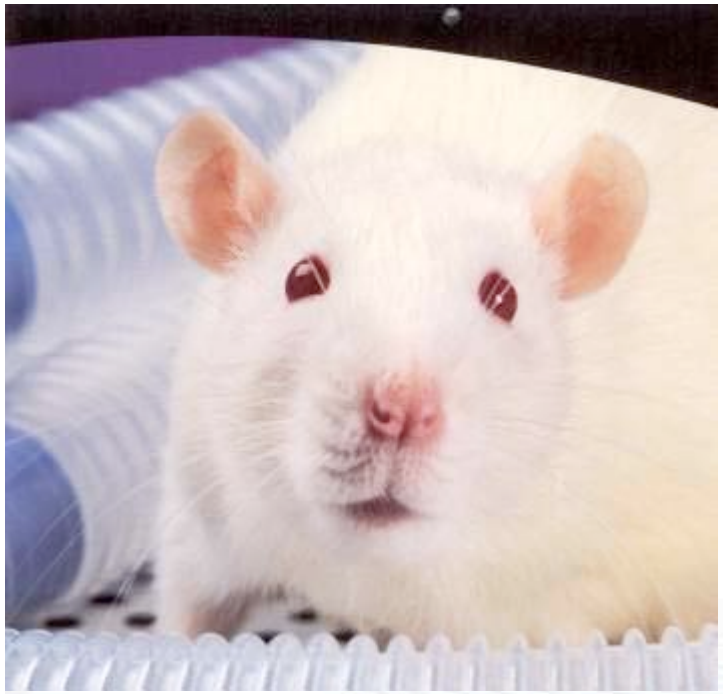
Testing multiple statistical hypotheses resulted in spurious associations: a study of astrological signs and health

PC Austin et al., J. Clin. Epid. 59, 964-969, 2006

Study:
All 10,674,945 residents of Ontario (18-100 years) in 2000.
Randomly assigned to equally sized derivation and validation cohorts and classified according to their astrological sign.
Derivation cohort searched for 223 of the most common diagnoses.
Results:
24 associations tested in validation cohort:
Leo ➔ gastrointestinal hemorrhage (P=0.0447)
Sagittarians ➔ humerus fracture (P=0.0123)

Conclusions: Testing of multiple, non-prespecified hypotheses increases the likelihood of detecting implausible associations.

In toxicology: 28d study ➔ 40 endpoints, cancer bioassay ➔ 60 endpoints
two-generation study ➔ 80 endpoints



We can not model all known human carcinogens in animals:

- **no animal model of cigarette smoke induced lung cancer,**
- **no rodent leukemia by benzene, and**
- **no genetic mutations in animals by arsenic**

[Silbergeld, 2004]

Problems of the cancer assay

- **Maximum tolerated dose (up to 10% of animals die from direct toxicity) = necrosis = inflammation = promotion**
- **Multiple testing: >60 endpoints**
- **Cost and through-put: Europe: in 30 years 14 of 4.500 new chemicals tested**
- **57% concordance of different protocols**
- **Variability: 200 instead of 50 animals, would mean 92% instead of 53% of substances positive [Gaylor 2004]**

- ***A chemical which is not positive has not been tested long enough.***

Regulatory "over-kill"?

- **TCDD (human carcinogenicity unclear)**
  regulated on high-dose animal data at 6fg/day "reference dose" (formerly "acceptable dose limit")
  comparison to alcohol:

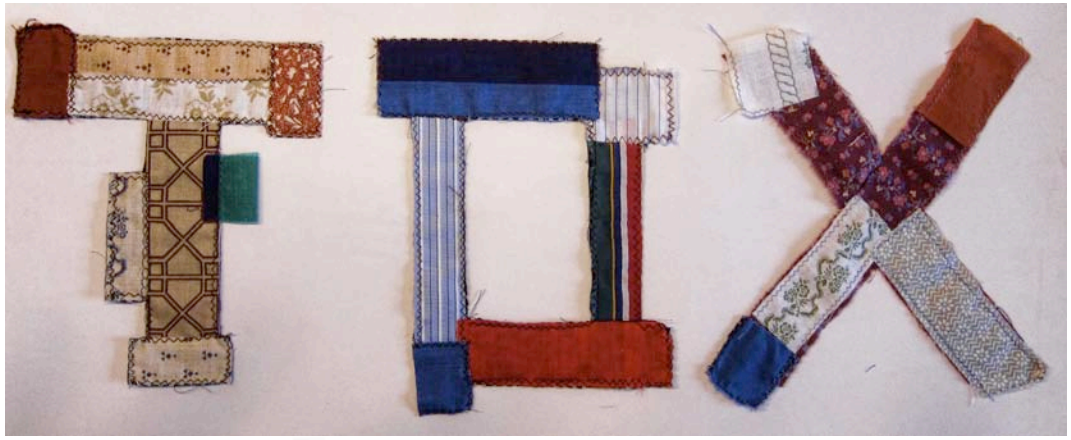
  one beer in 345 years

  [Ames et al., 1990]

Chemophobia?

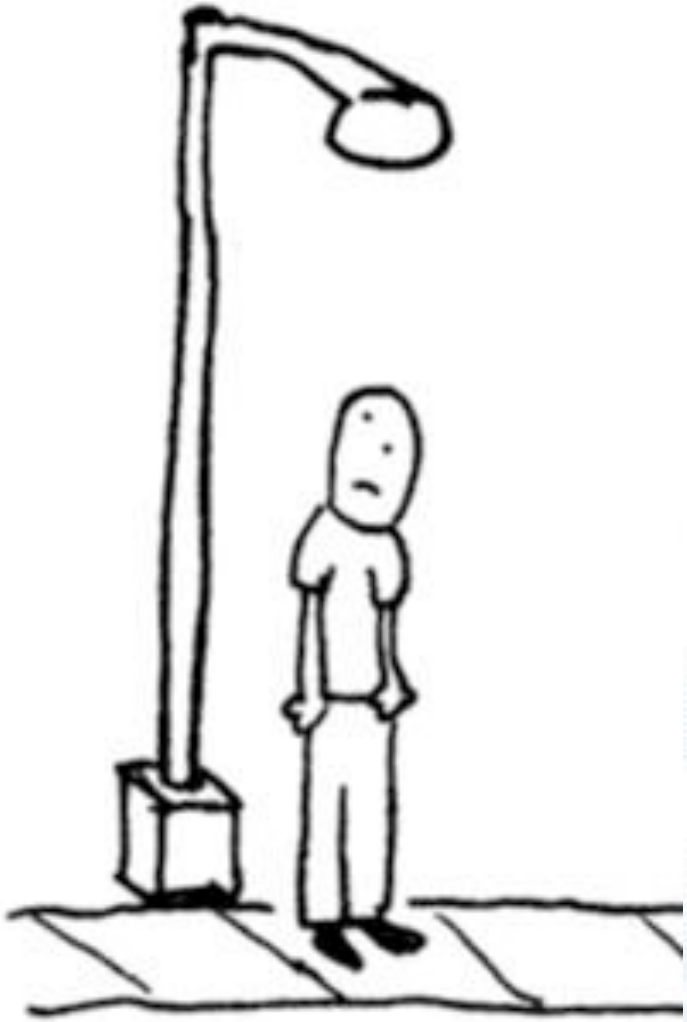
Chemicals are estimated to be cause of 2% of cancer cases

The dose makes the poison



The evolution of toxicology: patchwork

- **Every scandal gives one patch.**
- **Many patches are 50-80 years old.**
- **No way to remove a patch.**
- **Difficult to integrate new technologies.**
- **Every patch is of its own appearance and workmanship.**



A man is looking for his keys under the street light in the night.

“Did you lose them here?”

“No, but here I have light!”