# RELATIONSHIP BETWEEN COMPOSITION AND TOXICITY OF ENGINE EMISSION SAMPLES

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# BACKGROUND

## CHALLENGES:

**What are *really* the most important emissions to control (and to avoid in technology choices)?**

**Can we estimate the relative health *hazards* of different emissions from knowledge of their *composition*?**

## OPPORTUNITIES:

**We had:** **7 emission samples**

**Detailed physical-chemical characterization**

**Relative lung toxicity and mutagenicity data**

**We knew:** **Ingvar Eide at Statoil had succeeded in relating complex chemistry of petroleum samples to mutagenicity with *good predictive ability***

**We tried:** **Using Ingvar's approach to relate composition of the 7 samples to their relative toxicity**

# MUTAGENIC AND INFLAMMATORY POTENCY OF DIESEL & GASOLINE EMISSION SAMPLES WAS RANKED

(Seagrave et al. *Toxicol. Sci.* 70: 212-226, 2002)

1. PM and vapor-phase SVOCs collected on chassis dynamometer
2. Combined the 2 phases in original mass ratio for testing
3. Instilled into rat lungs and measured inflammation at 24 hours
4. Measured mutagenicity in bacteria (Ames TA98 and TA100 ± S9)

**Samples (7):**

| | |
|---|---|
| Gasoline (5) | G |
| Gasoline 30$^{o}$ | $G_{30}$ |
| White smoker gas. | WG |
| Black smoker gas. | BG |
| Diesel (3) | D |
| Diesel 30$^{o}$ | $D_{30}$ |
| High-emitter diesel | HD |

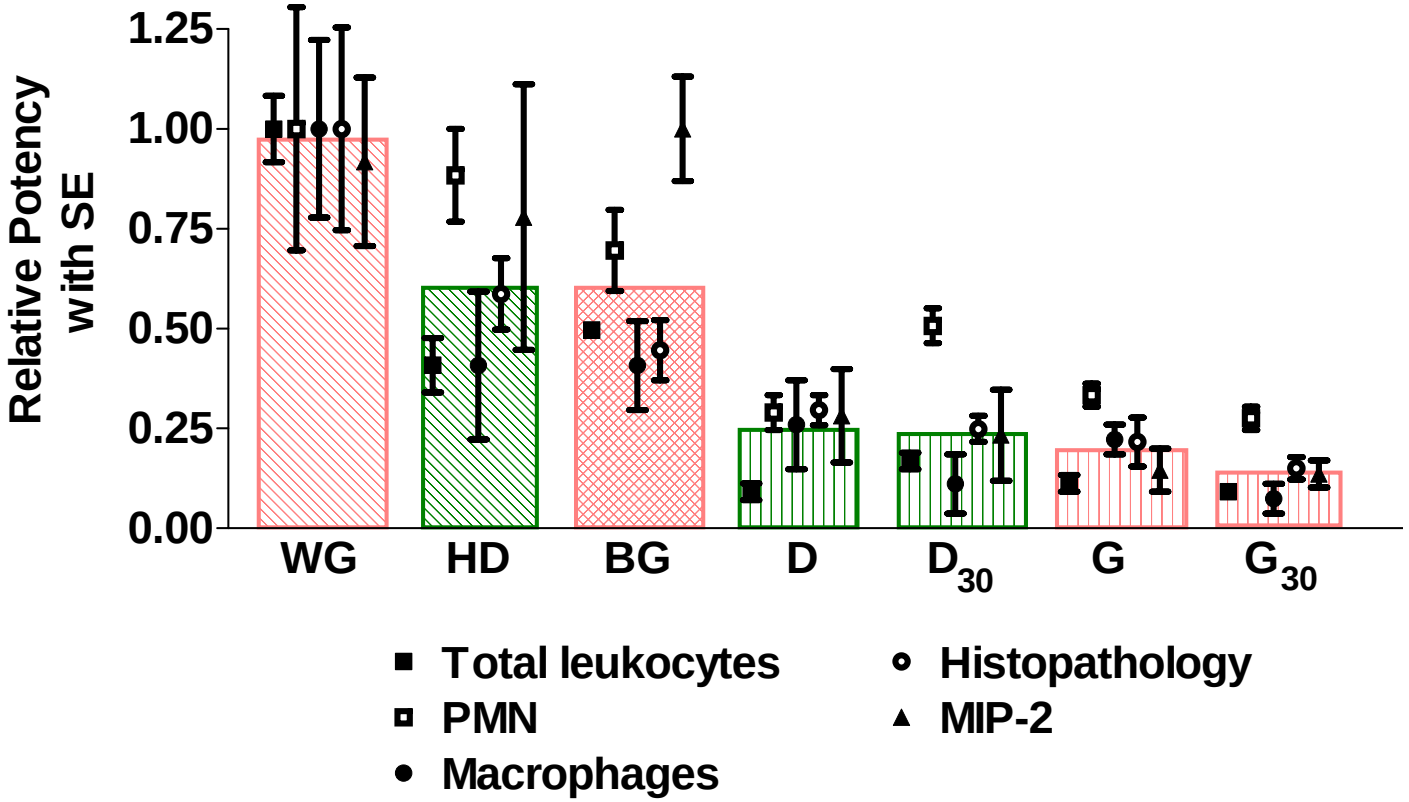
**Measures of inflammation:**

- Lung Lavage
  - Total leukocytes
  - PMNs
  - Macrophages
  - MIP-2
- Histopathology

# SAMPLES HAD A 5-FOLD RANGE OF LUNG TOXICITY PER UNIT OF MASS

## E.g., five measures of inflammation gave good agreement

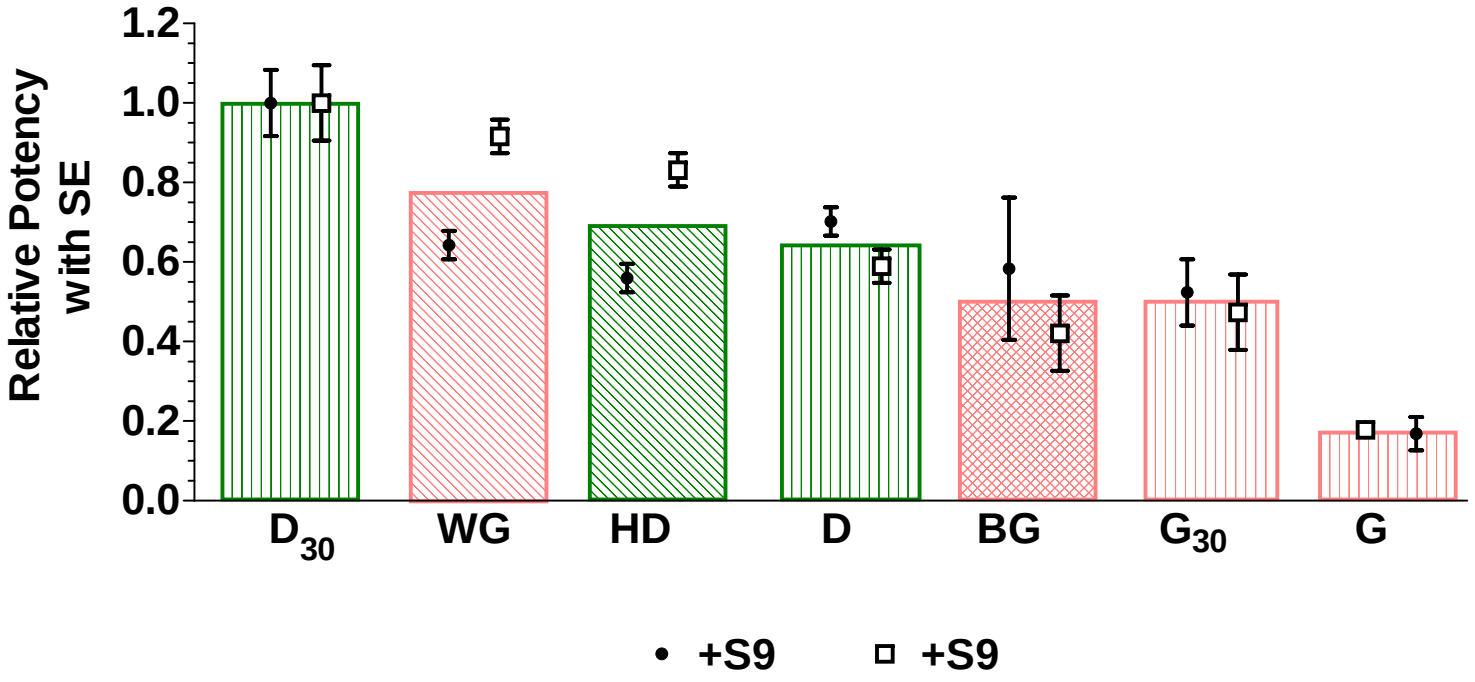
1. High-emitters not only contribute more mass, but are also more toxic per unit of mass
2. Normal-emitter diesel and gasoline have very similar toxicity per unit of mass



# SAMPLES ALSO HAD A 5-FOLD RANGE OF MUTAGENICITY, BUT WITH DIFFERENT RANKING THAN LUNG TOXICITY

## E.g., rankings by TA100 ± S9

1. Cold diesel highest, normal gasoline lowest
2. Not much difference among others



# RELATIONSHIPS BETWEEN COMPOSITION AND RESPONSE WERE EVALUATED BY PCA/PLS

**Strategy used successfully by Ingvar Eide, Statoil, to identify chemical compounds in petroleum samples driving mutagenicity**

- **PCA = Principal component analysis**
- **PLS = Partial least squares regression (aka: "projection to latent surfaces")**
- **Simca 10.0® software (Unimetrics)**

**Finds relationships between dependent (toxicity) and predictor (composition) variables by regression techniques involving simultaneous projection of Y and X variables to multi-dimensional planes**

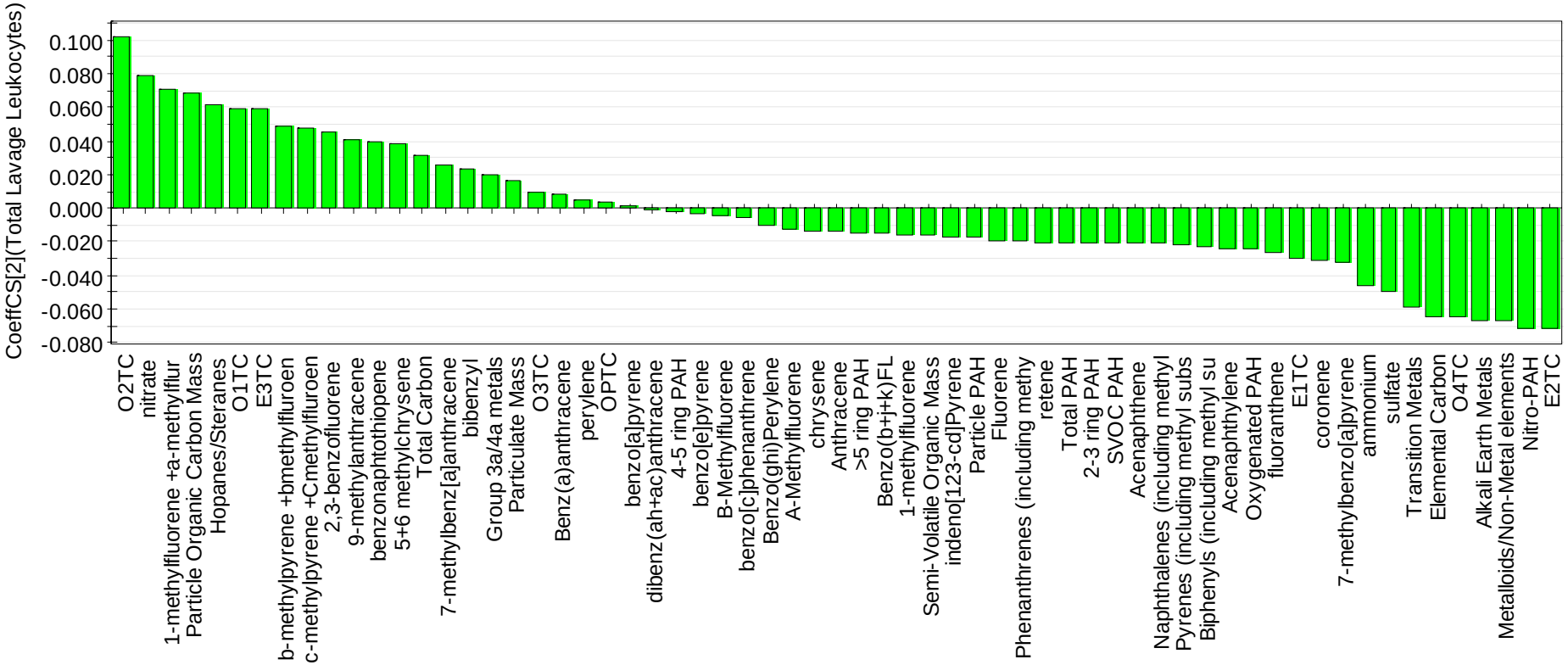
- **Minimizes key problems:**
  1. **Inter-correlated composition variables**
  2. **Greater number of variables than samples**

**PLS can be used to develop predictions of effect from composition**

- **Determines which, and number of, composition variables giving best prediction of the differences in toxicity**

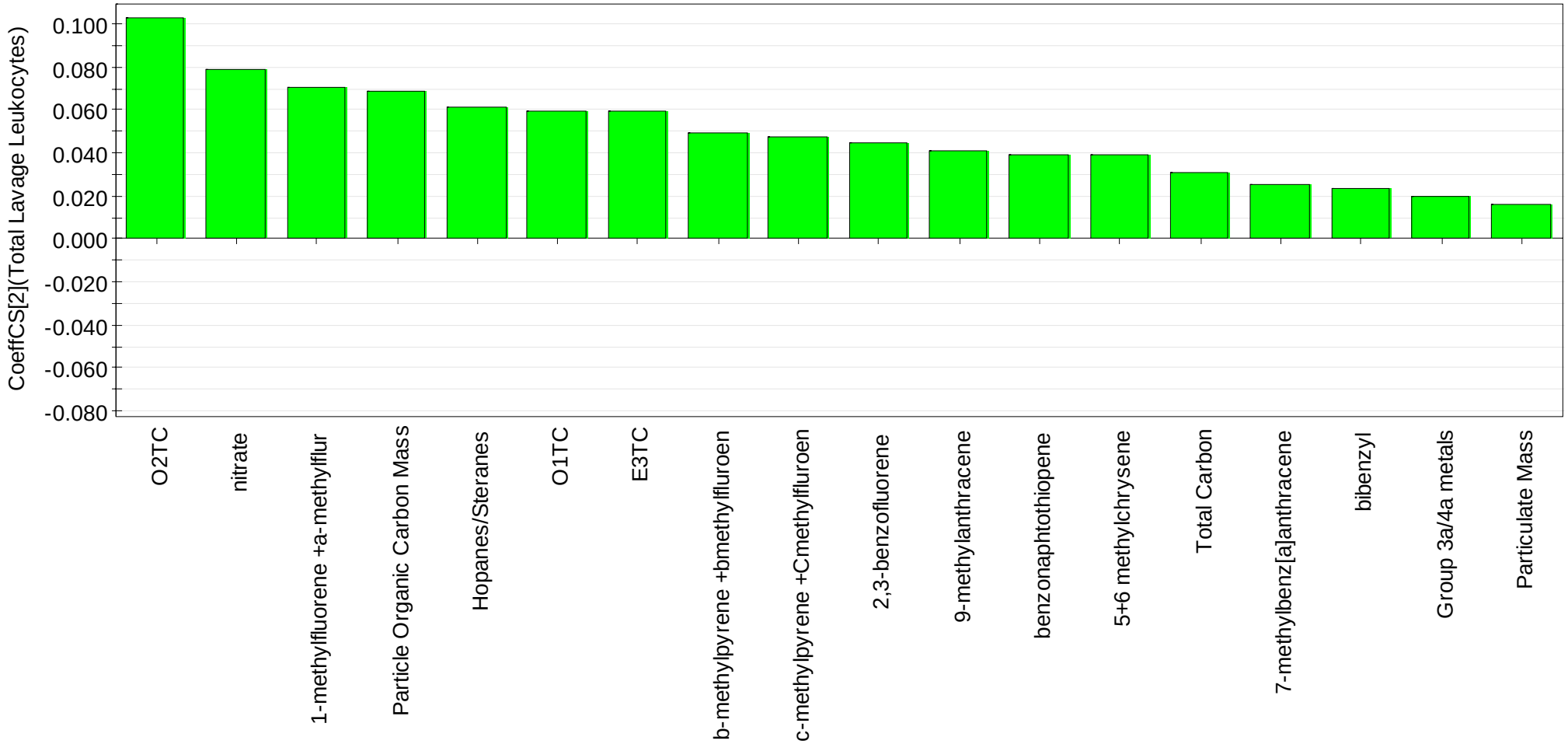
# RELATIVE ASSOCIATIONS BETWEEN PHYSICAL-CHEMICAL PROPERTIES AND INFLAMMATORY RESPONSE

## E.g., total lung lavage inflammatory cells

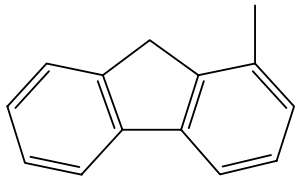



- **Shows classes & compounds varying most strongly with toxicity**
- **Does <u>not</u> prove causality**

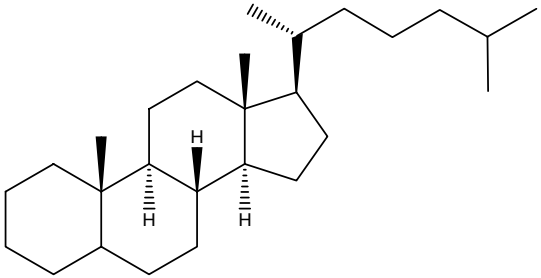
# TOP 18 PHYSICAL-CHEMICAL PROPERTIES TRACKING WITH INFLAMMATION



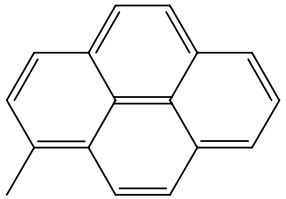
# ORGANIC SPECIES HAVING HIGHEST CORRELATION WITH INFLAMMATION



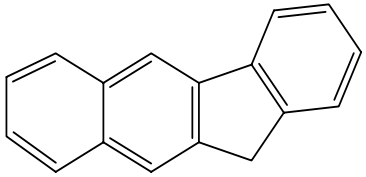
1-Methylfluorene
mw. 216



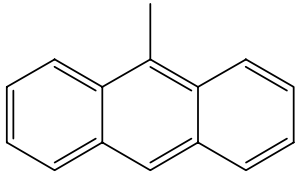

Representative Sterane
m.w. 372



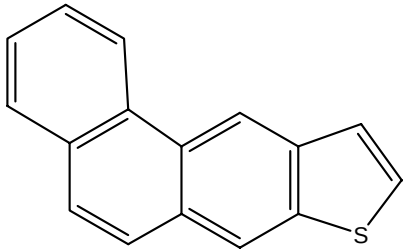
7-Methylpyrene
m.w. 216



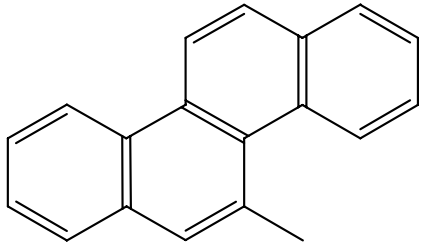
Benzofluorene
mw. 220



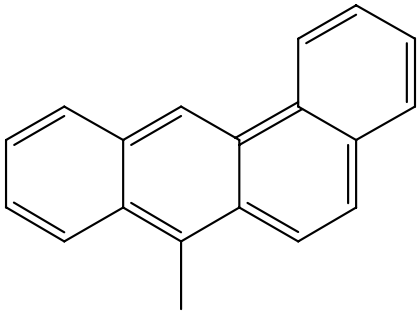
9-Methylanthracene
m.w. 192



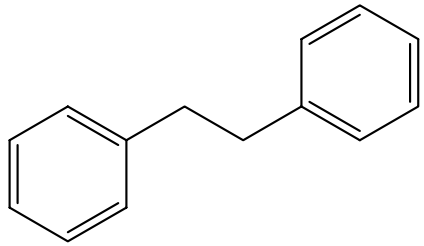

Benzonaphtothiopene
m.w. 234



5-Methylchrysene
m.w. 242



7-Methylbenz(a)anthracene
m.w. 242



Bibenzyl
m.w 182

**[SVOCs range ≈ from naphthalene (MW 128) to benz(a)anthracene (MW 228)]**

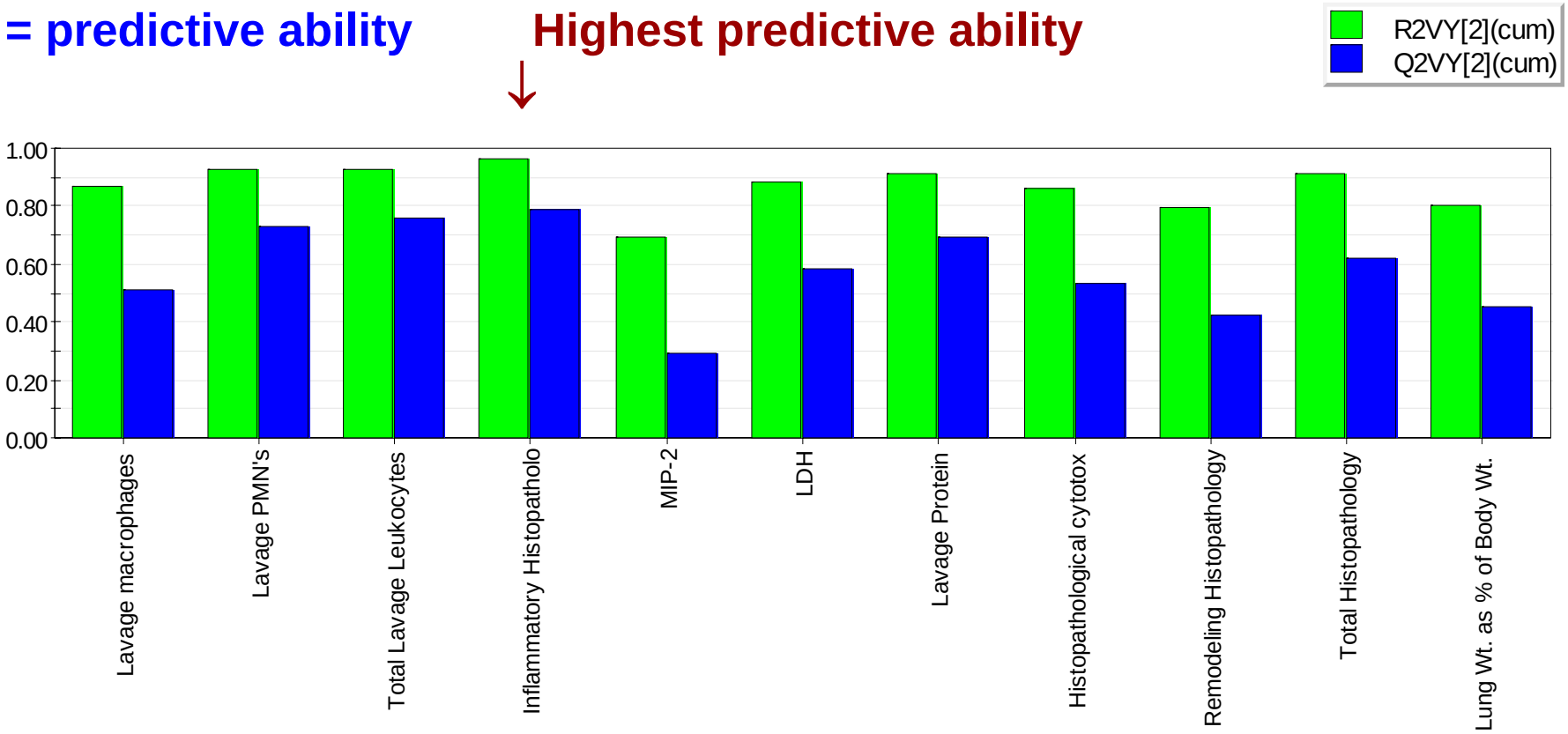
# QUALITY OF MODEL
# USING 63 COMPOSITION VARIABLES TO PREDICT EACH OF 11 LUNG TOXICITY PARAMETERS

**$R^2$ = goodness of fit**

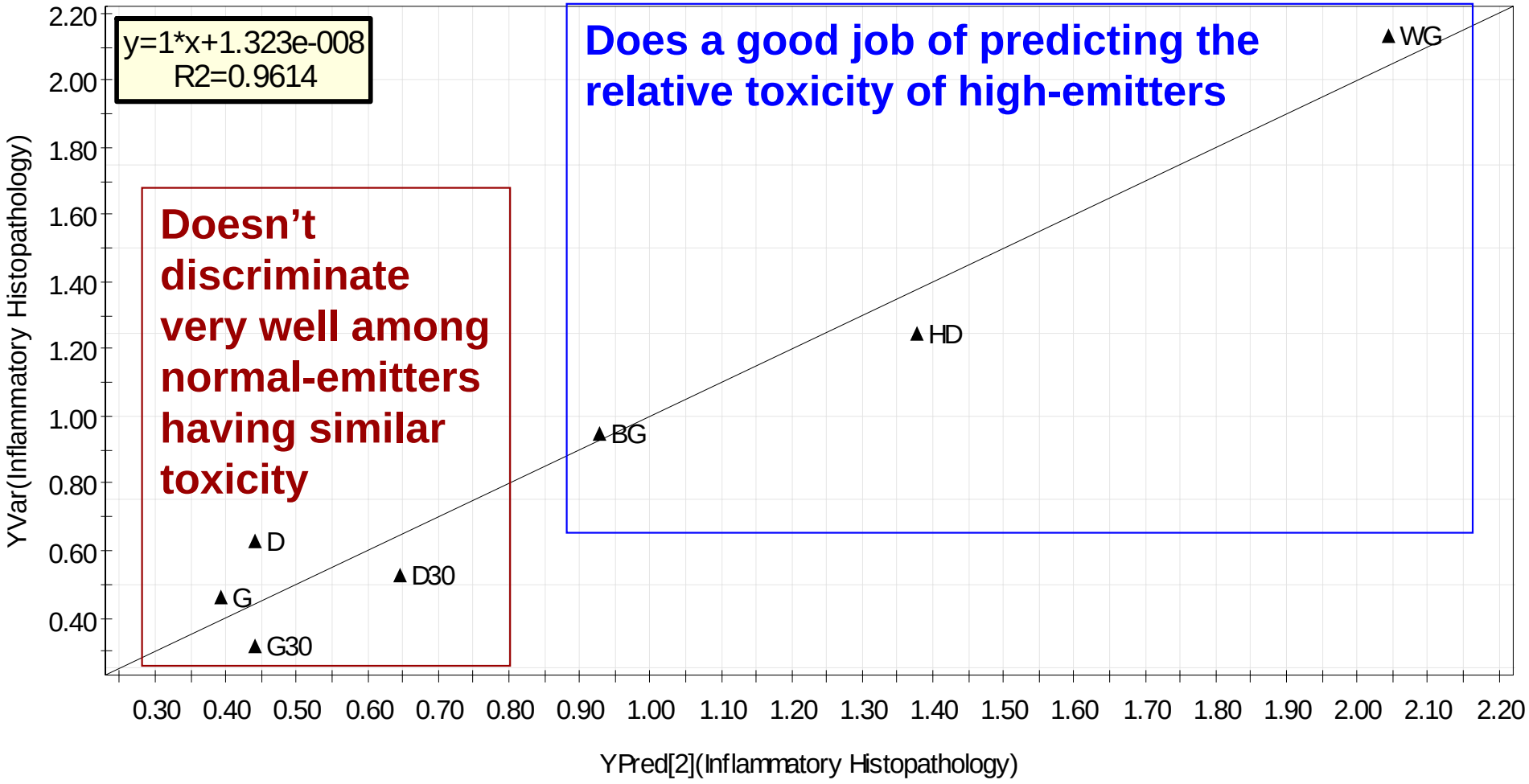
**$Q^2$ = predictive ability**

**Best fit**

**Highest predictive ability**

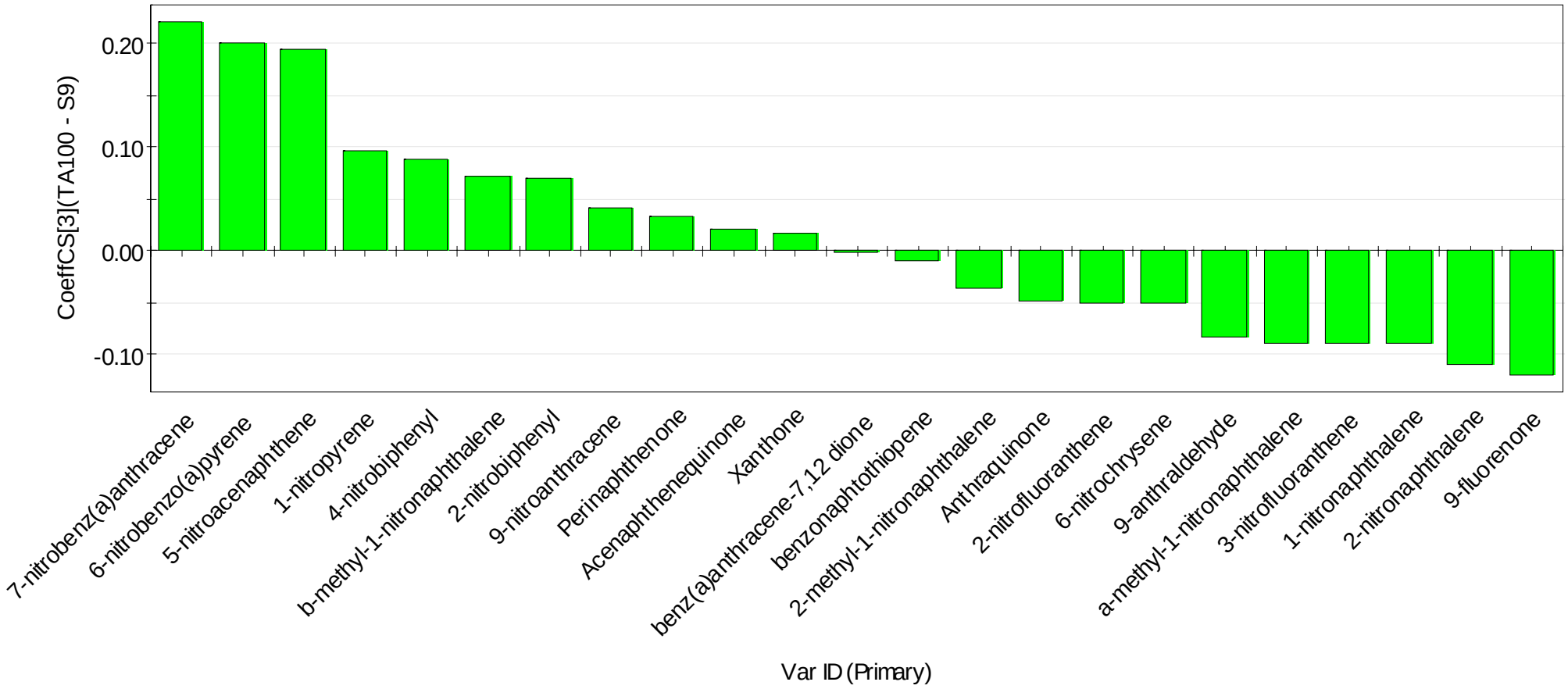


# OBSERVED vs PREDICTED RELATIVE HISTOPATHOLOGICAL EVIDENCE OF INFLAMMATION



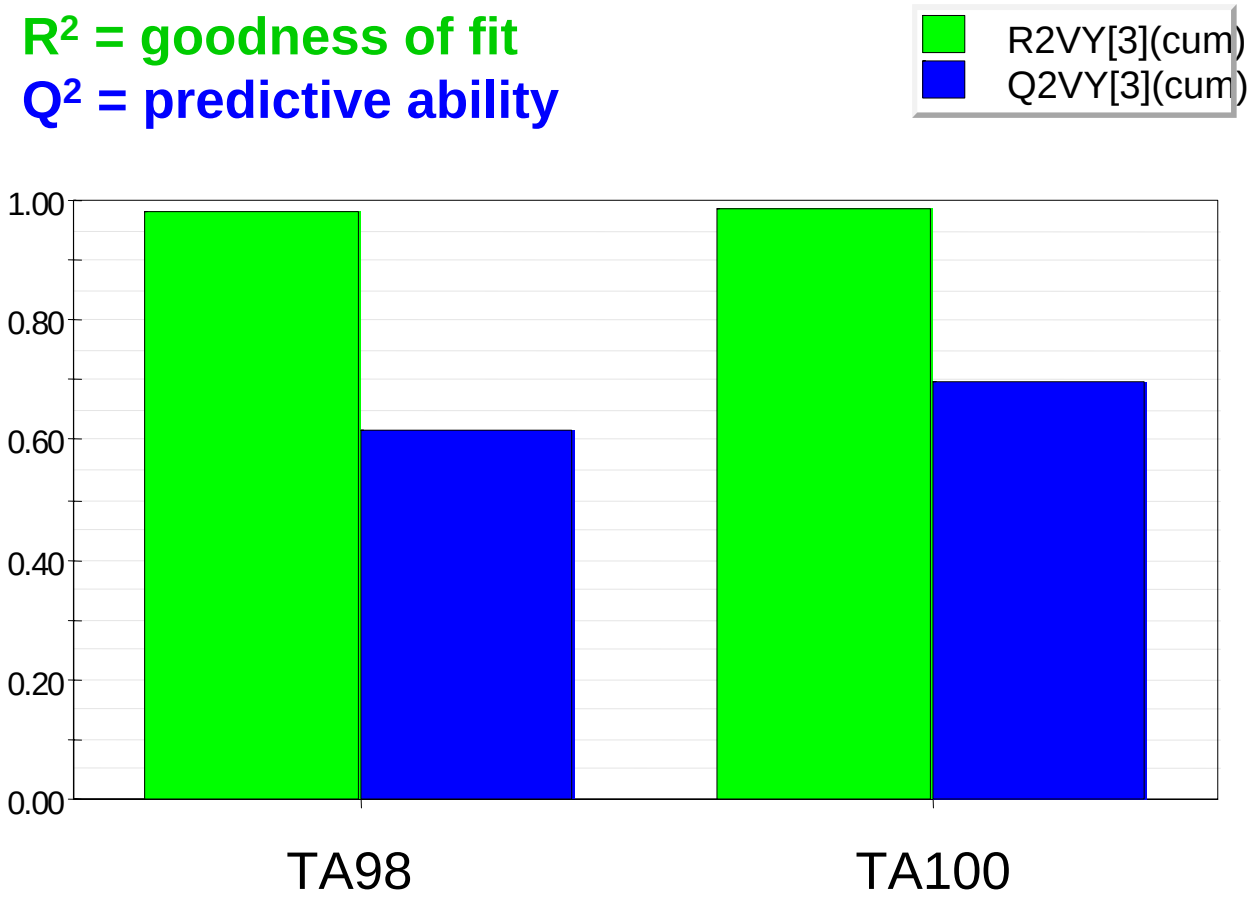
# RELATIVE ASSOCIATIONS BETWEEN PHYSICAL-CHEMICAL PROPERTIES AND MUTAGENICITY

## E.g., TA100 –S9



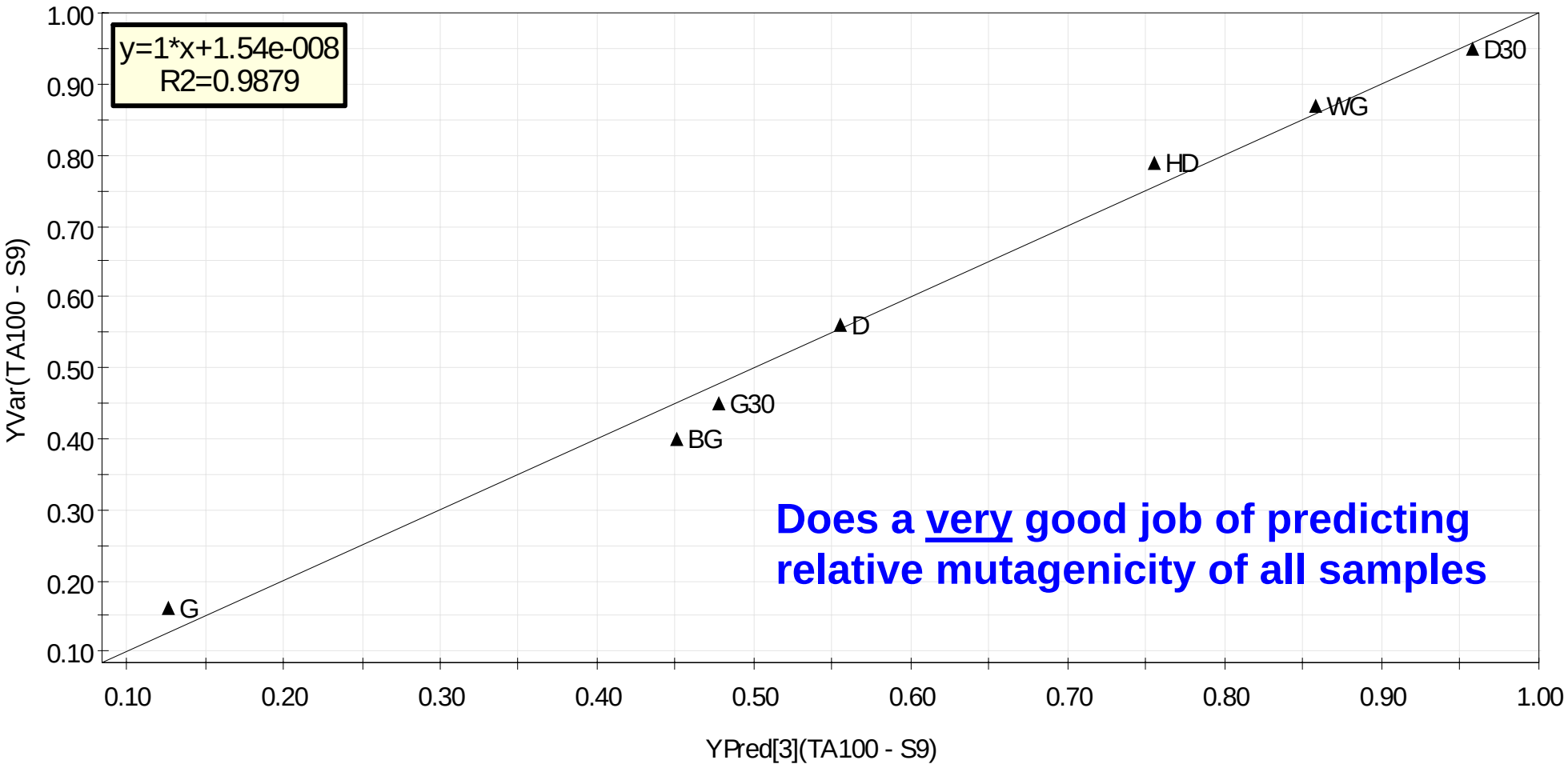
# QUALITY OF MODEL USING 23 NITRO- AND OXY-PAHs TO PREDICT MUTAGENICITY IN TA98 AND TA100

**$R^2$ = goodness of fit**
**$Q^2$ = predictive ability**

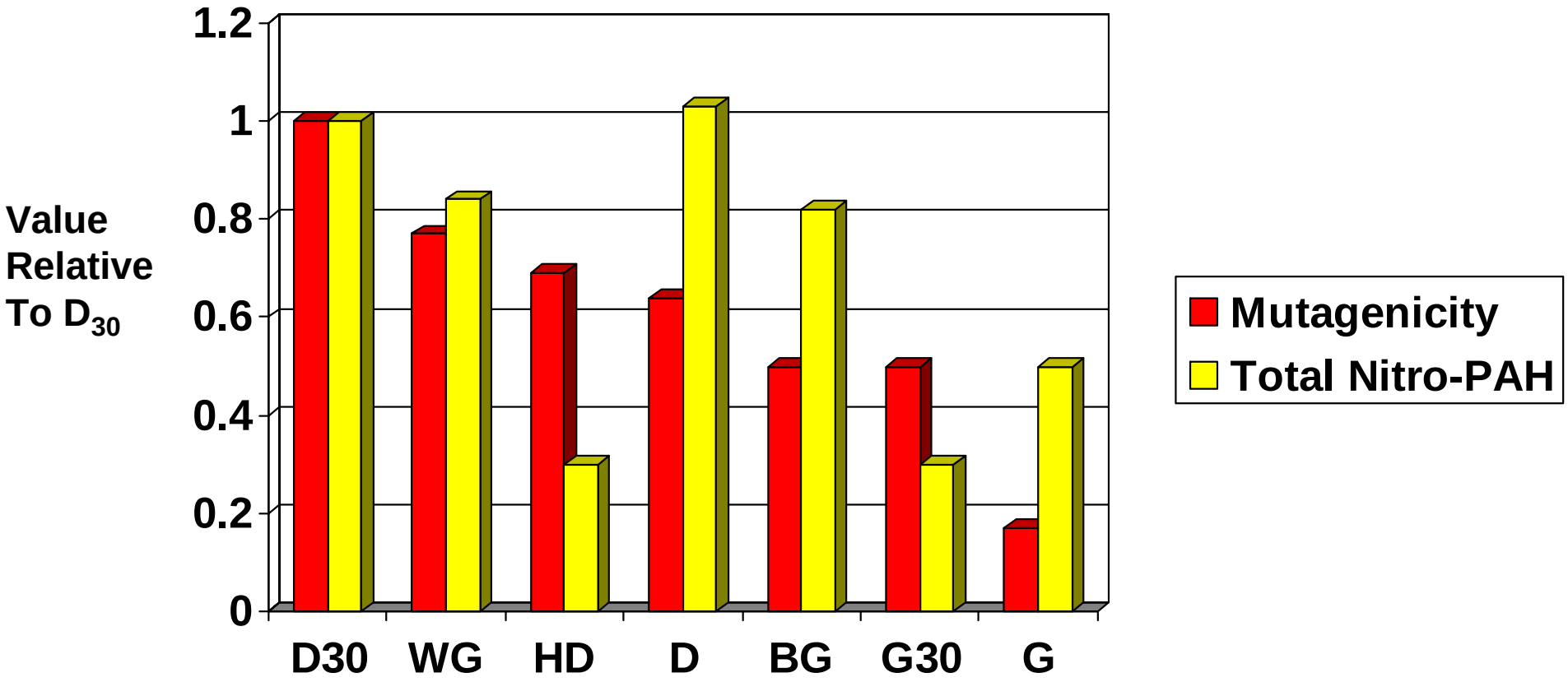



↑ **Best fit**
**Highest predictive ability**

# OBSERVED vs PREDICTED
# RELATIVE MUTAGENICITY IN TA100



# MUTAGENICITY WAS RELATED TO SPECIFIC NITRO-PAHs




**Top 8 compounds:**

- 7-nitrobenz(a)anthracene
- 6-nitrobenzo(a)pyrene
- 5-nitrocenaphthene
- 1-nitropyrene
- 4-nitrobiphenyl
- b-methyl-1-nitronaphthalene
- 2-nitrobiphenyl
- 9-nitroanthracene

**Validates PCA/PLS approach against years of "bio-directed fractionation"**

# SO NOW WHAT?

## Expand PCA/PLS analysis to more and different samples

The analysis is made more robust by increasing the number of samples and diversity of composition

See if composition-effect relationships hold for a broader range of engine emissions (and other air contaminants)

## Confirm health significance of results using “doped” samples

Necessary to move from “association” to “cause”

Thereby gain confidence in predicting hazards of new technologies

**We’re chuggin’ right along!**