

RELATIONSHIP BETWEEN COMPOSITION AND TOXICITY OF ENGINE EMISSION SAMPLES

Joe Mauderly, JeanClare Seagrave, and Jake McDonald

Lovelace Respiratory Research Institute, Albuquerque, NM

Ingvar Eide

Statoil, Trondheim, Norway

Barbara Zielinska

Desert Research Institute, Reno, NV

Doug Lawson

National Renewable Energy Laboratory, Golden, CO

Environmental Science and Health Impacts Program

DOE Office of FreedomCar and Vehicle Technology

James Eberhardt, Manager

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°	G₃₀
White smoker gas.	WG
Black smoker gas.	BG
Diesel (3)	D
Diesel 30°	D₃₀
High-emitter diesel	HD

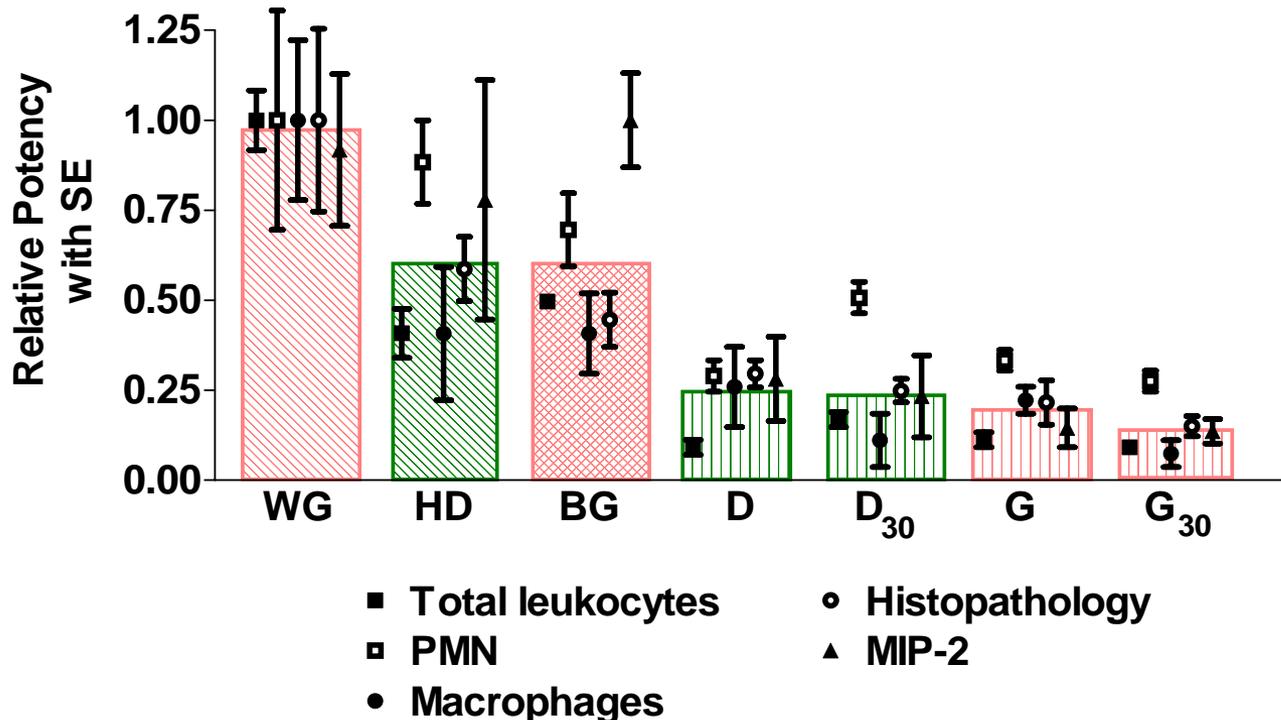
Measures of inflammation:

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

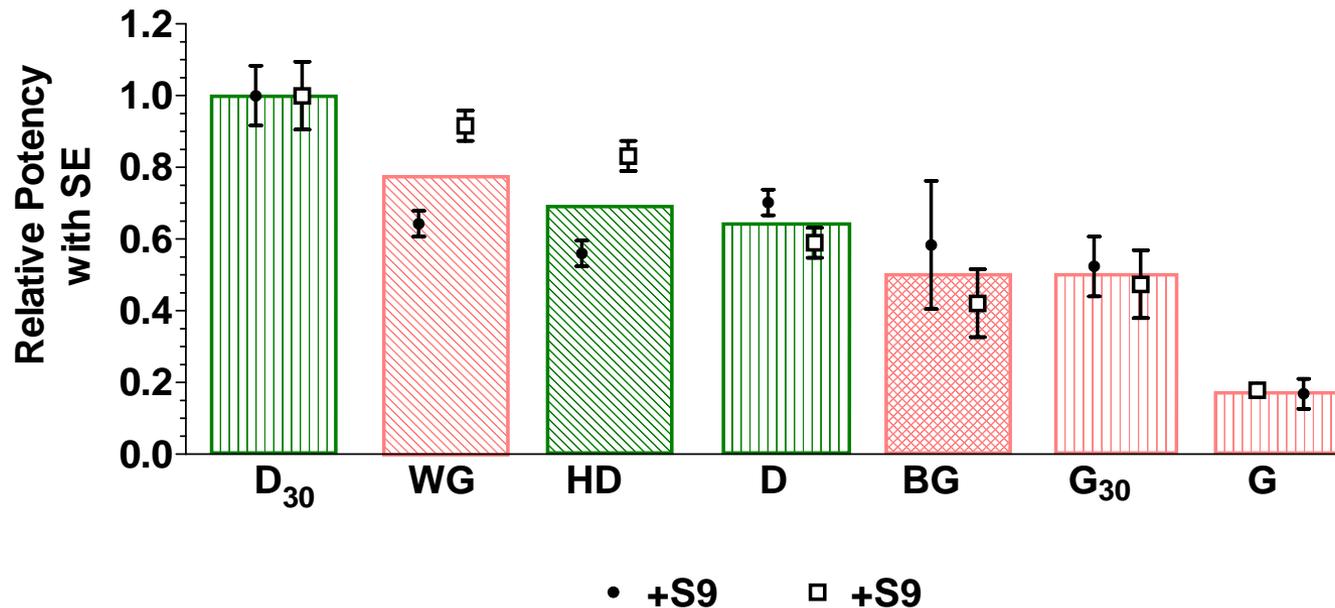
1. High-emitters not only contribute more mass, but are also more toxic per unit of mass
2. Normal-emitter diesel and gasoline have very similar toxicity per unit of mass



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:

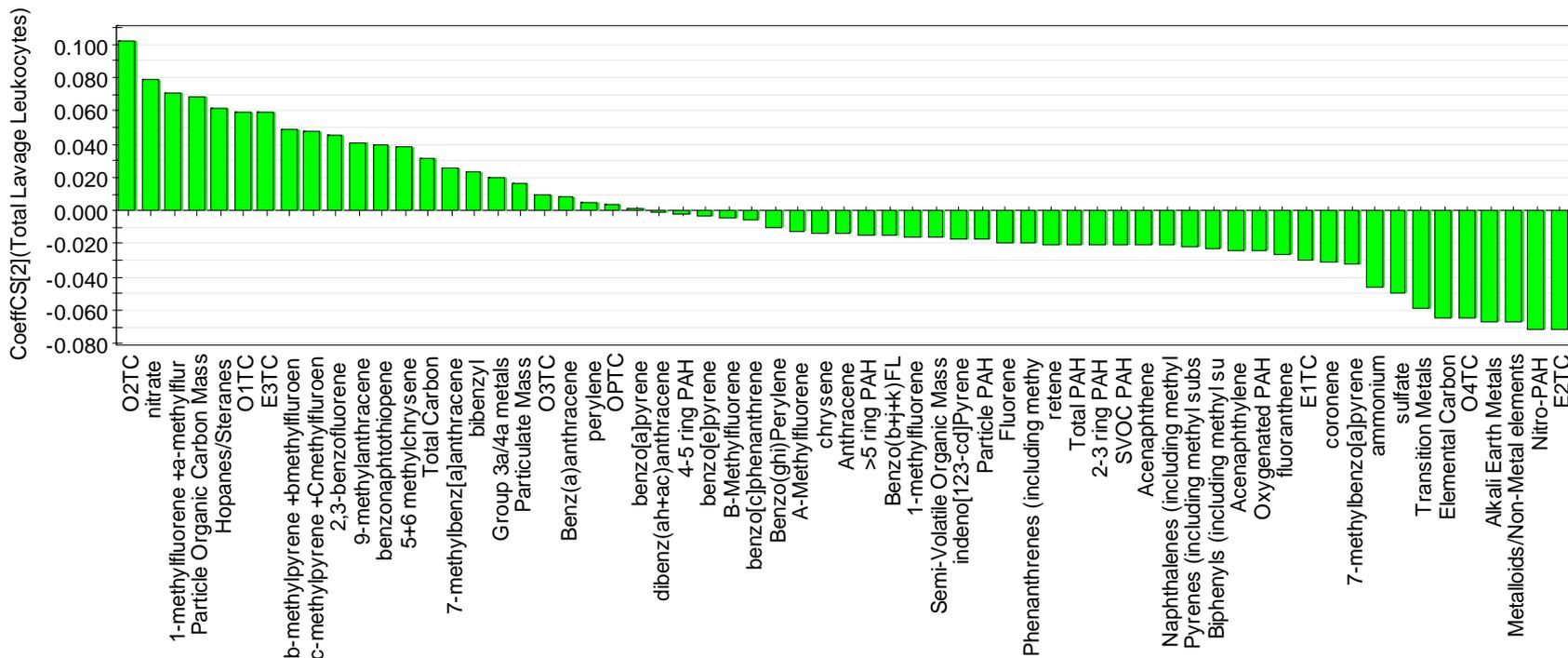
- 1. Inter-correlated composition variables**
- 2. Greater number of variables than samples**

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

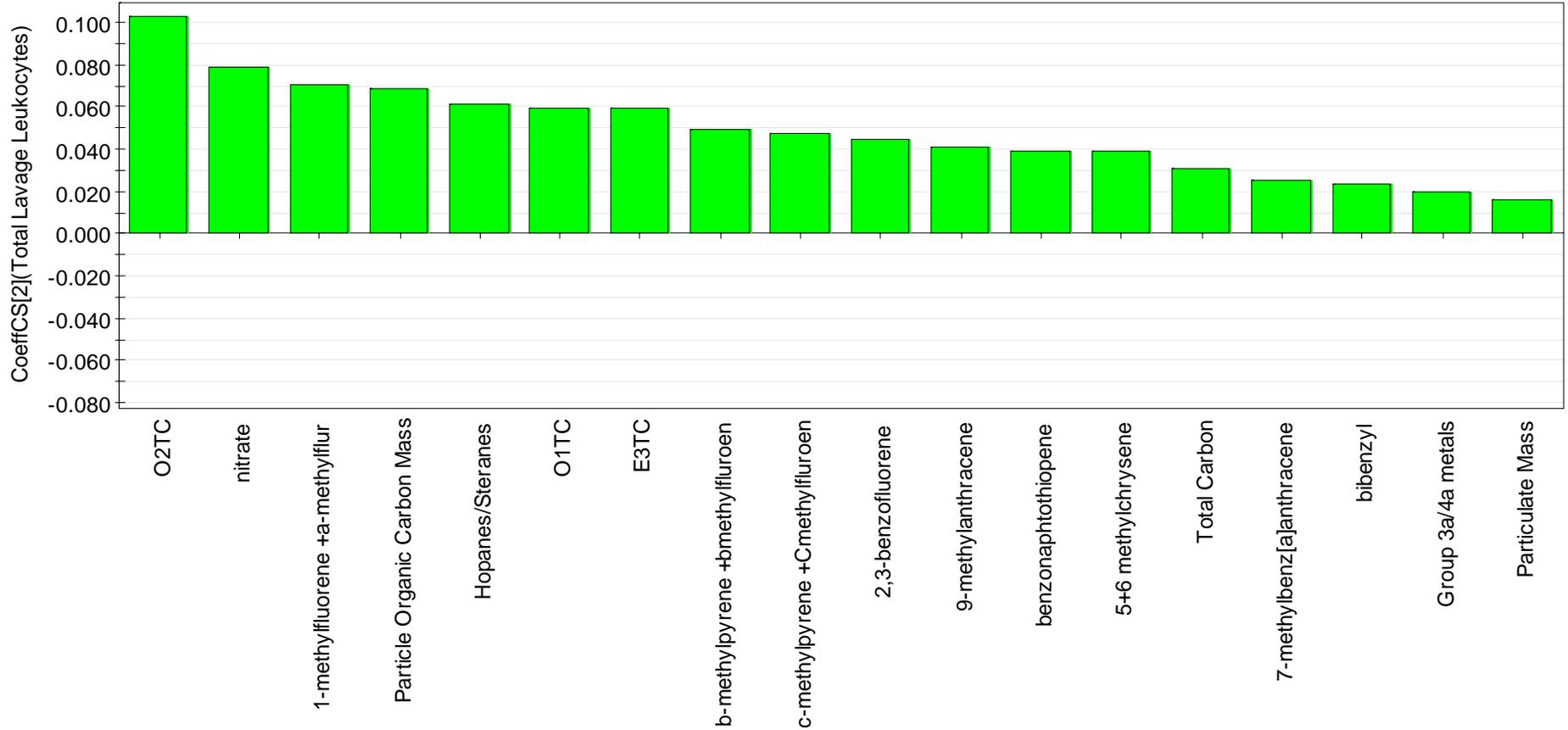
RELATIVE ASSOCIATIONS BETWEEN PHYSICAL-CHEMICAL PROPERTIES AND INFLAMMATORY RESPONSE

E.g., total lung lavage inflammatory cells

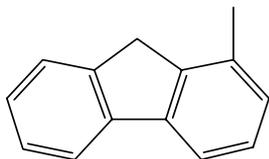


- Shows classes & compounds varying most strongly with toxicity
- Does not prove causality

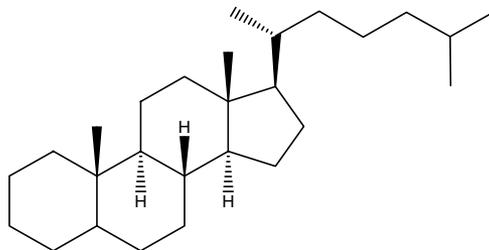
TOP 18 PHYSICAL-CHEMICAL PROPERTIES TRACKING WITH INFLAMMATION



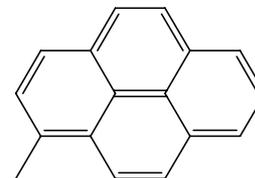
ORGANIC SPECIES HAVING HIGHEST CORRELATION WITH INFLAMMATION



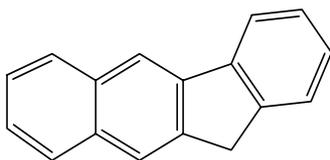
1-Methylfluorene
mw. 216



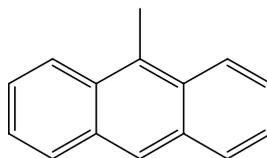
Representative Sterane
m.w. 372



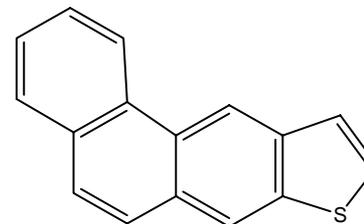
7-Methylpyrene
m.w. 216



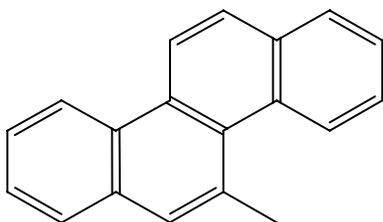
Benzofluorene
mw. 220



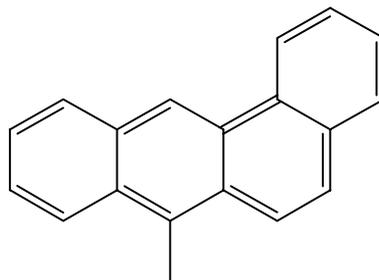
9-Methylanthracene
m.w. 192



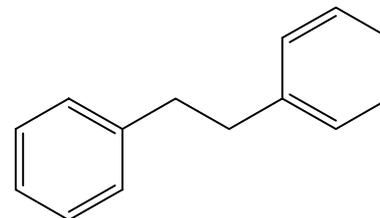
Benzonaphthothiophene
m.w. 234



5-Methylchrysene
m.w. 242



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



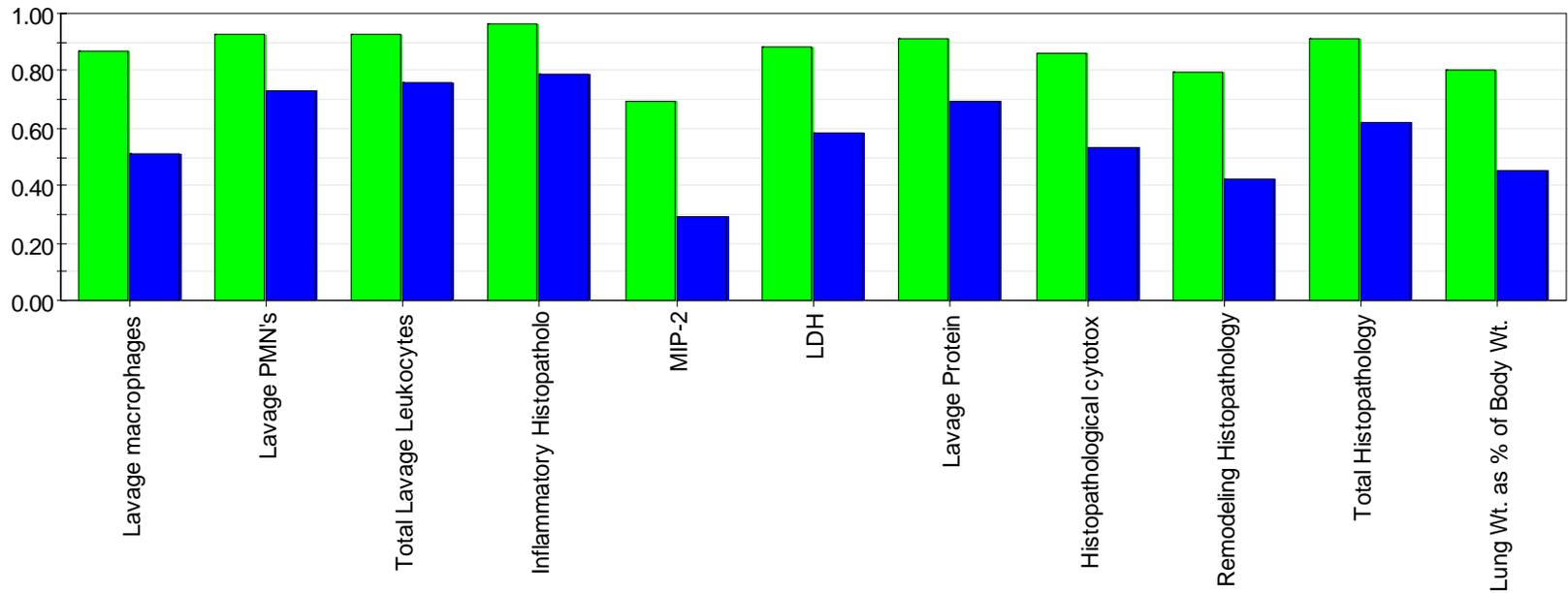
Bibenzyl
m.w. 182

[SVOCs range \approx from naphthalene (MW 128) to benz(a)anthracene (MW 228)]

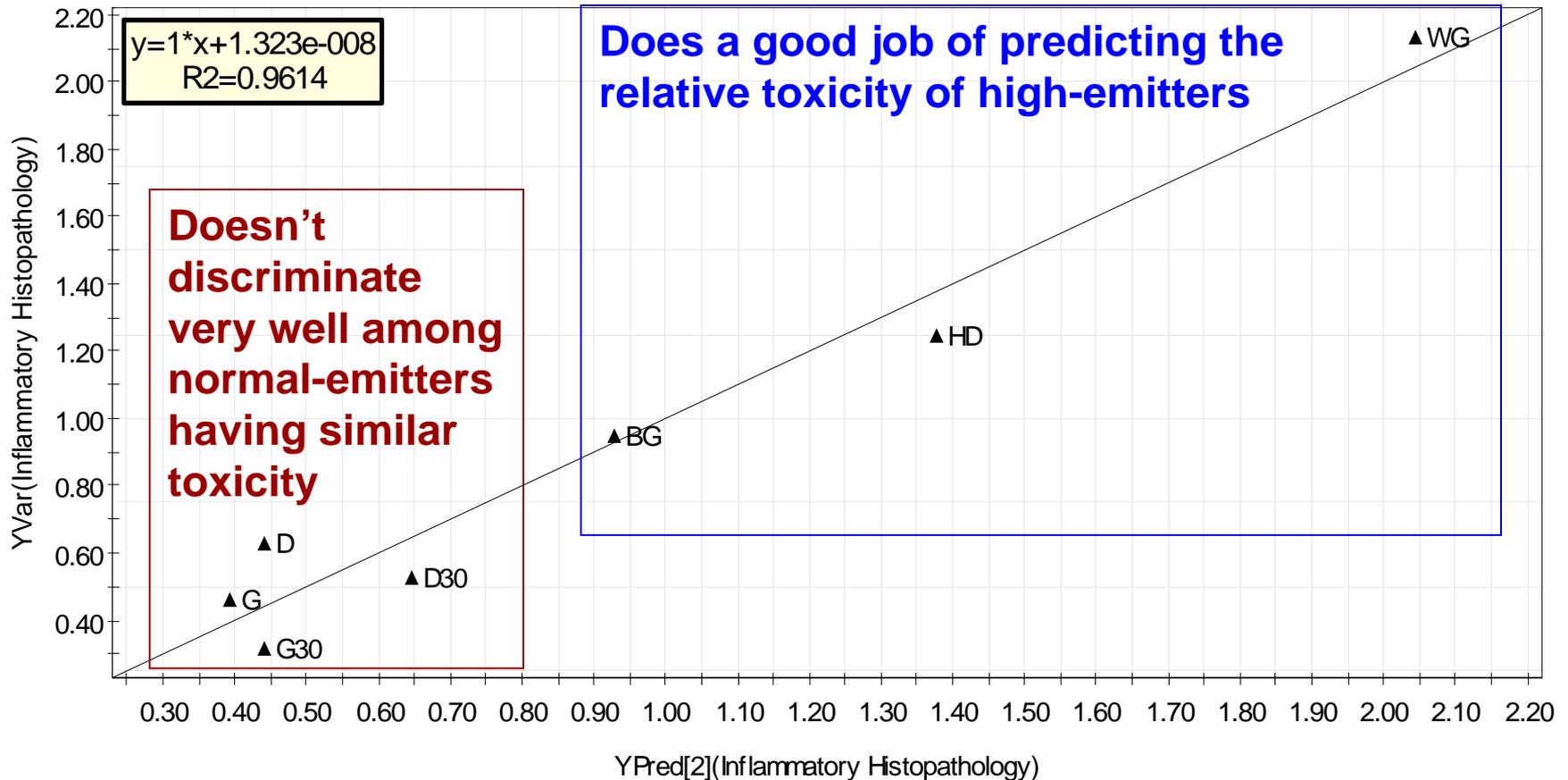
QUALITY OF MODEL USING 63 COMPOSITION VARIABLES TO PREDICT EACH OF 11 LUNG TOXICITY PARAMETERS

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

Best fit
Highest predictive ability

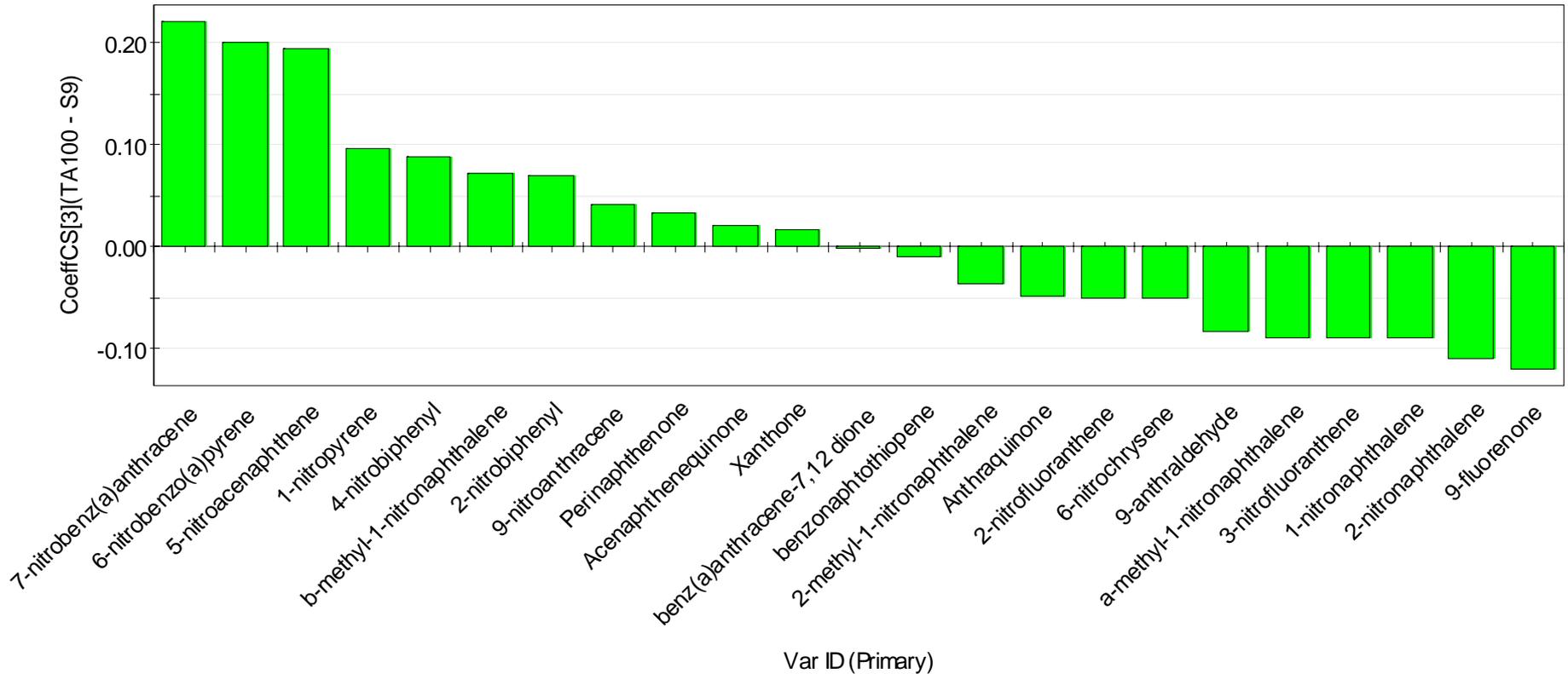


OBSERVED vs PREDICTED RELATIVE HISTOPATHOLOGICAL EVIDENCE OF INFLAMMATION



RELATIVE ASSOCIATIONS BETWEEN PHYSICAL-CHEMICAL PROPERTIES AND MUTAGENICITY

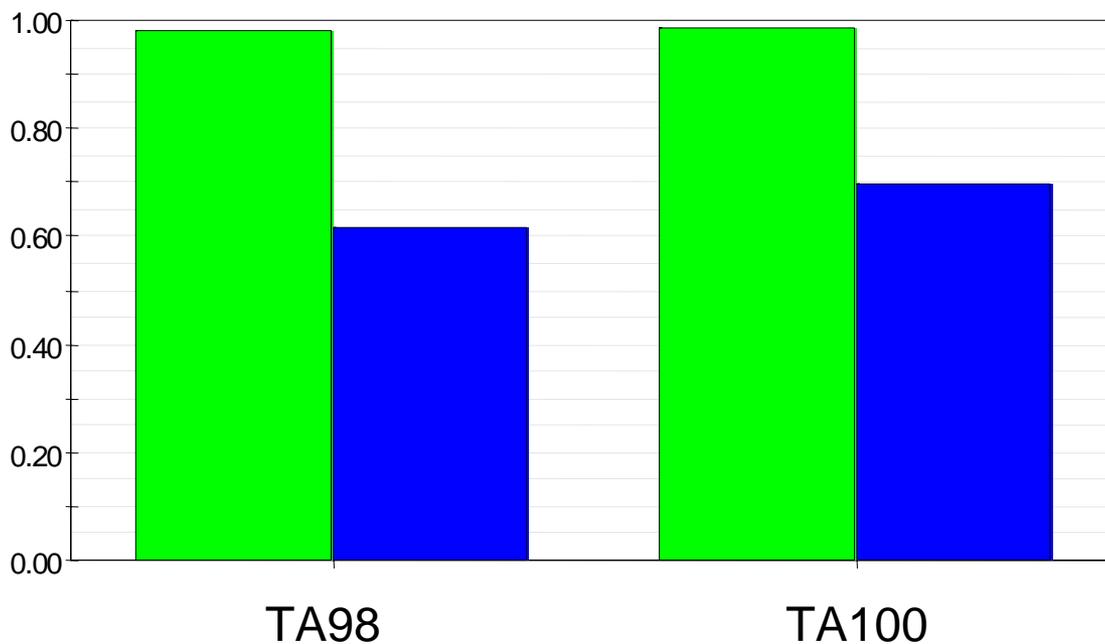
E.g., TA100 –S9



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

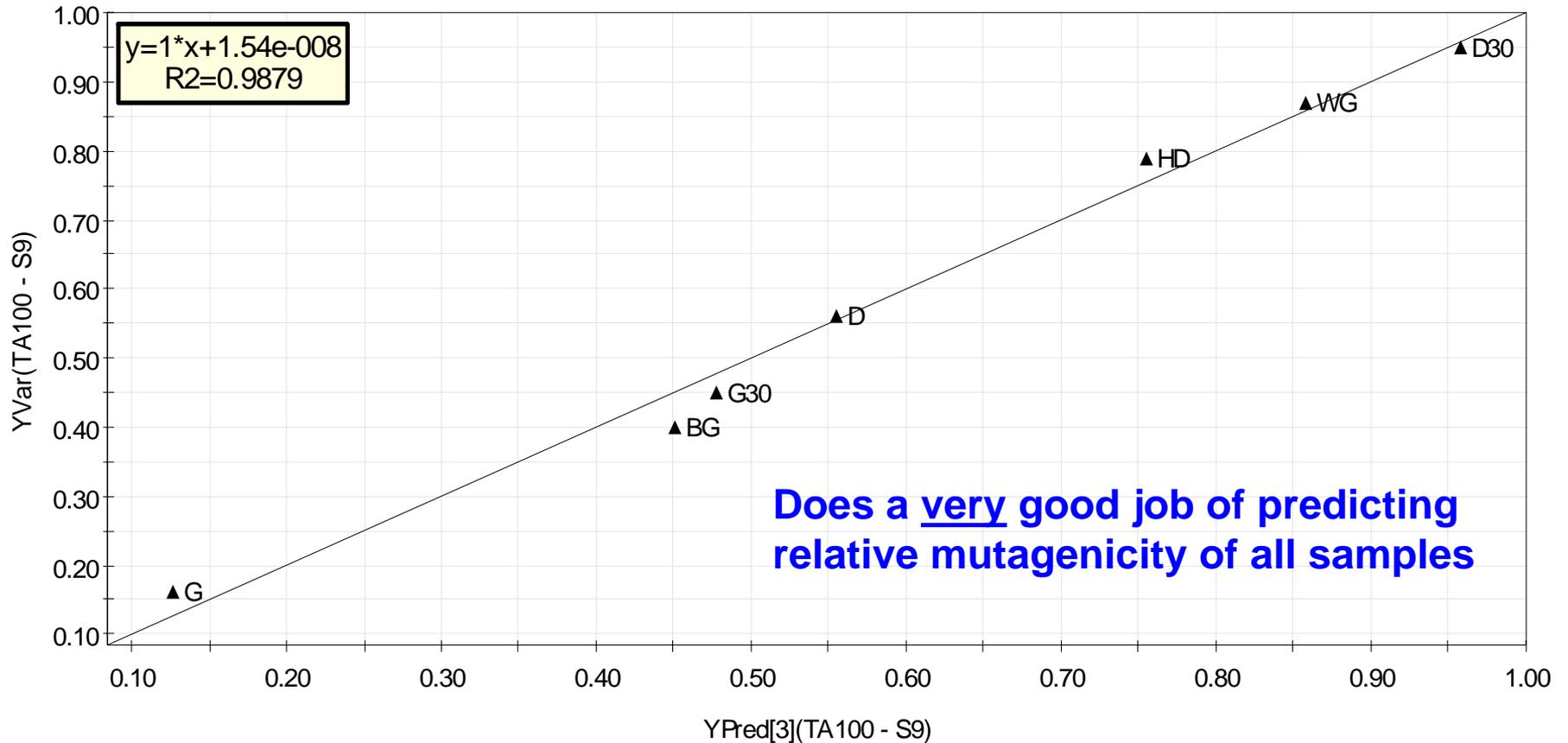
R^2 = goodness of fit
 Q^2 = predictive ability

 $R^2_{VY[3]}(\text{cum})$
 $Q^2_{VY[3]}(\text{cum})$

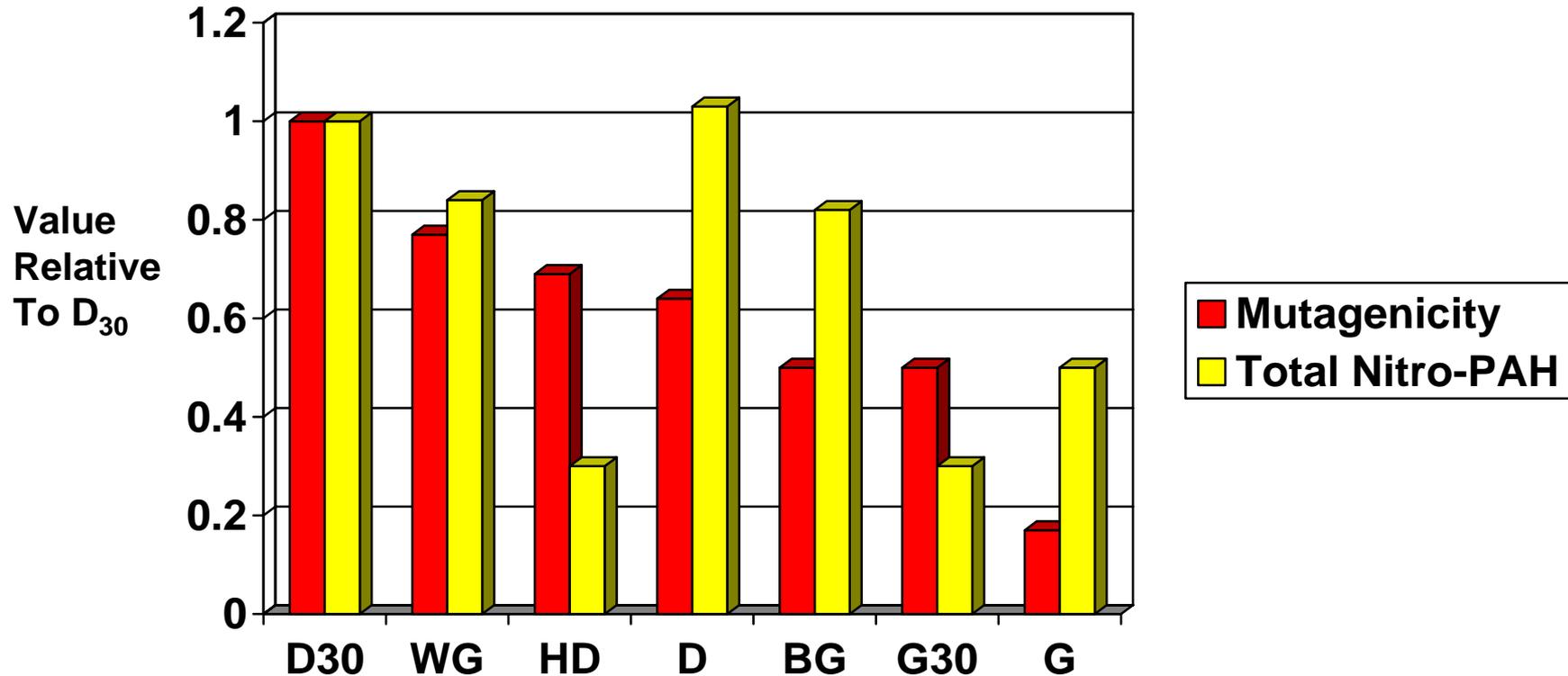


↑ **Best fit**
Highest predictive ability

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!

