Cancer / carcinogenicity: computational models

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Chemical Risk Assessment into the new century

- **Traditional toxicology** has been the major source of information
- **Rodent bioassay**: consistent and reliable indicator and predictor of human cancer risk
- **New regulatory policies** (e.g., REACH) tend to drastically reduce the number of new cancer bioassays
- Opportunities to accept **"alternative" approaches**, including (Q)SARs, Read-Across and Chemical Category

Evolution of theories on the early stages of carcinogenesis:

- **Somatic mutation and its variations (**epigenetic, chromosomal, stem-cell):

Cancer originates at the cellular level of biological organization

Carcinogens alter DNA structure or function in cells in tissues from which cancer arises

- **Tissue organization field:**

Cancer arises from disruption of tissue microachitecture

Mutations and genetic instability as consequence of disruption of the morphostat gradient

Baker et al., 2010, J.Clin.Oncol., 28: 3215-3218

Prevailing model: somatic mutation theory of cancer

Testing strategy for carcinogenicity pre-screening in REACH

- **Pivotal role of genotoxicity Short-Term Tests (STT)**

- Bioassay may be required in the case of high exposure plus genotoxicity evidence

- Implemented as **Tiered Approach**

In vitro {bacteria + mammalian cells} {gene mutations + chrom. damage}

In vivo {filter *in vitro* false positives}

STTs to predict carcinogenicity: what the data say ?

- **Mutagenicity =** *{correlates with}* **Carcinogenicity ?**
  Only within a limited area of the chemical space, i.e., **DNA-reactive** chemicals

- **DNA-reactive** chemicals induce **cancer, and a wide spectrum of mutations**

- **Ames test:** most predictive mutagenicity-based assay (80% positive predictivity)

Benigni R. *Exp.Opinion Drug Metab.Toxicol., 2012, 8: 1-11.*
Zeiger E. *Regulat.Pharmacol.Toxicol.* *1998;28:85-95.*

STTs to predict carcinogenicity: what the data say ?

- **Mammalian *in vitro* STTs** when Ames-negative :
  no correlation with carcinogenicity *(too many false positives)*

- No reliable ***in vivo* STTs** (e.g., micronucleus) *(too many false negatives)*

- Present tiered strategy: **genotoxic carcinogens may go undetected**

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Ames test *versus* rodent carcinogenicity

Carcinogenicity	Ames test: neg	Ames test: pos
Non carcinogens	233	76
Carcinogens: Non DNA-reactive	136	34
Carcinogens: DNA-reactive	79	277

Ames identifies DNA-reactive carcinogens

Results from 835 chemicals in ISSCAN v3a
http://www.iss.it/ampp/dati/cont.php?id=233&lang=1&tipo=7

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Ames mutagen: 80% probability of being a carcinogen

Results from 835 chemicals in ISSCAN v3a
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Backing up the STTs with **Structure-Activity** concepts

Structure-activity relationship concepts:

application to different issues, through different approaches

Coarse-grain **Structural Alerts**
(mechanistic classes, category formation, priorities)
- mechanistic explanation
- basis for software implementation

Fine-tuned Quantitative Structure-Activity Relationships (**QSAR**)
of congeneric classes

Hybrid (??) non-local, or global QSARs

Toxtree 2.5: Rulebase for carcinogens / mutagens

Structure-based approach consisting of:

- Updated compilation of *Structural Alerts* (mostly genotox, some nongenotox)

-Three mechanistically-based *QSARs* for congeneric classes
(aromatic amines, aldehydes)

Open-source, freely available: *http://ecb.jrc.it/qsar/qsar-tools/index.php?c=TOXTREE*

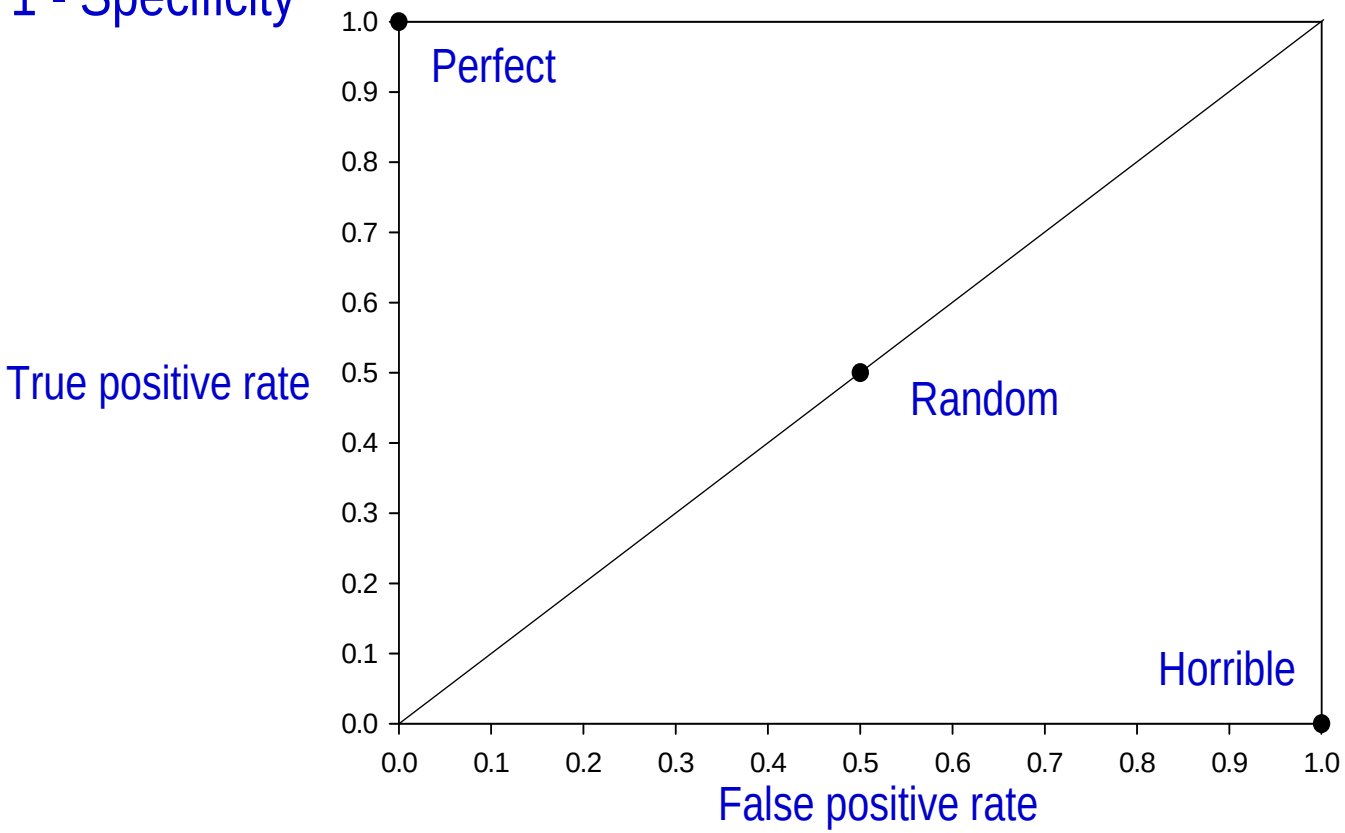
ROC graph: A simple, graphical way of comparing predictions with results

True positive rate = (Positives predicted as positive) / (Real positives)

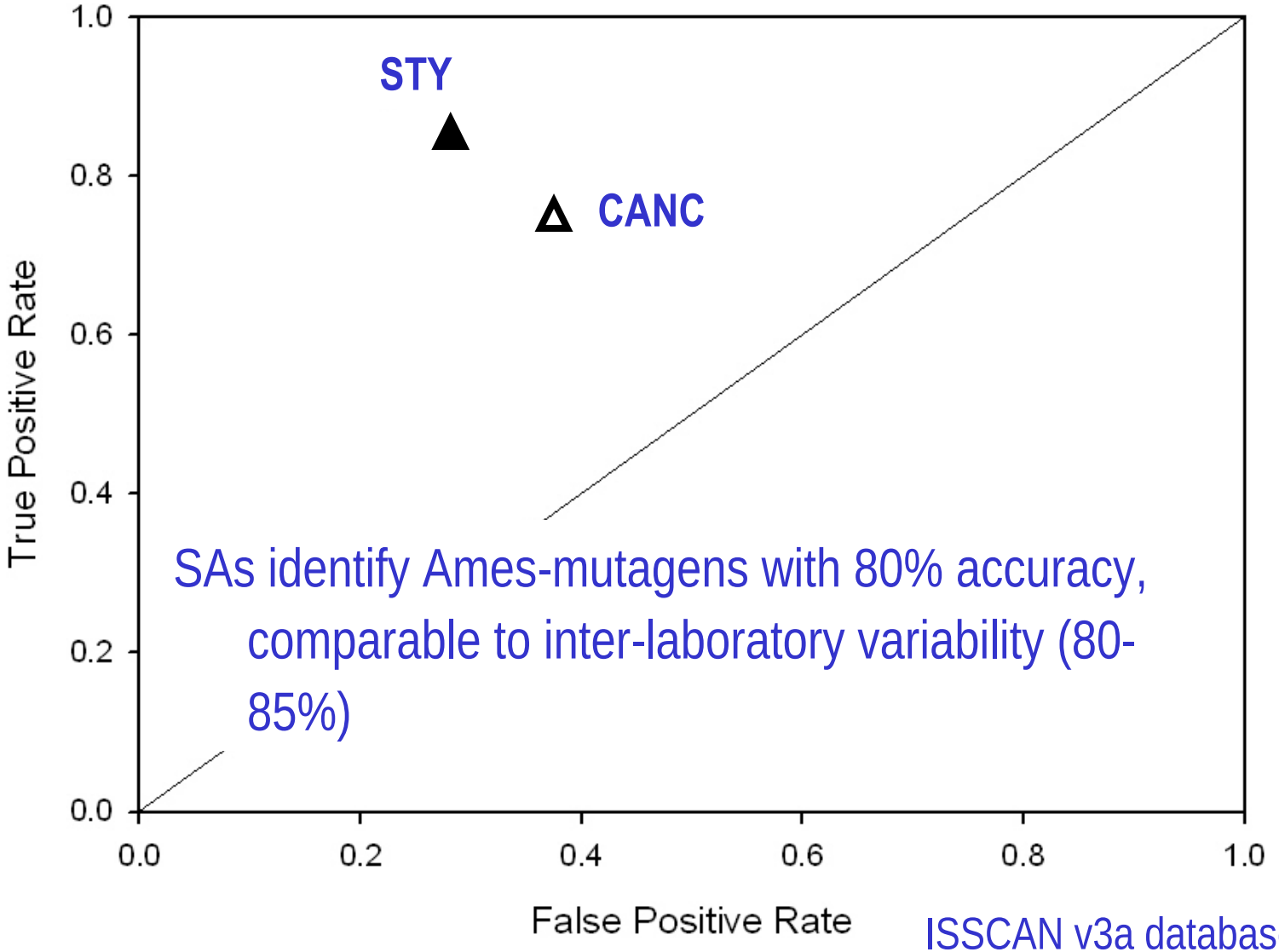
= Sensitivity

False Positive Rate = (Negatives predicted as positive) / (Real negatives)

= 1 - Specificity



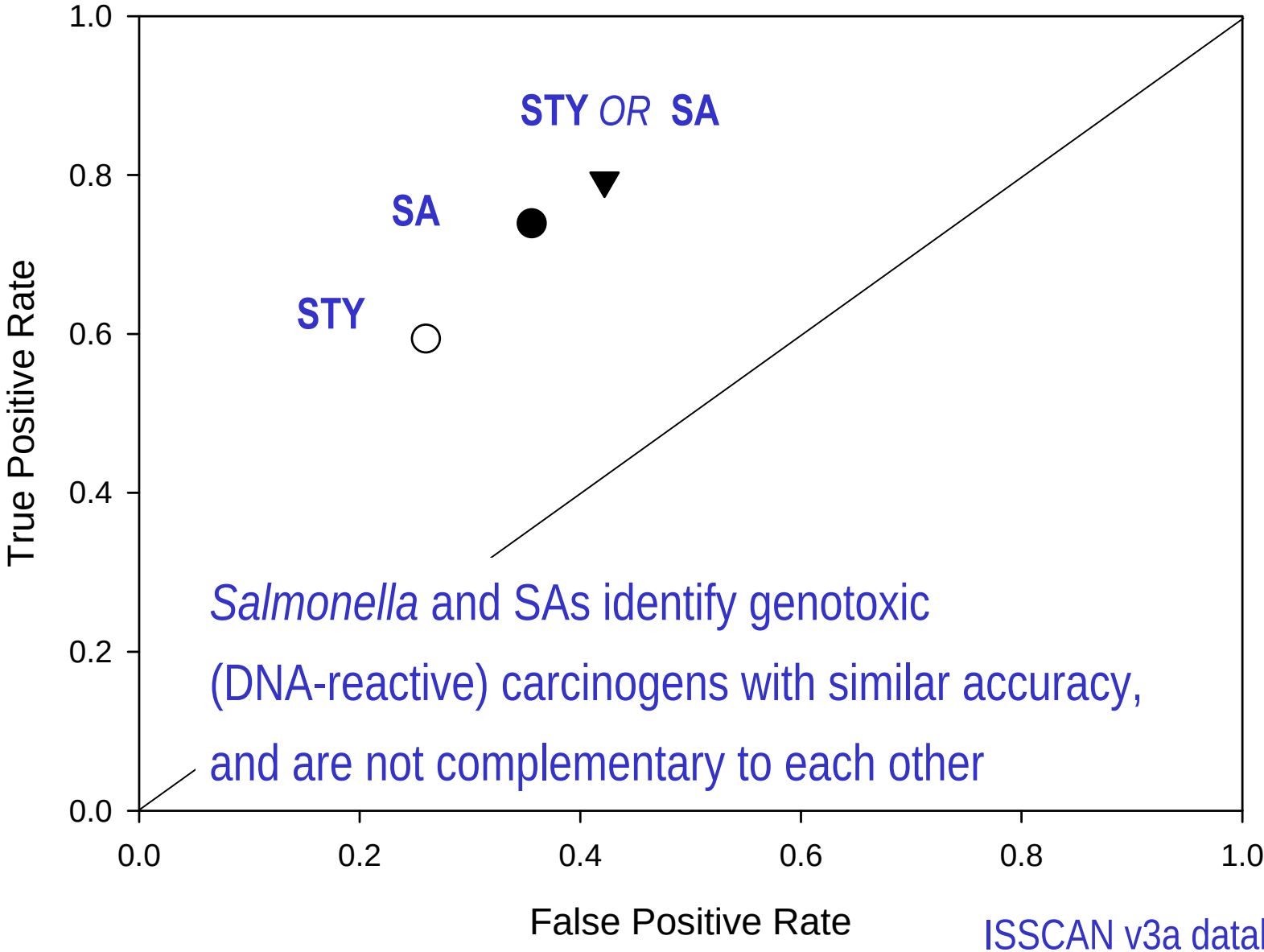
Toxtree SAs: agreement with Carcinogenicity and *Salmonella* (STY)



SAs identify Ames-mutagens with 80% accuracy, comparable to inter-laboratory variability (80-85%)

ISSCAN v3a database

Carcinogenicity prediction: *Salmonella* (STY) *OR* **SAs**



Salmonella and SAs identify genotoxic (DNA-reactive) carcinogens with similar accuracy, and are not complementary to each other

ISSCAN v3a database

A success story in science:
understanding DNA-reactivity (and SAs implementation)

- Reliable enough to predict *Salmonella* results, and identify many carcinogens
- Identify human carcinogens
- Basis for successful priority setting in NTP bioassays (70% carcinogens among structurally suspect chemicals, only 10% among high exposure chemicals)
- Contribution to reduce DNA-reactive carcinogens among synthetic chemicals (pesticides, pharmaceuticals)

Testing strategy for carcinogenicity pre-screening in REACH

- Pivotal role of genotoxicity short-term tests
- Bioassay may be required in the case of high exposure plus genotoxicity evidence
- **Nongenotoxic carcinogens:**
  negative in genotoxicity tests, go undetected

Nongenotoxic carcinogens: an overlooked issue

- theory on nongenotoxic carcinogenicity is much less developed than that on the genotoxic mechanisms

- nongenotoxic carcinogens are often considered to represent a lesser risk for human health

however

- nongenotoxic carcinogens are **a remarkable proportion of recognized human carcinogens** (17 - 27% of IARC Group 1)

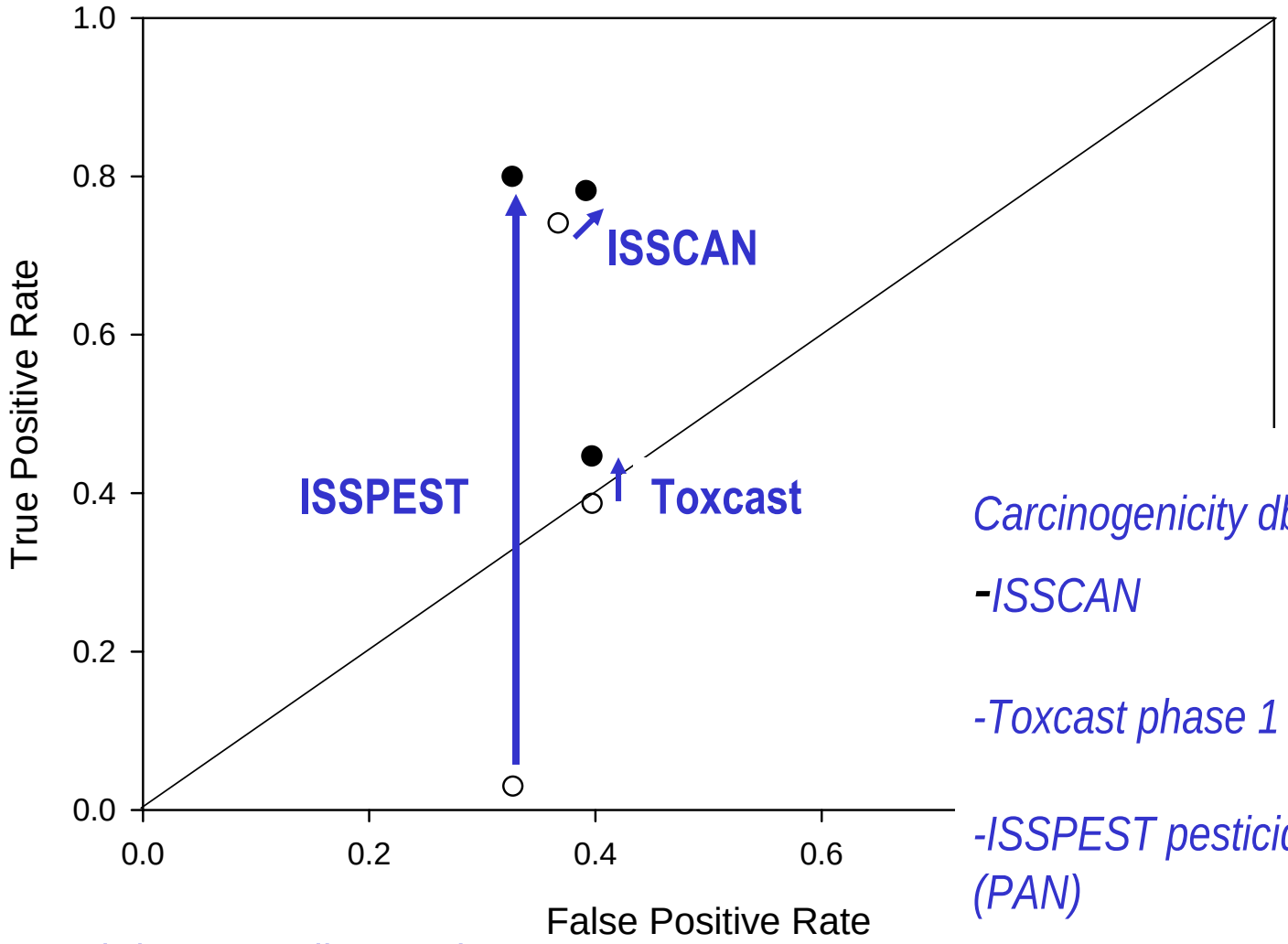
Hernandez et al., 2009, Mutat.Res., 682: 94-109.

Alternative approaches to the identification of nongenotoxic carcinogens ?

Expanded list of SAs at the ISS (analysis of mechanistic knowledge)

Carcinogenicity prediction:

Old (mainly genotoxic) *versus* Expanded SAs rulebase



Carcinogenicity dbs:

-ISSCAN

-Toxcast phase 1

-ISSPEST pesticides (PAN)

Implementation: OECD (Q)SAR Toolbox and Toxtree

Alternative approaches to the identification of nongenotoxic carcinogens ?

Cell transformation: non-mutagenicity *in vitro* assays for non-DNA-reactive carcinogens

ISSCTA: a database of Cell Transformation Assays results

In **ISSTOX** *http://www.iss.it/ampp/dati/cont.php?id=233&lang=1&tipo=7*
and **OECD (Q)SAR Toolbox**

n = 370 (including inorganics and organics)

Systems

primary normal diploid cells: Syrian Hamster Embryo cells assay (pH 7)
Syrian Hamster Embryo cells assay (pH 6.7)

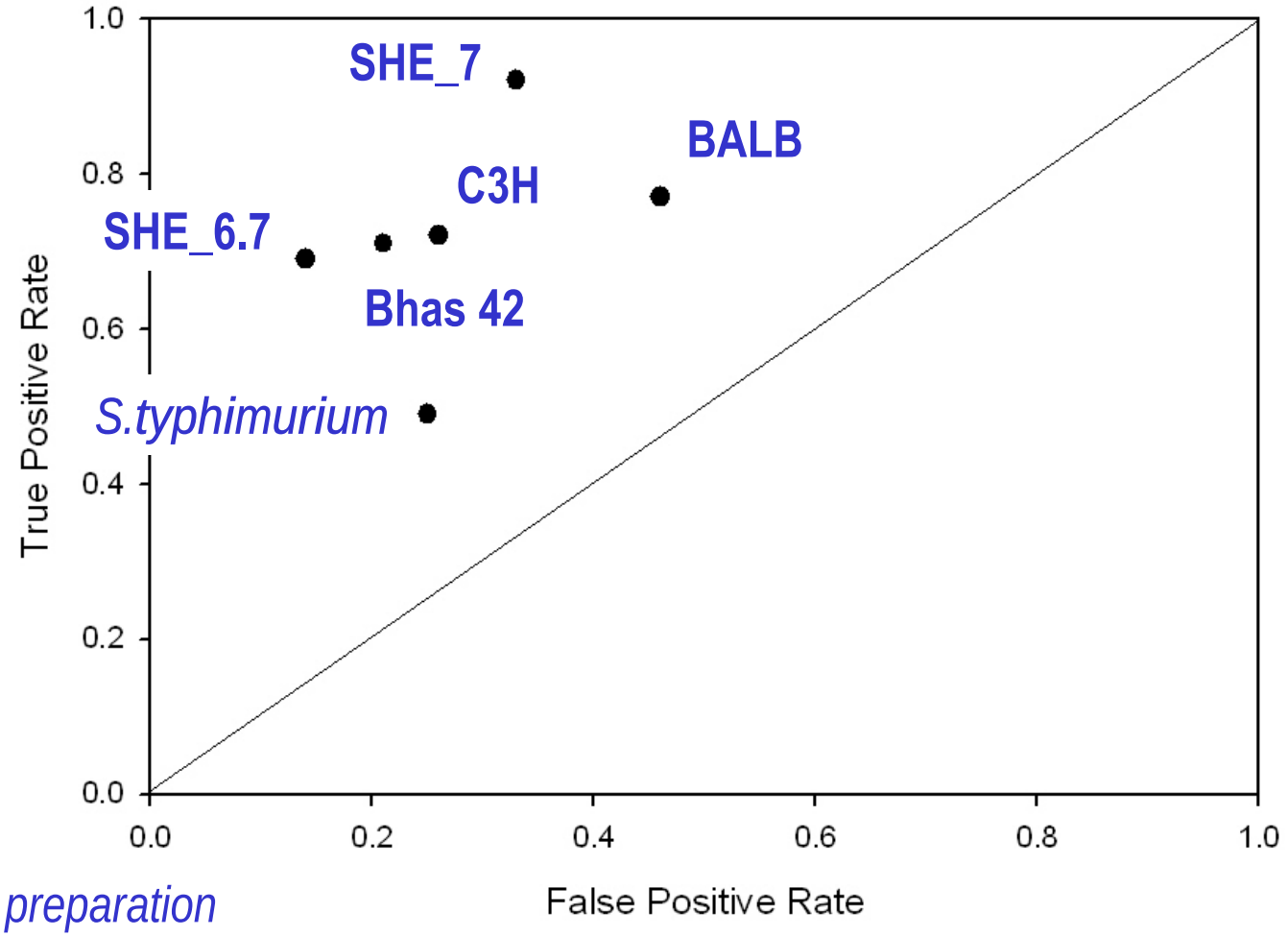
immortalized aneuploid mouse cells: BALB/c 3T3
C3H10T1/2
Bhas 42

Data: *OECD vol.31, 2007*

Sakai et al., 2010 Mutat.Res. 702:100-122

Cell Transformation Assays: carcinogenicity prediction

A new analysis on ISSCTA, including inorganics and Bhas 42



Benigni et al., 2012, in preparation

SHE pH≥7 Cell transformation *versus* rodent carcinogenicity

Carcinogenicity	SHE neg	SHE pos
Negatives	36	18
Non DNA-reactive	6	70
DNA-reactive	5	59

Carcinogens

Sensitive to DNA-reactive and non-DNA-reactive carcinogens

Chemicals n = 194, from OECD vol. 31

Benigni et al., 2012, in preparation

Efficient, alternative testing strategy with tools available today?

in vitro **assays** {*Salmonella* and Cell transformation}
sensitive to both DNA-reactive and non DNA-reactive carcinogens

Structural Alerts to predict / rationalize experimental results

A tiered approach to carcinogens identification

Tiered Approach A			Tiered Approach B		
	Noncarc	Carc		Noncarc	Carc
Initial Sample	36	86	Initial Sample	52	130
	After Tier 1			After Tier 1a	
STY Negative	27	41	SAgeno Negative	32	66
				After Tier 1b	
			SA nongeno Negative	27	43
	After Tier 2			After Tier 2	
SHE Negative	17	8	SHE Negative	17	5
% initial sample	47 %	9%		33%	4%

only ~ 5-10% undetected carcinogens

Benigni et al., 2012, in preparation

…considerations I.

Tiered Approach A			Tiered Approach B		
	Noncarc	Carc		Noncarc	Carc
Initial Sample	52	130			
			After Tier 1a		
	32	66			
			After Tier 1b		
	27	43			
			After Tier 2		
SHE Negative	17	8	SHE Negative	17	5
% initial sample	47 %	9%		33%	4%

Given experimental variability, how much can we improve?

only ~ 5-10% undetected carcinogens

…considerations II.

Tiered Approach A			Tiered Approach B		
	Noncarc	Carc		Noncarc	Carc
Initial Sample	36	86	Initial Sample	52	130
	After Tier 1			After Tier 1a	
STY Negative	27	41	S-		6
	After Tie				
SHE Negative	17				
% initial sample	47 %	9%			

only ~ 5-10% undetected carcinogens

Salmonella: probe for a large category of effects (DNA-reactivity, protein-reactivity, wide range of disturbances to cell physiology…)

…considerations III.

Tiered Approach A		
	Noncarc	Carc
Initial Sample	36	86
	After Tier 1	
		41
		9%

Tiered Approach B		
	Noncarc	Carc
Initial Sample	52	130
	After Tier 1a	
SAgeno Negative	32	66
	After Tier 1b	
SA nongeno Negative	27	43
	After Tier 2	
SHE Negative	17	5
	33%	4%

SAs for genotox detect efficiently DNA-reactive chemicals

only
un
car

…considerations IV.

Tiered Approach A		
	Noncarc	Carc
Initial Sample	36	86
	After Tier 1	
		41
		9%

Tiered Approach B		
	Noncarc	Carc
Initial Sample	52	130
	After Tier 1a	
SAgeno Negative	32	66
	After Tier 1b	
SA nongeno Negative	27	43
	After Tier 2	
SHE Negative	17	5
	33%	4%

only
un
car

A remarkable portion of nongenotox mechanisms can be coded through SAs

…considerations V.

Tiered Approach A			Tiered Approach B		
	Noncarc	Carc		Noncarc	Carc
Initial Sample	36	86	Initial Sample	52	130
	After Tier 1			After Tier 1a	
		41	SAgeno Negative	32	66
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			SA nongeno Negative	27	43
				After Tier 2	
			SHE Negative	17	5
		9%		33%	4%

only
un
car

Cell Transformation: Model for systemic component in carcinogenesis ?

(How) can it be modeled theoretically?

…final considerations

- The **present strategies** for carcinogenicity pre-screening **do not defend** adequately **human health,** and need **to be updated**

- **Simpler and more efficient alternative strategies available**

- **Reliable ground for refinements**:

  - Improve specificity

  - Expand SAs for nongenotoxic carcinogens to decrease reliance on experiments (mechanistic knowledge plus data mining)

  - Global theoretical models of CTAs

  - More CTAs (SHE) experiments

Freely-available tools generated at the ISS

- **Structural Alerts** for - canc / *in vitro* mut
  - *in vivo* micronucleus

Toxtree

http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree

OECD (Q)SAR Toolbox

http://www.oecd.org/document/54/0,3746,en_2649_37465_42923638_1_1_1_37465,00.html

- **Curated toxicological databases**

ISSTOX

http://www.iss.it/ampp/dati/cont.php?id=233&lang=1&tipo=7

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Chiara Battistelli *Mauro Colafranceschi*

Predictions reliability

	Predictivity	
	Positive	**Negative**
Genotox *(ISSCAN db)*		
Ames Test	.80	.52
SA_geno	.77	.59
SA_geno AND Ames Test	.84	.46
Overall *(ISSCTA db)*		
SHE_7	.88	.77
SA_geno_nongeno	.77	.43
SA_geno_nongeno AND SHE_7	.93	.48

Predictions reliability

		Predictivity	
		Positive	Negative
Tier A	*(STY and SHE_7)*	.80	.68
Tier B	*(SA_tot and SHE_7)*	.78	.77

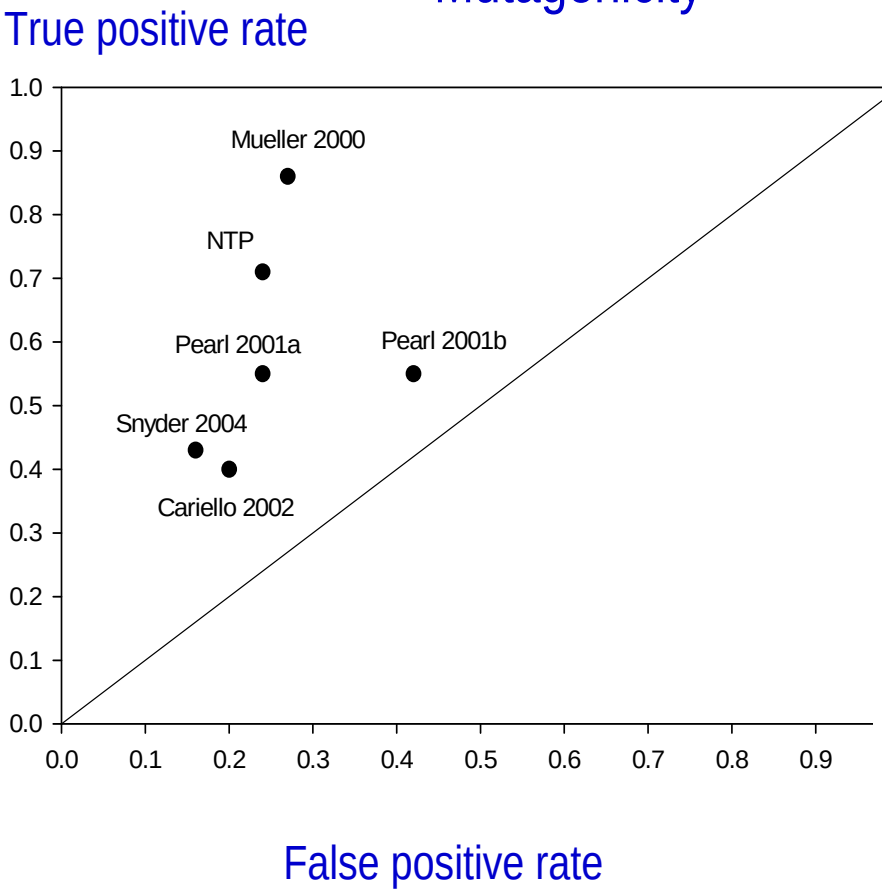
Local, mechanistically-based QSARs for congeners:

- scientifically interpretable, good internal statistics, but vary for their external predictivity
- **QSARs for potency: predictions 30 – 70 % correct**
- **QSARs for activity: predictions 70 – 100 % correct**
- Estimating intervals more reliable than estimating data points
- **Internal validation measures do not correlate with external predictivity**

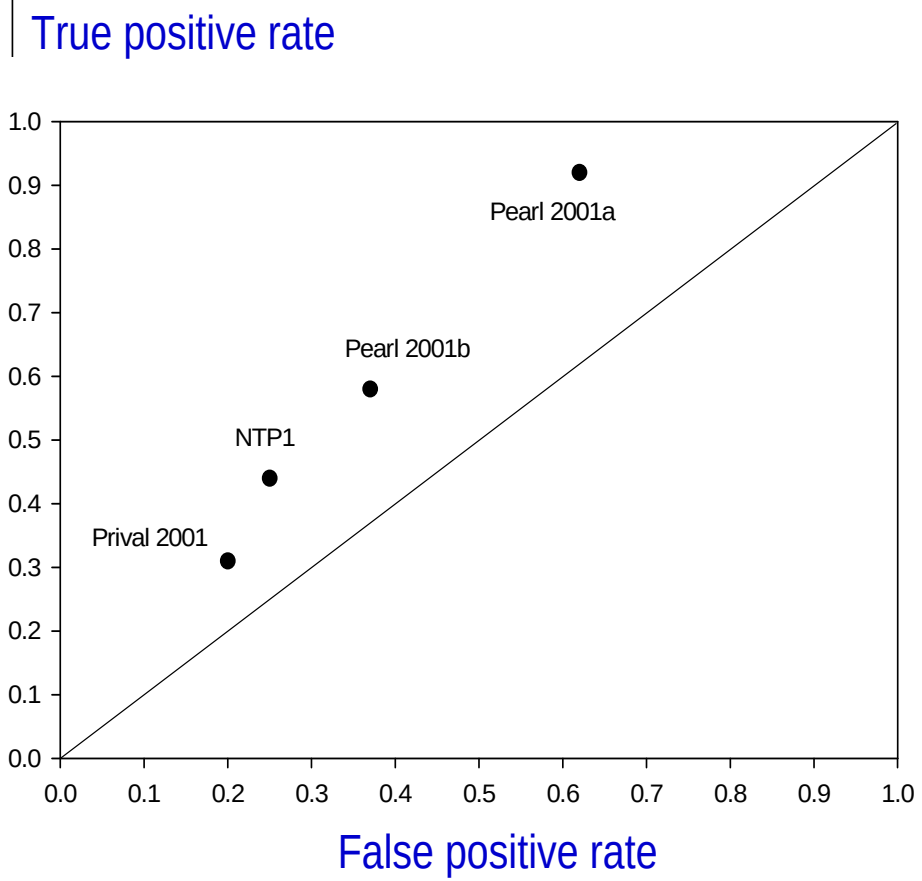
Benigni,R. and Bossa,C. (2008): Predictivity of QSAR. J.Chem.Inf.Model., 48:971-980

TOPKAT: External Validation

Mutagenicity

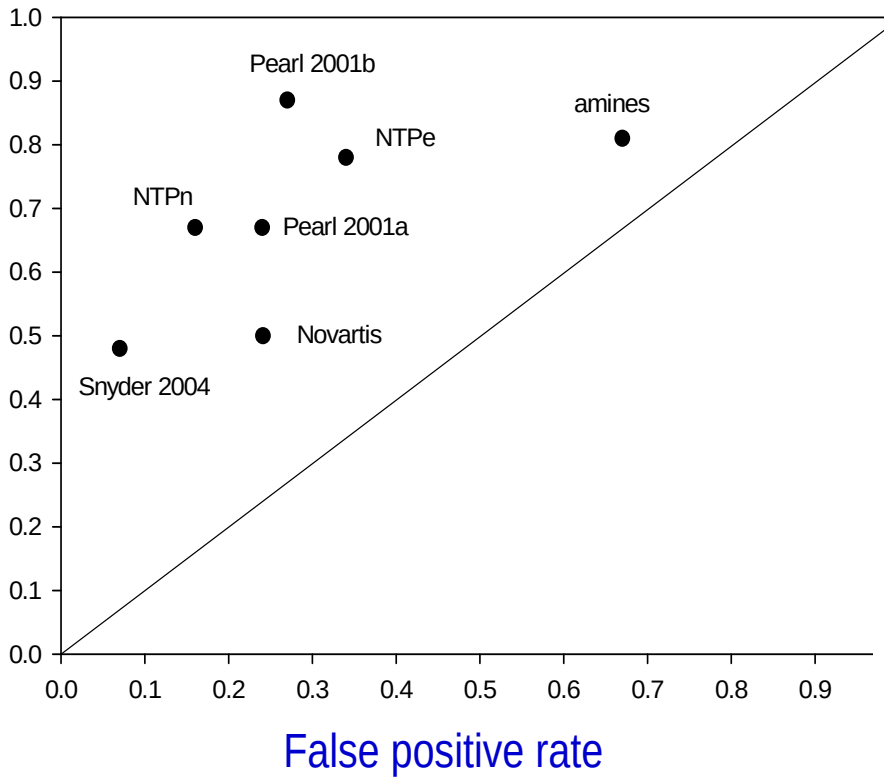


Carcinogenicity

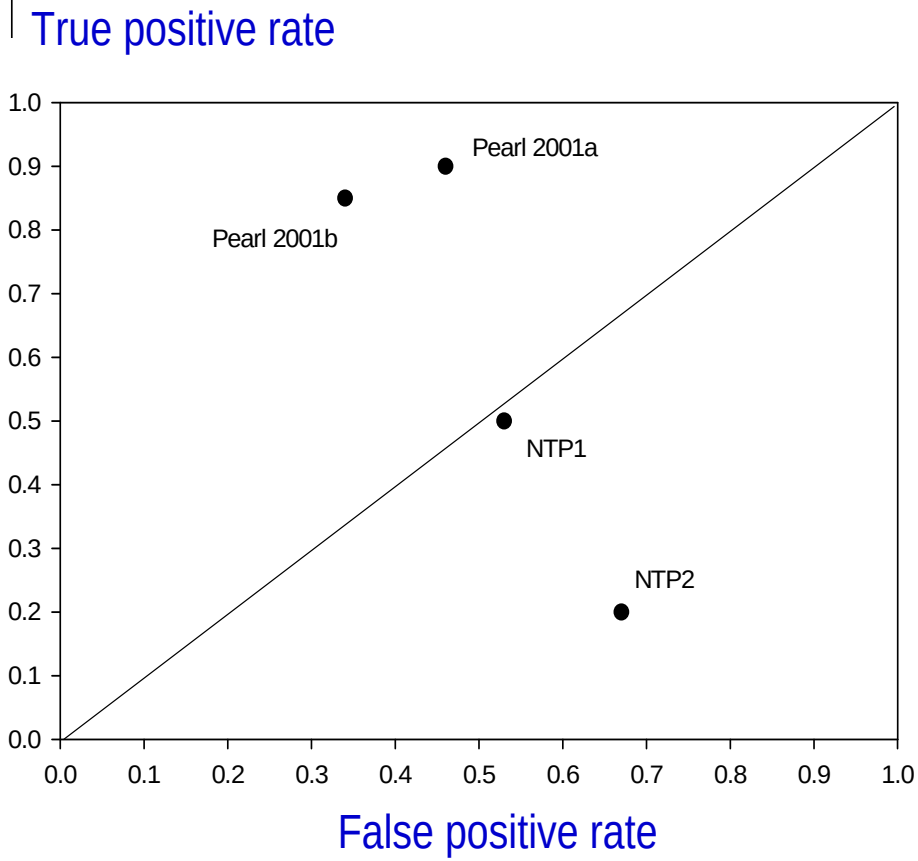


MULTICASE: External Validation

Mutagenicity



Carcinogenicity



Do “nongenotoxic carcinogens" lack genotoxic activity (and viceversa)?

Cyproterone acetate and **tamoxifen**:

liver carcinogens that had been thought to act by nongenotoxic mechanisms. Later studies indicated that are genotoxic and have tumor-initiating activity.

Steroidal estrogens: nongenotoxic, but also DNA-adducts

Inducers of **Oxidative stress**: also DNA damage

DNA-reactive chemicals can interact with proteins as well

Animal studies demonstrate that **tumor promoters** can cause cancer in the absence of an initiating agent

Do “nongenotoxic carcinogens" lack genotoxic activity (and viceversa)? **An issue for risk assessment**

…classification of carcinogens into genotoxic and non-genotoxic or into initiating or promoting agents may not only be unhelpful but even an impediment to risk assessment.

Once we can accumulate enough relevant mechanistic information about individual chemicals, it will be more reasonable to use this information directly for risk estimation, without expending efforts in classifying them… *Yamasaki 1995*

Classification systems based on labeling chemicals as genotoxic or nongenotoxic and on presumed mechanisms of action for each class lead to ambiguous reconstructions of the carcinogenic process….

… the existence or absence of threshold dose-responses cannot be determined from current knowledge of carcinogenic mechanisms.

The prudent policy for protecting public health is the one that considers the dose-response of all potential contributing effects of each specific chemical *(in each specific stage of carcinogenesis*)… *Melnick et al. 1996*

Mechanistic (Q)SAR:

- **Parameters that can be interpreted mechanistically**
- **Models that can be read by humans**

- Common ground for modelers, toxicologists and regulators
- Additional tool for minimizing chance (statistical) traps
- Intelligible information for synthesizing safer chemicals
- Basis for developing a QSAR science

... A mechanistic interpretation, if possible *(OECD)*

... We do mechanistic QSAR; we leave the Las Vegas approach to others.... *(Corwin Hansch)*

…a more general perspective

- Most **reductionist approaches** are weak predictors of reality

(e.g., chromosomal aberrations and other STTs,
DNA-hypomethylation in tissues,
omics in pharmacology)

- Two **success stories**

- **Ames test** maps a large family of DNA- (and protein-) reactive carcinogens
- **Cell Transformation** as model for cell-cell and cell-stroma interplay in cancer tissues (not a single-cell condition)

Higher level pathways: sum of pathways and their interactions

- Balance of details and generality as **challenge** in describing **complex systems**

In a fluctuating (stochastic) environment

is governed by the three fundamental laws of conservation:

i) ***mass***,
ii) ***energy*** &
iii) ***momentum***

Courtesy of Kumar Selvarajoo

Resulting in Navier–Stokes equations

$$\rho\left(\frac{\partial \mathbf{v}}{\partial t} + \mathbf{v}.\nabla\mathbf{v}\right) = -\nabla p + \nabla.\mathrm{T} + f$$

v : flow velocity, ρ : fluid density, p : pressure, T : stress tensor, f : body forces/volume