In silico, in vitro, in vivo as alternatives to the carcinogenicity bioassay for genotoxic chemicals

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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

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

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

Implementing the somatic mutation theory

- After the success of the **Millers**' electrophilic (DNA-) reactivity theory and of **Ames test**, development of > 100 **Short-Term Tests** (STT)

- Hypothesis on **complementarity of STTs**

  to cover the spectrum of cancer-relevant factors:

  *different **genetic endpoints** (gene mutation, chromosomal damage)*
  *different **cells** (bacterial, mammalian)*
  ***animals** (ADME)*

- Implemented into regulations: **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 = 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 ?

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- 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

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Backing up the STTs with **Structure-Activity** concepts

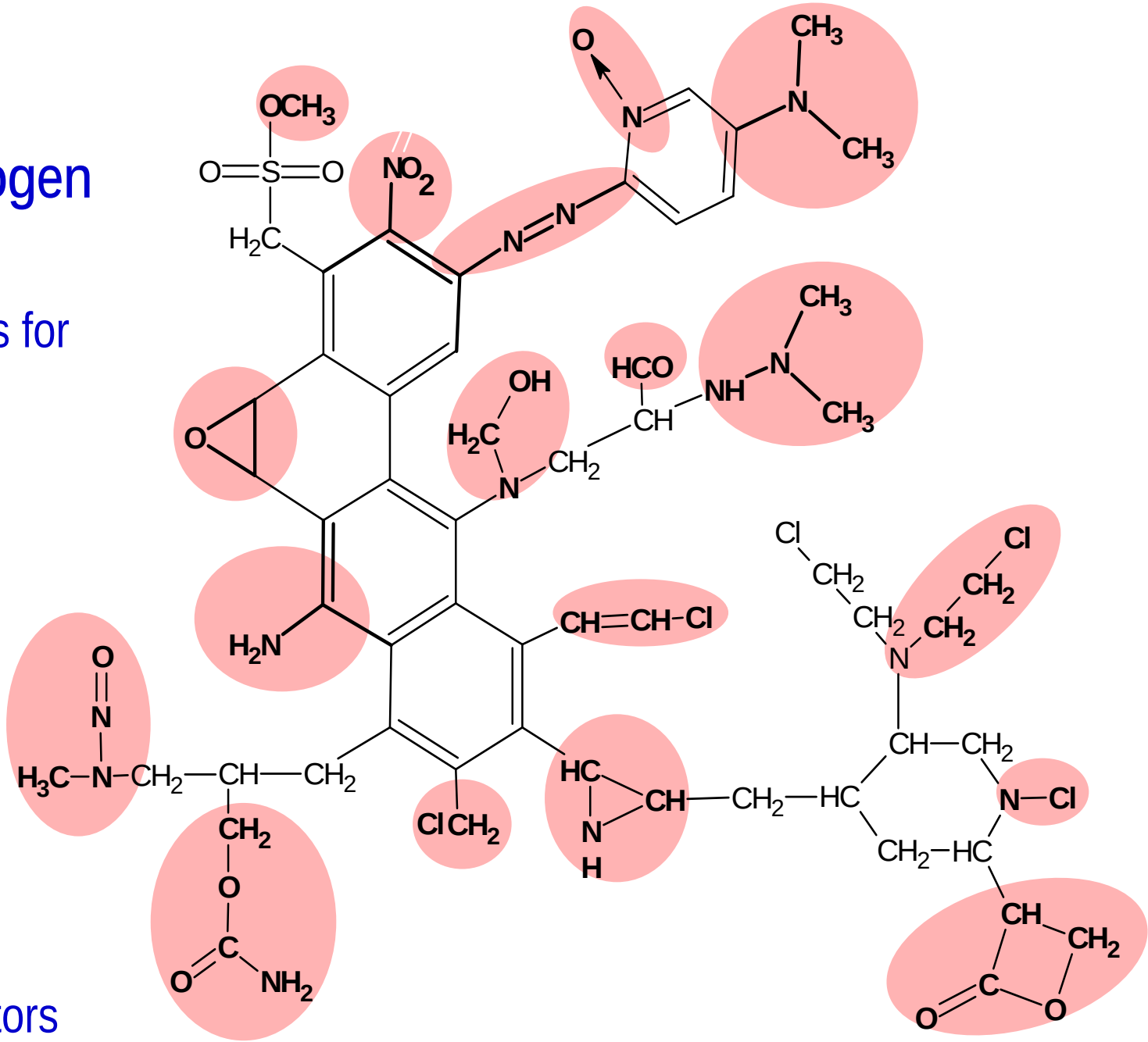
Structure-activity relationship concepts:

application to different issues, through different approaches

Coarse-grain	**Structural Alerts** (mechanistic classes, category formation, priorities)
Fine-tuned	Quantitative Structure-Activity Relationships (QSAR) of congeneric classes
Hybrid (??)	non-local, or global QSARs

Ashby's Poly-carcinogen

- Structural alerts for carcinogenicity;
- DNA-reactive functionalities



Some alerts accompanied by detoxifying (modulating) factors

Ashby (1995) Environ.Mutag. 7: 919-921

Toxtree – Institute for Health and Consumer Protection - (JRC-IHCP), European Commission - Windows Internet Explorer

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

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Preferiti | Toxtree — Institute for Health and Consumer...

European Commission

JOINT RESEARCH CENTRE

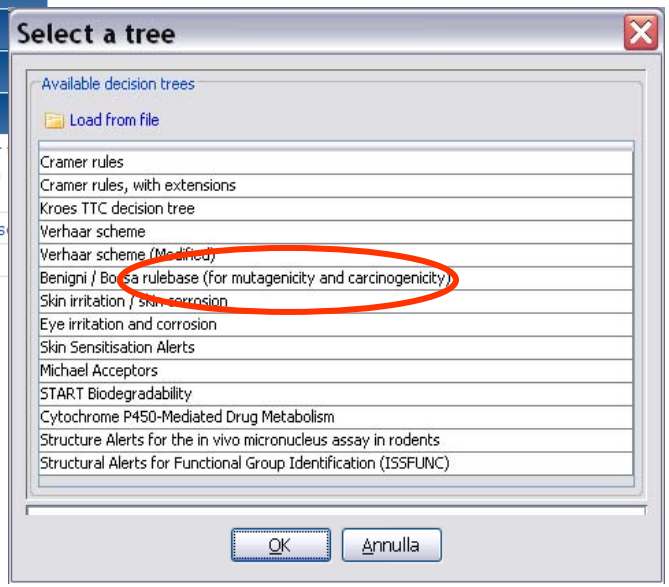
Institute for Health and Consumer Protection (IHCP)

European Commission > JRC > IHCP > Our Laboratories > Computational Toxicology and Modelling > QSAR Tools > Toxtree

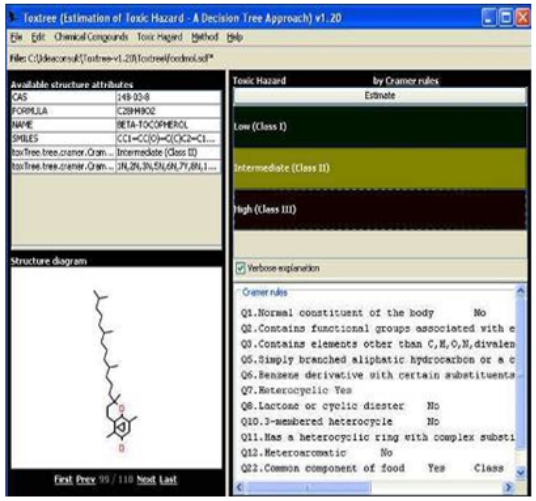
- Computational Toxicology and Modelling
- Background
- Information Sources
- Publications
- QSAR Tools
  - Stat4tox - Software for Statistical Evaluation of In Vitro Assays
  - Danish (Q)SAR Databas
  - Toxtree

Toxtree

Toxtree is a flexible and user-friendly open-source application that places chemicals into categories and predicts various kinds of toxic effect by applying decision tree approaches. implemented:



- the Cramer classification scheme
- an Extended Cramer scheme
- the Kroes TTC decision tree
- the Verhaar scheme for aquatic modes of action
- rulebases for skin and eye irritation and corrosion
- the Benigni-Bossa rulebase for mutagenicity and carcinogenicity
- the ToxMic rulebase for the in vivo micronucleus assay
- structural alerts for identification of Michael Acceptors
- the START rulebase for persistance / biodegradation potential
- structural alerts for the identification of organic functional groups (ISSFUNC rulebase)



Toxtree was developed by Ideaconsult Ltd (Sofia, Bulgaria) under the terms of a JRC contract. The software is made freely available as a service to scientific researchers and anyo based estimation methods in the assessment of chemical toxicity.

Toxtree (Version 2.5.0) (August 2011)

Toxtree 2.5.0 is a standalone software application that can be run on the Microsoft Windows operating system as well as other platforms with the Java 2 Runtime Environment (Sta

Many Toxtree rulebases included in the OECD (Q)SAR Toolbox

Toxtree: Rulebase for mutagens / carcinogens

Structure-based approach consisting of:

- Compilation of *Structural Alerts* (genotox and non-genotox)

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

Toxtree (version 2.5)

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

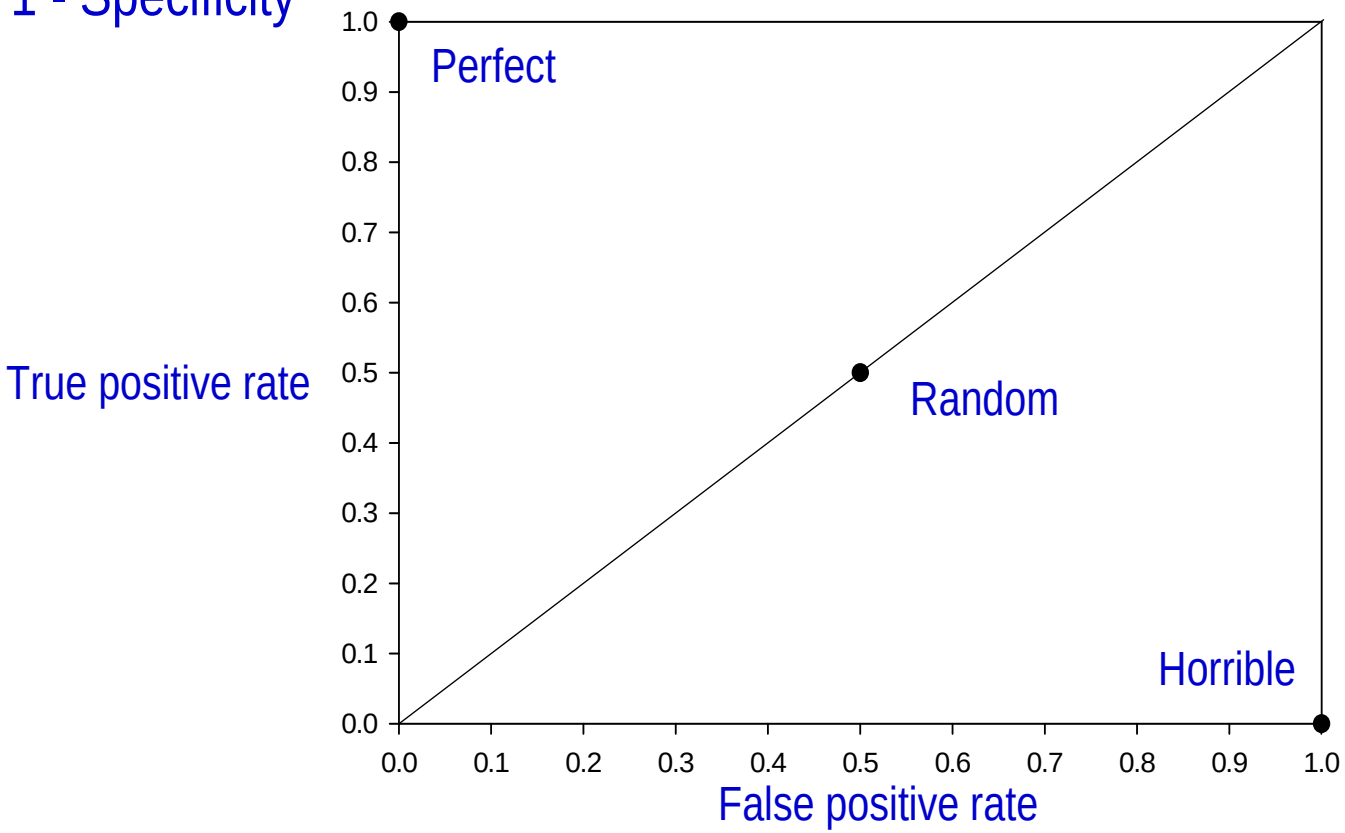
ROC graph: A simple, graphical way of comparing predictions with results

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

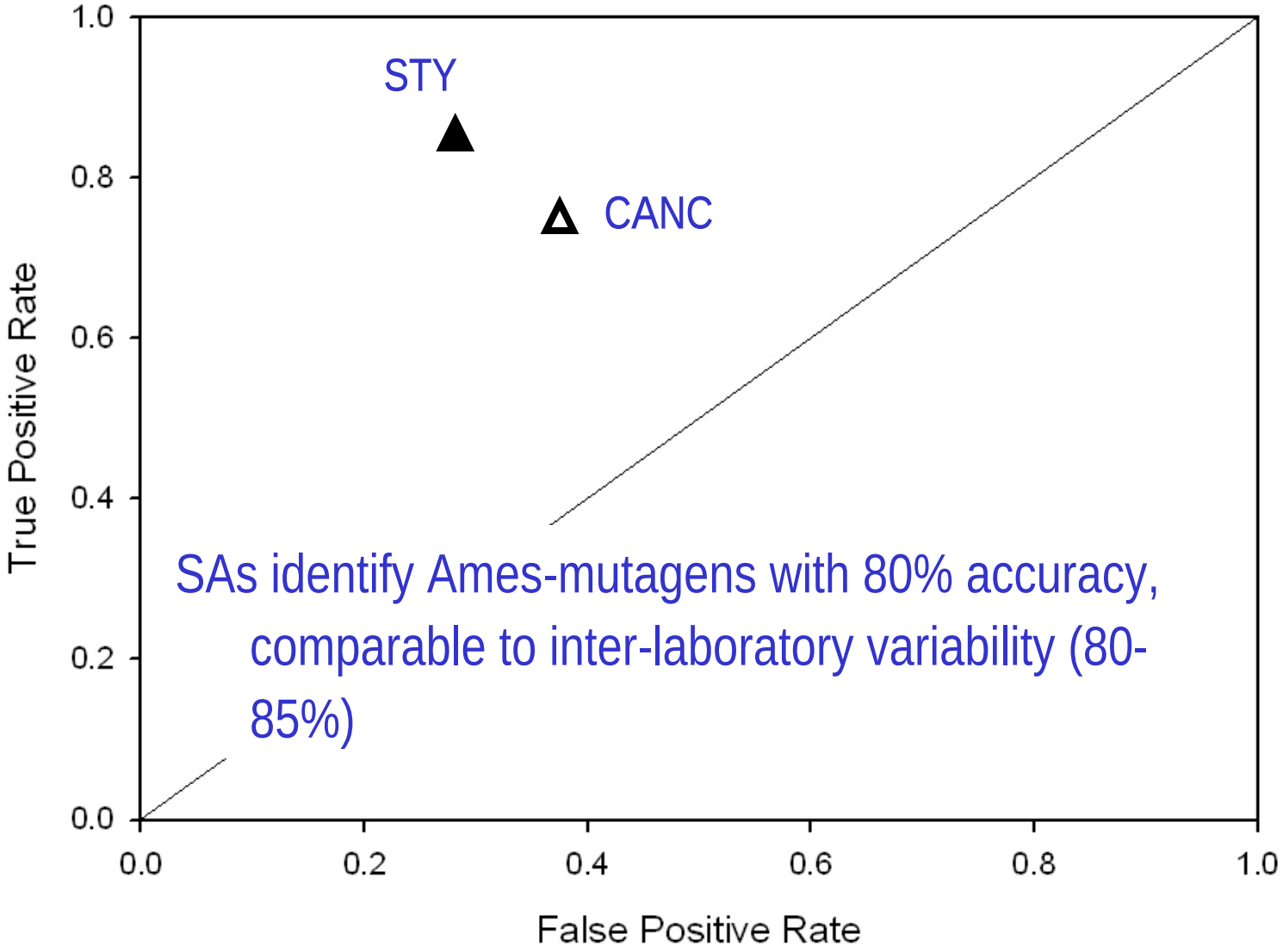
= Sensitivity

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

= 1 - Specificity



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



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

Positive Predictivity: Which reliability for toxicity estimates?

Mechanism	Number of substances	SA→Canc	SA→STY	STY→Canc
Acylating	6	0.67	1.0	0.80
Alkylating (direct)	199	0.73	0.60	0.83
Alkylating (indirect)	234	0.86	0.76	0.92
Intercalating	36	0.83	0.86	0.88
Amino-arylating	223	0.75	0.78	0.81
Overall (ISSCAN)	1141	0.77	0.67	0.80

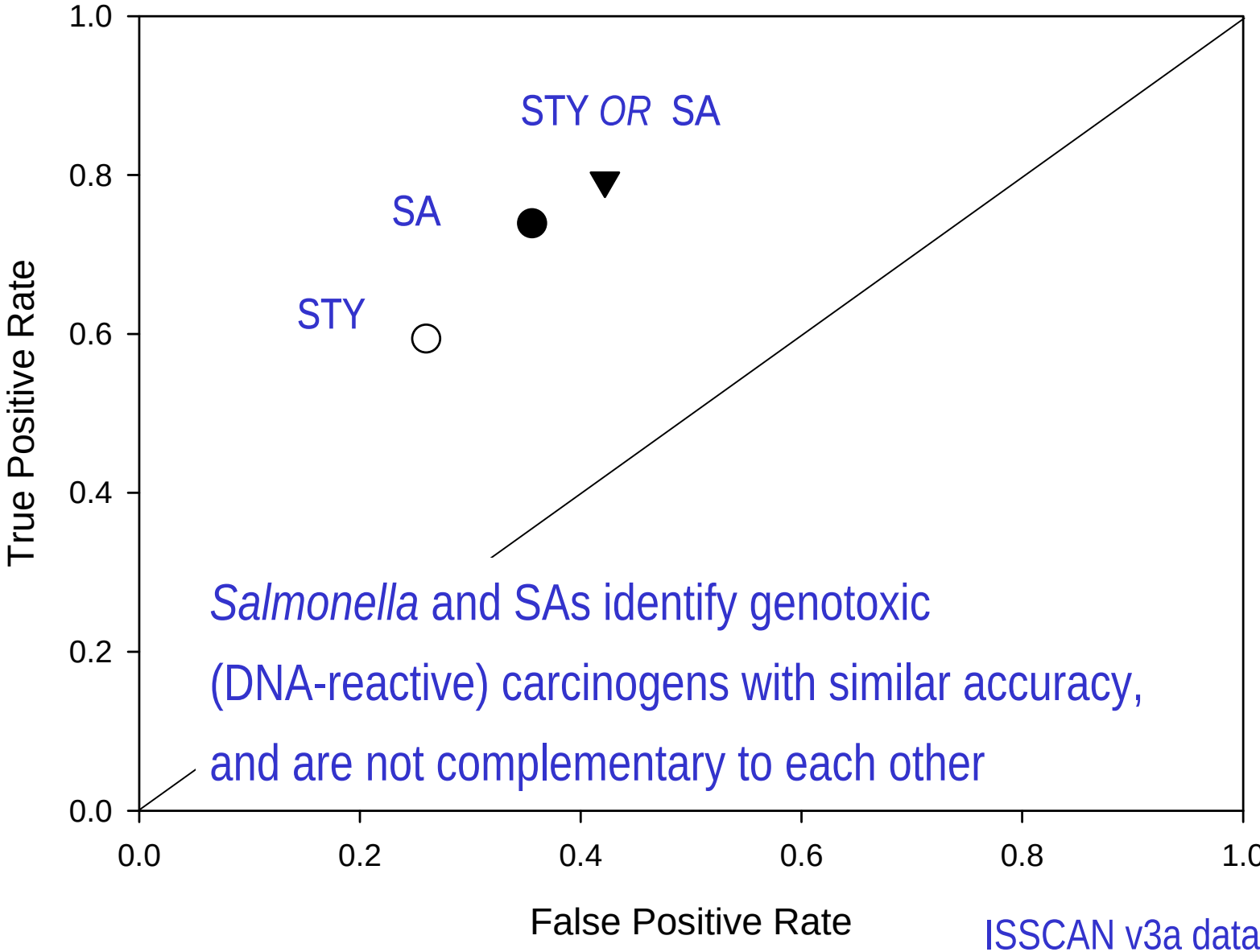
Knowledge on DNA-reactivity (as coded by SAs):

- 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)

Identifying genotoxic carcinogens through alternative approaches

- *in vitro* assay {*Salmonella*}
- Structural Alerts to predict / rationalize experimental results

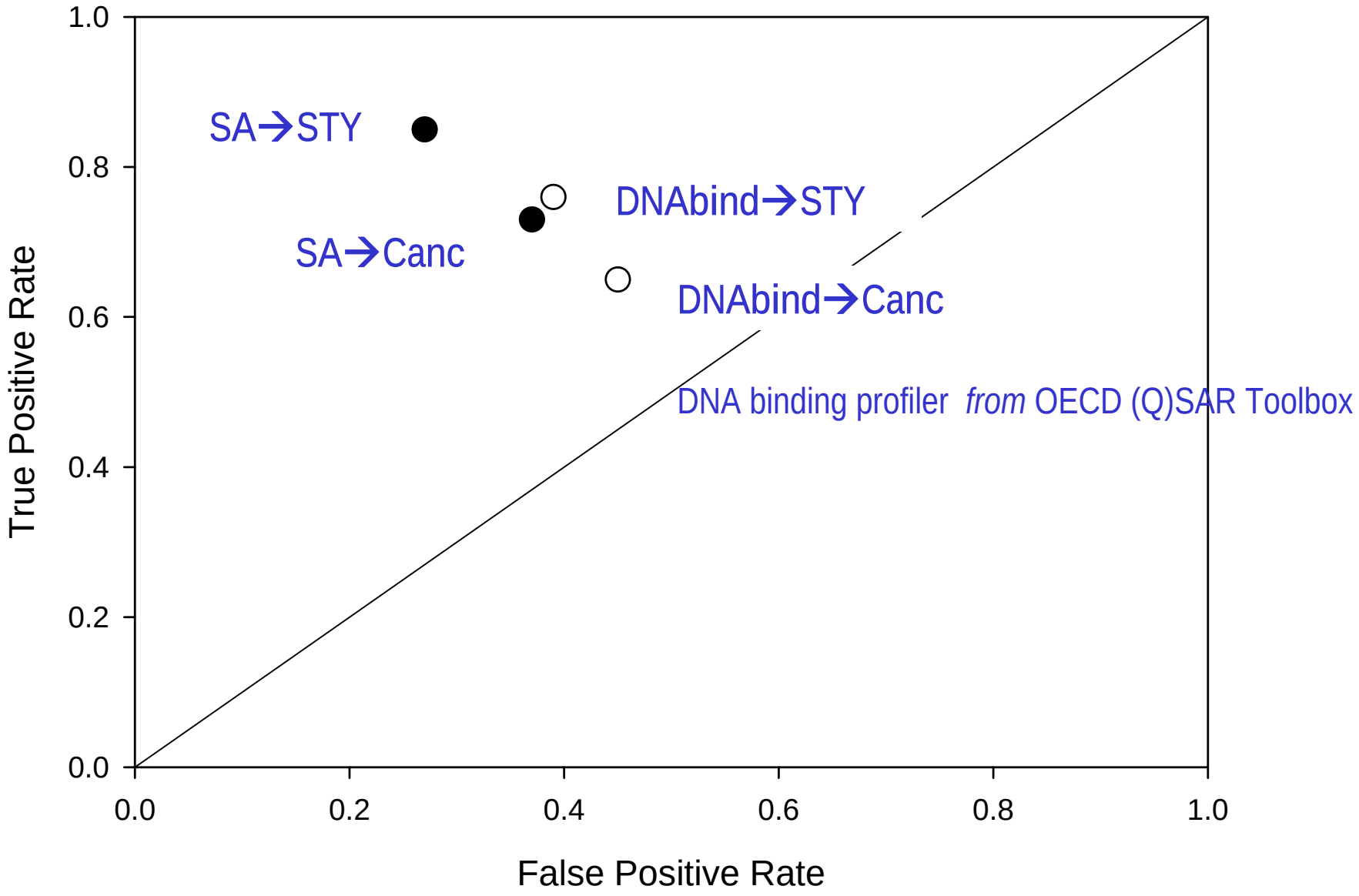
Carcinogenicity prediction: *Salmonella* (Ames) *OR* SAs



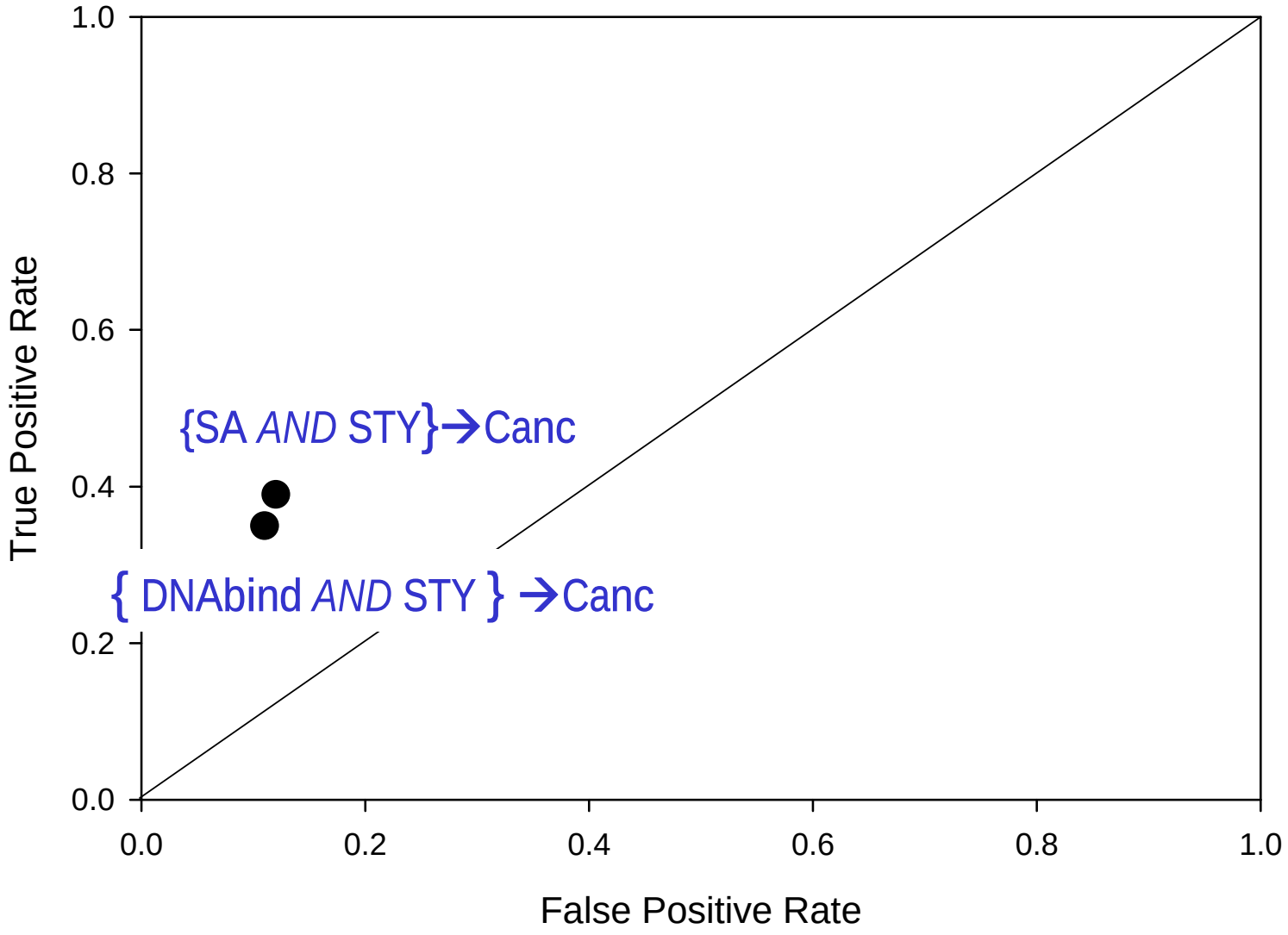
Salmonella and SAs identify genotoxic (DNA-reactive) carcinogens with similar accuracy, and are not complementary to each other

ISSCAN v3a database

SAs for genotoxic carcinogenicity *versus* DNA-binding potential

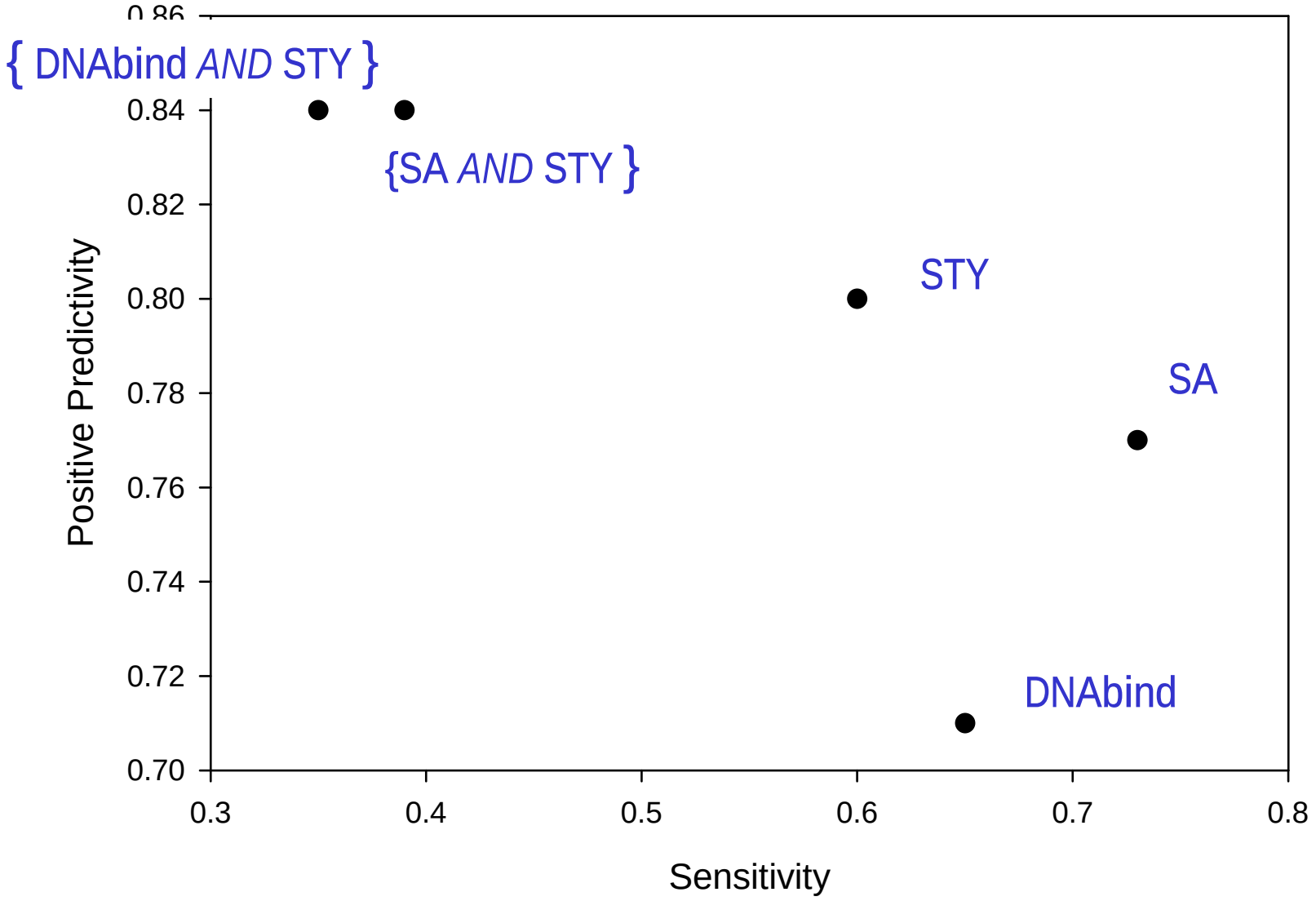


Two-tiered approach: Structural considerations *AND* Biological confirmation



	Positive predictivity
Individual systems	
Ames Test	0.80
Structural Alerts	0.77
DNA binding	0.71
Tiered approaches	
DNA binding AND Ames Test	0.84
Structural Alerts AND Ames Test	0.84

Positive Predictivity and Sensitivity: inverse functions



Identifying genotoxic carcinogens through alternative approaches:

- *in vitro* assay {*Salmonella*}
- Structural Alerts to predict / rationalize experimental results

A flexible process (different estimates can be attached to the chemicals, with different coverage / reliability)

High reliability can be attained

Acknowledgements

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