United States Environmental Protection Agency

EPA's ToxCast™ Program for Predicting Hazard and Prioritizing Toxicity Testing of Environmental Chemicals

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This work was reviewed by EPA and approved for presentation but does not necessarily reflect official Agency policy

Office of Research and Development
National Center for Computational Toxicology

http://www.epa.gov/comptox/toxcast

Outline

- EPA ToxCast Program
  - Developing new approaches to screening and prioritization of environmental chemicals
  - Addresses toxicity data gap
  - Reduces animal use
  - Anticipates NRC report on Toxicity Testing of the 21st Century

- ACToR – Aggregated Computational Toxicology Resource
  - Collecting all the world's publicly available *in vitro* and *in vivo* data on environmental chemicals
  - Includes deep yet-to-be-released toxicology data from EPA

ACToR and ToxCast
Addressing the Toxicity Data Gap

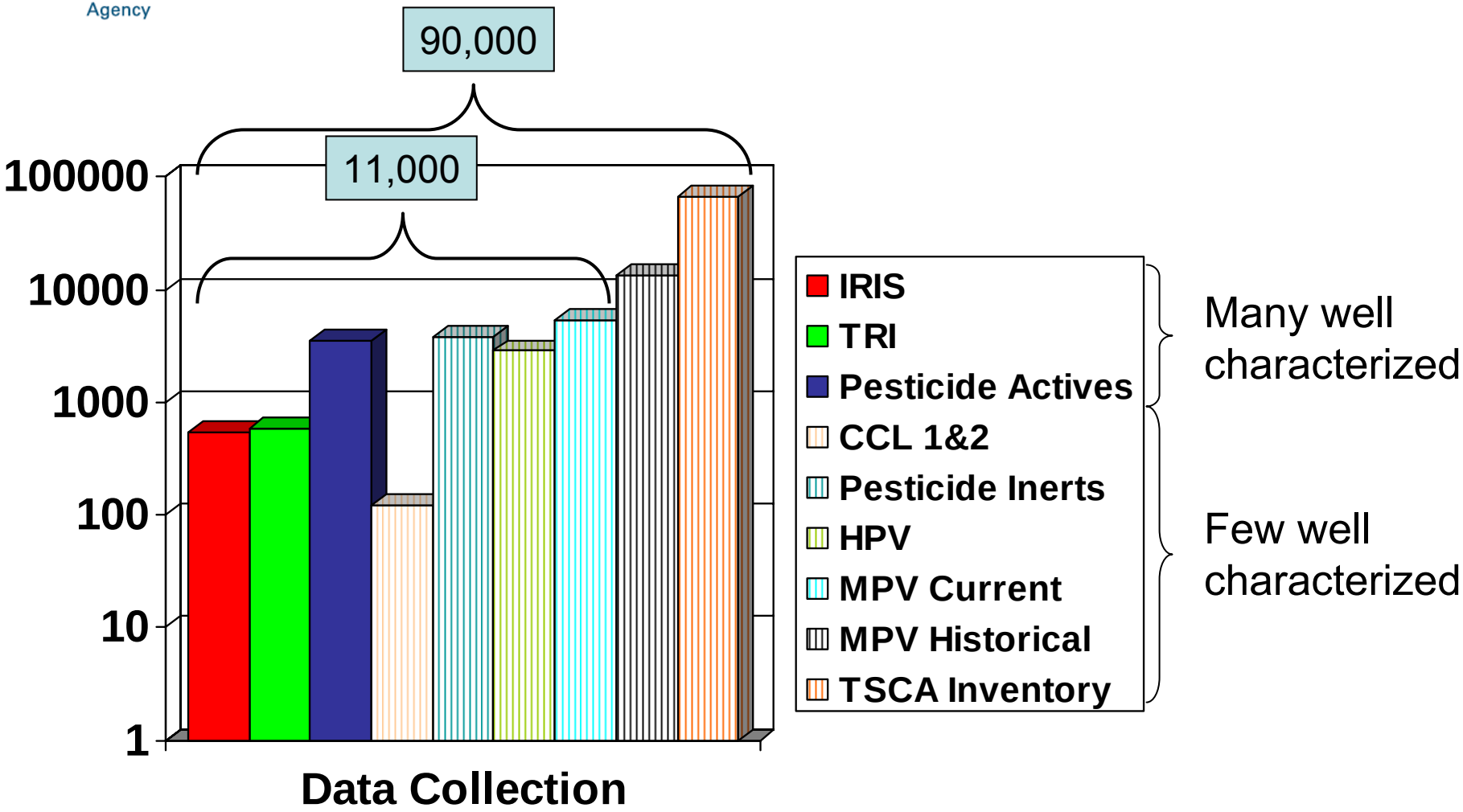
Complete toxicity package includes:

- Acute*
- Subchronic
- Chronic / Cancer
- Developmental *
- Reproductive *
- Immunotox
- Neurotox
- Genotox *
- Dermal
- Respiratory
- Nephrotox
- Endocrine
- Cardiotox
- Hepatotox
- Ecotox*

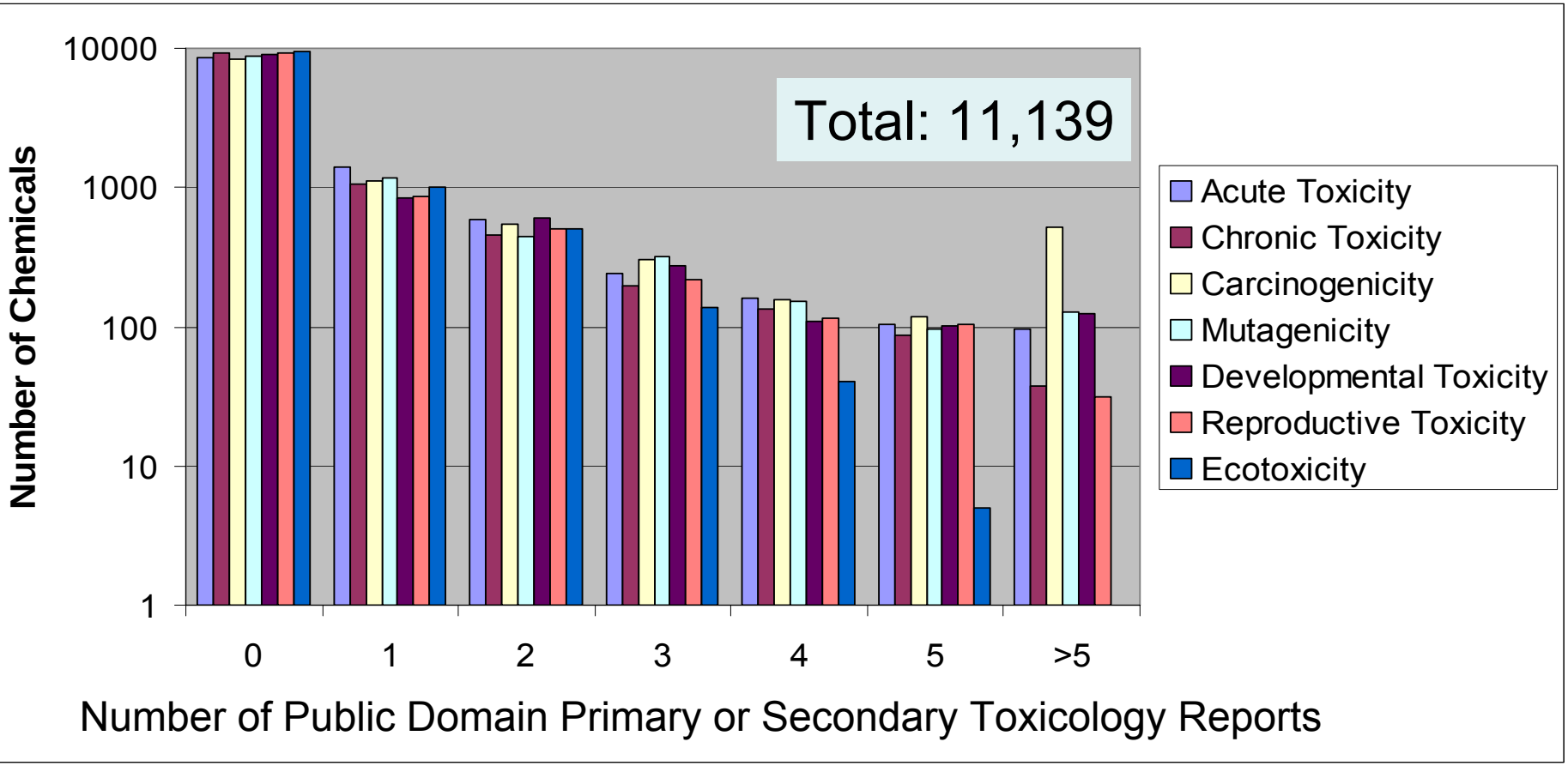
"Pesticide Food Use Actives" require all - $Ms/chemical

(*) Required for TSCA substances

The World of Environmental Chemicals



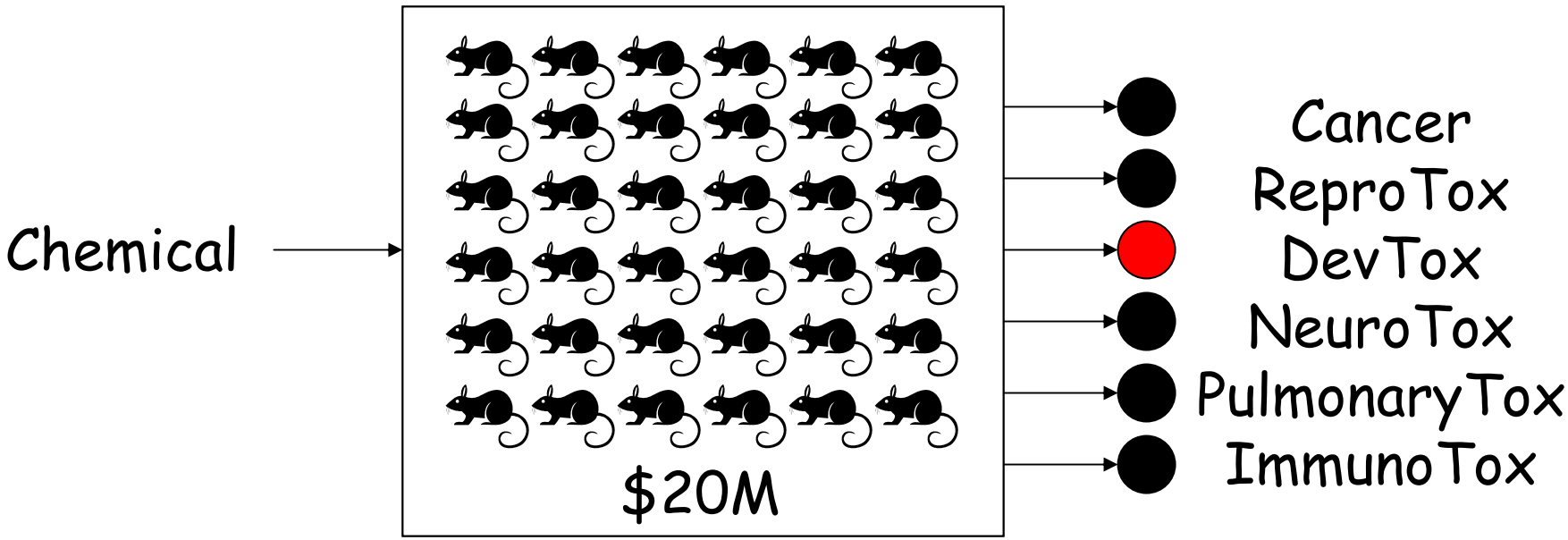
Current Data Coverage / Data Gaps for Widely-used Environmental Chemicals



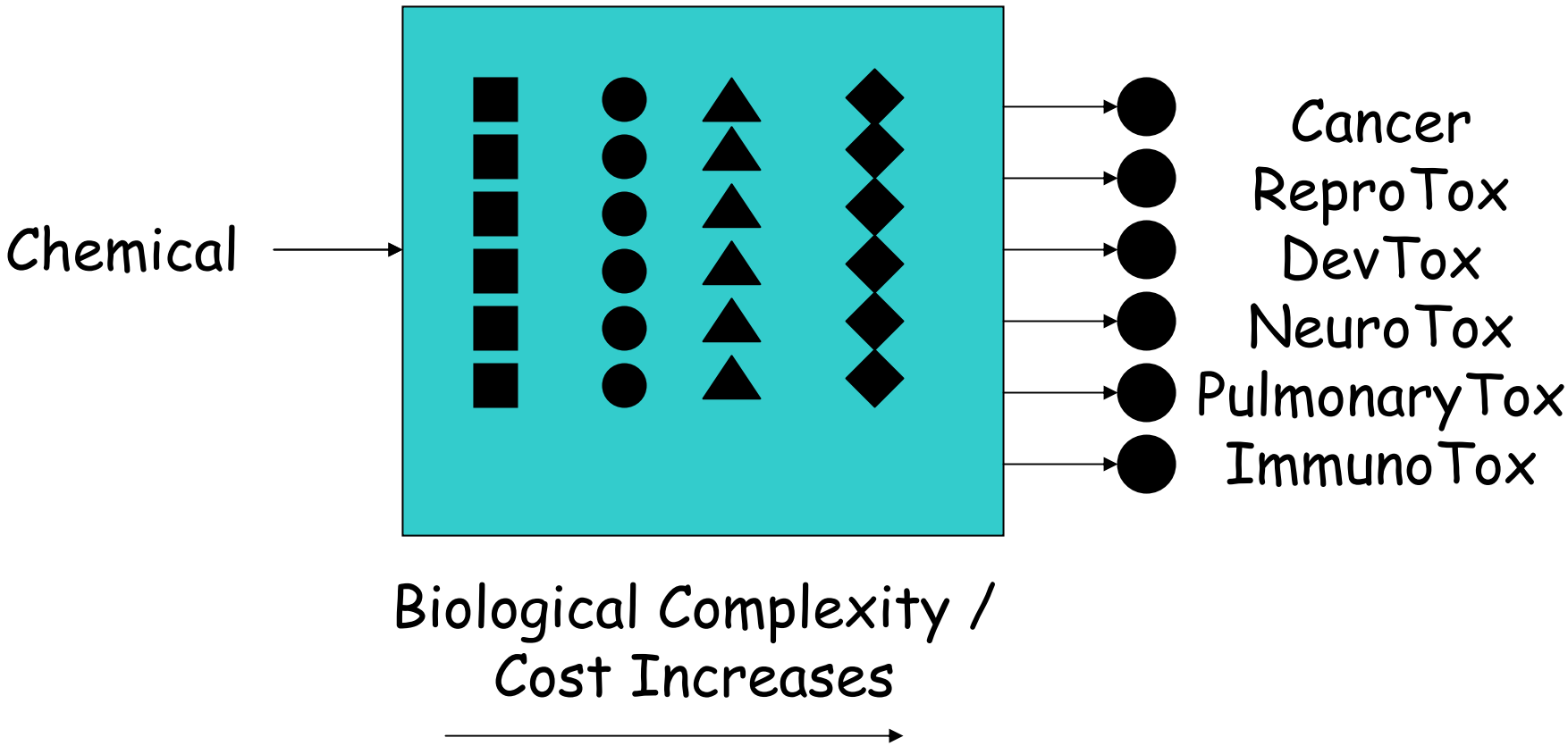
<10% have data for most tests

ACToR is the one-stop shop for this data

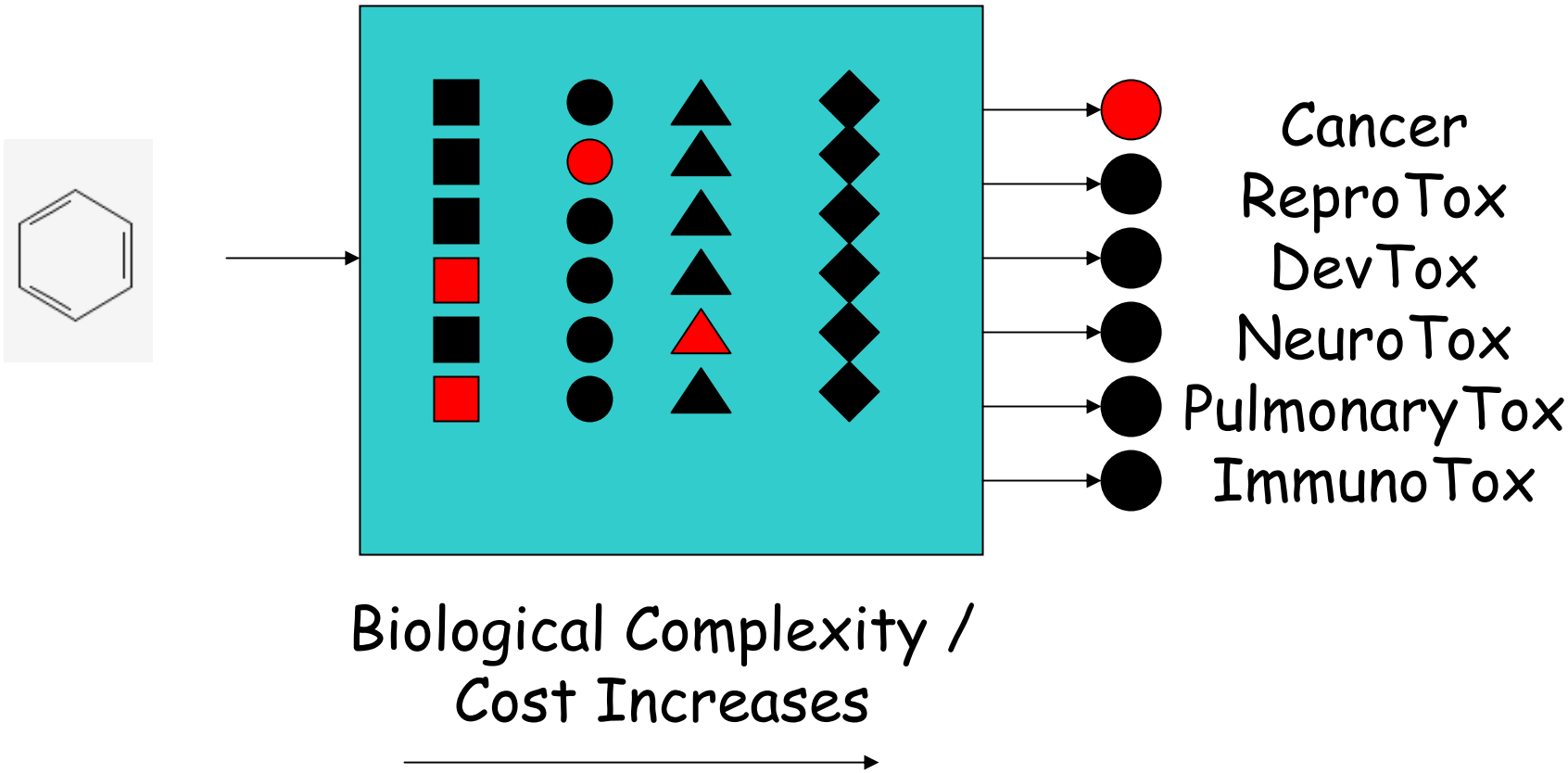
Toxicity Prediction Today



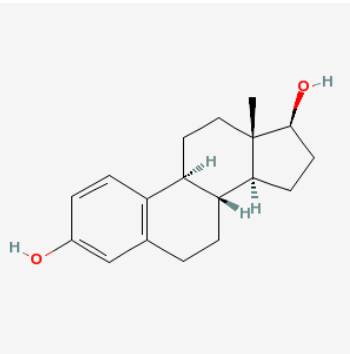
Toxicity Prediction Tomorrow: Find Pattern that Predicts Toxicity using Inexpensive Assays

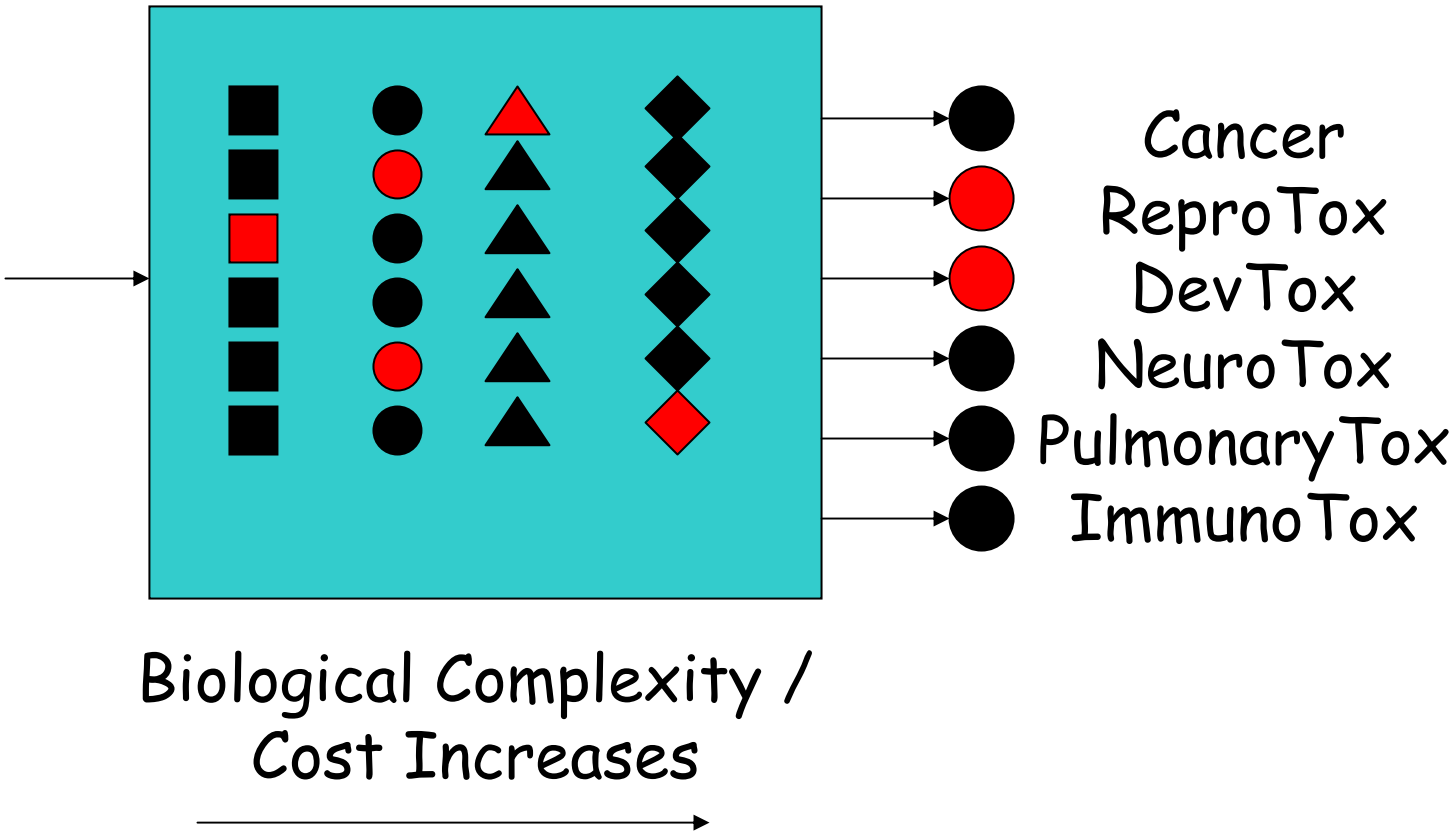


Toxicity Prediction Tomorrow: Find Pattern of Assays that Predicts Tox

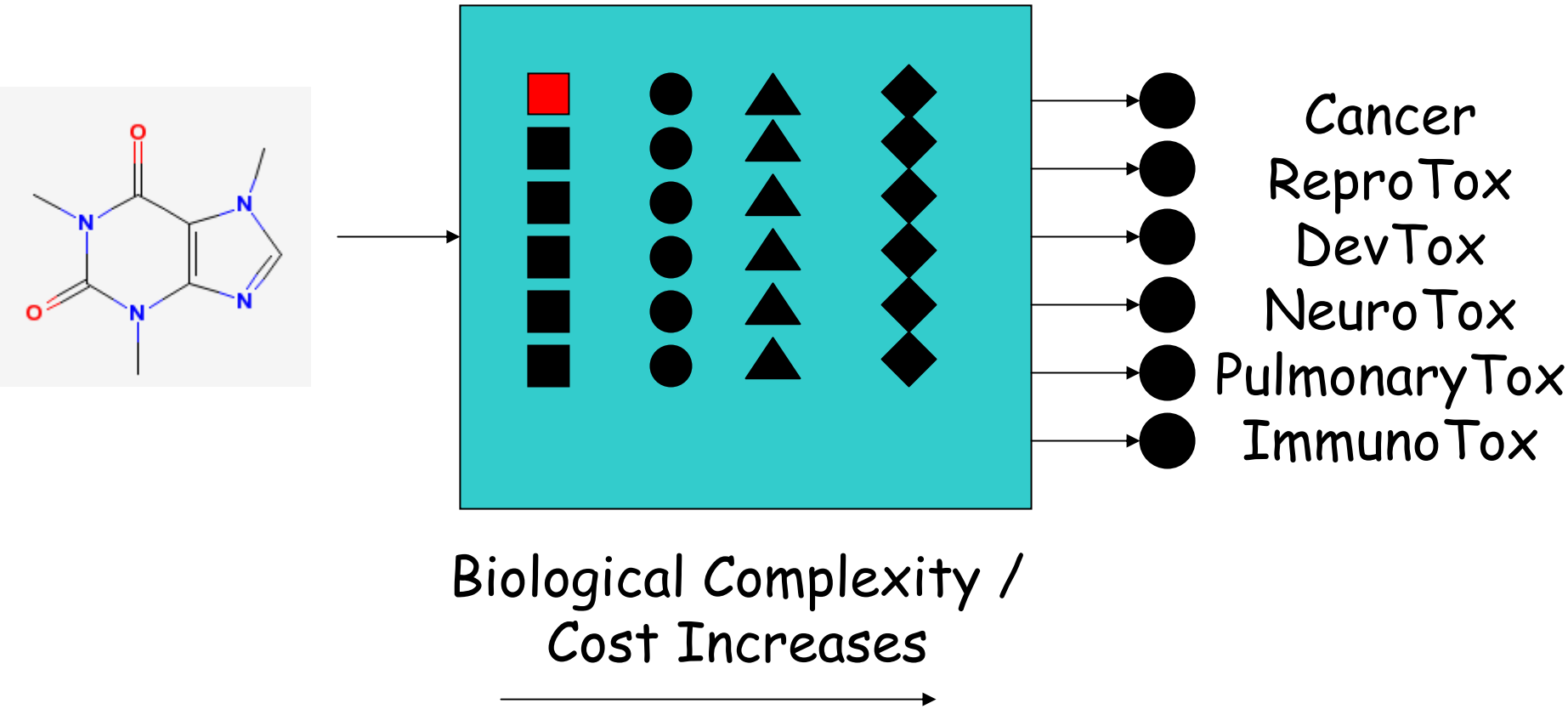


Toxicity Prediction Tomorrow: Find Pattern of Assays that Predicts Tox





Toxicity Prediction Tomorrow: Find Pattern of Assays that Predicts Tox

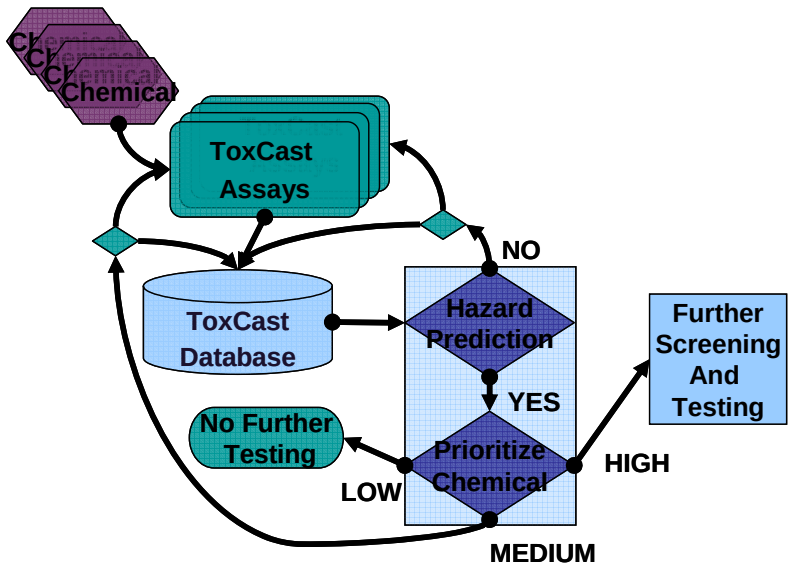


ToxCast Background

- Research program developed by EPA's National Center for Computational Toxicology
- Formulated to address chemical screening and prioritization needs
- Based on experience of the pharmaceutical industry
- Comprehensive use of current technology
- Phased approach to evaluate utility
- Committed to stakeholder involvement and release of data to public domain

Phased Development of ToxCast Program

Phase	Number of Chemicals	Chemical Criteria	Purpose	Number of Assays	Cost per Chemical	Target Date
I	>300	Data Rich (pesticides)	Signature Development	>400	$20k	FY07-08
II	>1000	Expanded Structure and Use Diversity	Evaluation and Extension	>300	$15-20k	FY08-09
III	Thousands	Data poor	Prediction and Prioritization	>300	$10-15k	FY10-12



- Delivers an affordable, science-based system for categorizing chemicals
- Increasing confidence as database grows
- Identify potential mechanisms of action
- Refine and reduce use of animals in hazard identification and risk assessment

Chemical Classes Investigated in ToxCast Phase I

291 pesticide actives with complete toxicity datasets
30 Carbamates (plus one metabolite)
33 Organophosphates (plus several metabolites)
12 Pyrethroids
12 Triazines (plus one metabolite)
17 Azole Fungicides (plus one metabolite)
13 Organochlorines
7 Phthalates (and several metabolites)
14 HPVs, 11 HPV challenge
56 of 73 chemicals proposed for Tier 1 EDSP

ToxRefDB

Reference In Vivo Toxicology Database

- Office of Pesticide Programs - Data Evaluation Records (DER)
  - 20 years of key primary toxicology data for industry submitted toxicology studies
  - Complete data package for >300 chemicals
  - High quality data with significant QC
  - Incorporate into relational model with expert-developed controlled vocabularies
  - Integrate other primary toxicology sources (NTP, OPPT, European agencies)

First effort to capture, tabulate and mine this unique resource

STUDY TYPE: Combined chronic toxicity/oncogenicity feeding – Rat
OPPTS 870.4300 [§83-5]

DP BARCODE: D257223 SUBMISSION CODE: S564270
P.C. CODE: 111901 TOX. CHEM. NO.: 497AB

TEST MATERIAL (PURITY): Imazalil (purity 97.4%)

CITATION: Van Deun, K. 1999. Combined oral chronic toxicity/carcinogenicity study with Imazalil in the SPF Wistar rat. Dept. Toxicology, Janss Foundation, 2340 Beerse, Belgium. Laboratory report number, 3817, June 8, 1999. MRID 44858001. Unpublished.

EXECUTIVE SUMMARY:

In a chronic toxicity/oncogenicity study (MRID 44858001), Imazalil (97.4% a.i.) was administered in the diet to groups of 50 male and 50 female H substrain (SPF) Wistar-derived rats at concentrations of 0, 50, 200, 1200, or 2400 ppm (equivalent to 0.0, 2.7, 10.8, 65.8, and 134.8 mg/kg/day fo 3.6, 14.6, 85.2, and 168.8 mg/kg/day for females) for two years. All rats were observed daily for clinical signs of toxicity and morbidity, weighed w consumption monitored biweekly. Blood and urine samples were collected after 6, 12, and 18 months of treatment and at study end. Surviving ra sacrificed after 104 weeks of treatment. All rats were necropsied and the tissues and organs inspected grossly and microscopically for toxicity-re the carcinogenic potential of Imazalil.

Male and female rats in the 1200 ppm and 2400 ppm groups had slightly increased RBCs and slightly decreased MCVs and MCHs that reached significance at most measurement intervals. The differences, however, were within 6% of controls and the historical control limits for rats of this a species. No significant treatment-related effects of biological relevance were found for most clinical chemistry parameters. However, plasma trig activities of ALKP, AST, and ALT of male rats in the 2400 ppm group and female rats in the 1200 and 2400 ppm groups were often statistically de remained within historical control limits. The effects were considered related to malnutrition.

The effect of treatment on the liver (males and females) and thyroid (males only) were confirmed microscopically, but had distinct sex-related etio incidence of clear cell and basophilic foci was equivocal while eosinophilic foci were significantly increased for male rats in the 2400 ppm group. the 2400 ppm group, the incidences of clear cell and basophilic foci were significantly decreased but the incidence of eosinophilic foci was unaffe incidence of hepatocyte fatty vacuolation was increased only in male rats of the 1200 ppm and 2400 ppm groups while the incidence of pigmenta increased only in females of the 200, 1200, and 2400 ppm groups. In addition, the location of hepatocellular hypertrophy was distinctly different. the 1200 and 2400 ppm groups had significant increases in centriacinar and periacinar hypertrophy while male rats only had centriacinar hypertro the incidence of thyroid follicular cell hyperplasia was increased only in male rats of the 1200 and 2400 ppm groups.

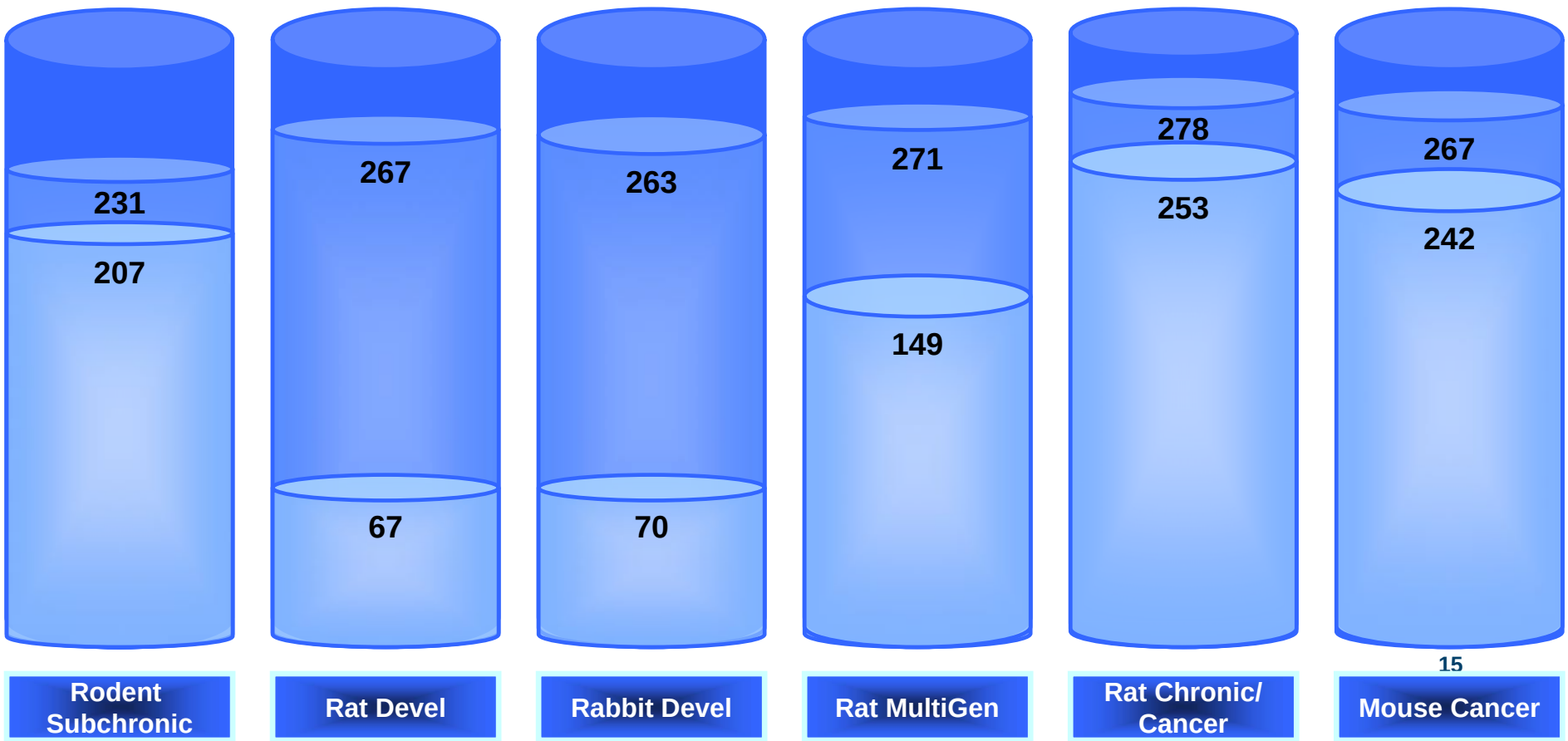
The lowest observed adverse effect level (LOAEL) for male and female rats was 1200 ppm (65.8 and 85.2 mg/kg/day, respectively) with a corresponding no observed adverse effect level (NOAEL) of 200 ppm (10.8 mg/kg/day males, 14.6 mg/kg/day females). These are based found on body weight, weight gain, and the macro- and microscopic effects noted in the liver of all rats and the thyroid of male rats.

Male rats had a significant increase in the incidence of hepatocellular adenomas and thyroid follicular neoplasia while no increase was found for f These results indicate a difference in the disposition of Imazalil between the sexes increases hepatic and thyroid neoplasia in male rats, likely thr in metabolic activation of the test material.

ToxRefDB Data Entry Status

ToxCast Chemicals	320
Unique Chemicals	308
Pesticide Actives	291

Current as of November 1, 2007



High-Throughput Screening Assays

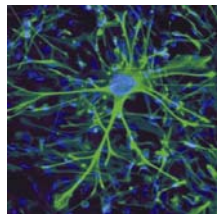
batch testing of chemicals for pharmacological/toxicological endpoints using automated liquid handling, detectors, and data acquisition

LTS	MTS	HTS	uHTS

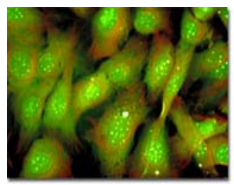
10s-100s/yr

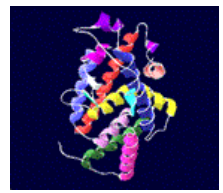
Gene-expression

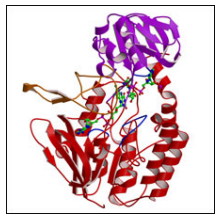
10s-100s/day



1000s/day







10,000s-
100,000s/day

Human Relevance/
Cost/Complexity

Throughput/
Simplicity

ToxCast HTS Assays

United States Environmental Protection Agency

BioFocus DPI
A Galápagos Company

NovaScreen
a Caliper Life Sciences company

Phylonix
Zebrafish Assays for Drug Screening

United States Environmental Protection Agency
ToxCast™
Computational Toxicology

BioSeek

attagene
The Home of TFomics™

Nine contracts and one IAG providing chemical procurement, biochemical assays, cellular reporter assays and genomics, complex human cell responses, and model organisms; capacity to screen up to 10,000 chemicals in over 400 assays by 2012

Cellumen

NCGC
NIH Chemical Genomics Center

ACEA Biosciences, Inc.

IVAL

Expression Analysis

EPA United States Environmental Protection Agency

NOVASCREEN a Caliper Life Sciences company

Biochemical and Cellular High Throughput Screeening (HTS)

Number of Assays

30 Cytochrome P450s

81 GPCRs

22 Ion Channels

28 Kinases

24 Nuclear Receptors

19 Phosphatases

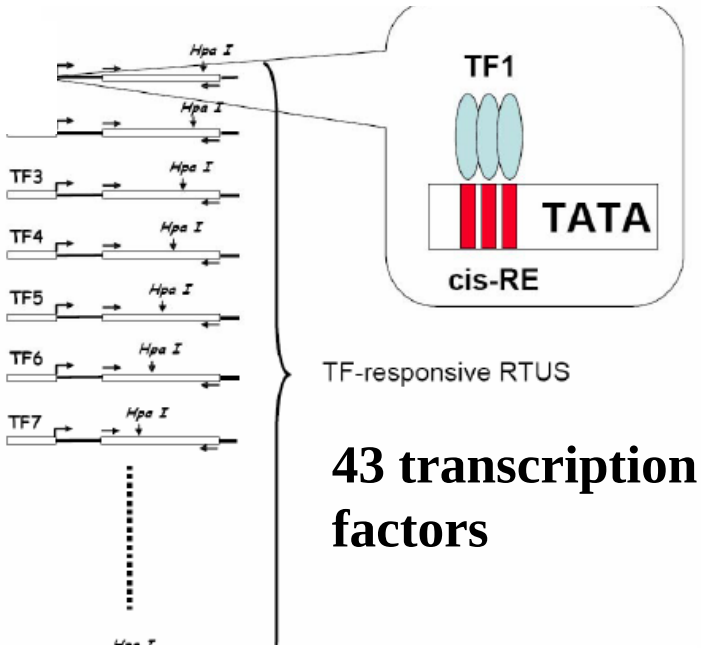
9 Transporters

27 Other Enzymes

Transcription Factor Activity Profiling

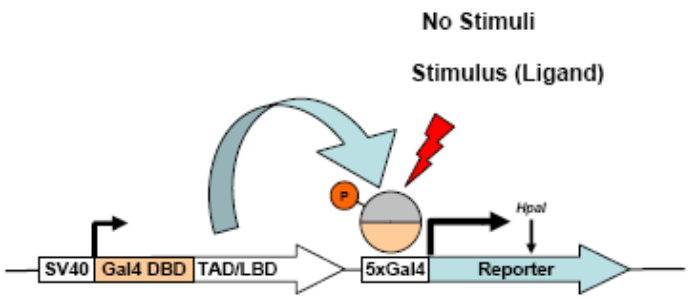
attagene
The Home of TFomics™

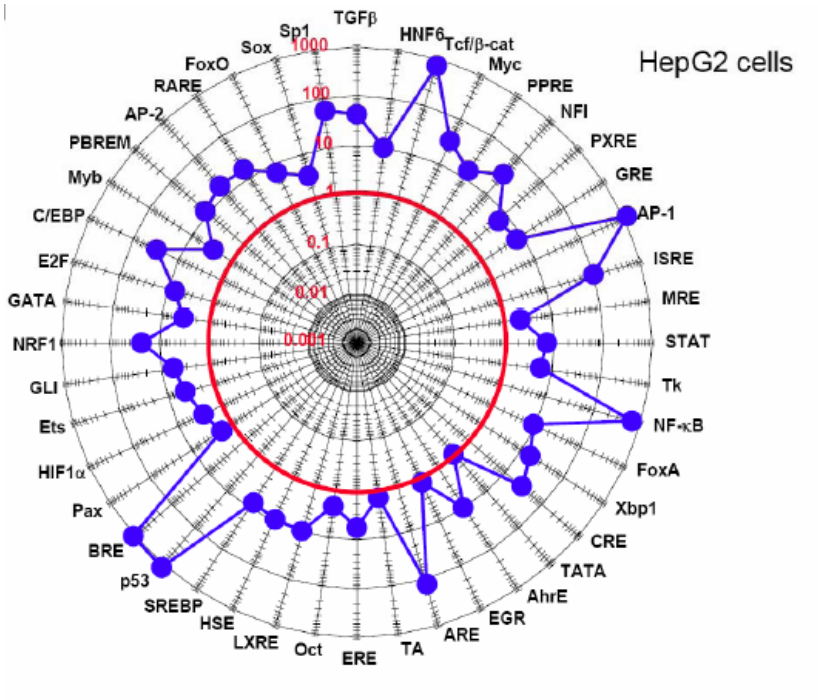
Cis-Factorial™ Biosensors



43 transcription factors

Trans-Factorial™ Biosensors





24 nuclear receptors

Nuclear Receptor Activation

NIH Chemical Genomics Center

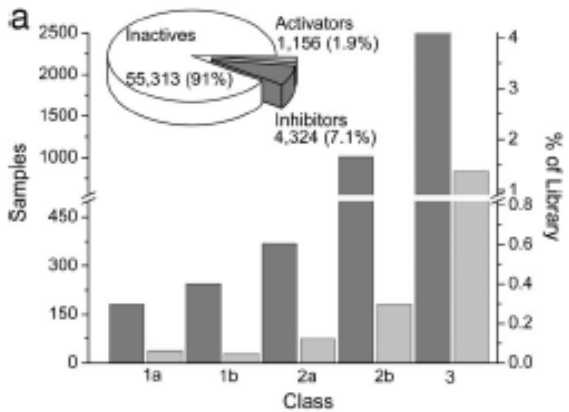
PNAS

Quantitative high-throughput screening: A titration-based approach that efficiently identifies biological activities in large chemical libraries

James Inglese*, Douglas S. Auld, Ajit Jadhav, Ronald L. Johnson, Anton Simeonov, Adam Yasgar, Wei Zheng, and Christopher P. Austin

NIH Chemical Genomics Center, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892-3370

Communicated by Francis S. Collins, National Institutes of Health, Bethesda, MD, May 31, 2006 (received for review April 12, 2006)



PNAS August 2006 vol 103 no 31 11473-11478

Reporter gene assays

From Invitrogen:
- AR
- ERa
- FXR
- GR
- LXRb
- PPARd
- PPARg
- RXR
- TRb
- VDR
- RORa

Considering from other sources:
- PPARa
- PPARb/d
- PPARg
- PXR
- CAR
- FXR
- RXRa
- ERRa
- TRa
- PR
- ERa
- ERb
- AR
- GR
- MR
- LXRa
- LXRb
- VDR
- RORa

EPA United States Environmental Protection Agency

EXPRESSION ANALYSIS

Gene Expression Profiling by Microarray and PCR

Human BeadChips assay up to 48,000 transcripts.
Mouse BeadChips assay up to 47,000 transcripts.
RatRef BeadChips assay up to 22,000 transcripts.
Customized chips- up to 1400 genes in 96well format.
Individual or multiplexed PCR (≤ 48 transcripts in parallel).

Toxicogenomic Profiling of Hepatocyte – Kupffer Cell Co-Cultures

- Testing human, mouse and rat
- Coordinating with Virtual Liver project
- Optimized ratio of Hepatocyte – Kupffer cells
- Concentration response
- Focusing analysis on Nuclear Receptor mediated gene expression pathways

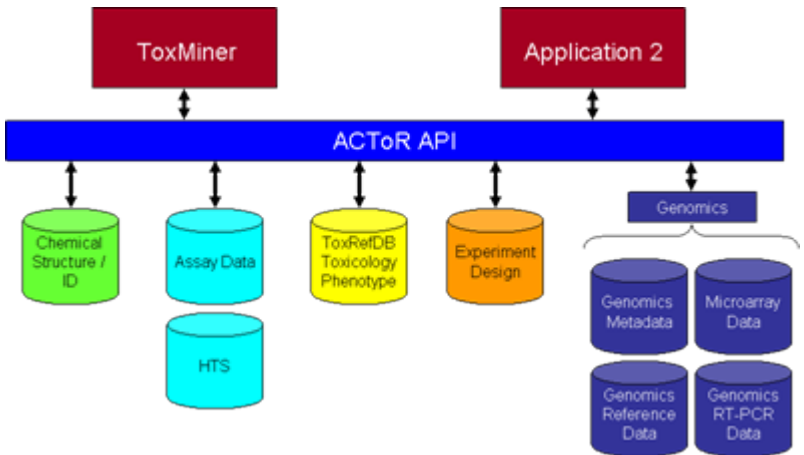
Table 1. Endpoints contained within Phase I signature development

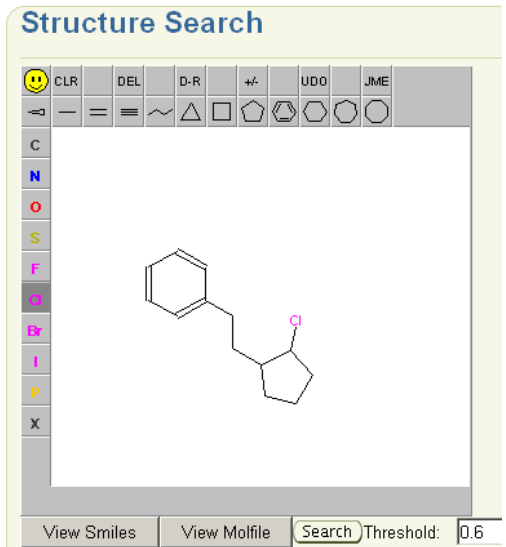
Assay Type	Number of Assays	Number of Unique Endpoints	Assay Source	Comment	Source	Reference
Biochemical	+200	+200	Mostly human and rat	Enzyme inhibition, Ion channels, GPCRs, Cytochromes	NovaScreen Biosciences	www.novascreen.com
Transcription Factor Profiling	2	+60	HepG2 cells (human liver)	Nuclear receptors and other transcription factors	Attagene	US Patent Application 20060160108 Populations of reporter sequences and methods of their use; www.attagene.com
Nuclear receptor activation	+20	+20	Human and rodent	Reporter gene assay over 15 concentrations	NIH Chemical Genomics Center	Inglese et al 2006. Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries. Proc Natl Acad Sci USA 103:11473-8; www.ncgc.nih.gov
Transcriptomics	1	+20,000	Primary hepatocytes-Kupffer cell co-cultures	Illumina microrrays	In Vitro ADMET Laboratories and Expression Analysis	Shi et al 2006. The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. Nat Biotechnol. 2006 Sep;24(9):1151-61; www.expressionanalysis.com
Kinetic Cell Growth	1	Kinetic	A549 cells (human lung)	Real time recording of electrical impedance	ACEA Biosciences	Xing et al 2006. Microelectronic cell sensor assay for detection of cytotoxicity and prediction of acute toxicity. Toxicol In Vitro 20:995-1004; www.aceabio.com
Cytotoxicity and Bioactivation	1	6	Primary human liver, lung and kidney cells	Shared metabolism across cell types	In Vitro ADMET Laboratories	Li AP 2007. Human hepatocytes: isolation, cryopreservation and applications in drug development. Chem Biol Interact 168:16-29; www.invitroadmet.com
Complex cell culture	8	87	Primary human cells	Many cell signaling pathways	Bioseek	Berg et al 2006. Characterization of compound mechanisms and secondary activities by BioMAP analysis. J Pharmacol Toxicol Methods 53:67-74; www.bioseekinc.com
High content screening	1	11	HepG2 cells (human liver)	Fluorescence imaging of cells	Cellumen	Giuliano et atl 2006. Systems cell biology based on high-content screening. Methods Enzymol 414:601-19; www.cellumen.com
Fish development	1	11	Zebrafish (Dana rerio)	Teratogenesis	Phylonix	Parng et al 2007. Neurotoxicity assessment using zebrafish. J Pharmacol Toxicol Methods 55:103-112; www.phylonix.com
TOTAL	>235	>20,395				

ACToR

Aggregated Computational Toxicology Resource

- Aggregating the world's chemical structure, bioassay and toxicology data for environmental chemicals
  - >150 sources of data – all public
  - Over 15M chemicals
- Manage all ToxCast Data
- Public release planned by June 2008
- Prototype Intranet: http://134.67.216.45:22722/servlet/ActorPrototype13

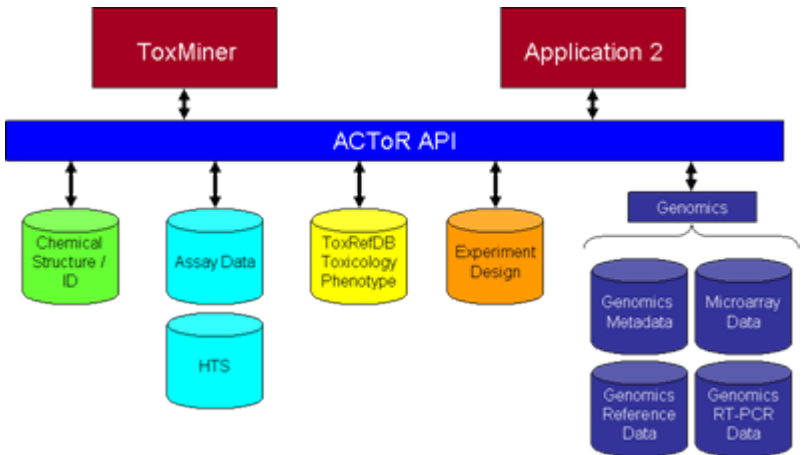


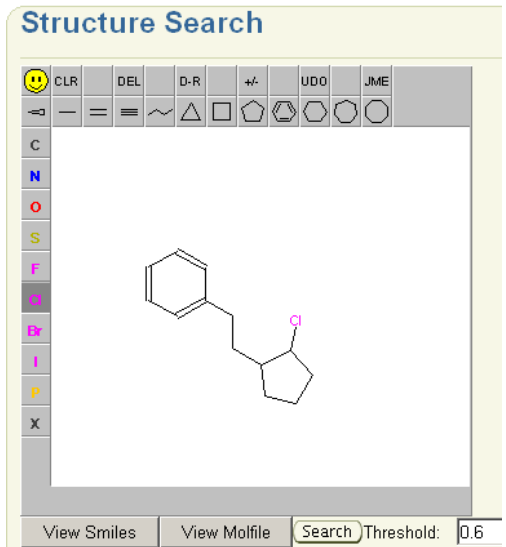


ACToR

Aggregated Computational Toxicology Resource

- Chemical Identity (Name / CAS)
- Chemical Structure
- In vitro & cell-based assay data
- In vivo chemical toxicity data
- Browsing by data source
- Searching by name, CAS, structure





ACToR Assay Types

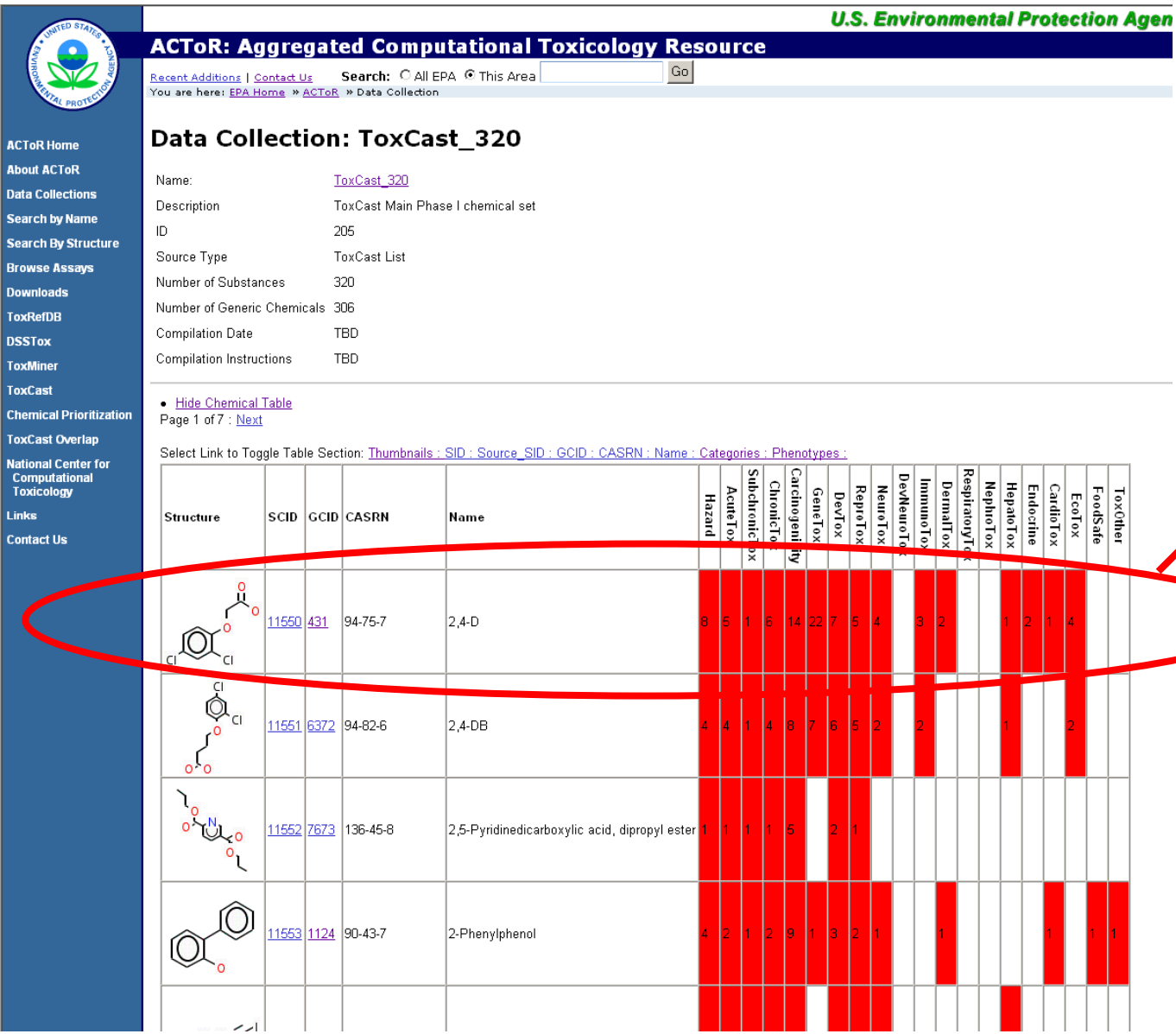
- PhysicoChemical (logP, MW)
- Biochemical (Ki - ToxCast, PubChem)
- Cellular (Cytotoxicity - ToxCast, PubChem)
- Tissue (Tissue slice assay)
- In vivo toxicology
  - Tabular – primary (NTP, OPPIN)
  - Tabular – secondary (IRIS)
  - Summary calls (Scorecard, CalEPA)
  - Summary report via URL (INCHEM)
- Category (from OPPT, Health Canada)
- Regulation (TSCA, FIFRA)
- Description (IUR Usage Levels)

ACToR
Currently Loaded Data Collections

ATSDR_ToxFaq	ESIS_ORATS	NCI-Open_09-03	SRS_OP_RPT_0798
BIND	ESIS_PBT	NCTRER_DSSTOX	SRS_PBT
BioCyc	EU_EINECS	NIAID	SRS_PCB
CalEPA	EXTOXNET	NMRShiftDB	SRS_RCRA_Appendix_VIII
CambridgeSoft	FDAMDD_DSSTOX	NTP1408	SRS_RCRA_F_Waste
CCL	FlameRetardant_1997	NTPBSI_DSSTOX	SRS_RCRA_U_Waste
CERCLA	GRADN	NTPHTS_DSSTOX	SRS_SARA_110
CERHR	GRADN_Oct07	OECD_HPVC	SRS_SARA_302A
ChEBI	HCPSL_1999	OPPIN_Active	SRS_SDWA_NPDWR
ChemDB	HCPSL_2006	OPPIN_ActiveSupported	SRS_SDWA_NSDWR
ChemIDplus	HealthCanada_DSL	OPPIN_AntiMicrobial	SRS_TSCA_4_Tests
CPDBAS_DSSTOX	HPV	OPPIN_AntiMicrobialFoodUse	SRS_TSCA_6_Unreasonable_Risk
Danish_EPA	HPV101_2007	OPPIN_FoodUseActive	SRS_TSCA_6A_CCCR
DBPCAN_DSSTOX	HPVChallenge	OPPIN_Inert	SRS_TSCA_8A_CAIR
DrugBank	HPVCSI_DSSTOX	PAN	SRS_TSCA_8c_SARS
DTP/NCI	HPVIS_All	PCN	SRS_TSCA_8D_HSDR_a
EAFUS	HPVIS_Robust_Summaries	PDSP	SRS_TSCA_8D_HSDR_c
EDC73	INCHEM_CICAD	RBC	SRS_TSCA_8D_HSDR_d
EPA	INCHEM_EHC	RoC	SRS_TSCA_Inventory
EPA_CARC	INCHEM_IARC	Scorecard	SRS_TSCAInvSyns
EPA_DWC	INERTS	SIDS	SRS_TTO
EPA_DWSHA	IRISTR_DSSTOX	SIDS_V1	ToxCast_1408
EPA_HPVC	ITER_TERA	SRS_CAA_112R	ToxCast_320
EPA_PFS_Conventional	IUR_2002	SRS_CAA112_b_HON	TOXNET GENETOX
EPA_SRS_NTS	IUR_86_02	SRS_CAS-9CI	TOXNET Toxicology
EPA_TRI_PBT	KEGG	SRS_ChemIDStd	ToxRefDB
EPA_TSCA	KIDB	SRS_CWA_307A	TRI
EPAFHM_DSSTOX	MHLWJP	SRS_CWA_311	WHO_CPH
EPISUITE	MITI_Japan	SRS_HAP	WHO_DiscontinuedPesticides
ESIS_ESIS	MTDP	SRS_HDDs_HDFs	xPharm
ESIS_LPV	NCGC	SRS_NJ_RTK_HS	ZINC

ACToR Aggregates Data from Global Sources

United States Environmental Protection Agency



Data Source
CPDBAS_DSSTOX
HPVCSI_DSSTOX
IRISTR_DSSTOX
NCTRER_DSSTOX
NTPBSI_DSSTOX
NTPHTS_DSSTOX
ToxCast_320
CERCLA
EDC73
EPA_DWC
EXTOXNET
HPVChallenge
HPV
INCHEM_EHC
INCHEM_EHC
INCHEM_IARC
ITER_TERA
IUR_2002
IUR_86_02

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Downloads
ToxRefDB
DSSTox
ToxMiner
ToxCast
Chemical Prioritization
ToxCast Overlap
National Center for Computational Toxicology
Links
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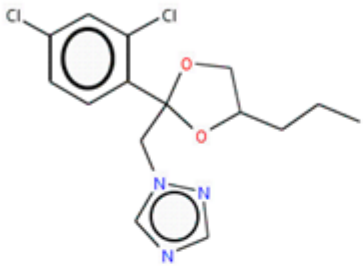
Chemical Summary: Propiconazole



GCID:	6467
CASRN:	60207-90-1
CID:	17172
CCID:	17172
Formula	C15H17Cl2N3O2
MW	342.2204
SMILES	Clc1ccc(c(Cl)c1)C3(Cn2ncnc2)OCC(CCC)O3
INCHI	InChI=1/C15H17Cl2N3O2/c1-2-3-12-7-21-15(22-12,8-20-10-18-9-19-20)13-5-4-11(16)6-14(13)17/h4-6,9-10,12H,2-3,7-8H2,1H3
QC Status	QUEUED

Substances Show

Synonyms Show

Notes Hide

SCID	Data Collection	Note
7508	IRISTR_DSSTOX	mixture of stereoisomers
11805	ToxCast_320	mixture of stereoisomers
1403526	EPA_SRS_NTS	ACD/Name-to-Structure Batch v. 8.05

MESH Hide

PubMed via MeSH:

- propiconazole

MSDS Show

External Search by Name and CASRN Show

Assay Data by Assay Category

- Show: PhysicoChemical (30)
- Show: Biochemical (0)
- Show: Genomics (0)
- Show: Cellular (0)
- Show: Tissue (0)
- Show: Organ (0)
- Show: Organism (0)
- Show: In vivo toxicology (tabular primary) (0)

Assay Name	Assay Component	Value	Units
IRIS	StudyType	Human Health Exposure Toxicity Review for Risk Assessment	
IRIS	Endpoint	cancer; acute; short-term; sub-chronic; chronic; developmental	
IRIS	Species	rodent; human; dog; rabbit	
IRIS	Oral_RfD_Assessed	1	
IRIS	Oral_RfD_CriticalEffects	gastric mucosal irritation	
IRIS	Oral_RfD_mg_per_kg_day	0.013	mg/kg-bw/day
IRIS	Oral_RfD_mmol_per_kg_day	3.8E-5	mmol/kg-bw/day
IRIS	Oral_RfD_Notes	NOEL (No observed effect level): 1.25 mg/kg-day	
IRIS	Oral_RfD_Confidence	High	
IRIS	Inhalation_RfC_Assessed	0	
IRIS	Inhalation_RfC_CriticalEffects	Not assessed under the IRIS program.	
IRIS	WtOfEvidence_Cancer_Assessed	0	
IRIS	WtOfEvidence_1986GuidelineCategories	Not assessed under the IRIS program.	
IRIS	DrinkingWater_OralSlope_Assessed	0	
IRIS	DrinkingWater_PrecursorEffect_TumorType	Not assessed under the IRIS program.	
IRIS	Inhalation_UnitRisk_Assessed	Not assessed under the IRIS program.	
IRIS	Inhalation_PrecursorEffect_TumorType	Not assessed under the IRIS program.	
IRIS	TotalAssessments	1	
WHO Classifications of Pesticide Hazard	Physical State	L	
WHO Classifications of Pesticide Hazard	Main Use	F	
WHO Classifications of Pesticide Hazard	LD50	1520.0	mg/kg
WHO Classifications of Pesticide Hazard	REMARKS	"JMPR 1988, 2005 "	

Toxicity Testing in the Twenty-first Century: A Vision and a Strategy

Committee on Toxicity Testing and Assessment of Environmental Agents

Board on Environmental Studies and Toxicology

Institute for Laboratory Animal Research

Division on Earth and Life Studies

NATIONAL RESEARCH COUNCIL

OF THE NATIONAL ACADEMIES

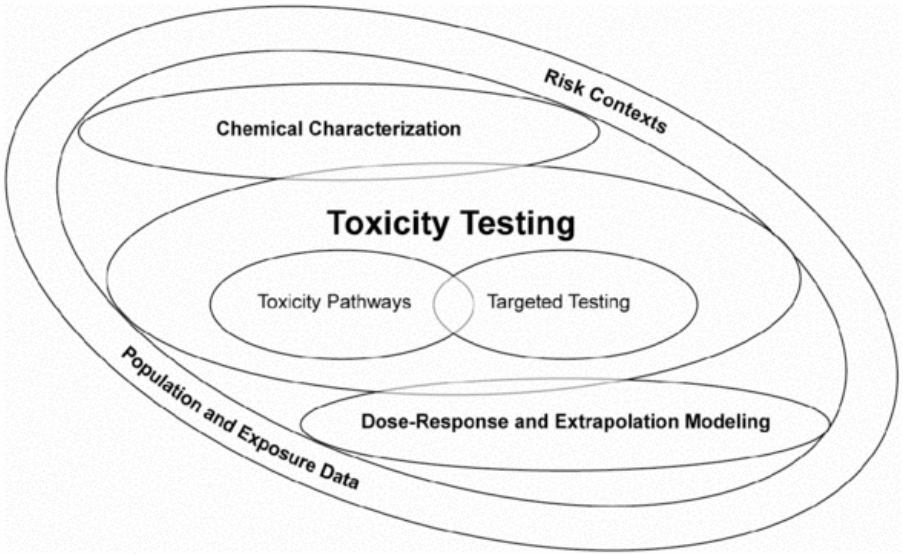
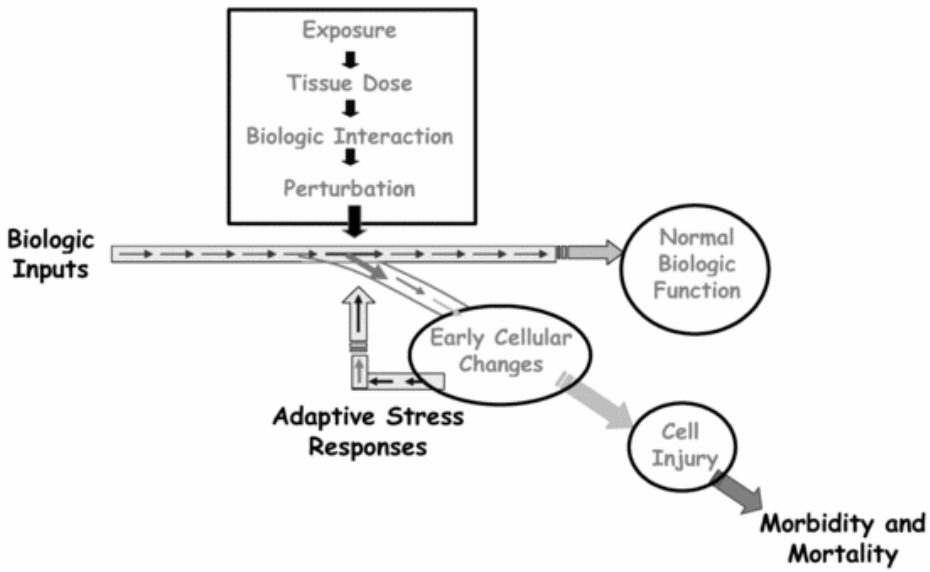


FIGURE S-1 The committee's vision for toxicity testing is a process that includes chemical characterization, toxicity testing, and dose-response and extrapolation modeling.



ToxCast / ACToR Collaborators

- ToxCast
  - Commercial Data Contractors
  - NCGC
  - OECD
  - CRADA Partners
    - AgChem, Industrial Chemical & Cosmetic companies
    - Assay providers
- ACToR / ToxRefDB
  - EPA Offices (OPP, OPPT, OW)
  - Environment Canada, RIVM, BfR
  - NTP
- Genomics
  - MAQC Project

- EPA STAR Bioinformatics Centers
  - NJ (UMDNJ / Princeton)
  - University of North Carolina

Summary – ToxCast and ACToR

- Address the toxicity data gap for environmental chemicals
- Address the need for screening and prioritization
- Provide large, high quality *in vivo* and *in vitro* data collections for others to use
- Provide a vehicle for collaborative efforts in analyzing and predicting chemical toxicity
- All data will become public in 2008

The ToxCast Team