NTP
National Toxicology Program

NTP Workshop: Role of Environmental Chemicals in the Development of Diabetes and Obesity January 11-13, 2011

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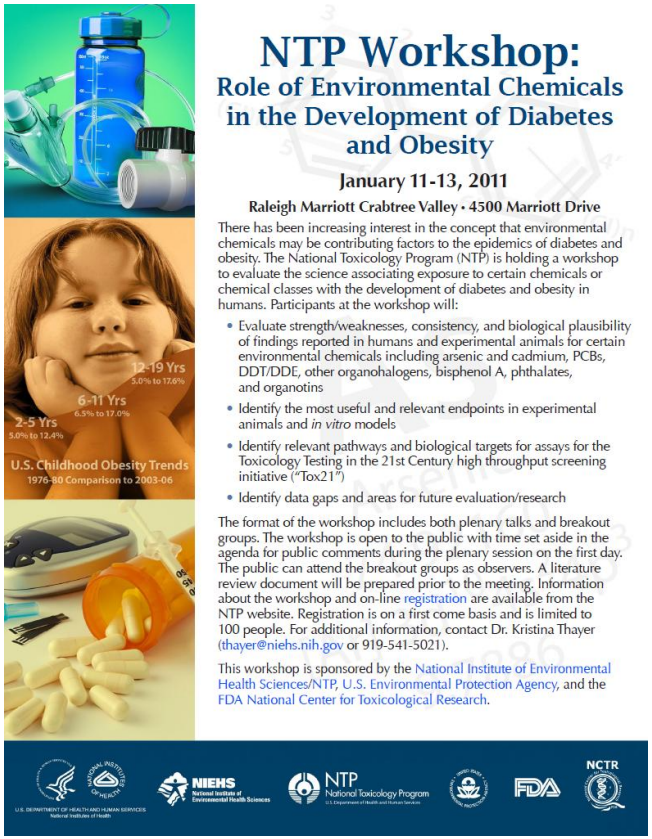
NIEHS/NTP



DEPARTMENT OF HEALTH & HUMAN SERVICES • USA

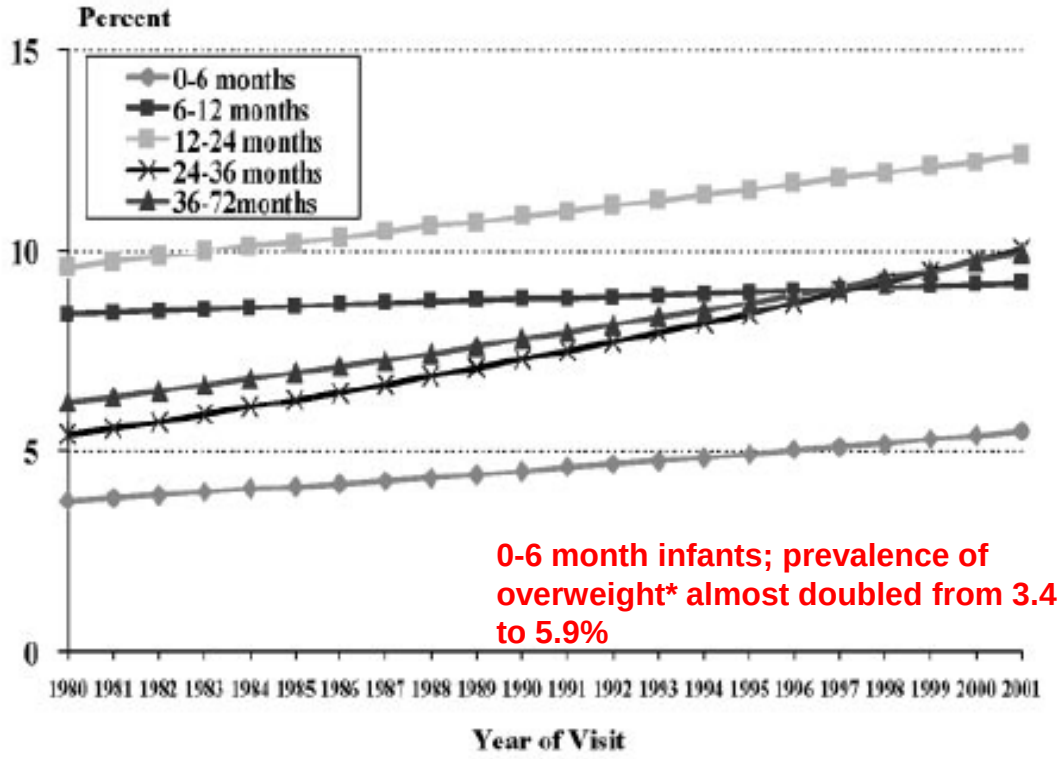
Outline

- Why a workshop on diabetes and obesity?
- Major conclusions
- Consideration of Tox21 data
  - Presentation format – making data accessible
  - Consistency of findings with published literature
  - Help establish biological plausibly
  - Generate testable hypothesis for targeted testing project
  - Additional assay targets to add

Diabetes & Obesity are Major Threats to Human Health

- **12.9% of people ≥20 of age in US estimated to have diabetes (NHANES 2005-2006, Cowie 2009)**
  - Undiagnosed in ~40% of diabetics
  - Another 29.5% with pre-diabetes (impaired fasting glucose or impaired 2-hour glucose tolerance)
    - ~40% with diabetes or pre-diabetes
- ~70% of type 2 diabetes risk attributed to overweight/obesity (Eyre 2004)
  - i.e., ~30% not accounted for by body weight
- Magnitude of problem supports consideration of “non-traditional” risk factors
  - Stress, gut microbiota, environmental chemicals, others

Trends for overweight infants and children



- ~9.5% infants and toddlers with excess weight* in NHANES 2007-2008

  Ogden CL (2010) JAMA 303(3): 242-249

Kim, J (2006). Obesity (Silver Spring) 14(7): 1107-1112. (data based on +366,000 well-child care visits at a HMO in Massachusetts)

* Weight-for-length/height ≥ 95th percentile

Overall Goals of Workshop

- Develop research agenda
  - Evaluate strength/weaknesses, consistency, and biological plausibility of findings reported in humans and experimental animals
  - Arsenic/other metals, POPs, BPA, organotins, phthalates, pesticides and nicotine
- Identify the most useful and relevant endpoints in experimental animals, *in vitro* models and screening systems
- Identify data gaps and areas for future evaluation/research.

Major Findings - Human Studies

- Maternal Smoking During Pregnancy
  - Workshop experts considered association between maternal smoking during pregnancy and childhood obesity likely causal
- Arsenic
  - Workshop experts considered association between arsenic and diabetes in areas of high arsenic exposure (i.e., Bangladesh/Taiwan) as limited to sufficient for an association
    - Most felt “sufficient” but data not sufficient to establish causality
- Organochlorines (PCBs, dioxins, DDT/DDE, others)
  - Workshop experts found several organochlorines were associated with diabetes in adults but data not sufficient to establish causality

Maternal Smoking During Pregnancy and Childhood Obesity

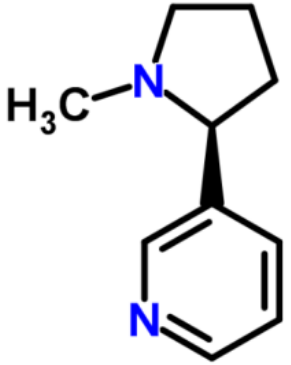
Reference	*Country*	*Study Design, Statistic*	*N in Analysis (Cohort)*	*Age in Years*	*Outcome (Prevalence)*
Adams, 2005	US, Wisconsin	retrospective, OR	252	3	overweight (40.9%)
Al Mamum, 2006	Australia, Queensland	prospective, OR	2,728 (3,253)	14	overweight (19.3%) obese (6.2%)
Bergmann, 2003	Germany, multi-site	prospective, OR	480 (918)	6	overweight (9.5%) obese (9.5%)
Chen, 2006	US, multi-site	prospective, OR	6,181 boys (14,486) 6,235 girls (14,612)	8	overweight/obese (11% boys+girls)
Dubois, 2006	Canada, Quebec	prospective, OR	1,550 (2,103)	4.5	obese (8.5%)
Hill, 2005	US, Pittsburgh	retrospective, $\chi 2$	288	8-11	smoking and BMI, $\chi 2$=9.94*
Iliadou, 2010	Sweden	prospective, OR	68,248 (124,203)	~18	overweight/obese (20%)
Karaolis-Danckert, 2008	Germany	prospective, β	370	2-6	rate of change in BMI, β=0.06*
Leary, 2006	UK	prospective, β	3,621	9.9	BMI, β=0.24*
Mizutani, 2007	Japan, Enzan City	prospective, OR	1,417 not reported (4,945)	5 33	overweight (11%) obese (2.7%) obese (10%)
Oken, 2005	US, Massachusetts	prospective, OR	746 (2,218)	3	overweight
Power, 2002	UK	prospective, OR	2,918 men 2,921 women	33	obese
Power, 2010	UK	prospective, OR	8,815 women	45	obese
Reilly, 2005	UK, England-Avon	prospective, OR	5,493	7	obese (8.6%)
Salsberry, 2005	US, national cohort	prospective, OR	3,022	6-7	obese (12%)
Syme, 2010	Canada, Quebec	cross-sectional	341 (508)	late puberty	body weight, 64 (exp) vs 60 kg*
Tome, 2007	Brazil, Ribeirao	prospective, OR	2,797	8-10	overweight
Toschke, 2002	Germany, Bavaria	cross-sectional, OR	6,579 (8,365)	5-6.9	overweight (10%) obese (3%)
Toschke, 2003	Germany, Bavaria	cross-sectional, OR	4,706 (4,974)	5-6.9	overweight (10.4%) obese (2.7%)
Von Kries, 2002	Germany, Bavaria	cross-sectional, OR	6,483	5-6.9	overweight (10%) obese (3%)
Von Kries, 2008	Germany, Bavaria	cross-sectional, OR	5,899	5-6.9	overweight (13.7%) obese (3.9%)
Whitaker, 2004	US, Ohio	retrospective, OR	5,089 (8,494)	4	obese (14.8%)
Wideroe, 2003	Norway & Sweden	prospective, OR	336 (482)	5	overweight (14.9%)

Nicotine

- Not in Phase I ToxCast™
  - Receptor target is (nAChRs)
- Tested at NCGC
  - Only “hit” was are-bla
- Some Phase 1 compounds interact with key receptor target

Name	CASRN	NVS_LGIC_hNNR_NBungSens (uM)
Acetamiprid	135410-20-7	5.7
Clothianidin	210880-92-5	30.0
Cyazofamid	120116-88-3	26.0
Imidacloprid	138261-41-3	9.7
Mepiquat chloride	24307-26-4	35.0
Thiacloprid	111988-49-9	4.9

Nicotine
54-11-5
$C_{10}H_{14}N_2$ (MW 162.23)



Use: Alkaloid found in the nightshade family of plants (Solanaceae) that constitutes approximately 0.6–3.0% of dry weight of tobacco. Considered the main factor responsible for dependence forming properties for tobacco use
Mechanism: binds to nicotinic acetylcholine receptors (nAChRs)
Assays for nAChRs included in ToxCast™:CHRNA4 and Chrna7

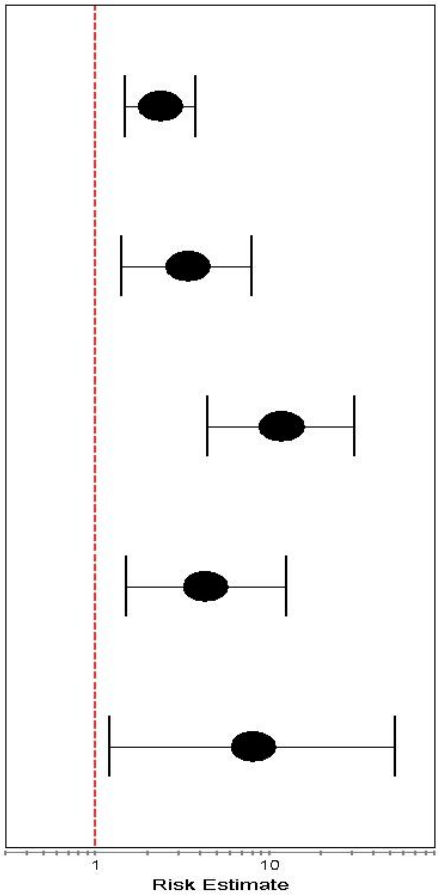
Pesticides

- Insufficient literature to reach conclusions
- Existing data support biological plausibility
  - Most animal studies on organophosphates (glycemic control > obesity/adiposity)
  - Human studies show associations with organochlorine pesticides
- Further research warranted

Trans-nonachlor

POPs: Trans-nonachlor with Diabetes

Reference	Study Design	Country and Cohort	N in Analysis (N in Cohort)	Health Outcome	Chemical	Exposure Comparison
Everett, 2010	cross-sectional, OR	US, NHANES 99-04	3,049	diabetes	trans-nonachlor	<14.5 vs ≥14.5 ng/g
Cox, 2007	cross-sectional, OR	US, HHANES 82-84	1308	diabetes (89)	trans-nonachlor	<1.00 vs. >1.80 ppb
Lee, 2006	cross-sectional, OR	US, NHANES 99-02	385 (2,106)	diabetes (54)	trans-nonachlor	114 ng/g vs. ND
Lee, 2010	nested case control, OR	US, CARDIA	85 (180)	diabetes (33)	trans-nonachlor	Q2 (110-174) vs. Q1 (≤109) pg/g *non-linear trend
Son, 2010	cross-sectional, OR	South Korea, Uljin Co.	51 (80)	diabetes (22)	trans-nonachlor	33.1 vs.8.4 ng/g lipid, p-trend=0.02



DDE

POPs: DDE with Diabetes

Reference	Study Design	Country and Cohort	N in Analysis (N in Cohort)	Health Outcome	Chemical	Exposure Comparison
Everett, 2010	cross-sectional, OR	US, NHANES 99-04	3,049	diabetes	p,p'-DDE	<168 vs ≥168.6 ng/g
Cox, 2007	cross-sectional, OR	US, HHANES 82-84	1306	diabetes (89)	p,p'-DDE	<22.81 vs. >58.60 ppb
Ukropec, 2010	cross-sectional, OR	Slovakia, general pop	819 (2047)	diabetes (102)	p,p'-DDE	Q5 (3605-22328) vs. Q1 (54-821) ng/g
Lee, 2006	cross-sectional, OR	US, NHANES 99-02	704 (2,106)	diabetes (53)	DDE	3,700 ng/g vs. ND
Rignell-Hydbom, 2007	cross-sectional, OR	Sweden, fisherman's wives	543	diabetes (8 high)	p,p'-DDE	per 100 ng/g ↑ (240ng/g lipid, cases), p-trend=0.002
Turyk, 2009a	prospective, RR	US, Great Lakes fish eaters	309 (471)	diabetes, incident (22)	DDE	5.4-49.2 vs. <2.2 ng/g wet weight, p-trend=0.008
Codru, 2007	cross-sectional, OR	US, Mohawks near Akwesasne	235 (352)	diabetes	DDE	544.6 vs 246.1
Rylander, 2005	cross-sectional, OR	Sweden, fishermen	196 (380)	diabetes (3)	p,p'-DDE	per 100 ng/g ↑ (1100ng/g lipid, cases), p-trend=0.04
Rylander, 2005	cross-sectional, OR	Sweden, fishermen's wives	184 (380)	diabetes (8)	p,p'-DDE	per 100 ng/g ↑ (990ng/g lipid, cases), p-trend=0.07
Lee, 2010	nested case control, OR	US, CARDIA	86 (180)	diabetes (23)	p,p'-DDE	Q2 (2154-3312 vs. Q1 (≤2153) pg/g
Son, 2010	cross-sectional, OR	South Korea, Uljin Co.	54 (80)	diabetes (25)	p,p'-DDE	667.4 vs. 162.2 ng/g lipid, p-trend<0.01
Rignell-Hydbom, 2009	nested case control, OR	Sweden, women in WHILA	39 pairs (371)	diabetes	p,p'-DDE	>4,600 ppt >7 years vs ≤4,600 at baseline
Turyk, 2009b	cross-sectional, OR	US, Great Lakes fish eaters	503	diabetes (61)	DDE	4.4-24.0 vs <1.2 ng/g (p trend = 0.005)

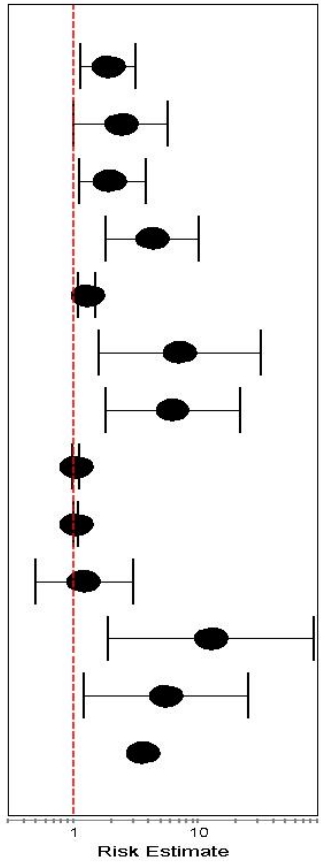


Table 9. ToxRef search results for chemicals that caused increased body weight (or body weight gain), increased glucose, or pancreatic effects. Those shaded red were tested in Phase 1 of ToxCast™

Chemical and CASRN	Chemical Class	Study Design*	Doses Tested (mg/kg-d)		Effect Doses (mg/kg-d)			Citation
			Lowest	Highest	↑ Body Weight	↑ Glucose	Pancreatic Pathology or Neoplasia	
Cymoxanil (57966-95-7)	aliphatic nitrogen	CHR, rat, feed	1.98	126			126	(Cox 1994b)
Cymoxanil (57966-95-7)	aliphatic nitrogen	CHR, mouse, feed	4.19	582			582	(Cox 1994a)
Acetochlor (34256-82-1)	amide	CHR, rat, feed	0.67	92.1			92.1	(Broadmeadow 1988)
Propyzamide (23950-58-5)	amide	SUB, rat, feed	2.5	289.2			254	(Anderson et al. 1989)
Abamectin (71751-41-2)	antibiotic	CHR, rat, feed	0.7	2.1	0.7			(Gordon 1985)
Emamectin benzoate (155569-91-8)	antibiotic	CHR, rat, feed	0.25	2.55	1.01			(Lankas 1994)
Azoxystrobin (131860-33-8)	antibiotic	SUB, rat, feed	20.4	448.6		223	444	(Milburn 1992)
Dicamba (1918-00-9)	aromatic acid	CHR, rat, feed	2.5	125	125			(Goldenthal 1985)
Quinclorac (84087-01-4)	aromatic acid	CHR, rat, feed	56	757			487	(Schilling 1988)
Ethofumesate (26225-79-6)	benzofuranyl alkylsulfonate	CHR, rat, feed	97	1466		332	1470	(Everett et al. 1991)
Diphenylamine (122-39-4)	bridged diphenyl	SUB, rat, feed	9.6	1323.8		650		(Krohmer 1992)
Carbofuran (1563-66-2)	carbamate	MGR, rat, feed	1	5	1			(EPA)
Sodium Dimethyldithiocarbamate (128-04-1)	carbamate	SUB, rat, gavage/intubation	0.5	250		250	250	(Marquis 1991)
Bifenazate (149877-41-8)	carbazate	CHR, rat, feed	1	9.7	1.2			(Ivett 1999)
Tetraconazole (112281-77-3)	conazole	SUB, rat, feed	0.7	28.7	23.9			(Mayfield et al. 1988b)
Propiconazole (60207-90-1)	conazole	CHR, rat, feed	3.6	100.6			18.1	(Hunter et al. 1982)
Sethoxydim (74051-80-2)	cyclohexene oxime	CHR, mouse, feed	4.48	142.85		4.85		(Takaori et al. 1981)
Tepraloxydim (149979-41-9)	cyclohexene oxime	CHR, rat, feed	5	272			272	(Mellert et al. 1997)
Halofenozide (112226-61-6)	diacylhydrazine	SUB, rat, feed	0.07	54.61		52.7		(Anderson et al. 1995)
Captafol (2425-06-1)	dicarboximide	SUB, rat, feed	174	174	174			(Brorby 1986)
Imazalil (35554-44-0)	imidazole	SUB, rat, feed	1.25	60		3.75		(Lina et al. 1983)
Disulfoton (298-04-4)	organophosphorus	CHR, mouse, feed	0.15	2.4	2.4			(Mobay Chemical Corp. 1983)
Malathion (121-75-5)	organophosphorus	CHR, rat, feed	4	868			29	(Daly 1996)
Parathion-methyl (298-00-0)	organophosphorus	CHR, mouse, feed	0.2	13.7	9.2			(Eiben 1991)
Propetamphos (31218-83-4)	organophosphorus	CHR, rat, feed	0.376	7.602			0.689, 7.6 (n)	(Luginbuehl 1980)
Tebupirimfos (96182-53-5)	organophosphorus	CHR, mouse, feed	0.52	43.57	38.8	38.8		(Eiben 1990)
Tebupirimfos (96182-53-5)	organophosphorus	SUB, rat, feed	0.2	4.9		0.4		(Eiben 1989)
Tribufos (78-48-8)	organophosphorus	CHR, mouse, feed	1.64	63.04	48			(Hayes 1989)

*CHR = chronic; SUB = subchronic; MGR = multigenerational study

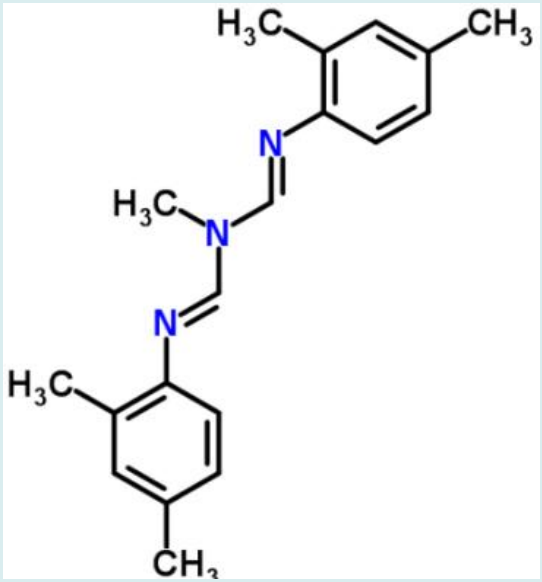
Amitraz

- Insecticide that causes hyperglycemia following poisoning incidents
- Also causes hyperglycemia and reduced insulin secretion in animal models (dogs, rats, mice, honey bees)
- α2-adrenoreceptor agonist
  - α2-adrenoreceptor antagonists block effects

Amitraz

33089-61-1

$C_{19}H_{23}N_3$ (MW 293.41)



Use: amidine insecticide

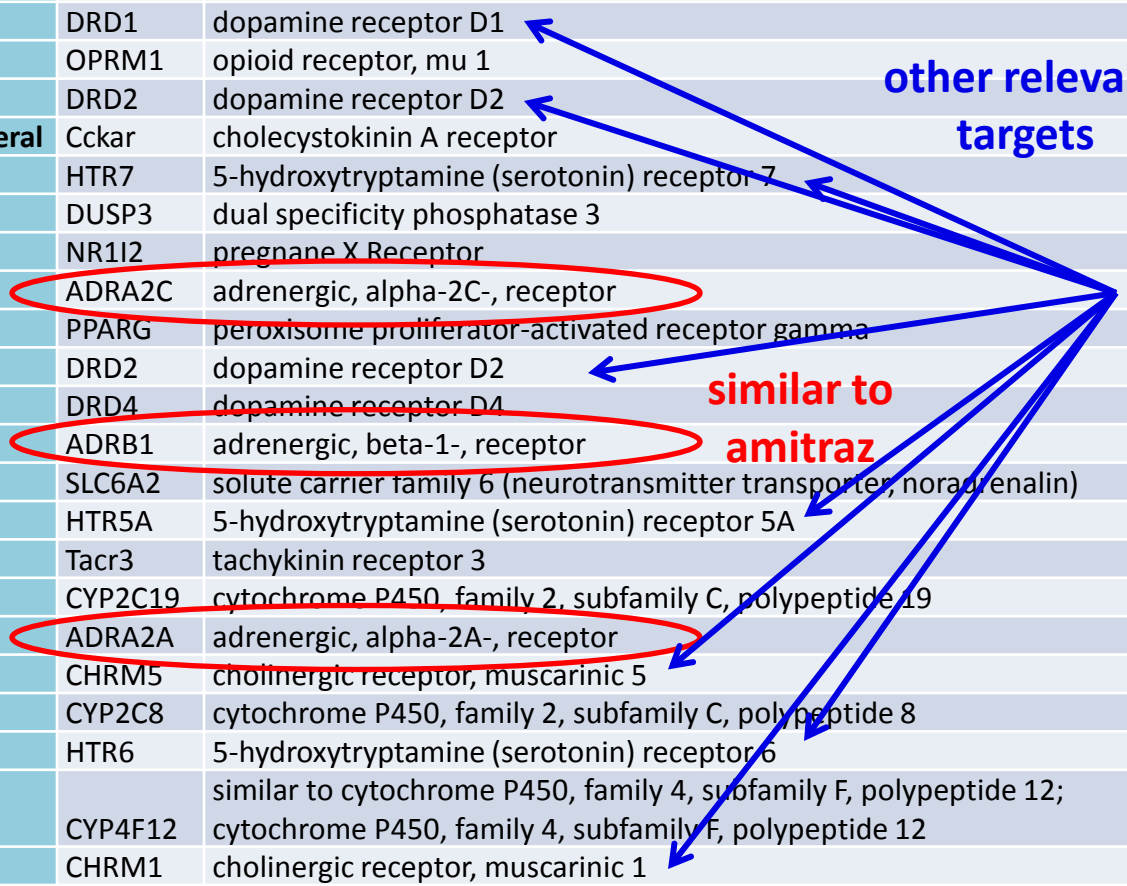
Mechanism: α2-adrenoreceptors agonist

Table 8. Pattern of screening data for Amitraz and other chemicals tested in Phase 1 of ToxCast™ that interacted with same assay targets[1]

CASRN	Name	adrenergic receptor, α-2A (ADRA2A)	adrenergic receptor, α-2A (Adra2a)	monoamine oxidase A (NVS ENZ rabI2C)	serotonin receptor 7 (HTR7)	adrenergic receptor, α-2b (Adra2b)	serotonin receptor 1A (Htr1a)
33089-61-1	Amitraz	0.05	0.06	0.16	0.45	1.03	1.8
43222-48-6	Difenzoquat metilsulfate	1.07	3.18	27.1	47.1	0.59	
155569-91-8	Emamectin benzoate	21.3	20.8		4	23.5	
68157-60-8	Forchlorfenuron	22.1	40		48.5		
67747-09-5	Prochloraz		1.83		39.4	4.7	
118134-30-8	Spiroxamine	6.82	29.7		14.4		
119446-68-3	Difenoconazole		2.36			29.5	
76-87-9	Fentin	5.79			0.2		
35554-44-0	Imazalil			42.4		12.4	
87820-88-0	Tralkoxydim	21.8				7.41	
2971-36-0	2,2-Bis(4-hydroxyphenyl)-1,1,1-trichloroethane (HPTE)				8.85		
71751-41-2	Abamectin				4.7		
314-40-9	Bromacil				47.2		
133-06-2	Captan				44.9		
120-32-1	Clorophene				16.5		
210880-92-5	Clothianidin					29.6	
120116-88-3	Cyazofamid					22.7	
52315-07-8	Cypermethrin				42.5		
85509-19-9	Flusilazole				38.8		
23422-53-9	Formetanate hydrochloride			1.94			
79983-71-4	Hexaconazole				41.9		
8018-01-7	Mancozeb		41.3				
51596-11-3	Milbemectin				6.84		

[1]Data presented as active concentration (AC_{50}) in μM. Based on assay targets most relevant for effects on glucose control (i.e., excludes whole cell toxicity, genes involved in immune/inflammation)

Appendix Table G. ToxCast™ results for the organotin fentin (CASRN 76-87-9)					
ToxCast™ Assay	Gene Symbol	Official Full Name	AC_{50} (µM)	E_{max}	UOM
ATG PPRE CIS	PPARA	peroxisome proliferator-activated receptor alpha	0.01	5.4	FC
ATG PPRE CIS	PPARD	peroxisome proliferator-activated receptor delta	0.01	5.4	FC
ATG PPRE CIS	PPARG	peroxisome proliferator-activated receptor gamma	0.01	5.4	FC
ATG RXRb TRANS	RXRB	retinoid X receptor, beta	0.01	2.4	FC
ATG NRF2 ARE CIS	NFE2L2	nuclear factor (erythroid-derived 2)-like 2	0.02	2.8	FC
ATG NURR1 TRANS	NR4A2	nuclear receptor subfamily 4, group A, member 2	0.02	5.3	FC
ATG PPARg TRANS	PPARG	peroxisome proliferator-activated receptor gamma	0.02	6.6	FC
NVS GPCR hDRD1	DRD1	dopamine receptor D1	0.15	95	% PC
NVS GPCR hOpiate mu	OPRM1	opioid receptor, mu 1	0.16	100	% PC
NVS GPCR hDRD2s	DRD2	dopamine receptor D2	0.17	99	% PC
NVS GPCR mCCKAPeripheral	Cckar	cholecystokinin A receptor	0.18	96	% PC
NVS GPCR h5HT7	HTR7	5-hydroxytryptamine (serotonin) receptor 7	0.20	92	% PC
NVS ENZ hPPVHR	DUSP3	dual specificity phosphatase 3	0.24	79	% PC
NVS NR hPXR	NR1I2	pregnane X Receptor	0.30	89	% PC
NVS GPCR hAdra2C	ADRA2C	adrenergic, alpha-2C-, receptor	0.43	94	% PC
NVS NR hPPARg	PPARG	peroxisome proliferator-activated receptor gamma	0.54	81	% PC
NVS GPCR hOpiate D2	DRD2	dopamine receptor D2	0.70	81	% PC
NVS GPCR hDRD4.4	DRD4	dopamine receptor D4	0.73	86	% PC
NVS GPCR hAdrb1	ADRB1	adrenergic, beta-1-, receptor	1.04	96	% PC
NVS TR hNET	SLC6A2	solute carrier family 6 (neurotransmitter transporter, noradrenalin)	1.14	85	% PC
NVS GPCR h5HT5A	HTR5A	5-hydroxytryptamine (serotonin) receptor 5A	1.84	100	% PC
NVS GPCR rNK3	Tacr3	tachykinin receptor 3	4.51	65	% PC
NVS ADME hCYP2C19	CYP2C19	cytochrome P450, family 2, subfamily C, polypeptide 19	4.64	59	% PC
NVS GPCR hAdra2A	ADRA2A	adrenergic, alpha-2A-, receptor	5.79	97	% PC
NVS GPCR hM5	CHRM5	cholinergic receptor, muscarinic 5	6.10	89	% PC
NVS ADME hCYP2C8	CYP2C8	cytochrome P450, family 2, subfamily C, polypeptide 8	6.36	62	% PC
NVS GPCR h5HT6	HTR6	5-hydroxytryptamine (serotonin) receptor 6	7.00	100	% PC
NVS ADME hCYP4F12	CYP4F12	similar to cytochrome P450, family 4, subfamily F, polypeptide 12; cytochrome P450, family 4, subfamily F, polypeptide 12	7.99	53	% PC
NVS GPCR hM1	CHRM1	cholinergic receptor, muscarinic 1	12.40	79	% PC
NVS GPCR gH2	Hrh2	histamine receptor H2	14.90	55	% PC
UOM = unit of measure; FC = fold change; % PC = % of positive control					



Correspondence Between Published Literature & ToxCast™

- ToxCast™ findings generally seemed to match strength of literature
  - Chemicals with strongest cases for biological plausibility would have been flagged, e.g., fentin and amitraz
  - Less consistent literature and less clear cut ToxCast “signal” for others, e.g., BPA, phthalates
- Provided biological support for organophosphate pesticides
- Suggested other chemicals should be tested based
  - “Hits” on signaling targets well-established to impact glucose homeostasis and adiposity, i.e., adrenergic receptors

HTS Profiling

Strategy to Identify Relevant Tox21 Targets

- Bioinformatics approach to look for genes associated with biological processes related to diabetes and obesity
  - Islet cell function, insulin sensitivity, feeding behavior, adipocyte differentiation, fatty acid metabolism
- Cross-reference with list of gene-based assays available in Tox21
  - Profiling results based on Phase 1 of ToxCast™
- Expert input to identify most relevant assay targets
  - Causal versus disease manifestation/therapeutic
  - Targeted testing to assess predictions
- Expert input to identify most relevant gene targets that are not currently included in Tox21

Bioinformatics

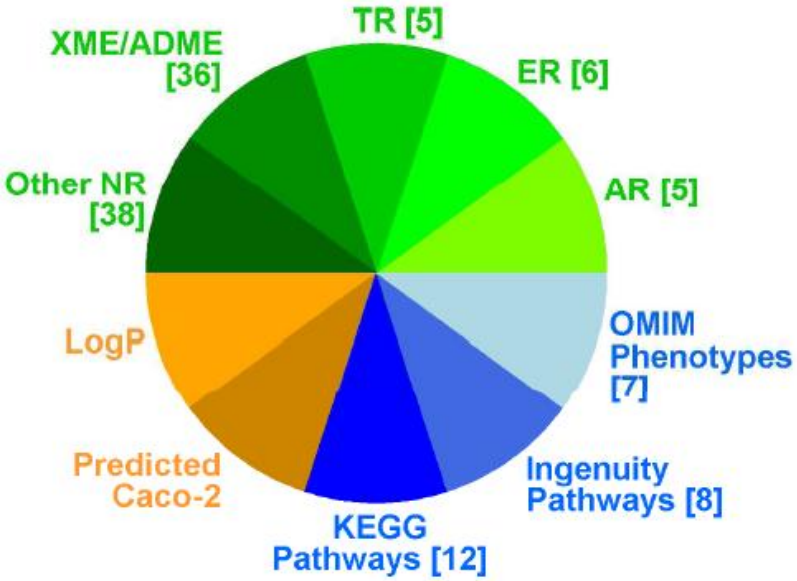
- CoPub
  - Uses text mining tools to identify relationships between gene, pathways/processes, diseases and drugs
  - Source: PubMed abstracts up to May 2010
  - Uses an R-scale score to quantify relationships
    - Describes the strength of a co-citation between two keywords (e.g. PNPLA3 and Fatty Liver)

Example result

Library Tested	SOURCE_NAME_AID	Name (gene symbol)- [homo sapiens]; EPA Gene Annotation; NCGC Gene Annotation; Hand	Official Full Name	CoPub-Pancreatic islets	CoPub-Islets of Langerhans	CoPub-Insulin secretion	CoPub-Insulin Processing	Average
TOXCAST	NVS_IC_rKATPCh	Kcnj11	potassium	41	45	52	52	47.5
TOXCAST	ATG_FoxA2_CIS	FoxA2	forkhead l	43	44	44	44	43.75
TOXCAST	NVS_ENZ_hInsR	INSR	insulin rec	39	37	43	43	40.5
TOXCAST	NVS_ENZ_hInsR_Activator	INSR	insulin rec	39	37	43	43	40.5
TOXCAST	ATG_Pax6_CIS	PAX6	paired box	41	40	36	36	38.25
TOXCAST	ATG_PPARg_TRANS	PPARG	peroxisom	36	31	39	39	36.25
TOXCAST	ATG_PPRE_CIS	PPARG	peroxisom	36	31	39	39	36.25
TOXCAST	BSK_SAg_CD38_down	CD38	CD38 mole	37	34	37	37	36.25
TOXCAST	BSK_SAg_CD38_up	CD38	CD38 mole	37	34	37	37	36.25
TOXCAST	NCGC_PPARg_Agonist	PPARG	peroxisom	36	31	39	39	36.25
TOXCAST	NVS_NR_hPPARg	PPARG	peroxisom	36	31	39	39	36.25
NTP	PPAR-gamma (CHO)	PPARG	peroxisom	36	31	39	39	36.25
Tox21	PPAR-gamma agonist	PPARG	peroxisom	36	31	39	39	36.25
Tox21	PPAR-gamma antagonist	PPARG	peroxisom	36	31	39	39	36.25
TOXCAST	NVS_GPCR_rVIPNon_Selective	Vip	vasoactive	36	36	36	36	36
TOXCAST	NVS_GPCR_bNPYNon_Selective	NPY	neuropep	34	37	36	36	35.75

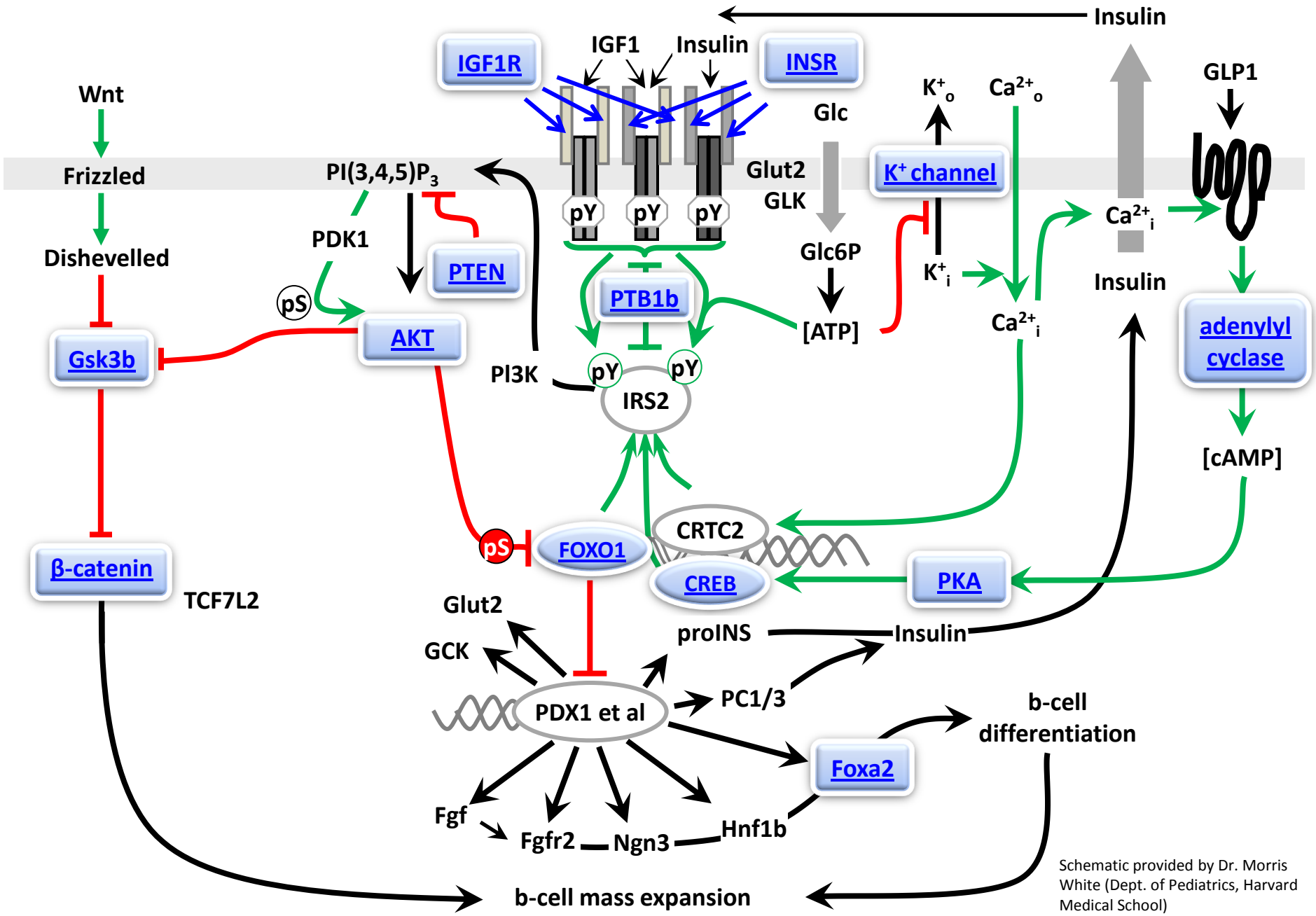
Used EPA's ToxPi™ to Visualize

- **Example: Prioritizing Endocrine-Disruptor Screening Using ToxPi (Reif 2010)**
  - **Visual of toxicity by weighted combinations of data (10 slices) from in vitro assays, AR/ER/TR interactions, chemical properties, pathways and XME/ADME**



The "slices" can be anything, i.e., genes, diseases, biological processes

Insulin Signaling in Pancreatic β-Cells



Schematic provided by Dr. Morris White (Dept. of Pediatrics, Harvard Medical School)

Sample Output From Signaling Hyperlinks to ToxCastDB

GSK3b

Assay: Novascreen Human GSK3b

Assay Id:	914
Source	Novascreen
Source Name AID	NVS_ENZ_hGSK3b
Name	Novascreen Human GSK3b
Description	Human GSK3b Fluorescein-peptide
Number of Substances	320
Number of Components	1
Species	Homo sapiens

Parameters

Parameter	Value
CATALOG NUMBER	200-0425
ASSAY CATEGORY	Enzyme Inhibition
ASSAY CATEGORY	In vitro (Biochemical)
ASSAY TARGET	GSK3b
ASSAY TARGET FAMILY	Kinase
ASSAY TARGET SOURCE	Recombinant
ASSAY GENE ID	2932
ASSAY GENE NAME	GSK3B
ASSAY TECHNOLOGY	Fluorescence -EMS
ASSAY REFERENCE COMPOUND	Staurosporine
ASSAY NOTE	KINASE
ASSAY SUBSTRATE NAME	CMGC group
ASSAY ATP CONCENTRATION (M)	NCCT_v2
ASSAY LIGAND NAME	1.5 E-06
ASSAY LIGAND CONCENTRATION (M)	1.20E-05
ASSAY BMAX	Fluorescein-peptide + ATP --> Fluorescein-phosphopeptide + ADP

Data

Name	CASRN	NVS_ENZ_hGSK3b (uM)
Mancozeb	8018-01-7	0.27
Maneb	12427-38-2	0.32
Metiram-zinc	9006-42-2	16.0

number of “actives” = 3

CREB

Assay: Attagene Factorial cis CRE

Assay Id:	16
Source	Attagene
Source Name AID	ATG_CRE_CIS
Name	Attagene Factorial cis CRE
Description	Factorial reporter gene assay
Number of Substances	320
Number of Components	1
Species	Homo sapiens

Parameters

Parameter	Value
ASSAY URL	Link Out EXIT Disclaimer
ASSAY CATEGORY	In vitro (Cellular)
ASSAY TARGET	cAMP Response Element
ASSAY TARGET FAMILY	Transcription Factor
ASSAY TARGET SOURCE	Cell line
ASSAY TARGET SOURCE TYPE	HepG2
ASSAY GENE ID	10488
ASSAY GENE NAME	CREB3
ASSAY TECHNOLOGY	Reporter gene assay
ASSAY MODE	DNA sequencer
ASSAY REFERENCE COMPOUND	Forskolin cAMP
ASSAY NOTE	"Multiplexed reporter gene assay; cAMP, cGMP, NO receptor, GPCR pathways"

Data

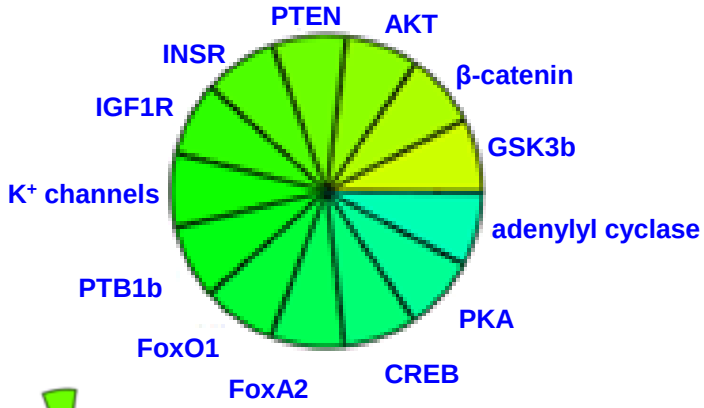
Name	CASRN	ATG_CRE_CIS (uM)
Alachlor	15972-60-8	3.4
Anilazine	101-05-3	59.0
Azinphos-methyl	86-50-0	27.0
Azoxystrobin	131860-33-8	46.0
Bendiocarb	22781-23-3	51.0
Bisphenol A	80-05-7	30.0
Bromoxynil	1689-84-5	40.0
Chlorpropham	101-21-3	31.0
Cyazofamid	120116-88-3	10.0
Cyprodinil	121552-61-2	23.0
Dazomet	533-74-4	49.0
Allethrin (d-cis,trans)	584-79-2	46.0
Dichloran	99-30-9	43.0

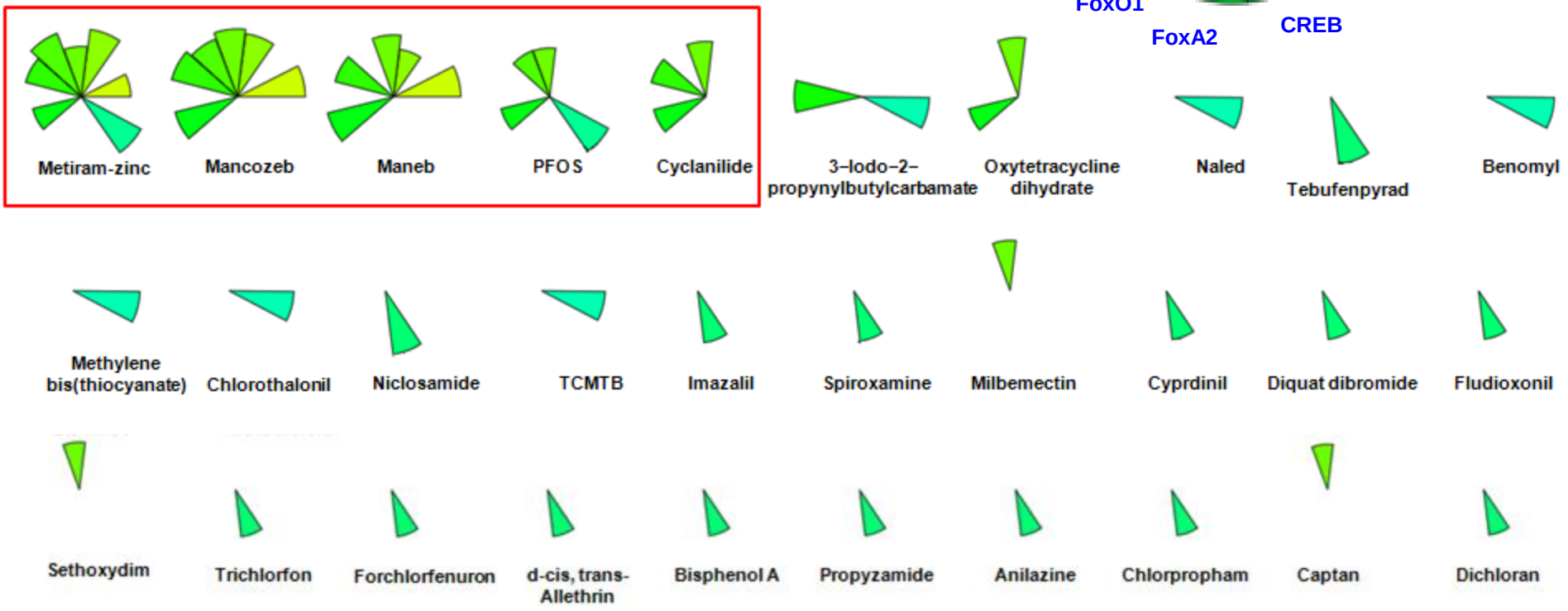
partial list:
number of total “actives” = 52

http://actor.epa.gov/actor/faces/ToxMiner/Home.jsp

EPA United States Environmental Protection Agency

ToxPi™ for Insulin Signaling in Pancreatic β Cells- Top 30 from 309 Chemicals in ToxCast Phase I





http://epa.gov/ncct/ToxPi/

EPA United States Environmental Protection Agency

Top 15 Targets For Biological Processes (DRAFT)

- Note variation between experts!
  - Suggest ToxCastDB evolve to let users create tailored ToxPi™ schemes

Islet cell function		Insulin sensitivity	Adipocyte differentiation		Feeding behavior	
A. Holloway	**M. White**	**M. White**	**B. Blumberg**	**J. Schlezinger**	**D. Clegg (mammalian)**	**S. Srinivasan (C. elegans)**
Fentin	Mancozeb	Metiram-zinc	Fludioxonil	**Tebupirimfos**	**Maneb**	**E. benzoate**
Milbemectin	(Z,E)-Fenpyroximate	Mancozeb	Prallethrin	**d-cis,trans-Allethrin**	**Mancozeb**	**Metiram-zinc**
HPTE	Metiram-zinc	d-cis,trans-Allethrin	**d-cis,trans-Allethrin**	**Cyazofamid**	**E. benzoate**	**Fentin**
Chlorpyrifos oxon	Maneb	Spiroxamine	**Cyazofamid**	Prallethrin	**Metiram-zinc**	**Milbemectin**
Cinmethylin	Fipronil	Prallethrin	**Flusilazole**	Tebufenpyrad	Bisphenol A	**PFOS**
E. benzoate	Spiroxamine	Niclosamide	Imazalil	**Flusilazole**	**Milbemectin**	**Mancozeb**
Prochloraz	**Imazalil**	PFOS	**Tebupirimfos**	Niclosamide	Cyazofamid	Forchlorfenuron
Flusilazole	Cyprodinil	Tebufenpyrad	Bromoxynil	(Z,E)-Fenpyroximate	**Fentin**	Clorophene
Imazalil	d-cis,trans-Allethrin	Bromoxynil	**Forchlorfenuron**	Trichlorfon	Fluazinam	**HPTE**
Chlorethoxyfos	PFOS	Cyclanilide	Fentin	Pyrimethanil	**HPTE**	Flusilazole
Bisphenol A	Fludioxonil	Fentin	Spiroxamine	Propargite	Niclosamide	Thidiazuron
Naled	Forchlorfenuron	Lactofen	Thidiazuron	Isazofos	PFOS	**Maneb**
Lactofen	Pyraclostrobin	Flusilazole	Fluazinam	Fenarimol	Chlorothalonil	Fludioxonil
Abamectin	Trichlorfon	Quinoxyfen	Phenoxyethanol	**Forchlorfenuron**	Fenthion	Carbaryl
Clorophene	Tetramethrin	Diclofop-methyl	2-Phenylphenol	Fenthion	Resmethrin	Diuron

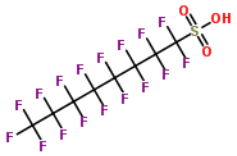
E. Benzoate = Emamectin benzoate; PFOS = Perfluorooctane sulfonic acid circle in red THOSE IN TOXREF DB - animation

Targeted Testing Project

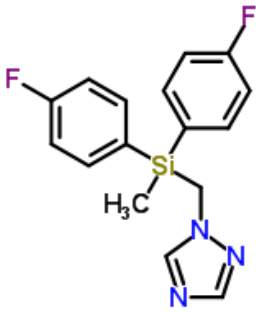
- Targeted testing on 10-20 chemicals
  - Top 10 predicted actives from expert developed ToxPis
  - 4 predicted to have no activity
  - Several predicted positives from bioinformatics (e.g., CoPub) approach?

Ten Most Frequent Predicted Positives Compounds Across Biological Processes

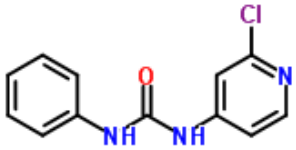
PFOS



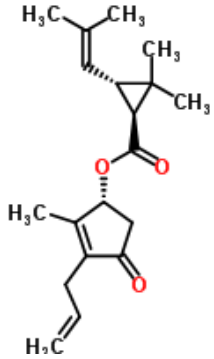
Flusilazole



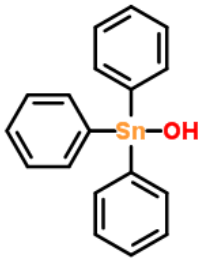
Forchlorfenuron



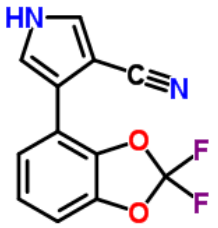
d-cis,trans-allethrin



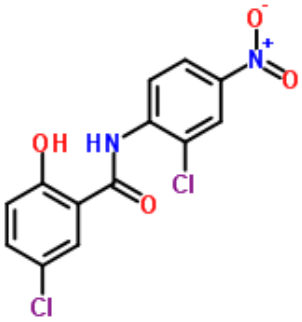
Fentin



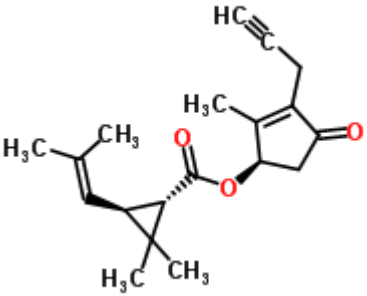
Fludioxonil



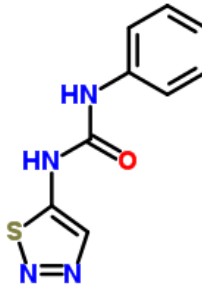
Niclosamide



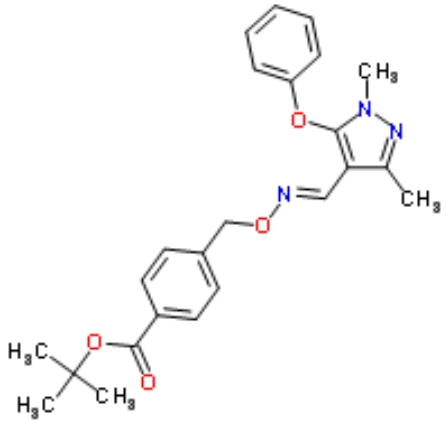
Prallethrin



Thidiazuron



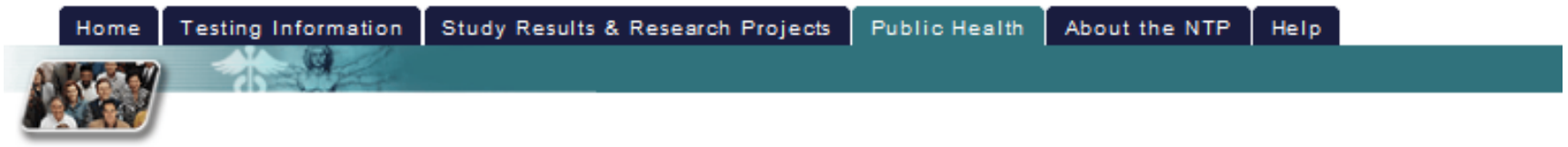
(Z,E)-Fenpyroximate



Predicted Negatives

	A	B	C	D	E	F	H	I	K	L	P	Q
1	GCID	CASRN	NAME	islet cell (Alison)	Adipocyte (Bruce)	Adipocyte (Jennifer)	Feeding behavior (Debbie)	Feeding behavior (Supriya)	Insulin Sensitivity (Morris)	islet cell (Morris)	Average Score	
2	12058	1007-28-9	6-Deisopropylatrazine	0	0	0	0	0	0	0	0	
3	12111	101-10-0	Cloprop	0	0	0	0	0	0	0	0	
4	12129	1596-84-5	Daminozide	0	0	0	0	0	0	0	0	
5	12135	1918-00-9	Dicamba	0	0	0	0	0	0	0	0	
6	12236	208465-21-8	Mesosulfuron-methyl	0	0	0	0	0	0	0	0	
7	12258	6923-22-4	Monocrotophos	0	0	0	0	0	0	0	0	
8	12311	84087-01-4	Quinclorac	0	0	0	0	0	0	0	0	
9	12084	10043-35-3	Boric acid	0	0	0	0	0	0	0	0.007943475	

http://cerhr.niehs.nih.gov/evals/diabetesobesity/



NTP Workshop: Role of Environmental Chemicals in the Development of Diabetes and Obesity

NIEHS Environmental Factor Article: NTP workshop investigates links between chemicals and obesity

January 11-13, 2011 Workshop: Crabtree Marriott (4500 Marriott Drive, Raleigh, North Carolina 27612 USA; 1-800-909-8289)

- Workshop Announcement
- Federal Register Notice
- Final Agenda
- Workshop Presentations
- List of Breakout Group Members
- Breakout Group Assignments
- List of Workshop Attendees
- Draft Literature Review Documents
  - BPA and GeneGo legend
  - BPA - Appendix C, Mechanisms of action and biochemical/molecular interactions
  - Maternal smoking during pregnancy/Nicotine
  - Pesticides and Appendix B
  - Arsenic
  - Organotins and phthalates
  - Appendix tables of human studies of POPs
- Public Comments

- Plan to publish workshop reports in EHP