

Computational Embryology and Predictive Toxicology of Cleft Palate

Nisha S. Sipes¹, M. Shane Hutson², Nancy C. Baker¹, Thomas B. Knudsen¹

¹US EPA, ORD, National Center for Computational Toxicology

²Depts of Physics & Astronomy and Biological Sciences, Vanderbilt University



53rd Teratology Society Annual Meeting

June 25, 2013 Tucson, Arizona

Platform Session 4: In Vitro, Computational, and Alternative Methods and Models for Screening Developmental Toxicants

Disclosure

The authors of this research have no financial or other interests which pose a conflict of interest.

This research was funded by the United States Environmental Protection Agency, Office of Research and Development.

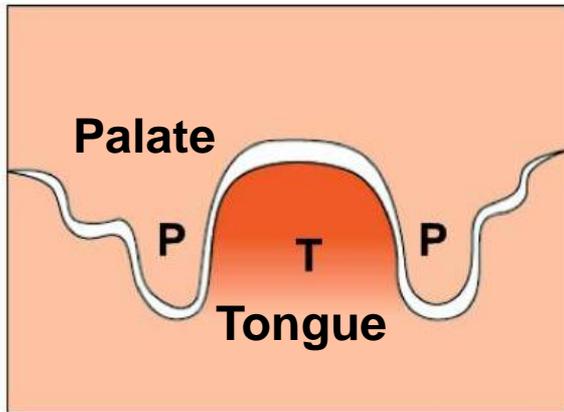
Disclaimer: views are those of the presenter and do not necessarily reflect Agency policy nor imply endorsement of software used here.

What is Cleft Palate (CP)?

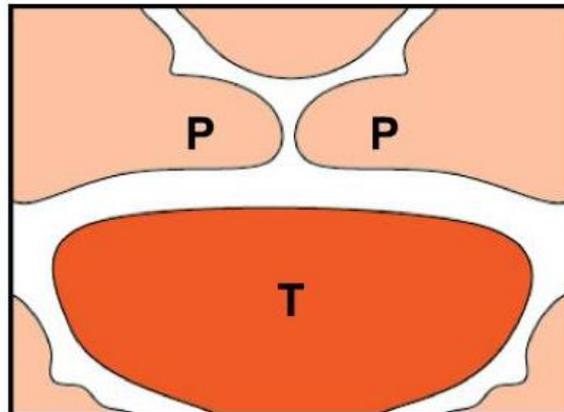
Disruption of secondary palate formation

Affects 1/700 live births worldwide

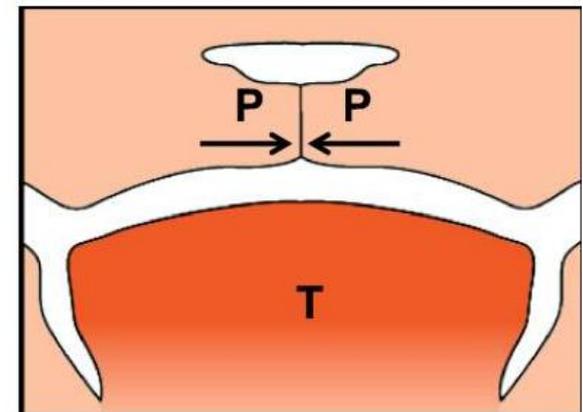
Mouse palatal development



Growth



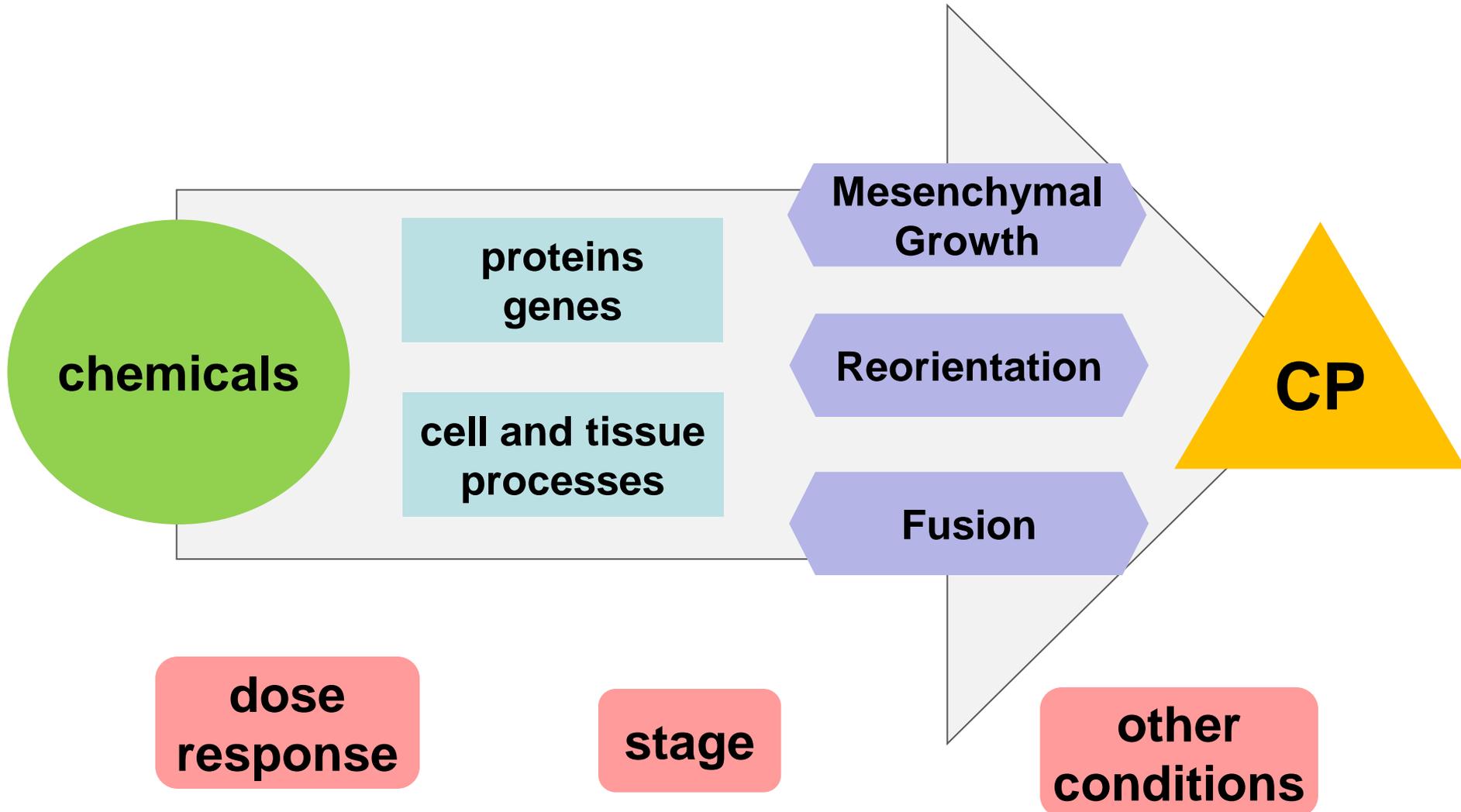
Reorientation



Fusion

What Do We Want to Know?

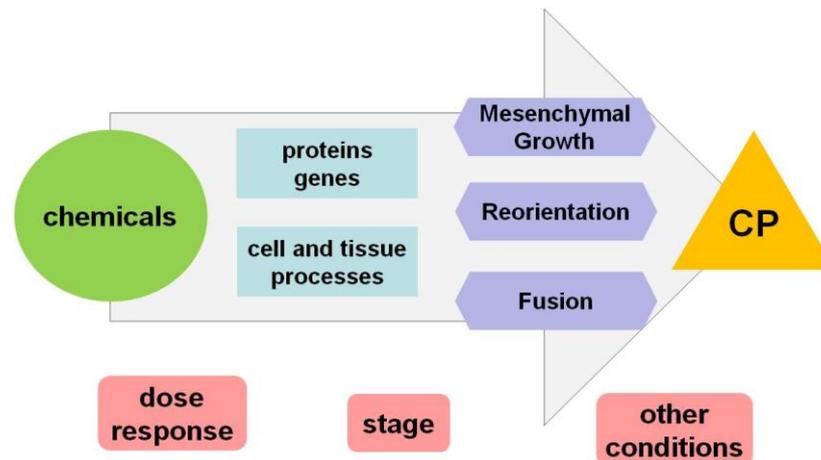
Environmental influences leading to CP



A Systems Biology Framework

Cleft Palate (CP)

1. Biological data from public literature (e-libraries)
2. High-throughput assay associations with CP
3. Adverse Outcome Pathway (AOP) elucidation
4. Multi-cellular/-scale computer simulation

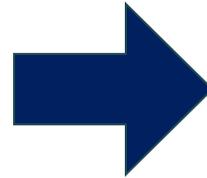


1. e-library Development

Literature Mining Using MeSH Annotations



> 20 million total articles



**effects/
diseases**

**proteins/
genes**

chemicals

**cell
processes**

1. e-library Development

Literature Mining Using MeSH Annotations

The image shows a screenshot of a PubMed article page. The browser address bar displays the URL www.ncbi.nlm.nih.gov/pubmed/21254359. The page header includes the NCBI logo and navigation links. The article title is "Folate pathway and nonsyndromic cleft lip/palate". The abstract text is visible on the left side. A list of MeSH Terms is displayed on the right side, enclosed in a green rectangular box. The terms are: 5-Methyltetrahydrofolate-Homocysteine S-Methyltransferase/genetics, Betaine-Homocysteine S-Methyltransferase/genetics, Carbon-Nitrogen Ligases/genetics, Cleft Lip/complications*, Cleft Lip/ethnology, Cleft Lip/genetics, Cleft Palate/complications*, Cleft Palate/ethnology, Cleft Palate/genetics, European Continental Ancestry Group/genetics, Folic Acid/metabolism*, Genes/genetics*, Genetic Predisposition to Disease*, Hispanic Americans/genetics, Humans, Methylenetetrahydrofolate Reductase (NADPH2)/genetics, Nitric Oxide Synthase Type III/genetics, Polymorphism, Single Nucleotide/genetics*, Reduced Folate Carrier Protein/genetics, and Thymidylate Synthase/genetics. At the bottom left of the page, there are buttons for "Publication Types, MeSH Terms" and "LinkOut - more resources".

MeSH Terms

- [5-Methyltetrahydrofolate-Homocysteine S-Methyltransferase/genetics](#)
- [Betaine-Homocysteine S-Methyltransferase/genetics](#)
- [Carbon-Nitrogen Ligases/genetics](#)
- [Cleft Lip/complications*](#)
- [Cleft Lip/ethnology](#)
- [Cleft Lip/genetics](#)
- [Cleft Palate/complications*](#)
- [Cleft Palate/ethnology](#)
- [Cleft Palate/genetics](#)
- [European Continental Ancestry Group/genetics](#)
- [Folic Acid/metabolism*](#)
- [Genes/genetics*](#)
- [Genetic Predisposition to Disease*](#)
- [Hispanic Americans/genetics](#)
- [Humans](#)
- [Methylenetetrahydrofolate Reductase \(NADPH2\)/genetics](#)
- [Nitric Oxide Synthase Type III/genetics](#)
- [Polymorphism, Single Nucleotide/genetics*](#)
- [Reduced Folate Carrier Protein/genetics](#)
- [Thymidylate Synthase/genetics](#)

Publication Types, MeSH Terms

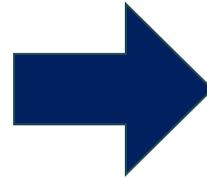
LinkOut - more resources

1. e-library Development

Literature Mining Using MeSH Annotations



> 20 million total articles



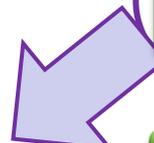
Cleft Lip / Palate

effects/
diseases

proteins/
genes

chemicals

cell
processes

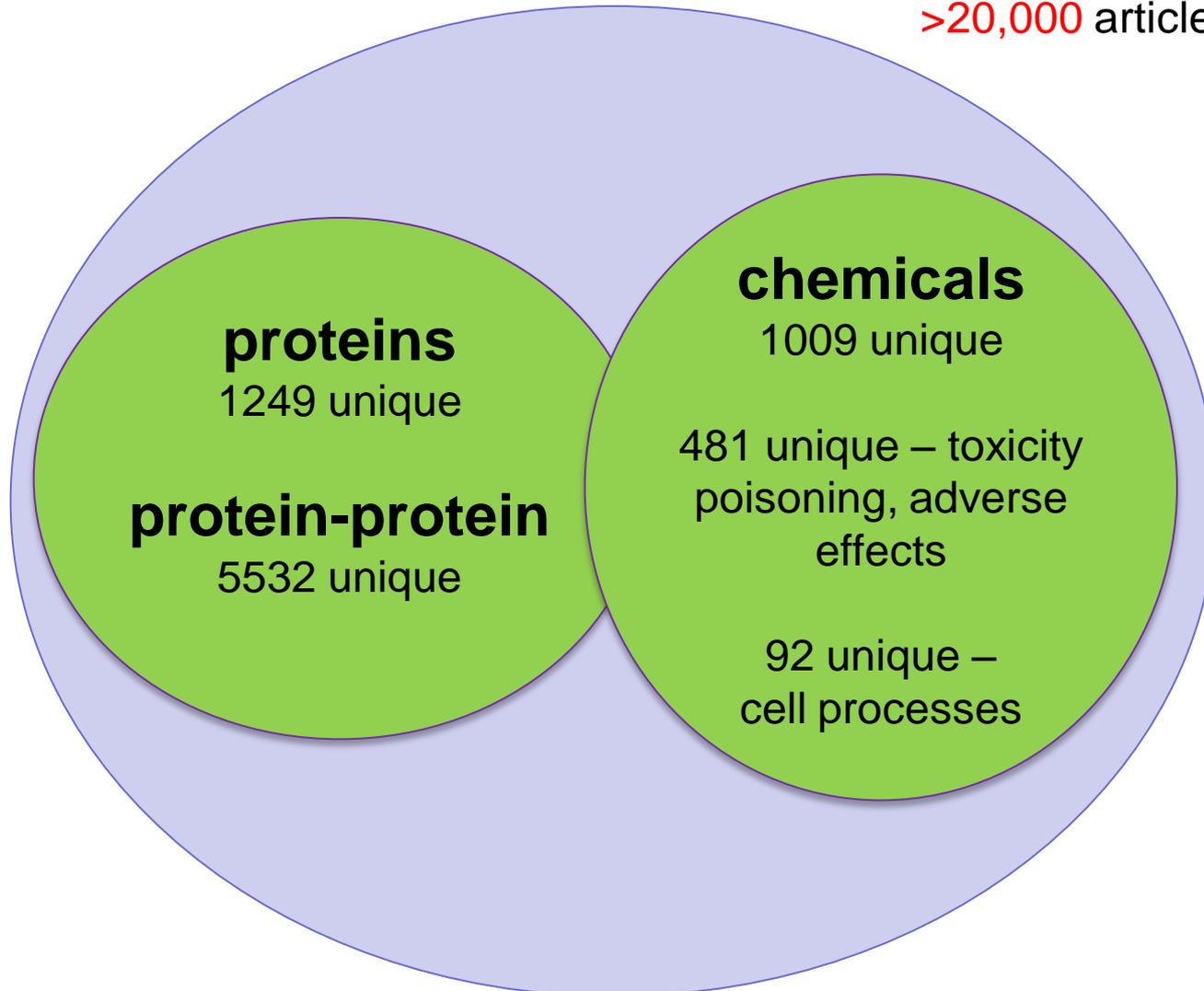


via computer languages and database software

1. e-library Development

Cleft Lip/Palate Articles

>20,000 articles



1. e-library Development

CLP Chemicals and Cell Processes

	A	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN
1	Cleft Palate Chemicals and Cell Processes																															
2	Article counts for co-occurring MeSH terms annotated with drug effects																															
3	Chemicals	G0 Phase	G1 Phase	G2 Phase	S Phase	Cell Death	Apoptosis	Anoikis	Autophagy	Cell Dedifferentiation	Cell Differentiation	Adipogenesis	Gametogenesis	Oogenesis	Spermatogenesis	Sperm Maturation	Hematopoiesis	Erythropoiesis	Hematopoiesis, Extramed	Leukopoiesis	Lymphopoiesis	Myelopoiesis	Neurogenesis	Cell Fusion	Cell Growth Processes	Cell Enlargement	Cell Proliferation	Cell Division	Cell Movement	Cell Aggregation	Cell Migration Inhibition	Chemotaxis
22	Betamethasone	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
23	bis(tri-n-butyltin)oxide	0	0	0	0	0	5	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	
24	Bromodeoxyuridine	1	1	1	3	10	10	0	0	0	14	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	17	28	4	0	0	
25	butylbenzyl phthalate	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	
26	Cacodylic Acid	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	6	0	0	0		
27	Caffeine	0	0	7	2	1	9	0	0	0	5	0	0	0	2	0	0	0	0	0	0	0	0	1	0	2	13	1	1	0		
28	Carbaryl	0	0	0	0	1	0	0	0	0	1	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	
29	Carmustine	0	0	2	0	2	3	0	0	0	1	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0	1	15	1	0	0	
30	Chlorpyrifos	0	0	0	0	6	10	0	0	0	14	1	1	2	2	0	0	0	0	0	0	0	4	0	0	0	4	4	1	0	0	
31	Corticosterone	0	0	0	0	3	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	0	
32	Cortisone	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
33	cycloamine	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

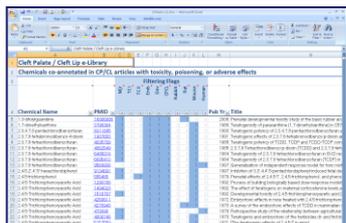
1. e-library Development

CLP Chemicals and Cell Processes

	A	B	C	D	F	G	H
1	Cleft Palate Chemicals - AOP Mat						
2	Cell Processes - detail view						
3	Filtering Flags						
4	ChemName	Process	Tox	Palate	PMID	PubYr	Title
1018	Caffeine	G2 Phase	1	0	1944378	1991	Effects of caffeine and cycloheximide during G
1019	Caffeine	S Phase	1	0	8613687	1996	UV-B-induced cell cycle perturbations, micronu
1020	Caffeine	S Phase	1	0	7506363	1994	A case of caffeine-mediated cancellation of mi
1021	Caffeine	Cell Death	1	0	8512808	1993	Caffeine potentiates the lethality of tumour ne
1022	Caffeine	Apoptosis	1	0	15039113	2004	1,2-bis(2-aminophenoxy)ethane-N,N,N'N'-tetra
1023	Caffeine	Apoptosis	1	0	10769661	2000	Sensitization and caffeine potentiation of cispl
1024	Caffeine	Apoptosis	1	0	12378022	2002	Caffeine induces apoptosis in human neurobla
1025	Caffeine	Apoptosis	1	0	9458292	1998	Enhancement of CDDP cytotoxicity by caffeine
1026	Caffeine	Apoptosis	1	0	12395097	2002	Caffeine-induced neuronal death in neonatal r
1027	Caffeine	Apoptosis	1	0	12884404	2003	Apoptosis induced by different doses of caffei
1028	Caffeine	Apoptosis	1	0	8299722	1994	Enhancement of CDDP cytotoxicity by caffeine
1029	Caffeine	Apoptosis	1	0	16709440	2007	The enigmatic effects of caffeine in cell cycle a
1030	Caffeine	Apoptosis	1	0	8512808	1993	Caffeine potentiates the lethality of tumour ne
1031	Caffeine	Cell Differentiation	1	0	22470550	2012	Exploring the caffeine-induced teratogenicity o
1032	Caffeine	Cell Differentiation	1	0	7948410	1994	In vitro study of teratogenic effects of caffeine
1033	Caffeine	Cell Differentiation	0	0	2885939	1987	Potentiating effect of caffeine on embryotoxic

2. High throughput links to CP

in vivo data



e-libraries (25)

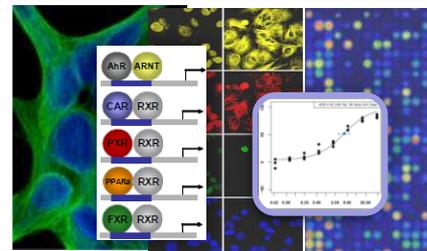
+



ToxRefDB (31)

**CP
teratogens**
**non-CP
teratogens**

in vitro testing



ToxCastDB

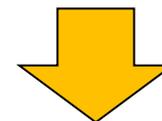
> 700 assays
Concentration response

2. High throughput links to CP

Assay Name	Description
APR_CellCycleArrest_72hr	cell-cycle arrest
BSK_CASM3C_Proliferation_down	decreased proliferation
BSK_CASM3C_SAA_up	serum amyloid
ATG_ERE_CIS	estrogen receptor
ATG_GR_TRANS	glucocorticoid receptor
ATG_RARa_TRANS	retinoic acid receptor
ATG_RARb_TRANS	retinoic acid receptor
ATG_RARg_TRANS	retinoic acid receptor
ATG_RXRa_TRANS	retinoid x receptor
ATG_RXRb_TRANS	retinoid x receptor
NVS_ADME_hCYP19A1	cytochrome p450
NVS_ENZ_hCASP5	caspase 5
NVS_ENZ_hElastase	elastase
NVS_ENZ_hFGFR1	fibroblast growth factor
NVS_ENZ_hPTPN11	phosphatase
NVS_ENZ_oCOX1	cyclooxygenase
NVS_GPCR_bH1	histamine GPCR
NVS_GPCR_gH2	histamine GPCR
NVS_GPCR_gLTD4	leukotriene GPCR
NVS_GPCR_hAdoRA1	adenosine GPCR
NVS_GPCR_hAdoRA2a	adenosine GPCR
NVS_GPCR_rAdra1_NonSelective	adrenergic GPCR
NVS_GPCR_rAdra1A	adrenergic GPCR
NVS_GPCR_rAdra1B	adrenergic GPCR
NVS_GPCR_rAdra2_NonSelective	adrenergic GPCR
NVS_GPCR_rSST	somatostatin GPCR
NVS_OR_gSIGMA_NonSelective	sigma receptor
NVS_IC_rCaBTZCHL	ion channel
NVS_IC_rNaCh_site2	ion channel

1/2 in e-libraries

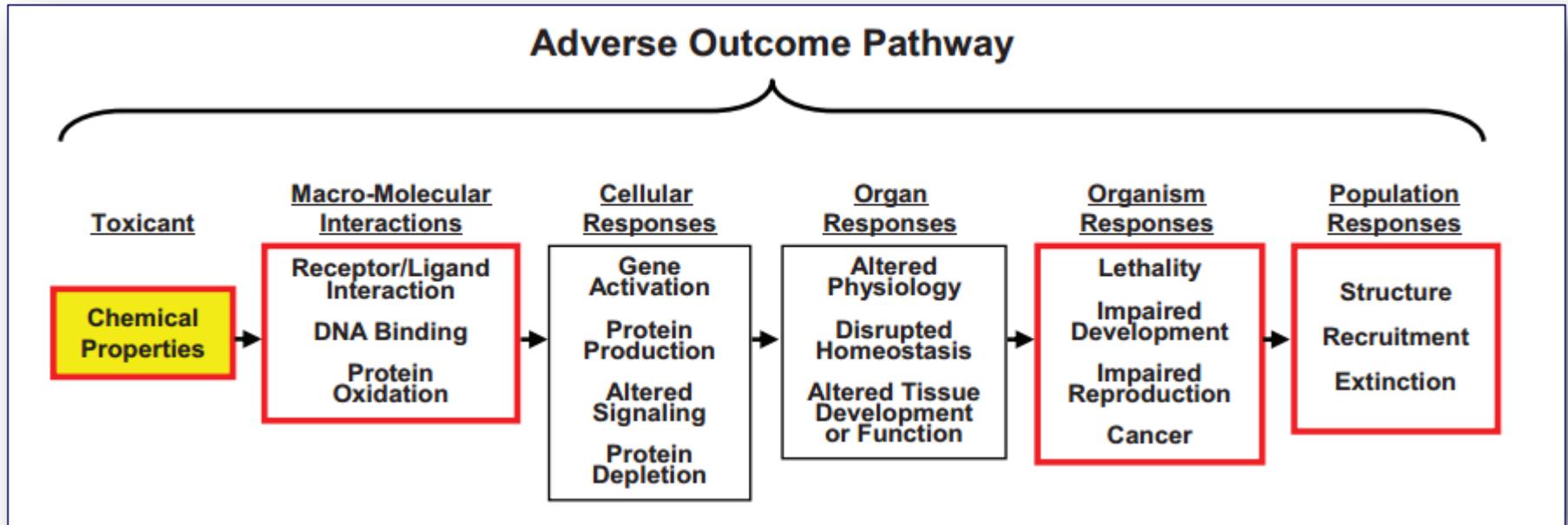
**Statistical
associations**



**Quantitative
chemical-assay
concentration
response**

3. Adverse Outcome Pathway (AOP)

conceptual and practical tool to capture multiscale descriptions of a toxicological process



Develop predicted target-to-outcome pathways of CP teratogens

CP teratogens

Molecular
Target pathways

3. Cleft Palate AOP Framework

Cellular
Cell-level behaviors

Tissue
Palatal shelf

Organ
Apposition

Outcome
Structural malformation

Pubmed search results for 'Cleft Palate'. The table shows columns for Chemical Name, PMID, and Pub. Title. The first few entries are:

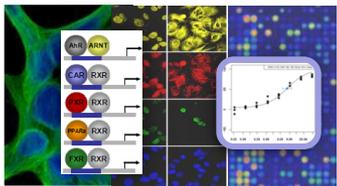
Chemical Name	PMID	Pub. Title
1,2-Dibromoethane	20020201	1,2-Dibromoethane is a potent inducer of the human c-myc gene...
1,2-Dichloroethane	20020201	1,2-Dichloroethane is a potent inducer of the human c-myc gene...
1,2-Dichloroethane	20020201	1,2-Dichloroethane is a potent inducer of the human c-myc gene...

1. e-libraries

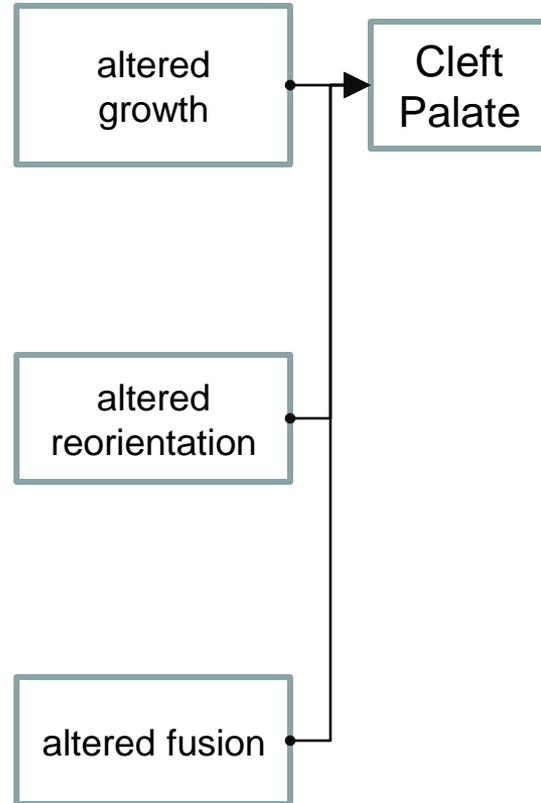
MGI Mammalian Phenotype Ontology website showing search results for 'cleft palate'. The table lists gene symbols and their associated phenotypes.

Gene Symbol	Phenotype
Ahr1 ^{tm1.1}	abnormal palate
Arnt ^{tm1.1}	cleft palate
Arnt ^{tm1.2}	cleft palate
Arnt ^{tm1.3}	cleft palate
Arnt ^{tm1.4}	cleft palate
Arnt ^{tm1.5}	cleft palate
Arnt ^{tm1.6}	cleft palate
Arnt ^{tm1.7}	cleft palate
Arnt ^{tm1.8}	cleft palate
Arnt ^{tm1.9}	cleft palate
Arnt ^{tm1.10}	cleft palate
Arnt ^{tm1.11}	cleft palate
Arnt ^{tm1.12}	cleft palate
Arnt ^{tm1.13}	cleft palate
Arnt ^{tm1.14}	cleft palate
Arnt ^{tm1.15}	cleft palate
Arnt ^{tm1.16}	cleft palate
Arnt ^{tm1.17}	cleft palate
Arnt ^{tm1.18}	cleft palate
Arnt ^{tm1.19}	cleft palate
Arnt ^{tm1.20}	cleft palate
Arnt ^{tm1.21}	cleft palate
Arnt ^{tm1.22}	cleft palate
Arnt ^{tm1.23}	cleft palate
Arnt ^{tm1.24}	cleft palate
Arnt ^{tm1.25}	cleft palate
Arnt ^{tm1.26}	cleft palate
Arnt ^{tm1.27}	cleft palate
Arnt ^{tm1.28}	cleft palate
Arnt ^{tm1.29}	cleft palate
Arnt ^{tm1.30}	cleft palate
Arnt ^{tm1.31}	cleft palate
Arnt ^{tm1.32}	cleft palate
Arnt ^{tm1.33}	cleft palate
Arnt ^{tm1.34}	cleft palate
Arnt ^{tm1.35}	cleft palate
Arnt ^{tm1.36}	cleft palate
Arnt ^{tm1.37}	cleft palate
Arnt ^{tm1.38}	cleft palate
Arnt ^{tm1.39}	cleft palate
Arnt ^{tm1.40}	cleft palate
Arnt ^{tm1.41}	cleft palate
Arnt ^{tm1.42}	cleft palate
Arnt ^{tm1.43}	cleft palate
Arnt ^{tm1.44}	cleft palate
Arnt ^{tm1.45}	cleft palate
Arnt ^{tm1.46}	cleft palate
Arnt ^{tm1.47}	cleft palate
Arnt ^{tm1.48}	cleft palate
Arnt ^{tm1.49}	cleft palate
Arnt ^{tm1.50}	cleft palate

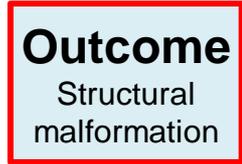
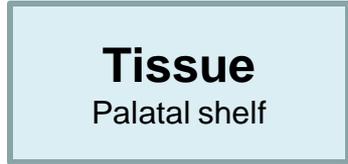
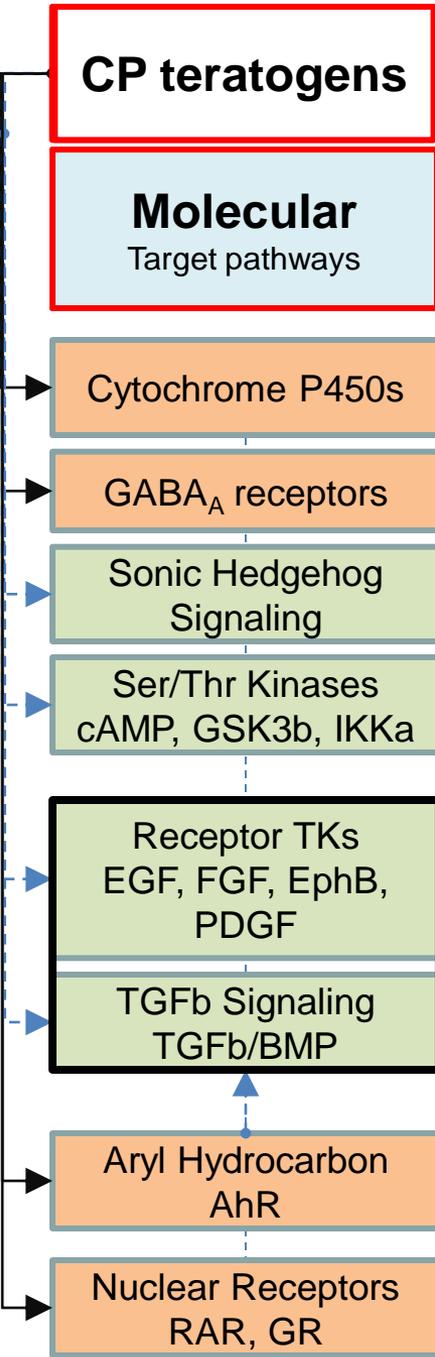
2. informatics. jax.org



3. ToxCast

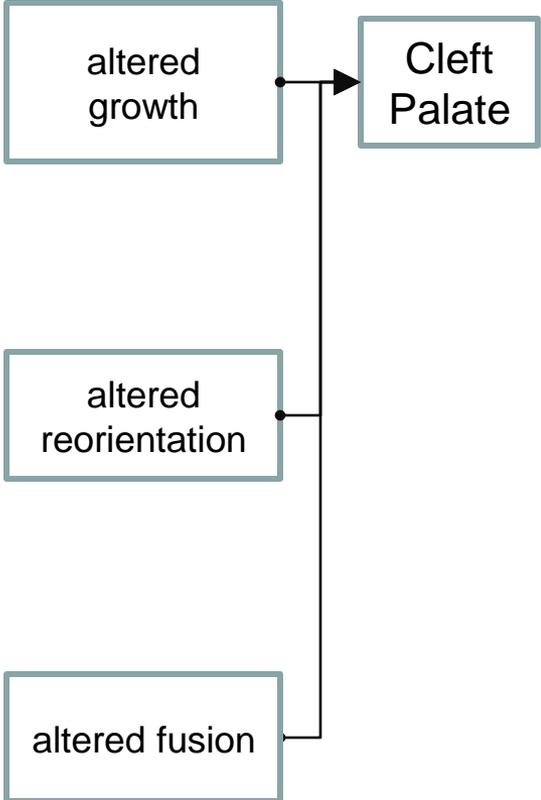


3. Cleft Palate AOP Framework

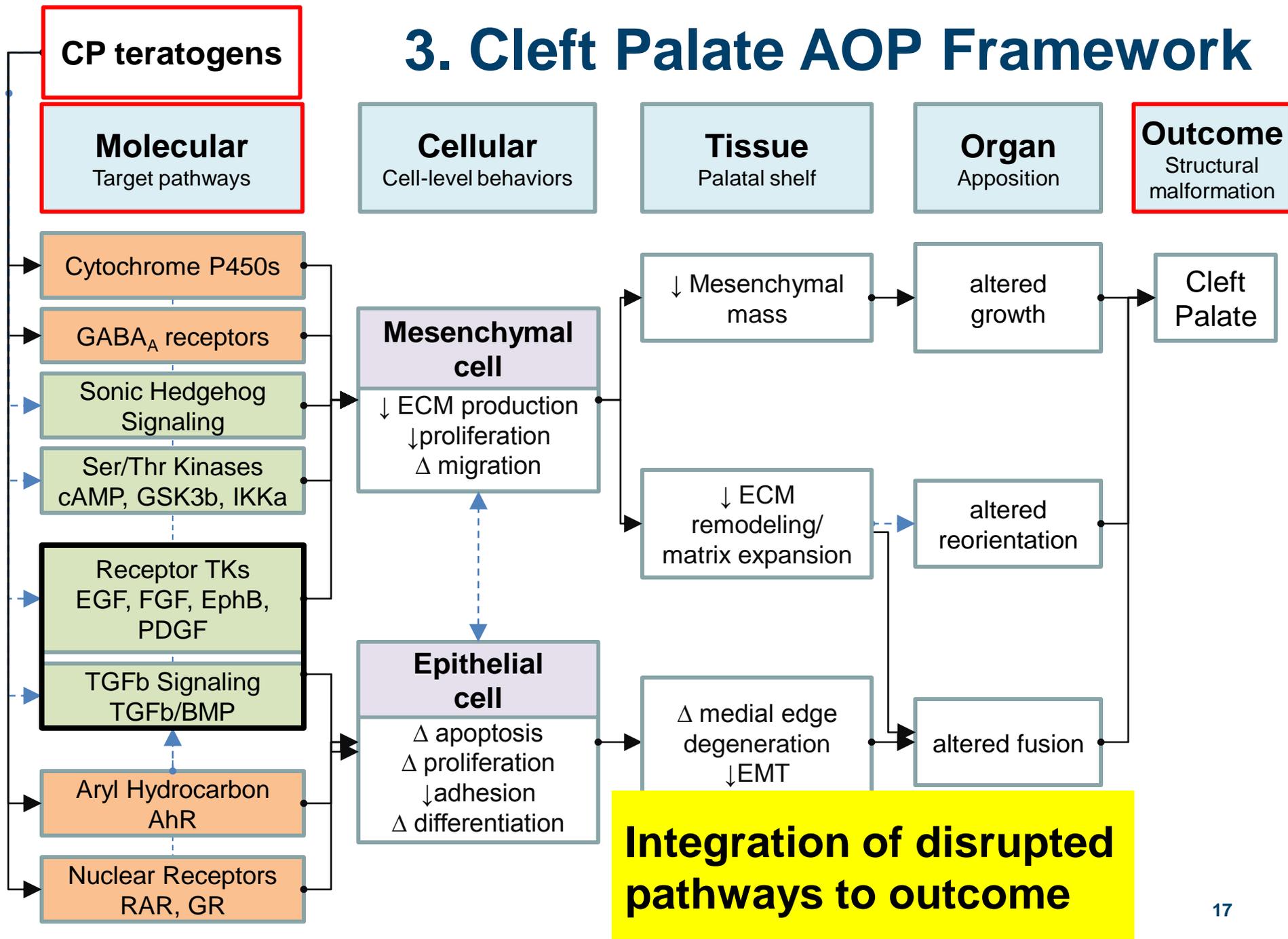


Chemical Name	PMID	Pub Title
1. 1,1-Dibromoethane	10000000	10000000
2. 1,1-Dichloroethane	10000000	10000000
3. 1,1-Dichloroethene	10000000	10000000
4. 1,1-Dichloroethane	10000000	10000000
5. 1,1-Dichloroethane	10000000	10000000
6. 1,1-Dichloroethane	10000000	10000000
7. 1,1-Dichloroethane	10000000	10000000
8. 1,1-Dichloroethane	10000000	10000000
9. 1,1-Dichloroethane	10000000	10000000
10. 1,1-Dichloroethane	10000000	10000000
11. 1,1-Dichloroethane	10000000	10000000
12. 1,1-Dichloroethane	10000000	10000000
13. 1,1-Dichloroethane	10000000	10000000
14. 1,1-Dichloroethane	10000000	10000000
15. 1,1-Dichloroethane	10000000	10000000
16. 1,1-Dichloroethane	10000000	10000000
17. 1,1-Dichloroethane	10000000	10000000
18. 1,1-Dichloroethane	10000000	10000000
19. 1,1-Dichloroethane	10000000	10000000
20. 1,1-Dichloroethane	10000000	10000000

e-libraries



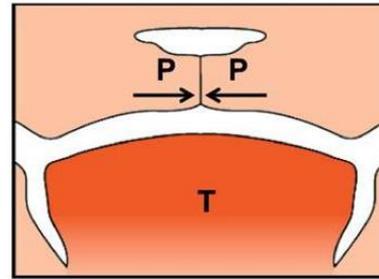
3. Cleft Palate AOP Framework



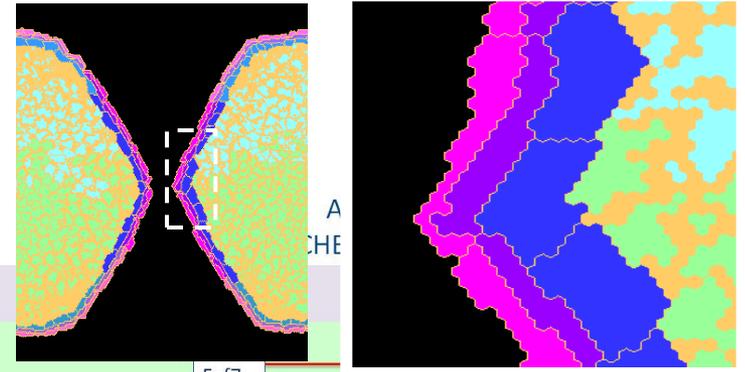
4. Multicellular Computer Simulation

- **Cell-level model**

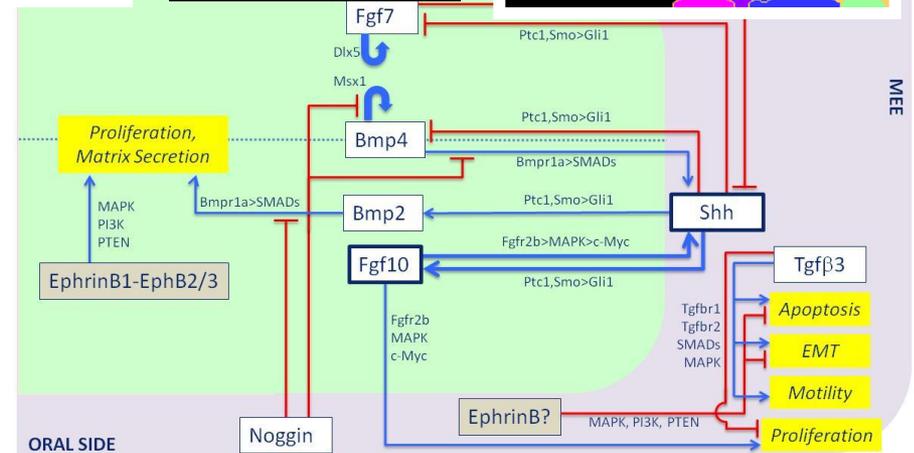
- Simulation is driven by biological networks and rules



Fusion



- **Gene, protein, pathway incorporation**
- **AOP visualization**



TIME

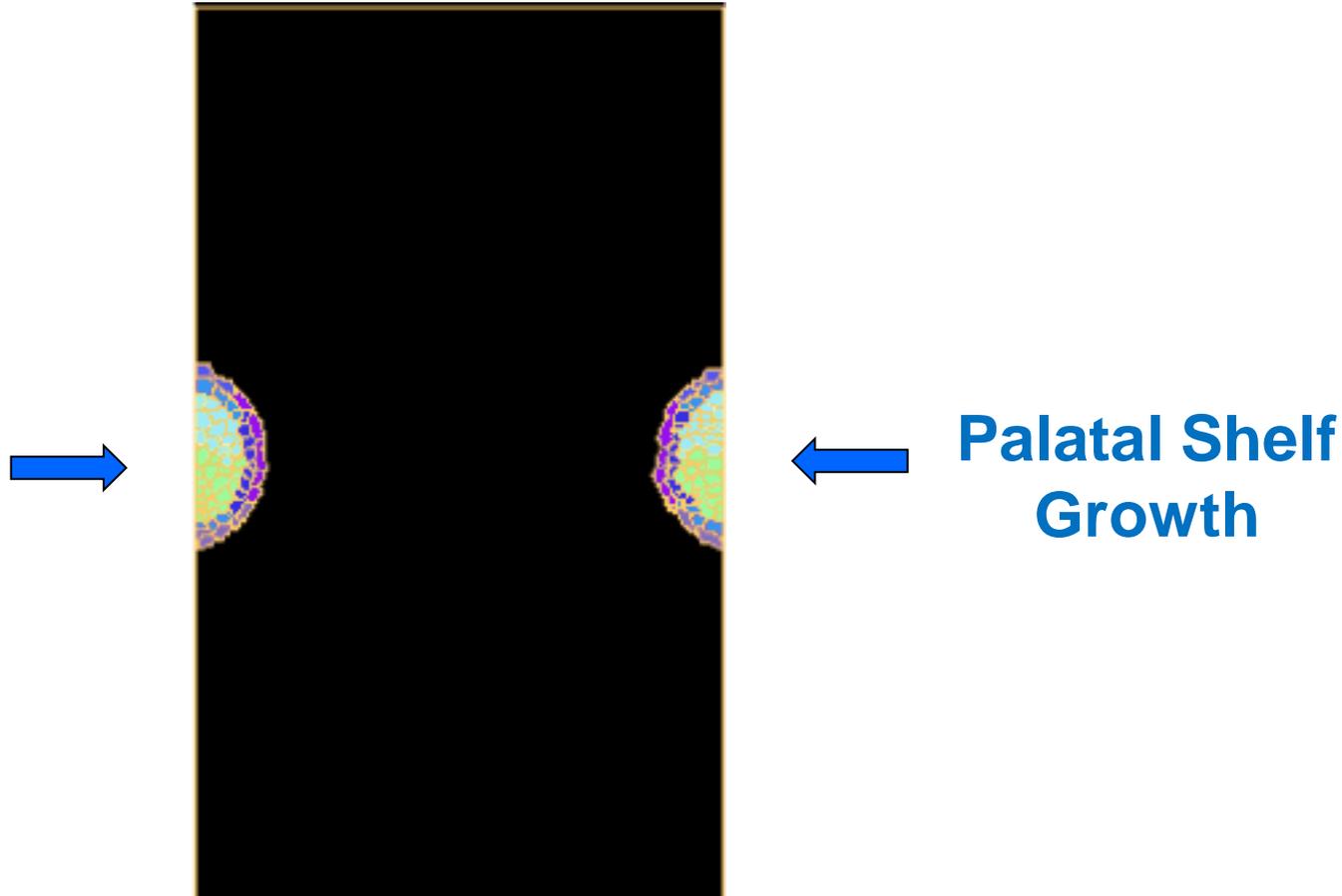
**DOSE
RESPONSE**

**STAGE
SPECIFICITY**

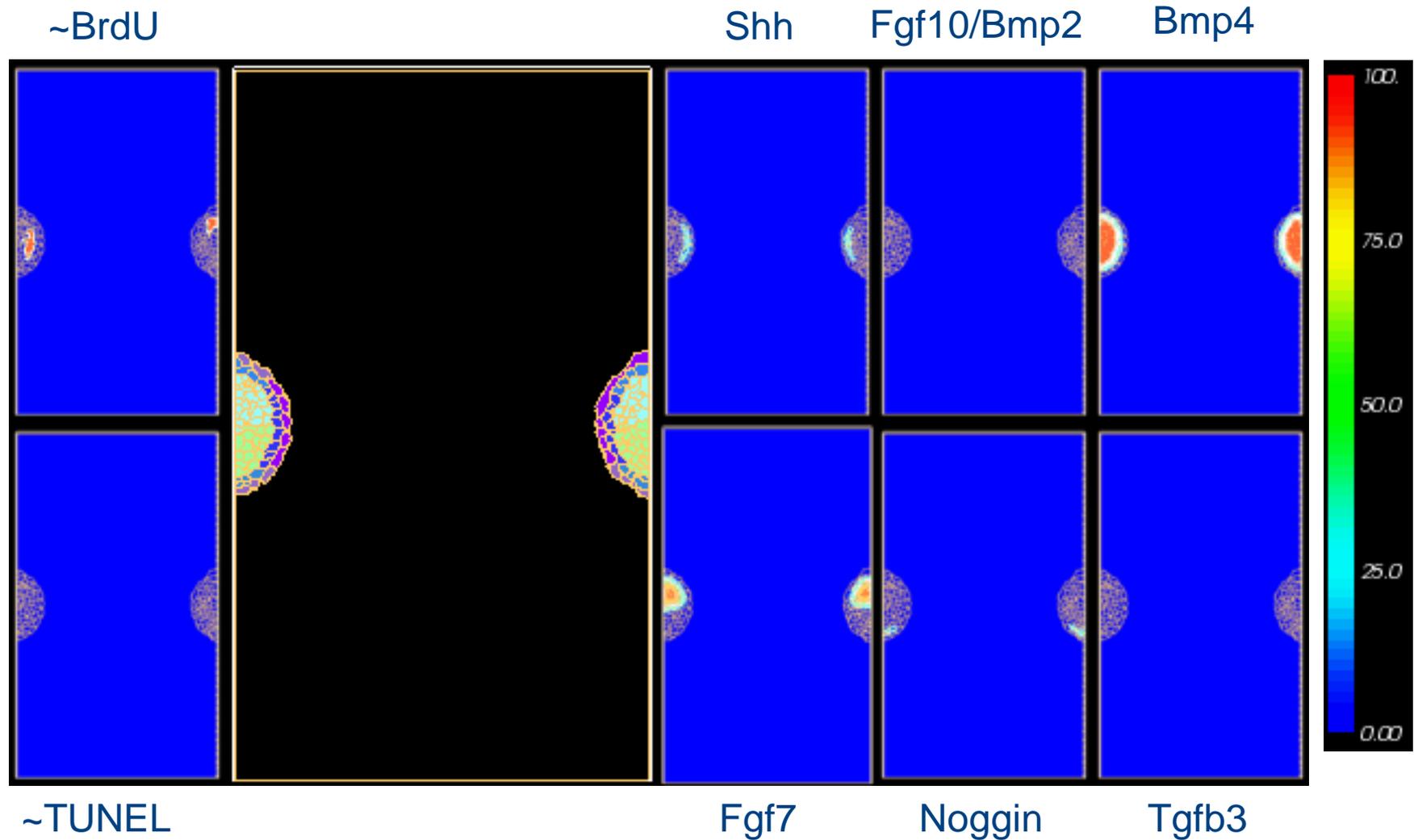
PREDICTIONS

4. Multicellular Computer Simulation

Normal Palatal Fusion



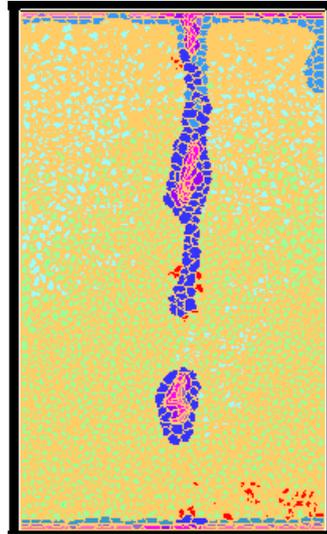
4. Multicellular Computer Simulation



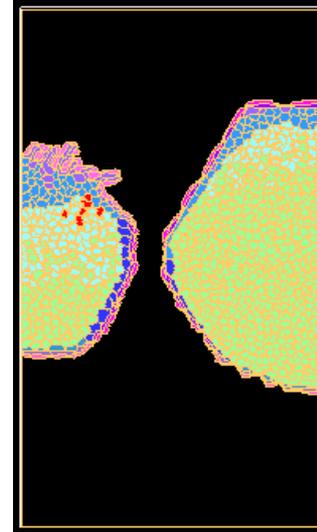
4. Multicellular Computer Simulation

in silico
knockouts

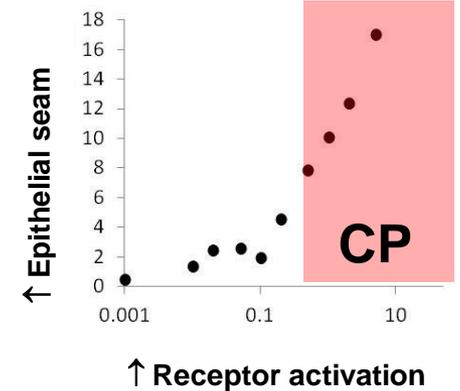
Tgfb3^{-/-}



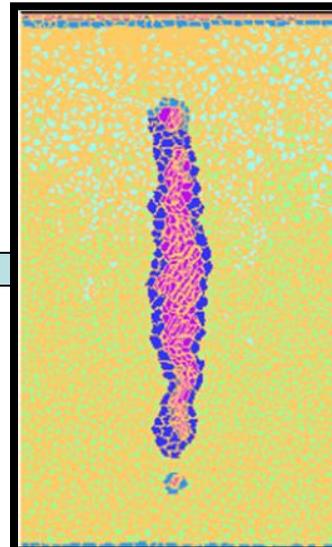
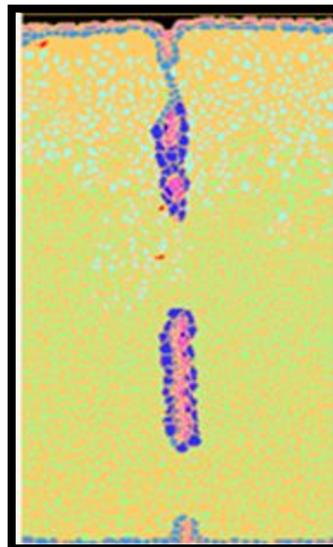
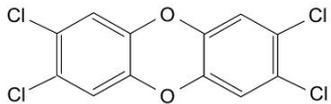
Fgf10^{-/-}



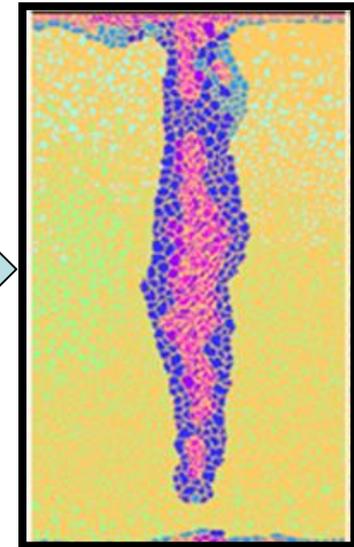
Chemical exposure



in silico
toxicant
exposure



highest dose



Summary

- ❖ Toxicity in the embryo is an expression of complex and interwoven events that follow from cellular perturbation
- ❖ Systems biology framework can be used to integrate information
 - ❖ e-libraries *publically available articles*
 - ❖ ToxCast assays *dose response*
 - ❖ AOPs *fill in sequence of events*
 - ❖ *in silico* computational models *predictive capability + time & dose*
- ❖ Systems-level models that recapitulate *in vivo* biology can be used to integrate information for making predictions about potential chemical hazards

Thank you!



<http://www.epa.gov/ncct/>

Virtual Embryo (NCCT)

Thomas Knudsen

Nancy Baker (LM)

Shane Hutson (Vanderbilt)

Maxwell Leung

Richard Spencer

Virtual Embryo (NHEERL)

Barbara Abbott

Kelly Chandler (NCCT)

Sid Hunter

Stephanie Padilla

Tamara Tal

ToxCast (NCCT)



THE
TERATOLOGY
SOCIETY
BIRTH DEFECTS RESEARCH • EDUCATION • PREVENTION
EST. 1960

**Postdoctoral Fellow
Travel Award**