National Institute of Environmental Health Sciences
Your Environment. Your Health.

Assessment of Latent Hazards at DNTP: Evidence and Evaluations

B. R. Berridge, DVM, PhD, DACVP
Scientific Director, DNTP
National Institute of Environmental Health Sciences

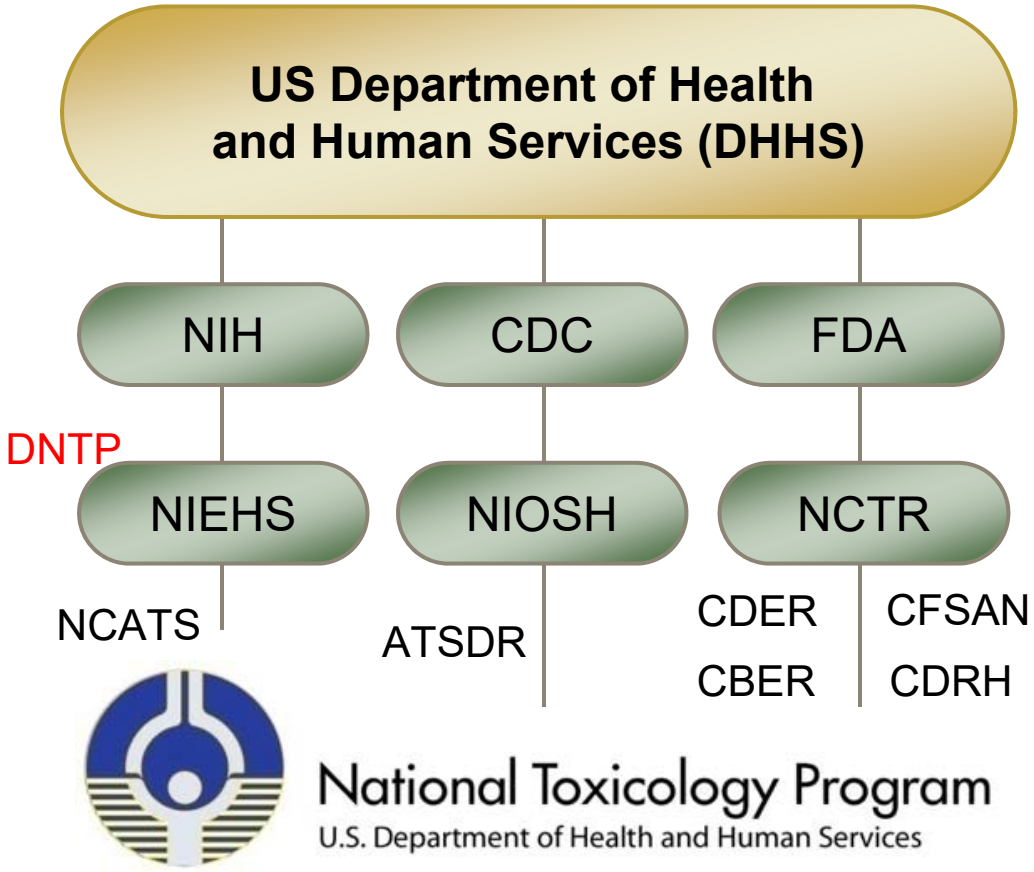
11 May 2020

Outline

- Brief intro to DNTP/NTP
- Definition and perspectives on latency
- Developmental latency
- Carcinogenic latency
- Latency of innovation

National Institute of Environmental Health Sciences
Your Environment. Your Health.

NTP/DNTP Continuum



National Toxicology Program
U.S. Department of Health and Human Services

http://ntp.niehs.nih.gov

Health and Environmental Sciences Institute

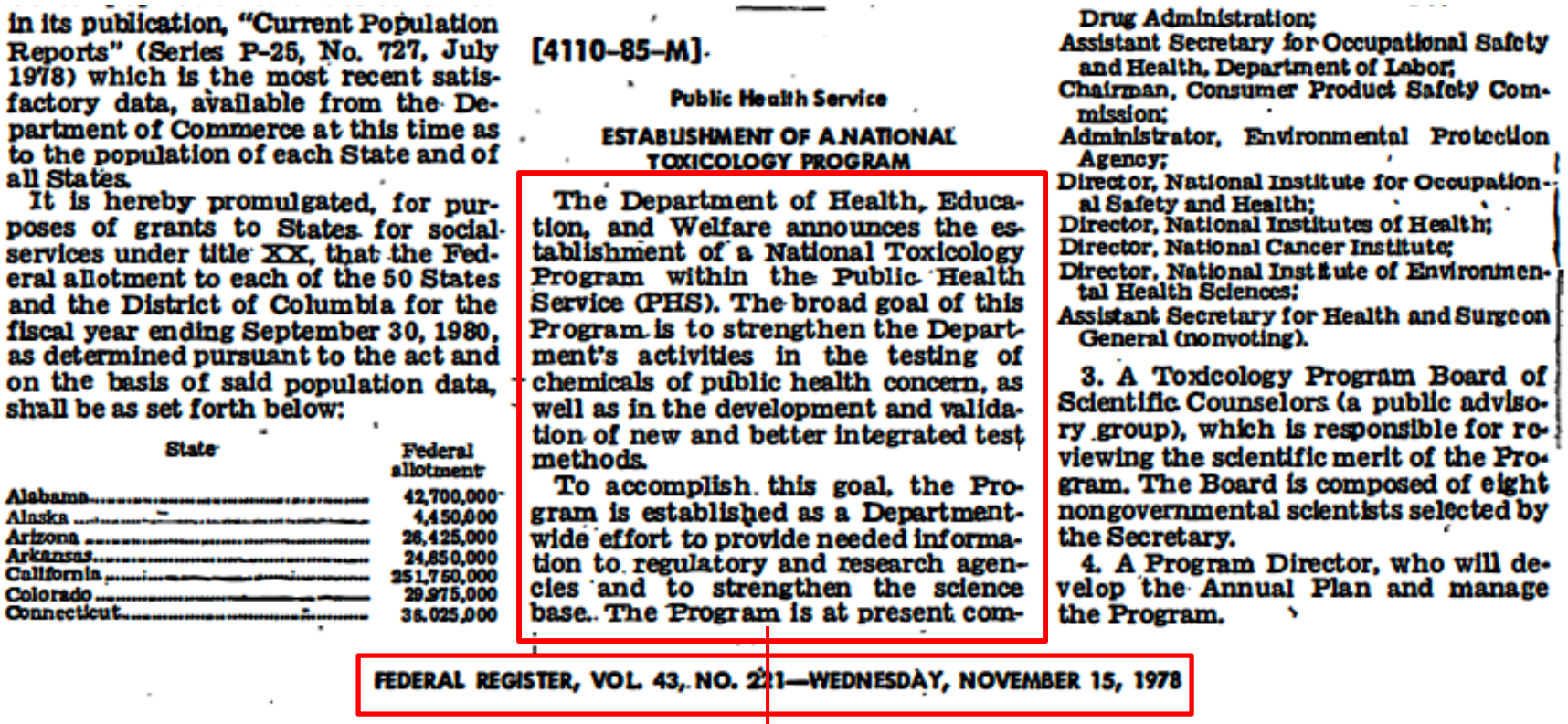
International Agency Research on Cancer

World Health Organization

EURL ECVAM
European Union Reference Laboratory for Alternatives to Animal Testing

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

NTP Mission



in its publication, "Current Population Reports" (Series P-25, No. 727, July 1978) which is the most recent satisfactory data, available from the Department of Commerce at this time as to the population of each State and of all States.

It is hereby promulgated, for purposes of grants to States for social services under title XX, that the Federal allotment to each of the 50 States and the District of Columbia for the fiscal year ending September 30, 1980, as determined pursuant to the act and on the basis of said population data, shall be as set forth below:

State	Federal allotment
Alabama	42,700,000
Alaska	4,450,000
Arizona	26,425,000
Arkansas	24,850,000
California	251,750,000
Colorado	29,975,000
Connecticut	35,025,000

[4110-85-M]

Public Health Service

ESTABLISHMENT OF A NATIONAL TOXICOLOGY PROGRAM

The Department of Health, Education, and Welfare announces the establishment of a National Toxicology Program within the Public Health Service (PHS). The broad goal of this Program is to strengthen the Department's activities in the testing of chemicals of public health concern, as well as in the development and validation of new and better integrated test methods.

To accomplish this goal, the Program is established as a Department-wide effort to provide needed information to regulatory and research agencies and to strengthen the science base. The Program is at present com-

Drug Administration;
Assistant Secretary for Occupational Safety and Health, Department of Labor;
Chairman, Consumer Product Safety Commission;
Administrator, Environmental Protection Agency;
Director, National Institute for Occupational Safety and Health;
Director, National Institutes of Health;
Director, National Cancer Institute;
Director, National Institute of Environmental Health Sciences;
Assistant Secretary for Health and Surgeon General (nonvoting).

3. A Toxicology Program Board of Scientific Counselors (a public advisory group), which is responsible for reviewing the scientific merit of the Program. The Board is composed of eight nongovernmental scientists selected by the Secretary.

4. A Program Director, who will develop the Annual Plan and manage the Program.

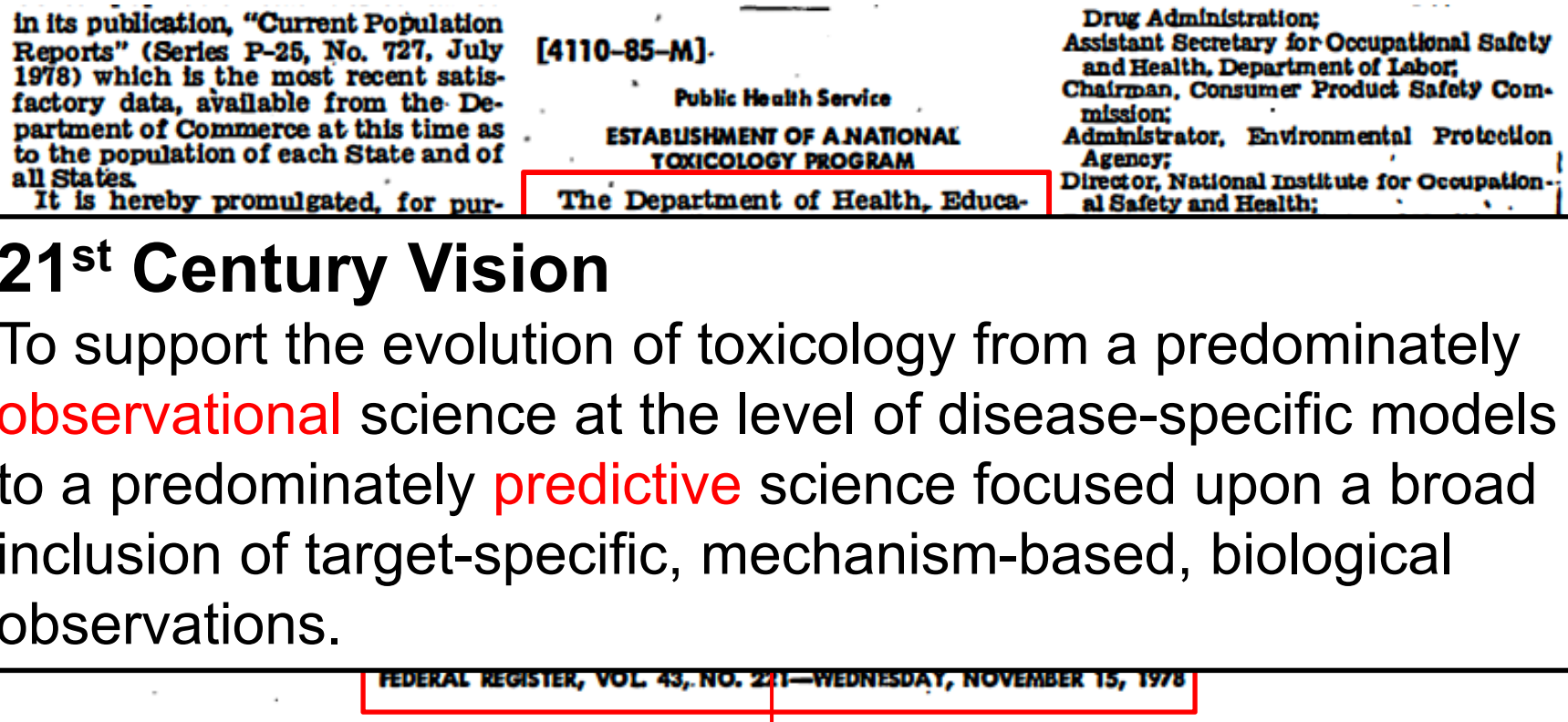
FEDERAL REGISTER, VOL. 43, NO. 221—WEDNESDAY, NOVEMBER 15, 1978

Historical strength of the Program

- "testing of chemicals"
- "development and validation of new and better integrated test methods
- "strengthen the science base"

Increasing focus

NTP Mission



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It is hereby promulgated, for pur-

[4110-85-M]

Public Health Service

ESTABLISHMENT OF A NATIONAL TOXICOLOGY PROGRAM

The Department of Health, Educa-

Drug Administration;
Assistant Secretary for Occupational Safety and Health, Department of Labor;
Chairman, Consumer Product Safety Commission;
Administrator, Environmental Protection Agency;
Director, National Institute for Occupational Safety and Health;

FEDERAL REGISTER, VOL. 43, NO. 221—WEDNESDAY, NOVEMBER 15, 1978

21st Century Vision

To support the evolution of toxicology from a predominately observational science at the level of disease-specific models to a predominately predictive science focused upon a broad inclusion of target-specific, mechanism-based, biological observations.

Historical strength of the Program

- "testing of chemicals"
- "development and validation of new and better integrated test methods
- "strengthen the science base"

Increasing focus

NTP Products, Research Areas, Resources

latent adjective

Save Word

la·tent | \ ˈlā-tᵊnt \

Definition of *latent* (Entry 1 of 2)

: present and capable of emerging or developing but not now visible, obvious, active, or symptomatic

// a latent infection

latency noun

Save Word

la·ten·cy | \ ˈlā-tᵊn(t)-sē \

plural **latencies**

Definition of *latency*

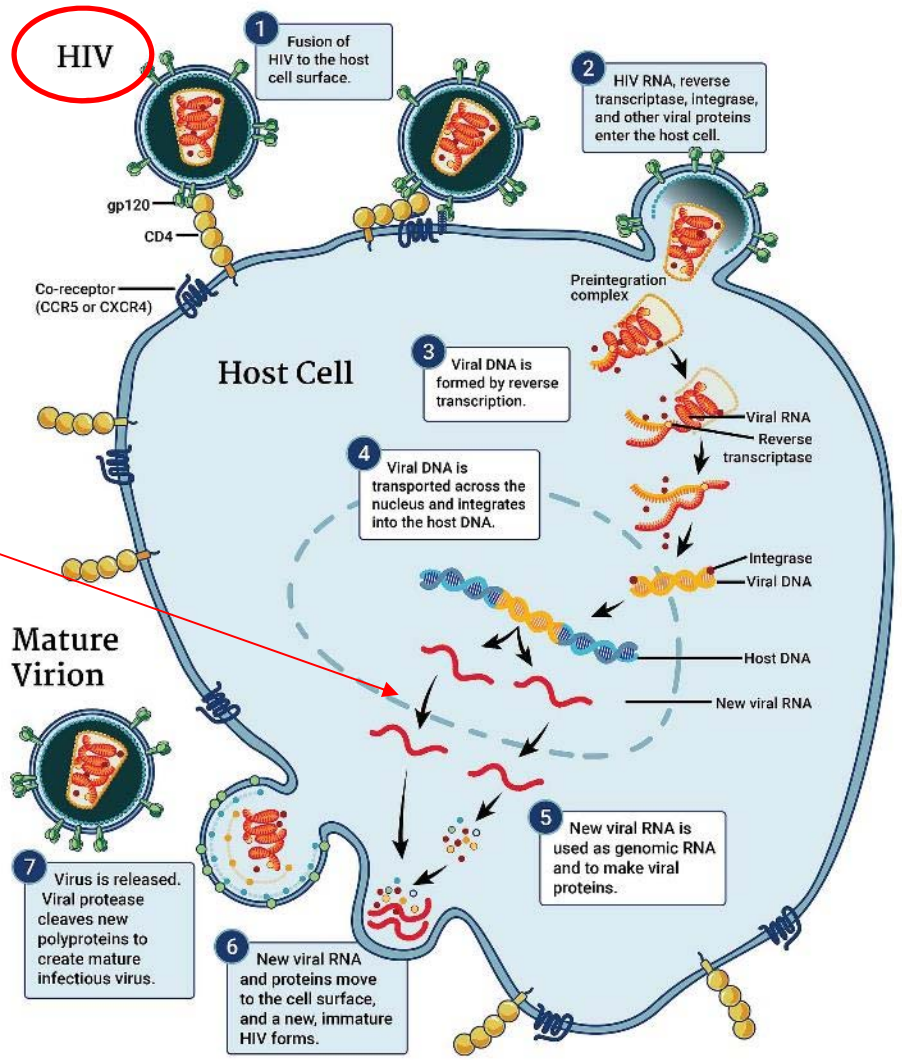
1 : the quality or state of being latent : DORMANCY

// latency is a characteristic common to all members of the troublesome herpes family

— Claudia Wallis

Viral latency

Point of latency



https://www.flickr.com/photos/niaid/5057022555/

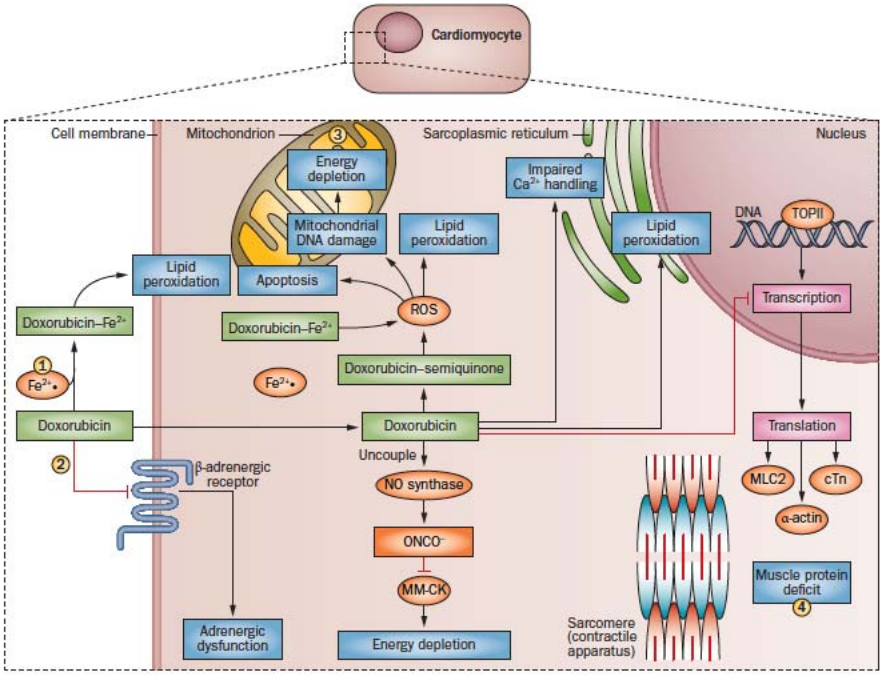
REVIEWS

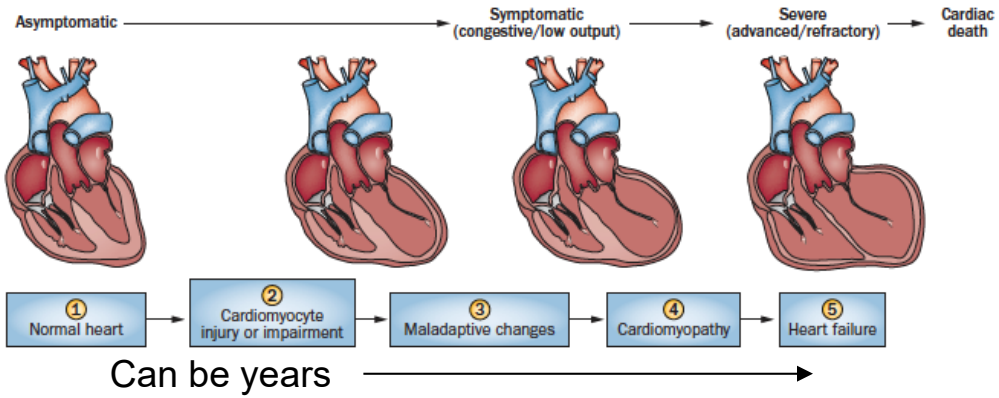
Treatment-related cardiotoxicity in survivors of childhood cancer

Steven E. Lipshultz, Thomas R. Cochran, Vivian I. Franco and Tracie L. Miller

Lipshultz, S. E. *et al. Nat. Rev. Clin. Oncol.* **10**, 697–710 (2013);

- Anthracylines are widely-used cancer chemotherapeutics
- Adult and childhood cancers
- Acute and chronic effects managed by cumulative dose thresholds
- Cardiotoxicity with heart failure a significant latent effect in both children and adults





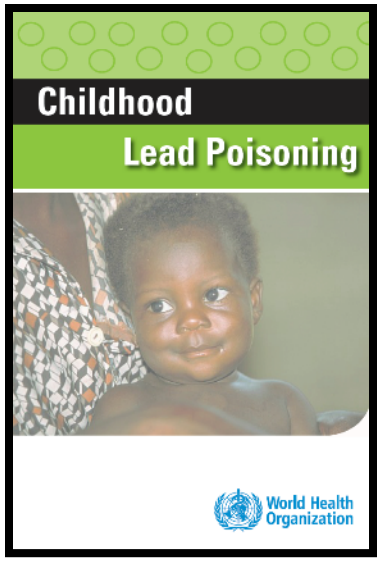
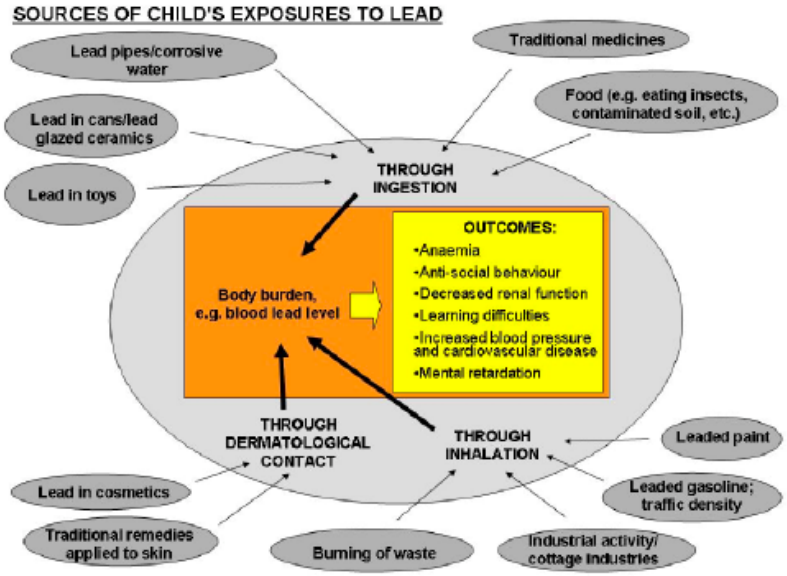


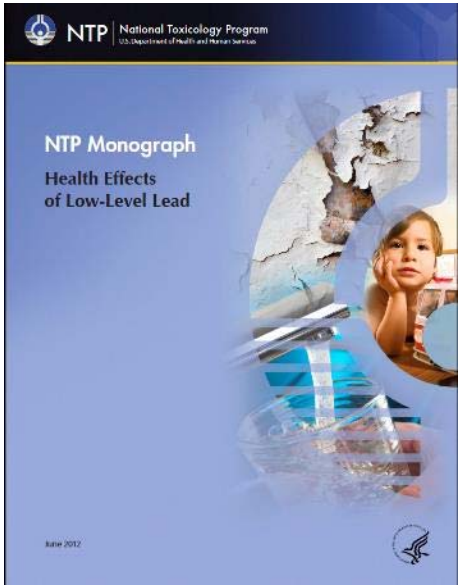
Fig. 1. Sources of children's exposure to lead



Children are now understood to be at particularly high risk of lead toxicity. From conception onward, children have a greater risk of exposure and greater susceptibility to the toxic effects of lead than do adults. There exist windows of vulnerability to lead in early life – during embryonic, fetal and early postnatal life – that have no counterparts in adult life (American Academy of Pediatrics Committee on Environmental Health, 2003).

Subclinical toxicity

The term *subclinical toxicity* denotes the concept that relatively low-dose exposure to lead at blood lead levels previously thought to be *safe* can cause harmful effects not evident in a standard clinical examination. Although they are not clinically obvious, the subclinical toxic effects of lead can be very damaging. The premise underlying the concept of subclinical toxicity is that there is a dose-related continuum of toxic effects in which clinically apparent effects have their asymptomatic (but still very real) counterparts (Landrigan, 1989) (Fig. 2).



Generally considered that there are no safe levels of lead for children!

Table 1.2: NTP conclusions on health effects of low-level Pb by major health effect areas

Health Area	Population or Exposure Window	NTP Conclusion	Principal Health Effects	Blood Pb Evidence
Neurological	Prenatal	*Limited*	Decrease in measures of cognitive function	Yes, <5 µg/dL
		Limited	Decreased IQ, increased incidence of attention-related and problem behaviors, decreased hearing	Yes, <10 µg/dL
	Children	*Sufficient*	Decreased academic achievement, IQ, and specific cognitive measures; increased incidence of attention-related and problem behaviors	Yes, <5 µg/dL
		Sufficient	Decreased hearing	Yes, <10 µg/dL
	Adults	*Sufficient*	Increased incidence of essential tremor	Yes, <10 µg/dL
		Limited	Psychiatric effects, decreased hearing, decreased cognitive function, increased incidence of ALS	Yes, <10 µg/dL
		Limited	Increased incidence of essential tremor	Yes, <5 µg/dL

878 THE NEW ENGLAND JOURNAL OF MEDICINE Apr. 22, 1971

ADENOCARCINOMA OF THE VAGINA*

Association of Maternal Stilbestrol Therapy with Tumor Appearance in Young Women

ARTHUR L. HERBST, M.D., HOWARD ULFELDER, M.D., AND DAVID C. POSKANZER, M.D.

There is a highly significant association between the treatment of the mothers with estrogen diethylstilbestrol during pregnancy and the subsequent development of adenocarcinoma of the vagina in their daughters (p less than 0.00001). Other factors

Birth Defects Res C Embryo Today. 2013 June ; 99(2): . doi:10.1002/bdrc.21035.

Exposure to Diethylstilbestrol during Sensitive Life Stages: A legacy of heritable health effects

Casey E. Reed[a] and **Suzanne E. Fenton**[a,*]

Table 2

Summary of hazard ratios for significant adverse health outcomes in women with in utero DES exposure compared to those without exposure. Reproduced from [21].

Adverse outcome	Exposed women (#/ total #)	Unexposed women (#/ total #)	Hazard Ratio (95% Confidence Interval)
Infertility	1144/3769	252/1654	2.37 (2.05–2.75)
Spontaneous abortion	916/2690	328/1291	1.64 (1.42–1.88)
Ectopic pregnancy	255/2692	36/1293	3.72 (2.58–5.38)
Loss of second-trimester pregnancy	201/2692	35/1293	3.77 (2.56–5.54)
Preterm delivery	624/2385	100/1238	4.68 (3.74–5.86)
Preeclampsia	216/2412	80/1159	1.42 (1.07–1.89)
Stillbirth	54/2385	16/1239	2.45 (1.33–4.54)
Neonatal death	57/2383	7/1238	8.12 (3.53–18.65)
Early menopause	181/3993	49/1682	2.35 (1.67–3.31)
Cervical intraepithelial neoplasia (CIN; grade ≥ 2)	208/4120	40/1785	2.28 (1.59–3.27)
Breast cancer at ≥ 40 yr	61/3693	21/1647	1.82 (1.04–3.18)
Clear-cell adenocarcinoma	4/4652	0/1926	∞

Latency complicates traditional views of toxicology

"All things are poison and nothing (is) without poison; only the dose makes that a thing is no poison."

Paracelsus (1493-1534)

LD_{50} view of toxicology

This approach might be better at identifying 'reagents' than human risks.

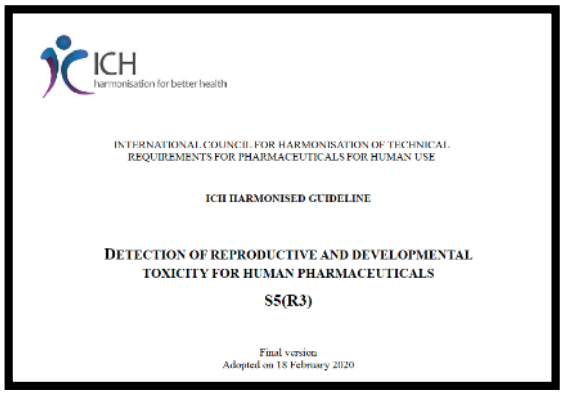
Subacute to chronic toxicity is more complex

Evolution of testing

LD50 → Carcinogenicity Testing →

Developmental Toxicity
Reproductive Toxicity
Sub-chronic Toxicity Studies
Chronic Toxicity Studies
Immunotoxicity

Development and reproduction as a sensitive state



ICH
harmonisation for better health

INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

DETECTION OF REPRODUCTIVE AND DEVELOPMENTAL TOXICITY FOR HUMAN PHARMACEUTICALS

S5(R3)

Final version
Adopted on 18 February 2020

1.1.1 Fertility and Early Embryonic Development (FEED) Study

The FEED study is designed to assess the maturation of gametes, mating behavior, fertility, preimplantation development of the embryo, and implantation. For females, this includes effects on the estrous cycle and tubal transport. For males, it includes detection of functional effects (e.g., epididymal sperm maturation) that cannot be detected by histological examinations of the male reproductive organs.

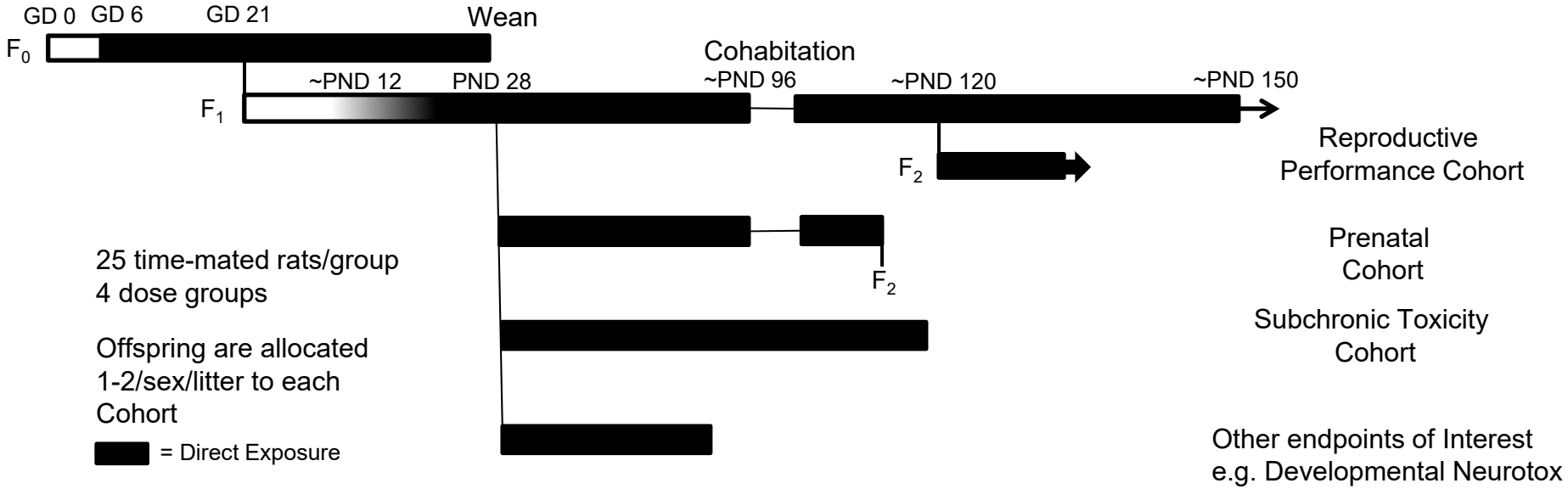
1.1.2 Embryo-Fetal Developmental (EFD) Toxicity Study

The EFD toxicity study is designed to assess maternal toxicity relative to that in non-pregnant females, and to evaluate potential effects on embryo-fetal survival, intrauterine growth, and morphological development.

1.1.3 Pre- and Postnatal Developmental (PPND) Toxicity Study

The PPND toxicity study is designed to assess enhanced toxicity relative to that in non-pregnant females, pre- and postnatal viability of offspring, altered growth and development, and functional deficits in offspring, including sexual maturation, reproductive capacity at maturity, sensory functions, motor activity, and learning and memory.

NTP Modified One-Generation Study Design



Continual exposure during all life stages

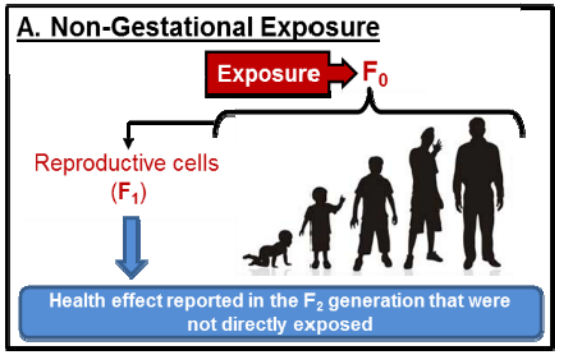
Multiple interrelated endpoints

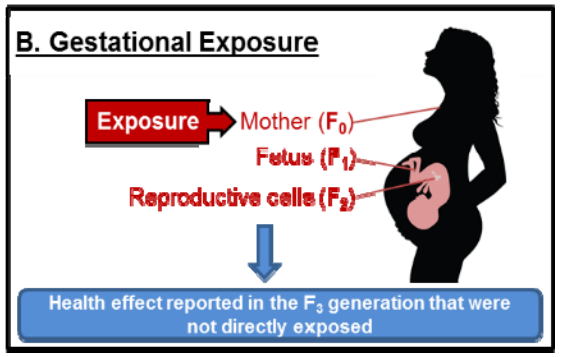
- $F_1 \approx F_2$ litter size?
- ↓perinatal F_1 [T] → delayed male sexual maturation
  → malformed [T]-dependent tissues →↓reproductive function→↓↑ F_2 litter size
  - ↓F_1 litter size → ↑ F_2 fetal malformations

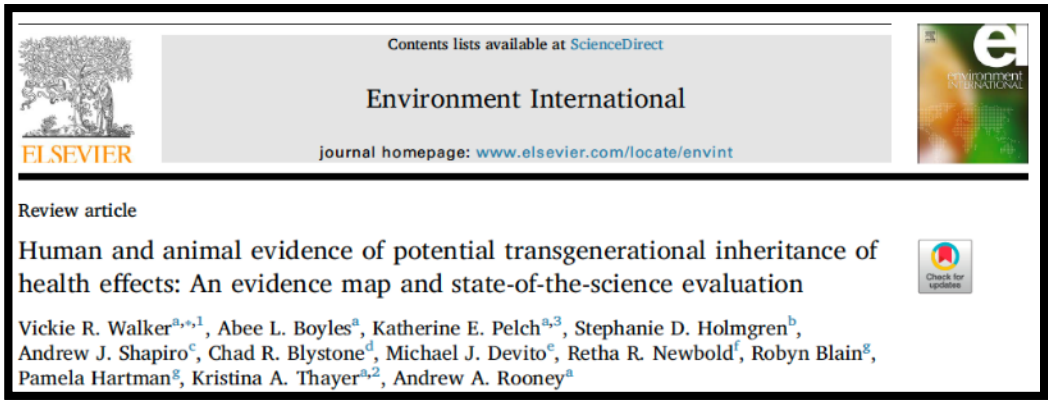
Blood levels of agent at multiple life stages

Transgenerational Latency

- Exposure stops
  - not continuous across generations
  - occurs in the F_0 generation in figures
- Health effect evaluated in generation(s) not directly exposed
  - F_2 for non – gestational exposure
  - F_3 for gestational exposure
- Note: the term “transgenerational” is not used consistently in literature
  - definition here was selected because it was used in NIEHS research grants







Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

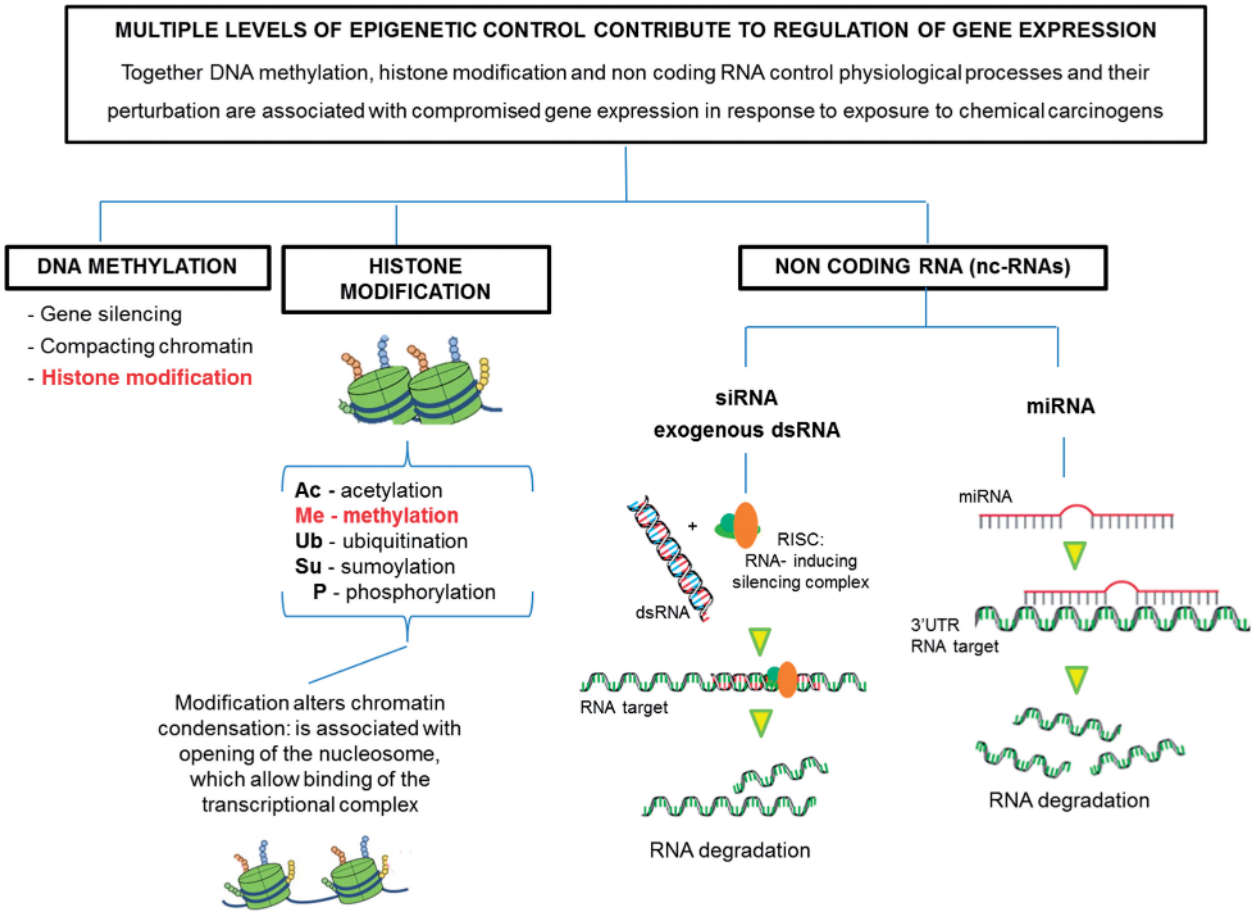
ELSEVIER

Review article

Human and animal evidence of potential transgenerational inheritance of health effects: An evidence map and state-of-the-science evaluation

Vickie R. Walker[a,*,1], Abee L. Boyles[a], Katherine E. Pelch[a,3], Stephanie D. Holmgren[b], Andrew J. Shapiro[c], Chad R. Blystone[d], Michael J. Devito[e], Retha R. Newbold[f], Robyn Blain[g], Pamela Hartman[g], Kristina A. Thayer[a,2], Andrew A. Rooney[a]

Epigenetics are heritable changes in gene expression that occur with no alteration in DNA sequence

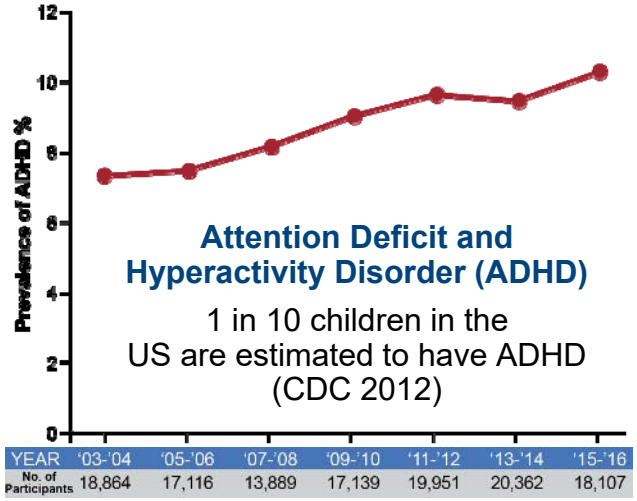


Toxicol Sci, Volume 148, Issue 1, November 2015, Pages 2–13, https://doi.org/10.1093/toxsci/kfv165.

Rise in Neurodevelopmental Disorders in the U.S

Increasing prevalence of learning and behavioral disabilities and neurodevelopmental disorders in children

Sources: Pediatrics, 128(5):1007-1022, 2011; American Psychiatric Associations, 2013



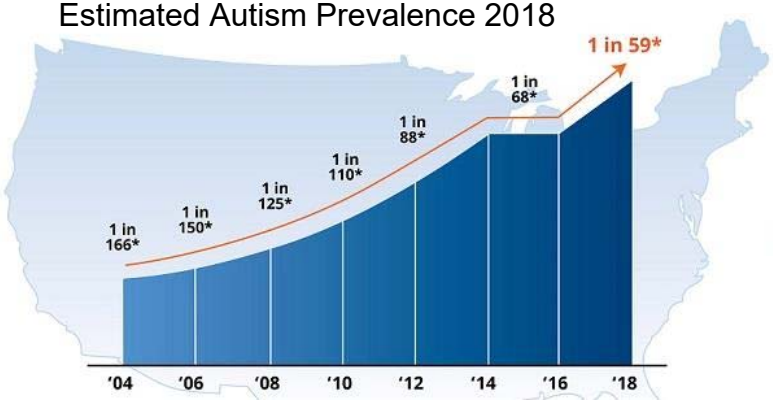
Attention Deficit and Hyperactivity Disorder (ADHD)

1 in 10 children in the US are estimated to have ADHD (CDC 2012)

Sources: abcnews.go.com, "ADHD rates in kids have increased over the past 20 years, new study says"; Journal of the American Medical Association (JAMA)

Autism Spectrum Disorder

Estimated Autism Prevalence 2018

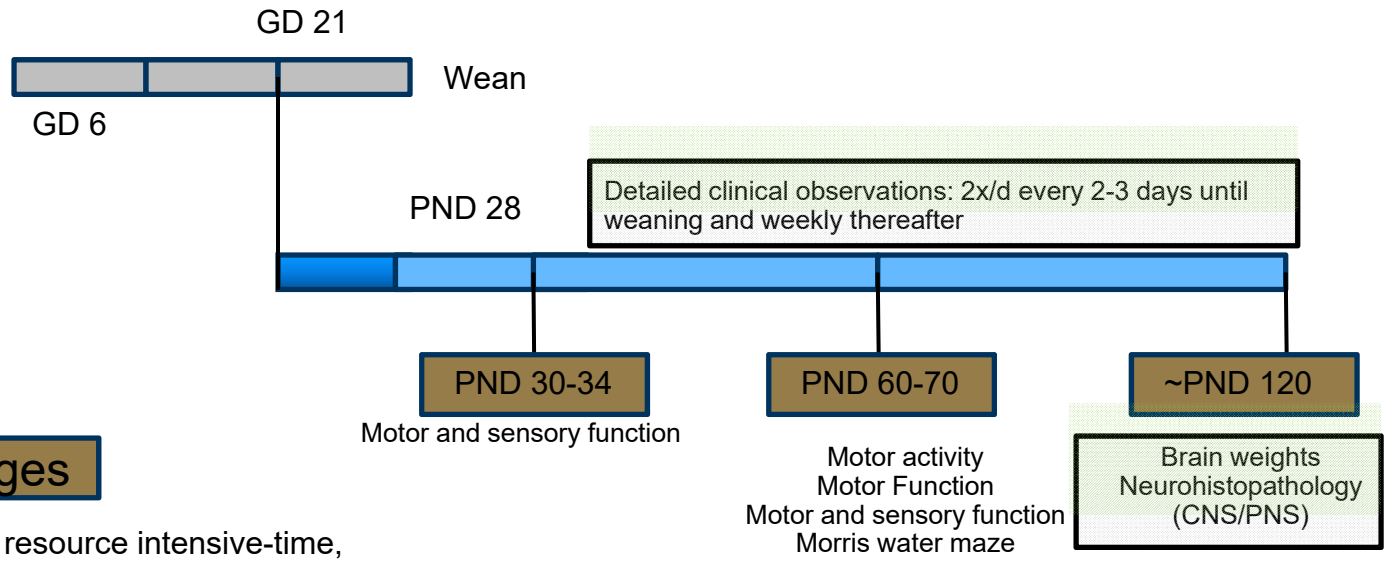


* Centers for Disease Control and Prevention (CDC) prevalence estimates are for 4 years prior to the report date (e.g. 2018 figures are from 2014)

Source: autismspeaks.org, "CDC increases estimate of autism's prevalence by 15 percent, to 1 in 59 children"

- Economic costs associated with neurodevelopmental disorders is staggering, estimated to be approximately $461 billion by 2025

Evaluating DNT in conjunction with other toxicities in littermates to increase power of detection



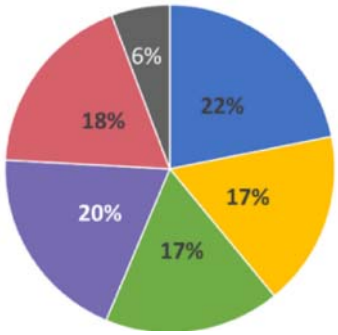
Challenges

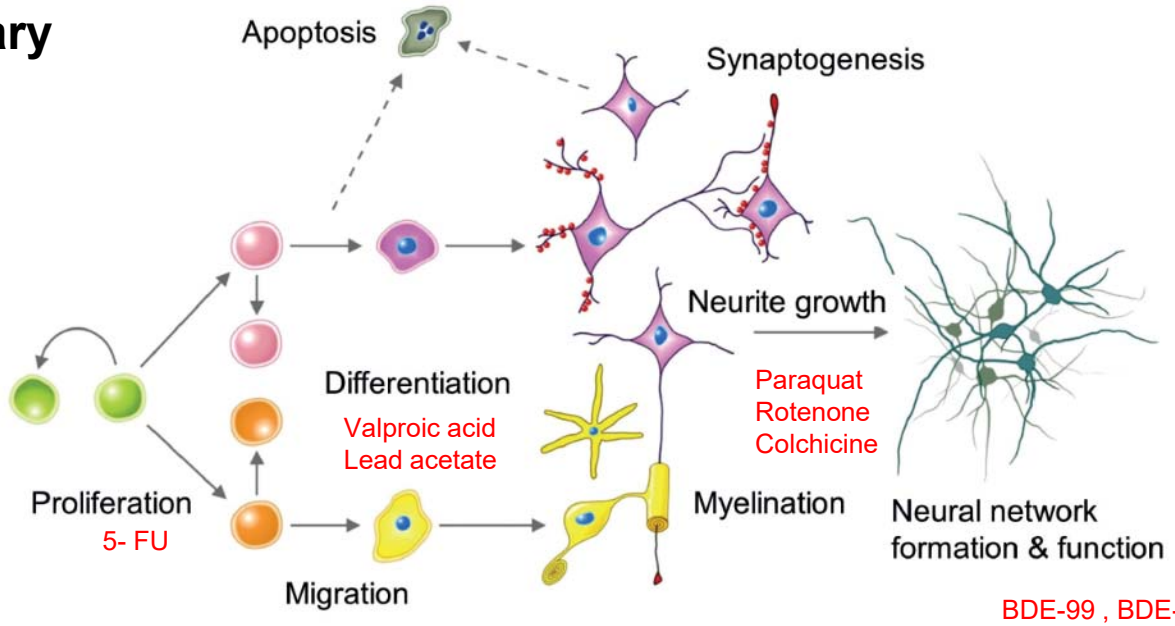
- DNT studies resource intensive-time, money and number of animals
- Limited potential for mechanistic understanding of neurodev. disorders
- Subjectivity and limited sensitivity

DNT Screening: Establishing a novel paradigm

Human-derived high content, cell-based functional assays

NTP Chemical Library





Aschner et al., 2017 & Mundy

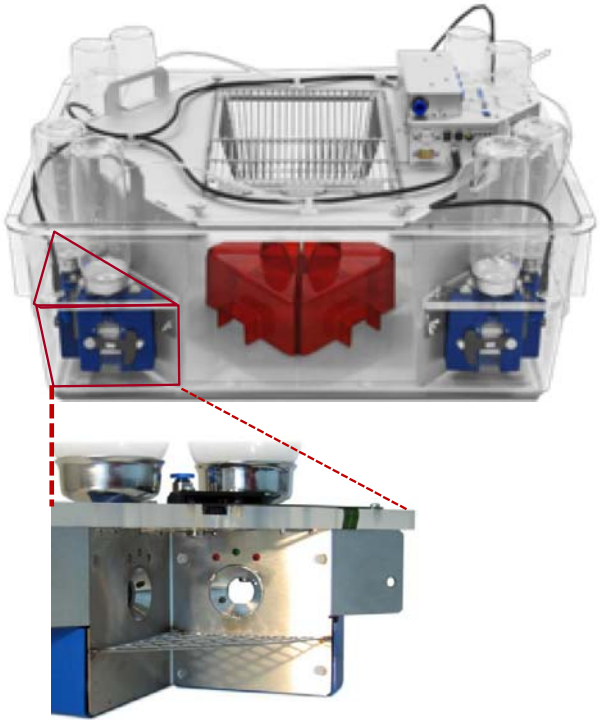
High readiness criteria

Behavior as a neurodevelopmental endpoint

Issue: Current behavioral methods are not very sensitive & do not represent natural behavior

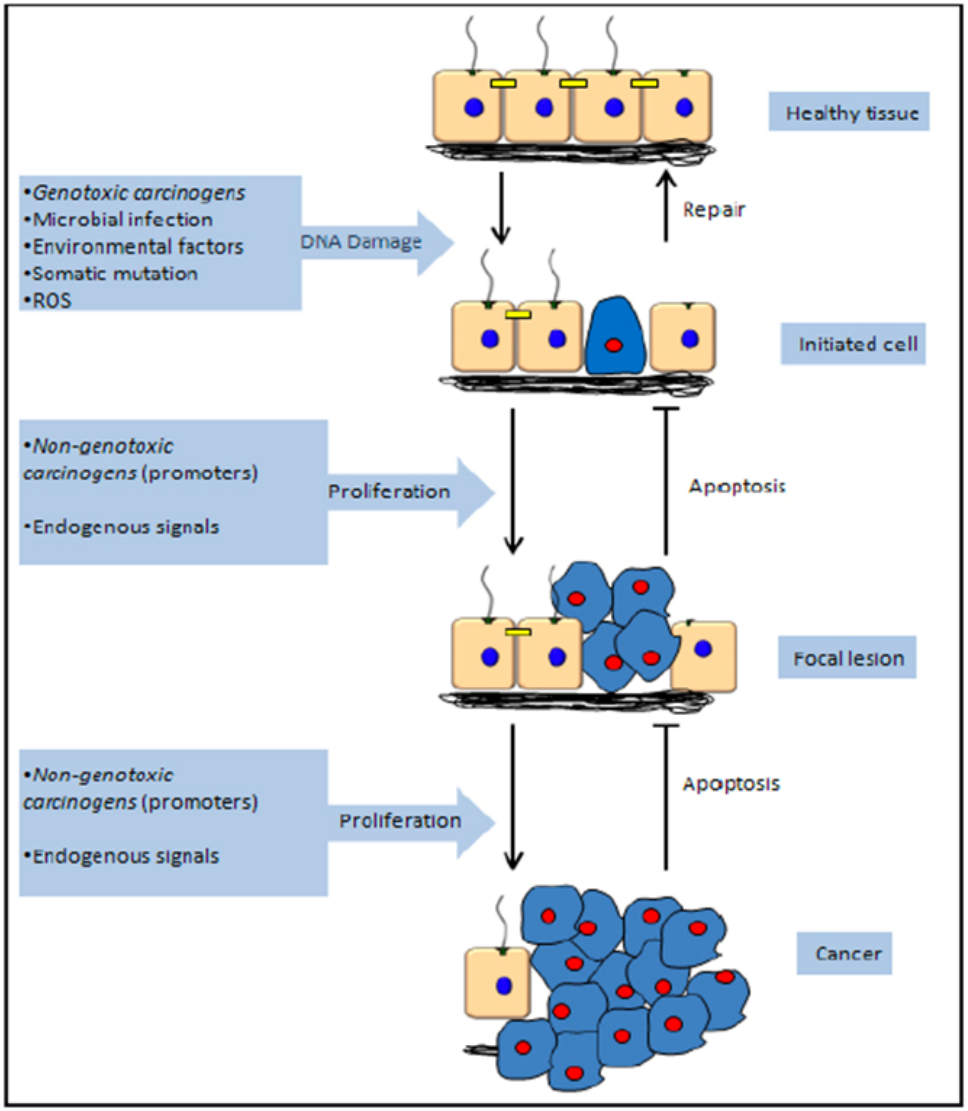
Proposed Solution: Explore use of automated home cage mon

- Social Housing under close-to-natural conditions
- No experimenter interference
- Full real-time access to recorded data
- Standardized data acquisition and -analysis



INTELLICAGE

Carcinogenesis as a latent hazard



Int. J. Mol. Sci. **2013**, *14*, 19416-19433;

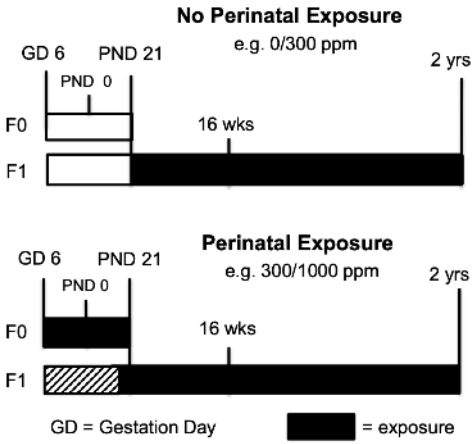
Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis

Table 1. Key characteristics of carcinogens.

Characteristic	Examples of relevant evidence
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone), formation of DNA and protein adducts
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

Abbreviations: AhR, aryl hydrocarbon receptor; ER, estrogen receptor; PPAR, peroxisome proliferator–activated receptor. Any of the 10 characteristics in this table could interact with any other (e.g., oxidative stress, DNA damage, and chronic inflammation), which when combined provides stronger evidence for a cancer mechanism than would oxidative stress alone.

Peri-/Prenatal exposure as a study design element in traditional carcinogenicity testing



No substantive difference in outcomes

Target Organs and Levels of Evidence for TR-598

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https://ntp.niehs.nih.gov/go/990433

TR-598 Toxicology and Carcinogenesis Studies of Perfluorooctanoic Acid (CASRN 335-67-1) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley SD) Rats

Chemical CASRN (Study Title)	Peer Review Date	Route/Exposure Levels	Study Laboratory
Perfluorooctanoic acid 335-67-1	12/12/2019	• Dosed-Feed MR: 0/0, 0/150, 0/300, 150/150, or 300/300; FR: 0/0, 0/300, 0/1000, 150/300, or 300/1000 ppm; 60/sex • Dosed-Feed MR: 0/0, 0/20, 0/40, 0/80, 300/0, 300/20, 300/40, or 300/80 ppm; 60/sex	Battelle Columbus Laboratory

Levels of Evidence

Male Rats: Clear Evidence

Type	Organ/Tissue (Lesion)	
	Postweaning	Perinatal + Postweaning
Neoplastic Lesions	• **Liver**: hepatocellular adenoma (0/50, 0/50, 7/50, 11/50); hepatocellular carcinoma (0/50, 0/50, 0/50, 0/50); hepatocellular adenoma or carcinoma (0/50, 0/50, 7/50, 11/50) • **Pancreas**: acinar cell adenoma (3/50, 28/50, 26/50, 32/50); acinar cell adenocarcinoma (0/50, 3/50, 1/50, 3/50), acinar cell adenoma or adenocarcinoma (3/50, 29/50, 26/50, 32/50)	• **Liver**: hepatocellular adenoma (0/50, 1/50, 5/50, 10/50); hepatocellular carcinoma (0/50, 0/50, 0/50, 4/50); hepatocellular adenoma or carcinoma (0/50, 1/50, 5/50, 12/50) • **Pancreas**: acinar cell adenoma (7/50, 18/50, 30/50, 30/50); acinar cell adenocarcinoma (0/50, 2/50, 1/50, 3/50), acinar cell adenoma or adenocarcinoma (7/50, 20/50, 30/50, 30/50)
Non-Neoplastic Lesions	• Liver: hepatocyte, cytoplasmic alteration; hepatocyte, hypertrophy; hepatocyte, single cell death; necrosis; pigment • Pancreas: acinus, hyperplasia	• Liver: hepatocyte, cytoplasmic alteration; hepatocyte, hypertrophy; hepatocyte, single cell death; necrosis; pigment • Pancreas: acinus, hyperplasia

Toxicologic Pathology
1-13

DOI: 10.1177/0192623318789398
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Exome Sequencing of Fresh-frozen or Formalin-fixed Paraffin-embedded B6C3F1/N Mouse Hepatocellular Carcinomas Arising Either Spontaneously or due to Chronic Chemical Exposure

Scott S. Auerbach[1], Miaofei Xu[1], B. Alex Merrick[1], Mark J. Hoenerhoff[1,2], Dhiral Phadke[3], Debra J. Taxman[3], Ruchir Shah[3], Hue-Hua L. Hong[1], Thai-Vu Ton[1], Ramesh C. Kovi[1,4], Robert C. Sills[1], and Arun R. Pandiri[1]

- Mechanism
- Translation
- Prediction

Table 2. Variants from Exome Sequencing of B6C3F1/N Mouse Hepatocellular Carcinomas Which Correspond to Known Mutations in Human Cancer-related Genes from Publicly Available Databases.

		Spontaneous						GBE								MEG								
		1		2		3		1		2		3		4		1		2		3		Database Match			
Gene	Mutation	FF	PE	FF	PE	FF	PE	FF	PE	FF	PE	FF	PE	FF	PE	FF	PE	FF	PE	FF	PE	COSMIC	IntOGen	NIEHS	SIFT Score
Acss3	G672V																	X	X			Yes			0.08
Bcl11a	A189T																			X	X	Yes			0.06
Braf	V637E																			X	X	Yes	Yes	Yes	0
Clta	N88S					X	X											X	X			Yes			0.28
Ctnnb1	D32N																	X				Yes	Yes	Yes	0
	D32Y																			X		Yes			0
	T41A													X								Yes			0
Dnahc5	E3279K															X	X					Yes			0.38
Elmo1	G125A																				X	Yes			0
Elk3	P88L																				X	Yes			0.06
Gnas	R926C																	X	X				Yes		0
Hras	Q61K	X	X	X	X																	Yes	Yes	Yes	0.1
	Q61R															X	X					Yes		Yes	0
Kif3c	E76K																				X	Yes			0.09
Lrp1b	R1646K																			X	X	Yes			0.85
Lyst	Y2952C																			X		Yes			0
Rbbp5	L399V																				X	Yes			0.08
Slc2a13	R601W																		X			Yes			0.01

Note: GBE = ginkgo biloba extract; MEG = methyleugenol; FF = fresh-frozen tissue; PE = formalin-fixed paraffin-embedded tissue; COSMI = Catalogue of Somatic Mutations in Cancer; IntOGen = Integrative Oncogenomics database.

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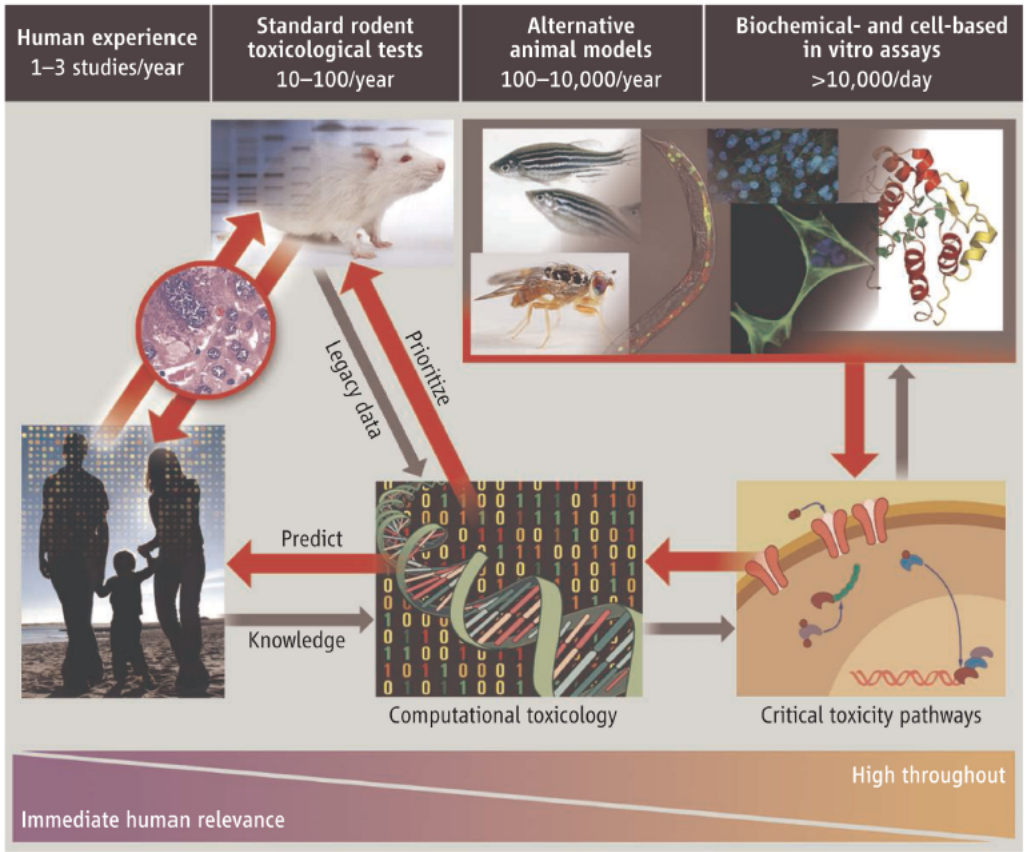
Transforming Environmental Health Protection

Francis S. Collins[1,*,†], **George M. Gray**[2,*], and **John R. Bucher**[3,*]

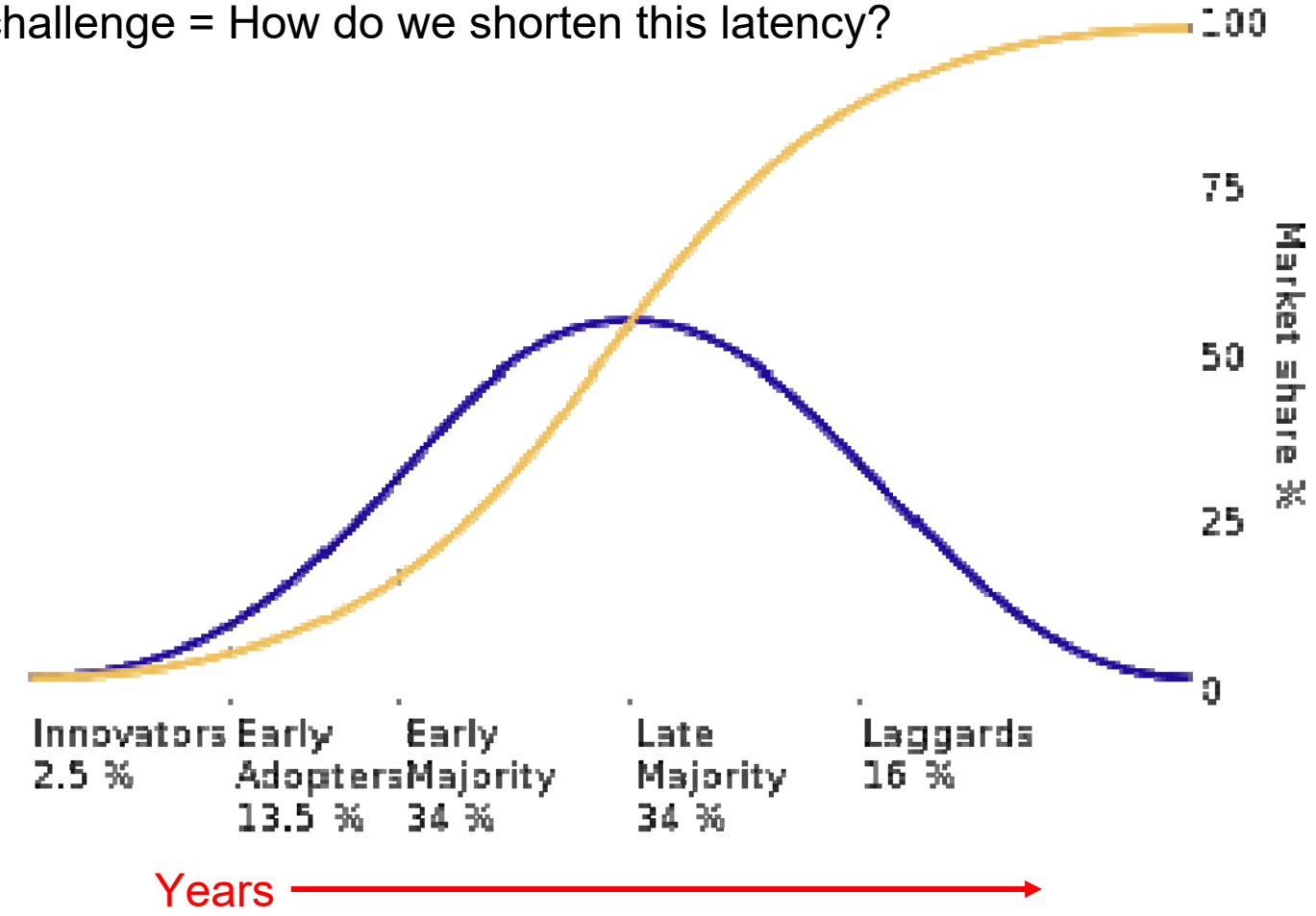
Drivers of innovation

- Large number of chemicals of potential concern
- Life stage susceptibilities
- Mechanistic understanding
- Cost and time
- Reduce animal use



Latency of Innovation

Strategic challenge = How do we shorten this latency?



https://en.wikipedia.org/wiki/Diffusion_of_innovations

Summary

- Latency is a significant concern in hazard assessment and influencing our approaches
- Study designs become more complex
- Complexity significantly increases when you consider latency in the context of human individuals and populations
- The future may be one where we are better at predicting latency than modeling it

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Thank You!