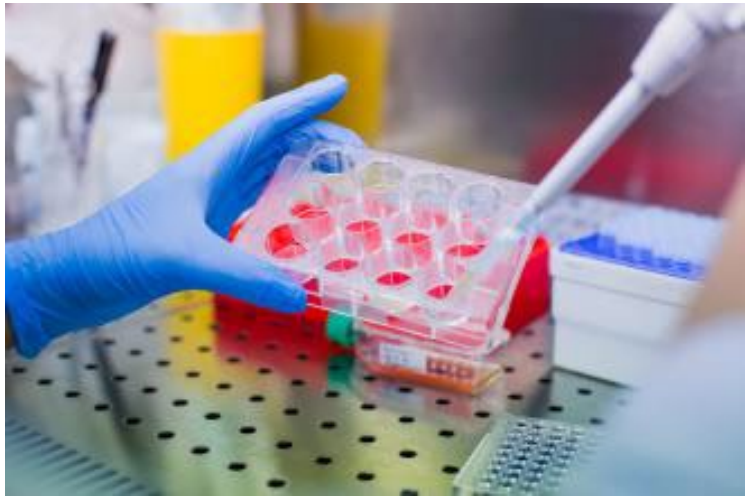
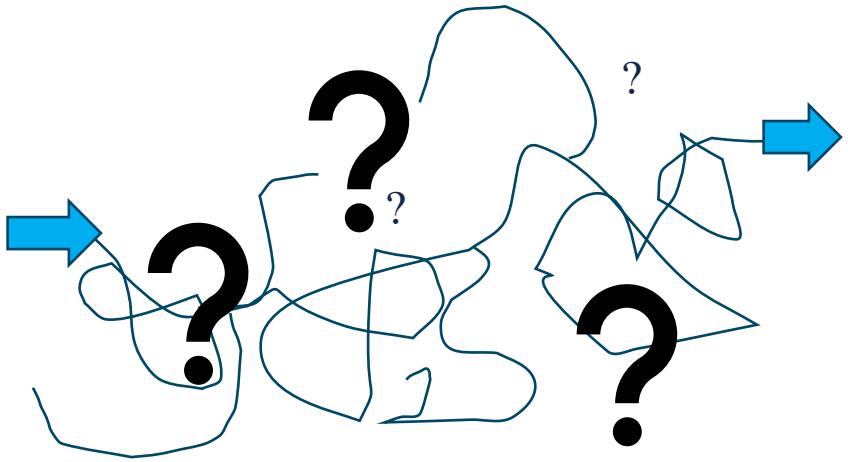
New Approach Methodologies (NAMs):
Scientific Challenges and Potential Solutions





Rashmi Joglekar, PhD, UCSF
and Jennifer Sass, PhD, NRDC

Feb 14, 2024

Children's Health Protection Advisory Committee (CHPAC) to EPA:

Some good advice (2021):

EPA's Promise:

- "(NAMs) have the potential to provide needed data and could be used to establish potential hazards or upgrade overall hazard identification. However, due to important limitations, **data from NAMs cannot be used to rule-out a specific hazard.**"
- "There are currently **no assays that can capture the most critical hazard endpoints for children's health** where complex biological systems are involved (such as reproductive and developmental toxicity; neurodevelopmental toxicity; placental development)."
- "Due to the limitations noted above, CHPAC recommends listed **NAMs be used for screening purposes and to indicate a hazard or upgrade concern for a hazard**, but conclusions about **the absence of hazard cannot be drawn solely based on NAMs** data."
- "Therefore, we advise that data from these alternative methods **should not be used to reduce default adjustment factors but could be used to add or increase such a factor**."

EPA Children's Health Protection Advisory Committee. Letter to EPA acting administrator on protecting children's health under amended TSCA: chemical prioritization. January 2021. Document ID EPA-HQ-OA-2022-0574-0011 .
https://www.regulations.gov/document/EPA-HQ-OA-2022-0574-0011

NAMs for endocrine toxicity – too many data gaps

- There are no NAMs for epigenetic and transgenerational effects, both of which are critical pathways for many adverse endocrine effects.
- There are no NAMs for the effects of the microbiome.
- There are no NAMs for the effects of inflammation and the immune system
- There are no NAMs for thyroid toxicity
- There are no NAMs for puberty effects

Table 2. EDSP Test Guidelines with Validated Alternatives

Each listing includes: assay/test name (test species); type of assay (in vitro or in vivo); and validated alternative status. All Tier 2 tests are *in vivo* assays. ER = estrogen receptor; AR = androgen receptor; STR = steroidogenesis; THY = thyroid.

EDSP Tier 1 Battery	Type	Tier 1 Battery Alternatives	Animals Used Per Assay [1]
OCSPP 890.1250 – Estrogen Receptor Binding (Rat Uterine Cytosol)	**In vitro**	**ER Pathway Model**	**13 (0)**
OCSPP 890.1300 – Estrogen Receptor Transcriptional Activation (Human Cell Line HeLa-9903)	**In vitro**	**ER Pathway Model**	**0**
OCSPP 890.1600 – Uterotrophic (Rat)	**In vivo**	**ER Pathway Model**	**18**
OCSPP 890.1150 – Androgen Receptor Binding (Rat Prostate Cytosol)	**In vitro**	**AR Pathway Model**	**10 (0)**
OCSPP 890.1200 – Aromatase (Human Recombinant)	In vitro	STR Model (Future)	0
OCSPP 890.1550 – Steroidogenesis (Human Cell Line – H295R)	In vitro	STR Model (Future)	0
OCSPP 890.1400 – Hershberger (Rat)	In vivo	AR/STR Model (Future)	48
OCSPP 890.1450 – Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Female Rats	In vivo	ER, STR, THY Models (Future)	45
OCSPP 890.1500 – Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats	In vivo	AR, STR, THY Models (Future)	45
OCSPP 890.1350 – Fish Short-Term Reproduction Assay	In vivo	ER, AR, STR Models (Future)	96
OCSPP 890.1100 – Amphibian Metamorphosis Assay (Frog)	In vivo	THY Model (Future)	320

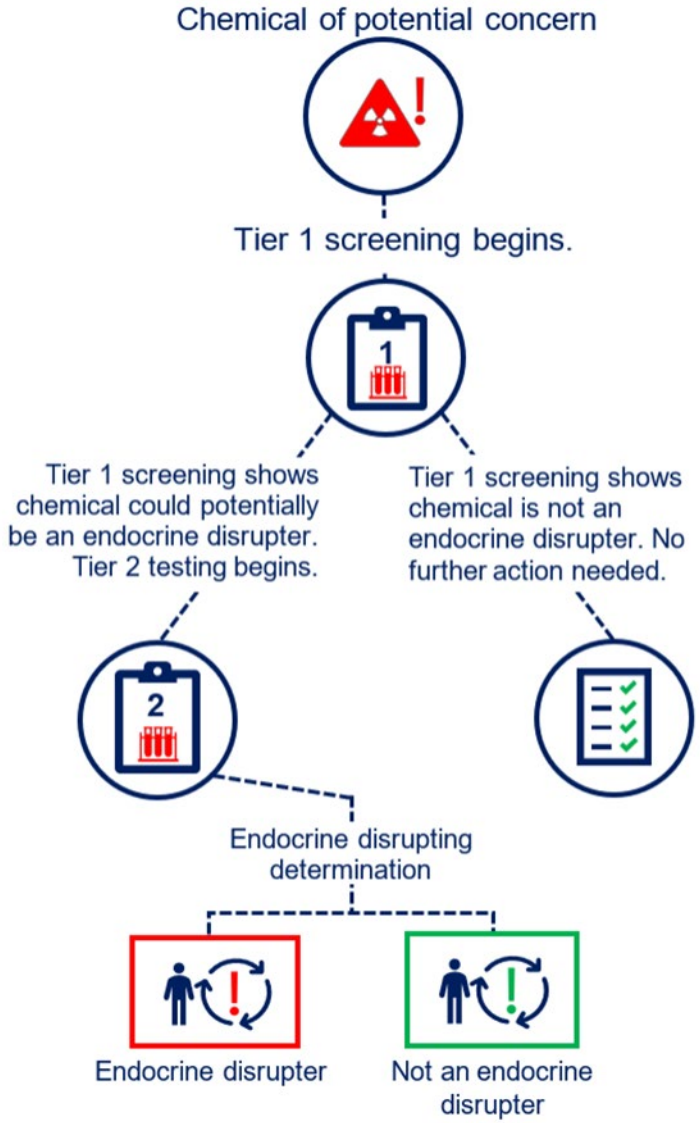
From EPA 2022 report on EDSP and NAMs here.

EPA's policy failure:

EPA's proposed framework for NAMs for endocrine effects will only use NAMs to classify chemicals as "not" endocrine disruptors.

Chemicals that trigger potential endocrine effects will go on to animal testing.

EPA should be using NAMs the other way around, to cut out animal testing by using NAMs to affirm toxicity endpoints like endocrine effects.



EPA OIG 2021. https://www.epa.gov/office-inspector-general/report-epas-endocrine-disruptor-screening-program-has-made-limited

NAMs for developmental neurotoxicity (DNT) – too many data gaps

- There are no NAMs for the effects of the microbiome.
- There are no NAMs for the effects of inflammation and the immune system
- There are no NAMs for thyroid toxicity
- There are no NAMs for impacts on brain microglia cells and neuroimmune molecules that sculpt neural circuitry and form a healthy brain or cause cell death.
- There are no NAMs for impacts on brain astroglial cells that provide nutrition to nerve cells and are critical to nerve cell growth and signaling.
- There are no NAMs for impacts on neuroendocrine hormones that are critical to development of brain regions that regulate growth and sex-specific behaviors.
- There are no NAMs for impacts on the gut microbiome that affects neurocognitive development, brain function and cancer risk.

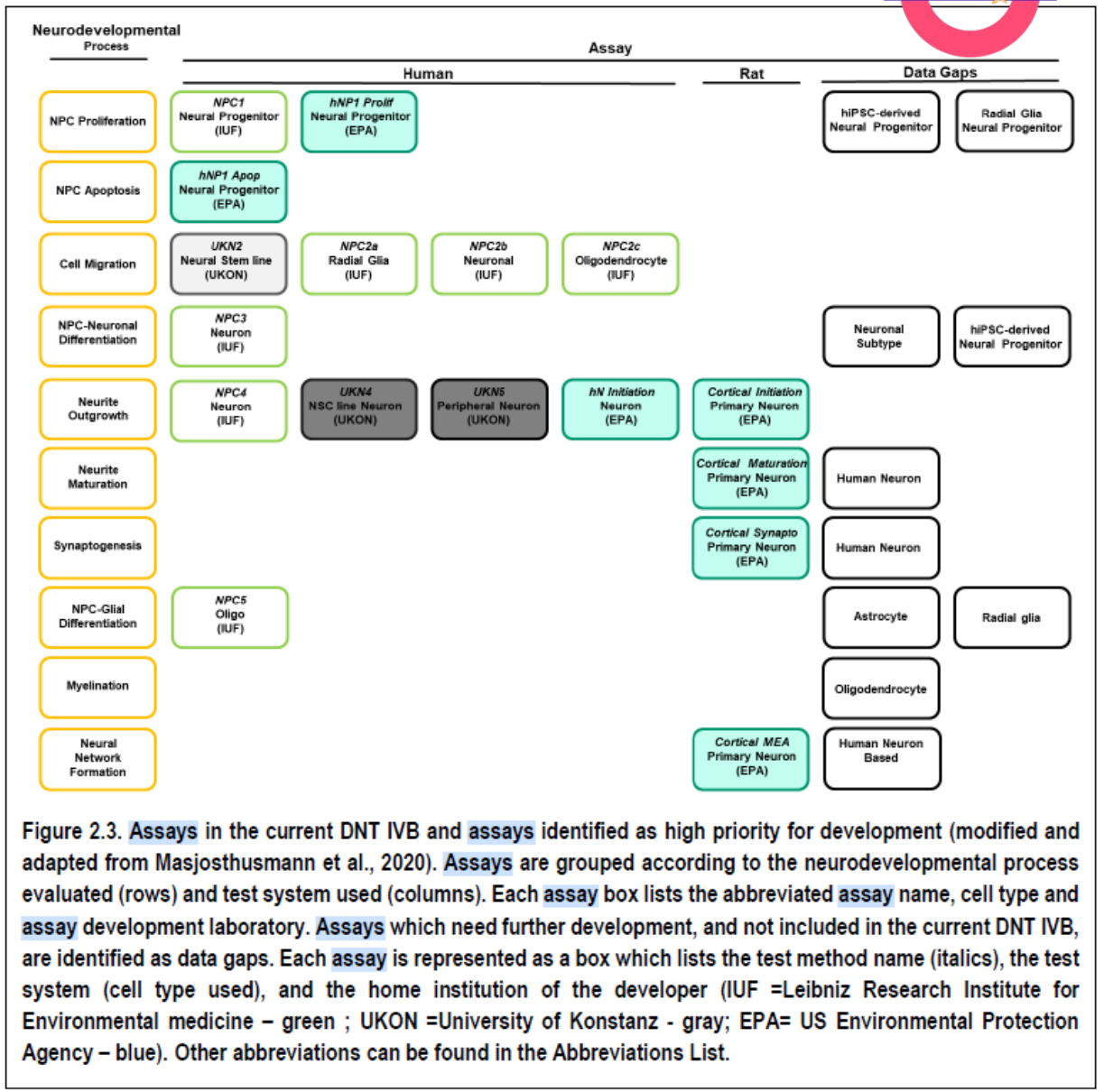
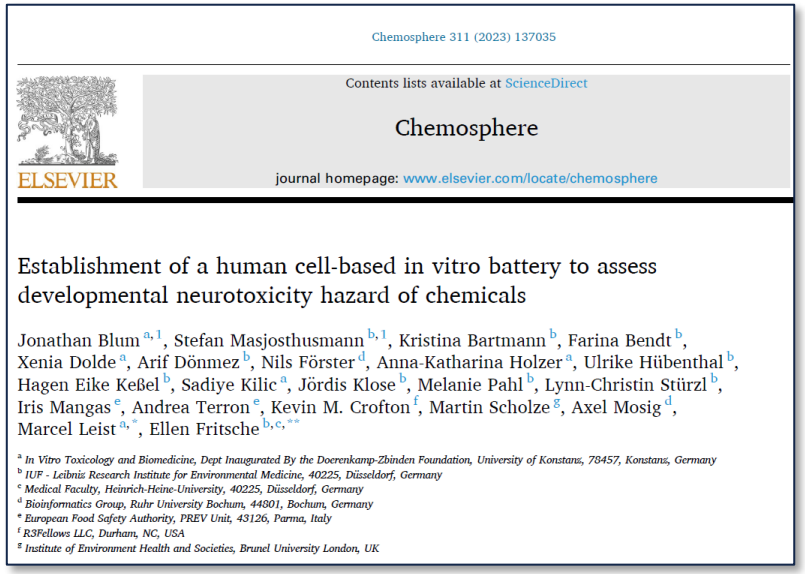


Figure 2.3. Assays in the current DNT IVB and assays identified as high priority for development (modified and adapted from Masjosthusmann et al., 2020). Assays are grouped according to the neurodevelopmental process evaluated (rows) and test system used (columns). Each assay box lists the abbreviated assay name, cell type and assay development laboratory. Assays which need further development, and not included in the current DNT IVB, are identified as data gaps. Each assay is represented as a box which lists the test method name (italics), the test system (cell type used), and the home institution of the developer (IUF =Leibniz Research Institute for Environmental medicine – green ; UKON =University of Konstanz - gray; EPA= US Environmental Protection Agency – blue). Other abbreviations can be found in the Abbreviations List.

Human-Derived DNT NAMs Battery Can't Detect Known Neurotoxicants



Chemosphere 311 (2023) 137035

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

Establishment of a human cell-based in vitro battery to assess developmental neurotoxicity hazard of chemicals

Jonathan Blum [a, 1], Stefan Masjosthusmann [b, 1], Kristina Bartmann [b], Farina Bendt [b], Xenia Dolde [a], Arif Dönmez [b], Nils Förster [d], Anna-Katharina Holzer [a], Ulrike Hübenthal [b], Hagen Eike Keßel [b], Sadiye Kilic [a], Jördis Klose [b], Melanie Pahl [b], Lynn-Christin Stürzl [b], Iris Mangas [e], Andrea Terron [e], Kevin M. Crofton [f], Martin Scholze [g], Axel Mosig [d], Marcel Leist [a, *], Ellen Fritsche [b, c, **]

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[b] IUF - Leibniz Research Institute for Environmental Medicine, 40225, Düsseldorf, Germany
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[d] Bioinformatics Group, Ruhr University Bochum, 44801, Bochum, Germany
[e] European Food Safety Authority, PREV Unit, 43126, Parma, Italy
[f] R3Fellows LLC, Durham, NC, USA
[g] Institute of Environment Health and Societies, Brunel University London, UK

- Blum et al 2023 (Fig 4) reported that:
- One-third (32%) of known DNT chemicals are missed or borderline by human-derived NAMs (9/28)
- One-seventh (14%) are missed by all DNT tests (4/28)

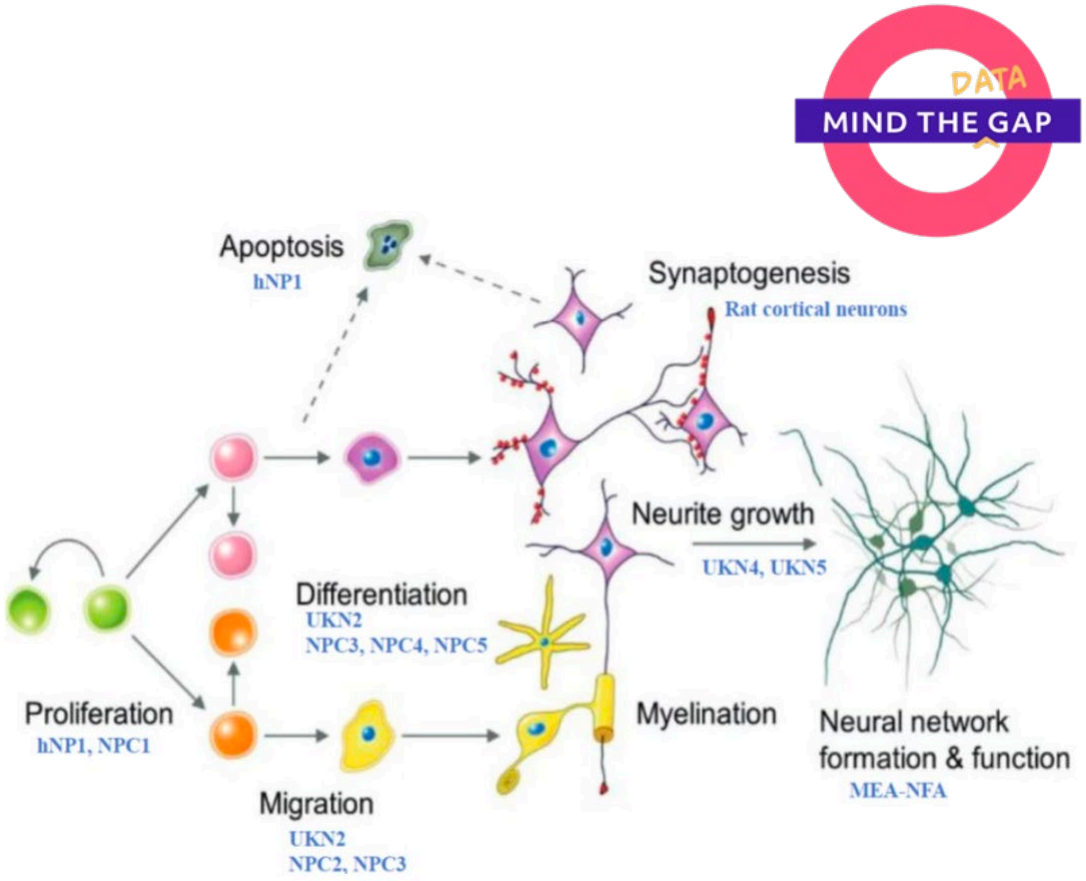
Positive controls	specific + brdl. + cytotox	specific + brdl.	specific
Cadmium chloride	TP	TP	TP
Chlorpyrifos	TP	TP	FN
Dexamethasone	TP	TP	TP
Hexachlorophene	TP	TP	TP
Lead (II) acetate trihydrate	TP	TP	TP
Manganese (II) chloride	TP	TP	TP
Methylmercury chloride	TP	TP	TP
PBDE 47	TP	TP	TP
PBDE 99	TP	TP	FN
(±) Ketamine hydrochloride	FN	FN	FN
5,5-Diphenylhydantoin	FN	FN	FN
Acrylamide	TP	TP	TP
all-trans-Retinoic acid	TP	TP	TP
Chlorpromazine hydrochloride	TP	TP	TP
Deltamethrin	TP	TP	TP
Domoic acid	FN	FN	FN
Haloperidol	TP	TP	TP
Maneb	TP	TP	FN
Methylazoxymethanol acetate	TP	TP	TP
Nicotine	FN	FN	FN
Paraquat dichloride hydrate	TP	TP	TP
PFOA	TP	FN	FN
PFOSK	TP	TP	TP
Sodium valproate	TP	TP	TP
Tebuconazole	TP	TP	TP
Tributyltin chloride	TP	TP	TP
Trichlorfon	TP	TP	TP
Triethyl-tin bromide	TP	TP	FN

FN= False Negative

TP = True Positive

EPA's Application of NAMs DNT Battery for OP Pesticides

- **2020**–Science Advisory Panel criticized battery's use in evaluating DNT potential of organophosphate pesticides (OPs) based on lack of biological coverage:
    - Missing key pathways in neurodevelopment
    - Lack of cellular heterogeneity
    - Missing maternal environment components, e.g. endocrine pathways, stress
    - Cannot predict behavioral outcomes
- **2020-present**—EPA moved forward with testing battery without providing formal response to SAP comments



EPA's Broken Promise—2023 Acephate Draft Risk Assessment

- **EPA** has relied on NAMs data to reverse long-standing science and policy positions, weaken regulatory protections, and put farmworkers and families at increased risk.

- **August 23rd, 2023—**EPA released updated Acephate DRA with updated risk values, waived default FQPA 10X child-protective safety factor
  - EPA concluded that acephate did not pose DNT risk based on flawed and underpowered NAMs
  - EPA also undermined epidemiologic and animal evidence
  - This determination was made for the *entire class of Ops.*

"Acephate …Great For Fire Ants. Not For Sale To California"

EPA's Broken Promise—2023 Acephate Draft Risk Assessment

1. NAMs tests showed no consistent DNT pattern so **the potential for OPs to cause DNT must be assessed on a chemical-specific basis** (no longer assessed as a Cumulative Assessment Group).

2. For the OP pesticide acephate EPA decided **positive NAMs were not 'true positives' (an undefined term) and thus treated acephate as negative for developmental neurotoxicity**, while acknowledging that it is positive for neurotoxicity.

3. **By law, EPA can eliminate or reduce the FQPA 10X "only if, on the basis of reliable data, such margin will be safe for infants and children."**

4. EPA's use of NAMs does NOT provide sufficiently reliable data to eliminate the FQPA 10X and still ensure there will be reasonable certainty of no harm to children.

"Acephate …Great For Fire Ants. Not For Sale To California"

EPA's Broken Promise—2023 Acephate Draft Risk Assessment

Results

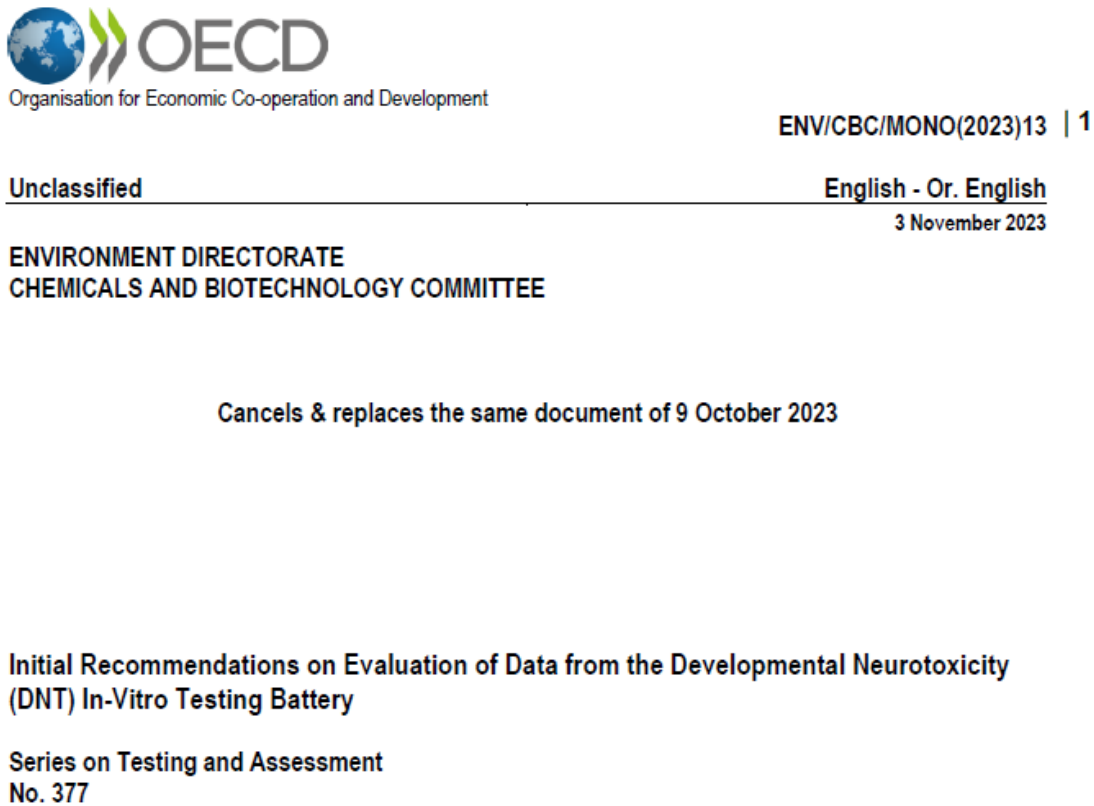
- Protections reduced by factor of 10x (many but not all risks still remain)
- Setting foundation to undermine OP DNT class determination
- Setting foundation to discount epidemiologic evidence
- Setting foundation to make regulatory decisions based on NAMs without consistent and scientifically vetted framework

"Acephate …Great For Fire Ants. Not For Sale To California"

Some more good advice (2023):

"At this time negative results from the DNT [NAMs] should not be interpreted as a lack of DNT potential. This is due to the uncertainties associated with the in vitro methods used, as well as the lack of coverage for some critical neurodevelopmental processes."

– OECD Nov 2023, p. 7



OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2023)13 | 1

Unclassified

English - Or. English

3 November 2023

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

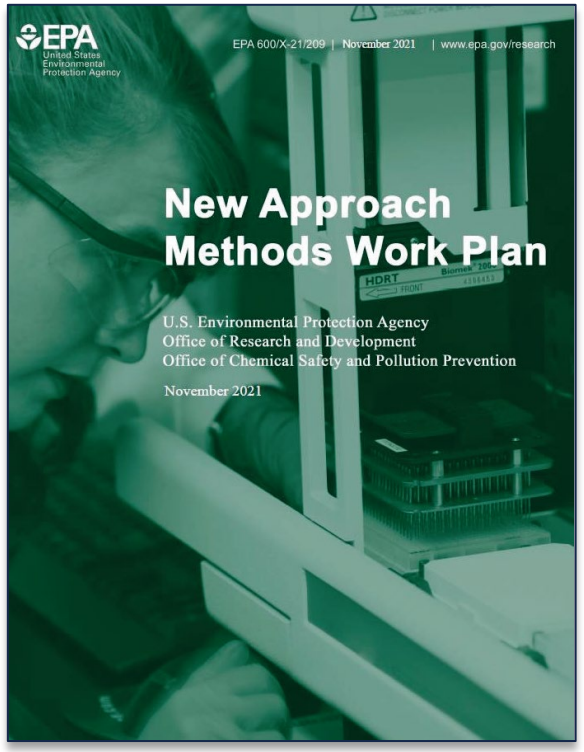
Cancels & replaces the same document of 9 October 2023

Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery

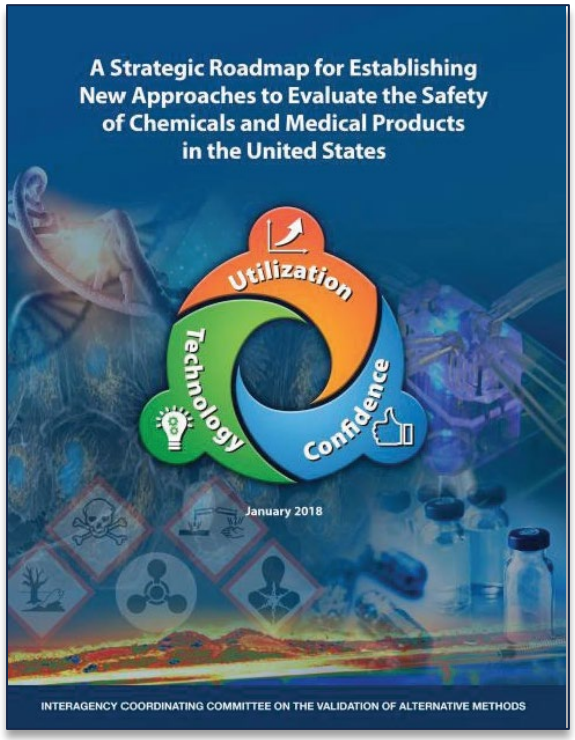
Series on Testing and Assessment
No. 377

Barriers to Using NAMs in Regulatory Decision Making

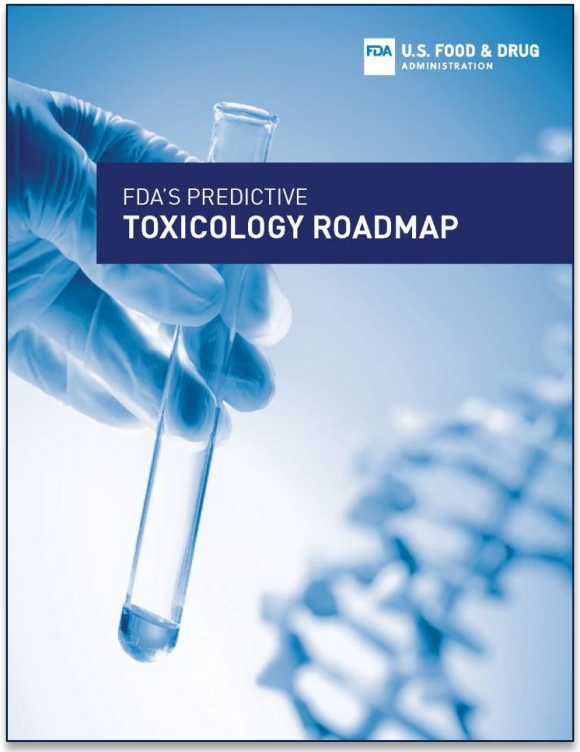
The Current Landscape:



EPA



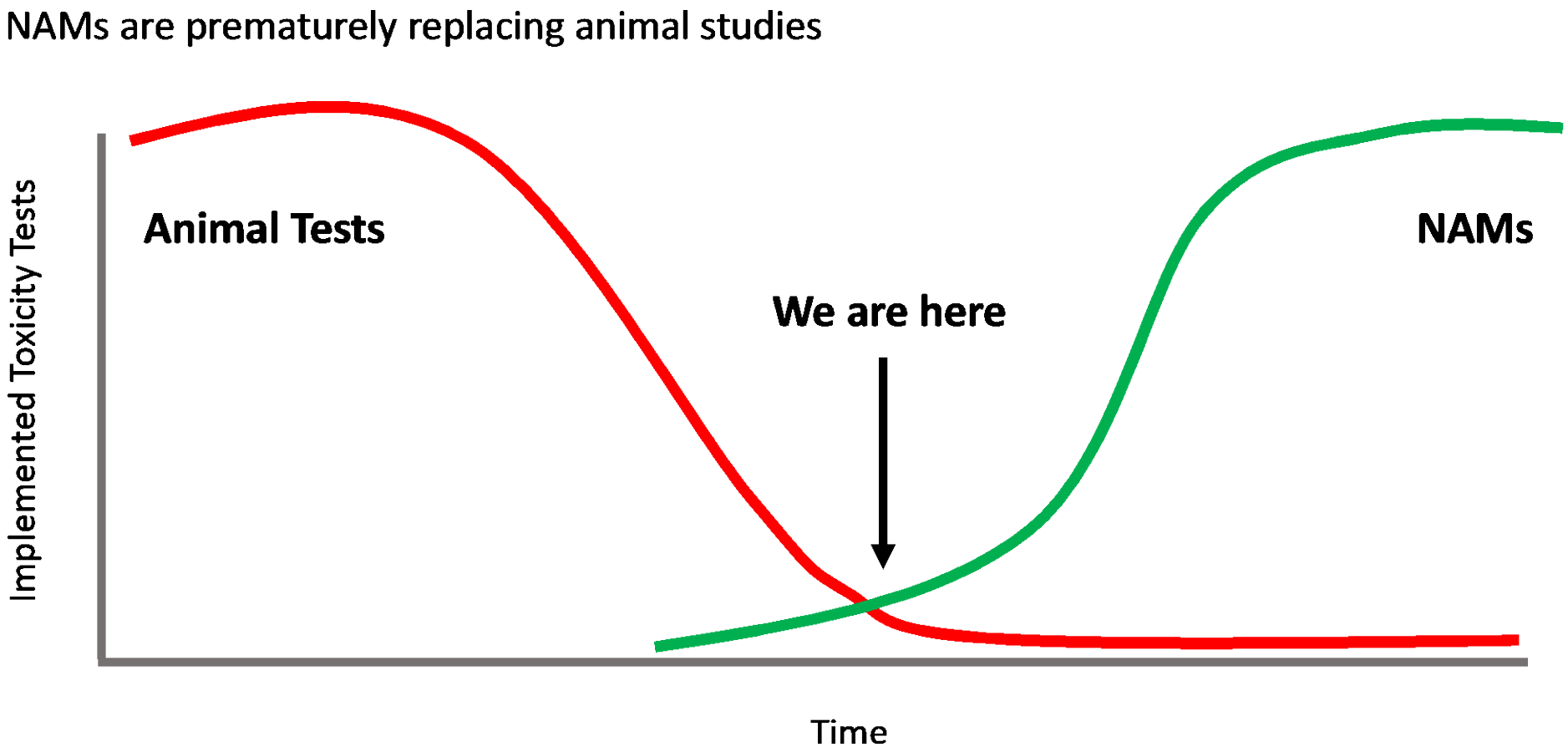
NIEHS



FDA

Barriers to Using NAMs in Regulatory Decision Making

The Current Landscape:



Barriers to Using NAMs in Regulatory Decision Making

The Law:

The 2016 amendments to the Toxic Substances Control Act (TSCA) direct EPA to encourage the "use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals" but require such assays to "provide information of equivalent or better scientific quality and relevance that will support regulatory decisions under this title."

Science problems:

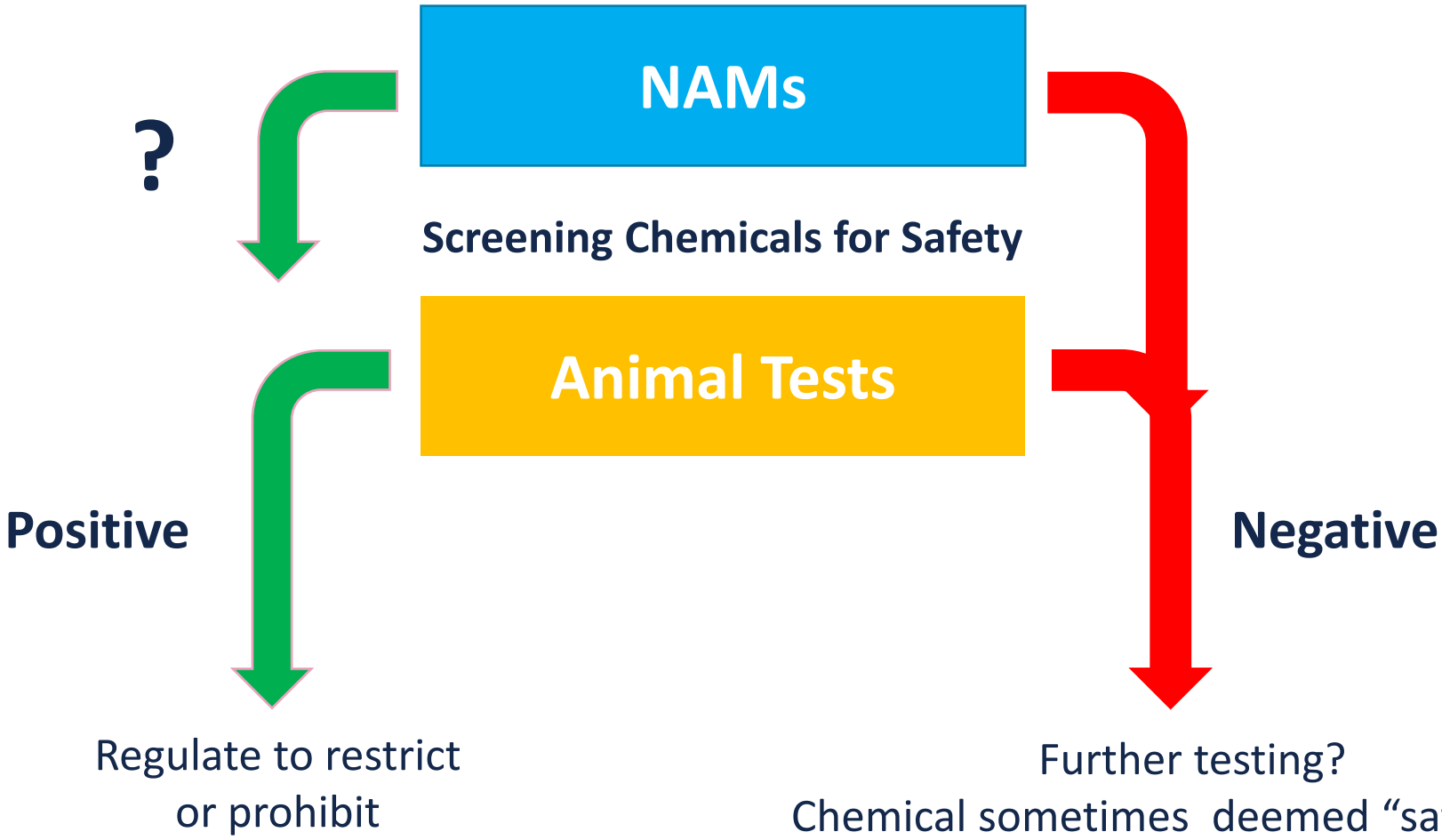
EPA's NAMs Work Plan (Dec 2021): "While considerable progress is being made in developing NAMs, there are still scientific challenges and information gaps that limit a complete reliance on NAMs for Agency decisions related to the assessment of a chemical's potential risk to human health and the environment."

EPA's Promise:

The Conclusion: Put simply, NAMs cannot reliably assess the risks of key health effects, such as endocrine toxicity, cancer and birth defects including developmental neurotoxicity, for which established validated rodent tests continue to provide reliable and actionable data to support risk assessment and risk management.

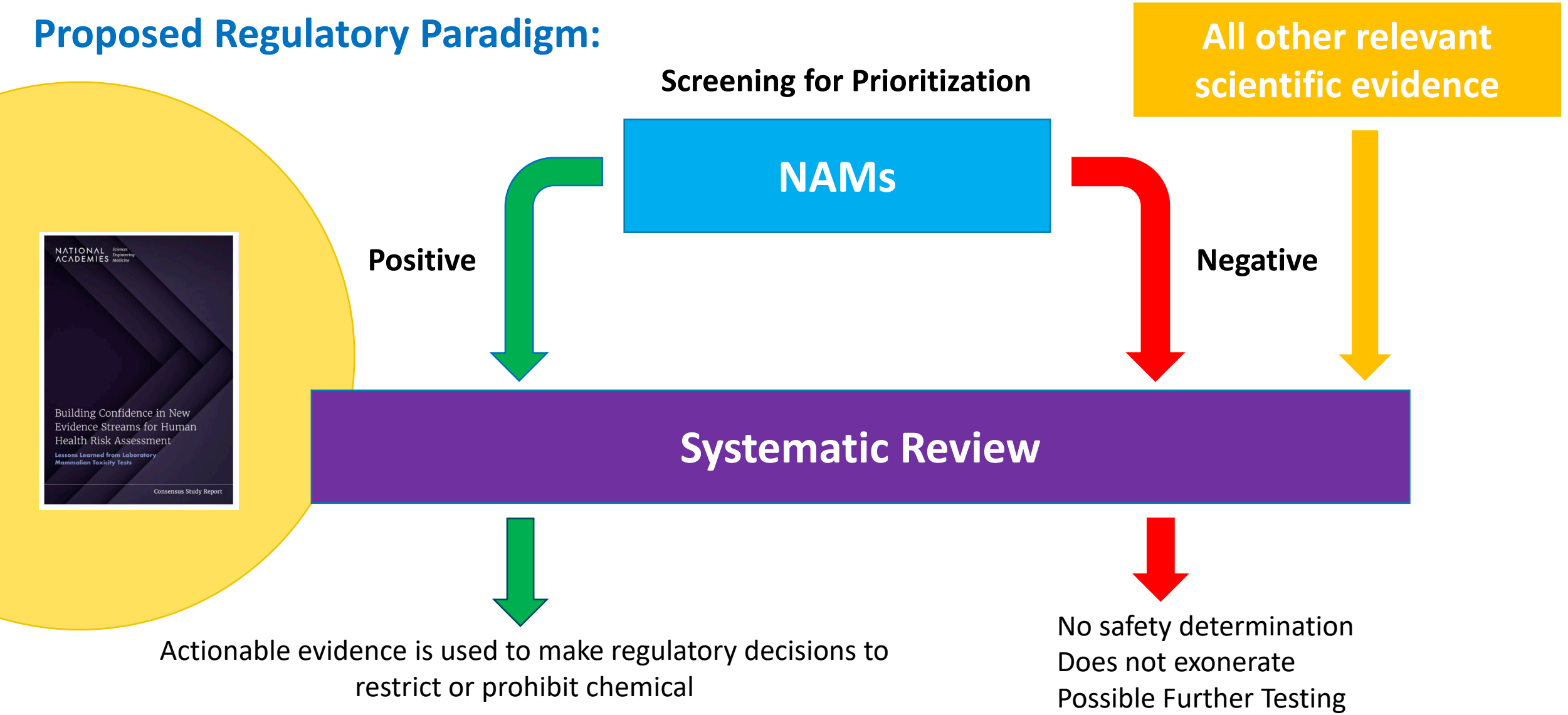
Barriers to Using NAMs in Regulatory Decision Making

Current Regulatory Paradigm:



A Path Forward: Developing Actionable Evidence and a Consistent Regulatory Framework for NAMs

Proposed Regulatory Paradigm:



A Path Forward: Developing Actionable Evidence and a Consistent Regulatory Framework for NAMs

- If there is sufficient existing data, do not delay regulation.

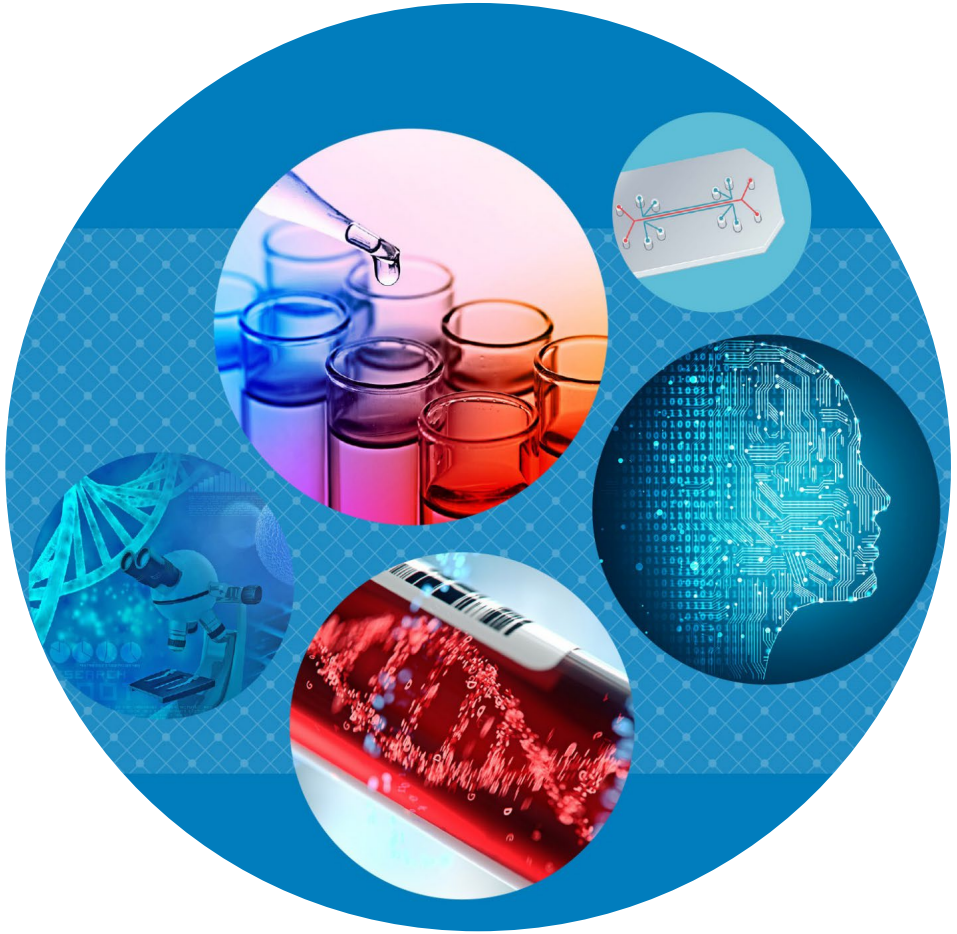


Image credit: US FDA

A Path Forward: Developing Actionable Evidence and a Consistent Regulatory Framework for NAMs

Chemical agents classified as *carcinogenic to humans* after 1990	Year classified as *carcinogenic to humans*	Year with *sufficient evidence* in animals	Time lag
PCBs	2016	1987	29
Benzo[a]pyrene	2010	1983	27
Diesel engine exhaust	2014	1989	25
Phenacetin	2012	1987	25
MOCA	2010	1987	23
ortho-Toluidine	2010	1987	23
1,3-Butadiene	2008	1986	22
NNN and NNK	2007	1985	22
Pentachlorophenol	2019	1999	20
Trichloroethylene	2014	1995	19
Formaldehyde	2006	1987	19

Chemical agents classified as *carcinogenic to humans* after 1990	Year classified as *carcinogenic to humans*	Year with *sufficient evidence* in animals	Time lag
Aristolochic acid	2012	2002	10
2,3,7,8-TCDD	1997	1987	10
Crystalline silica dust	1997	1987	10
Ethylene oxide	1994	1987	7
Beryllium/compounds	1993	1987	6
Cadmium/compounds	1993	1987	6
Lindane	2018	2018	0
1,2-Dichloropropane	2017	2017	0
PCB–126	2012	2012	0
2,3,4,7,8-PCDF	2012	2012	0
Etoposide	2012	–	–

Credit: Vince Cogliano, OEHHA, 2021

A Path Forward: Developing Actionable Evidence and a Consistent Regulatory Framework for NAMs

- If there is sufficient existing data, do not delay regulation.
- NAMs should generate ***actionable evidence*** that agencies can use to make regulatory decisions
  - A consistent regulatory framework should determine how to use positive and negative data from NAMs to inform risk evaluation, hazard-based decisions
- **NAMs should be used to enhance our understanding of:**
  - Mixture toxicity, cumulative risk
  - Human variability and susceptibility (e.g. nonchemical stressors)

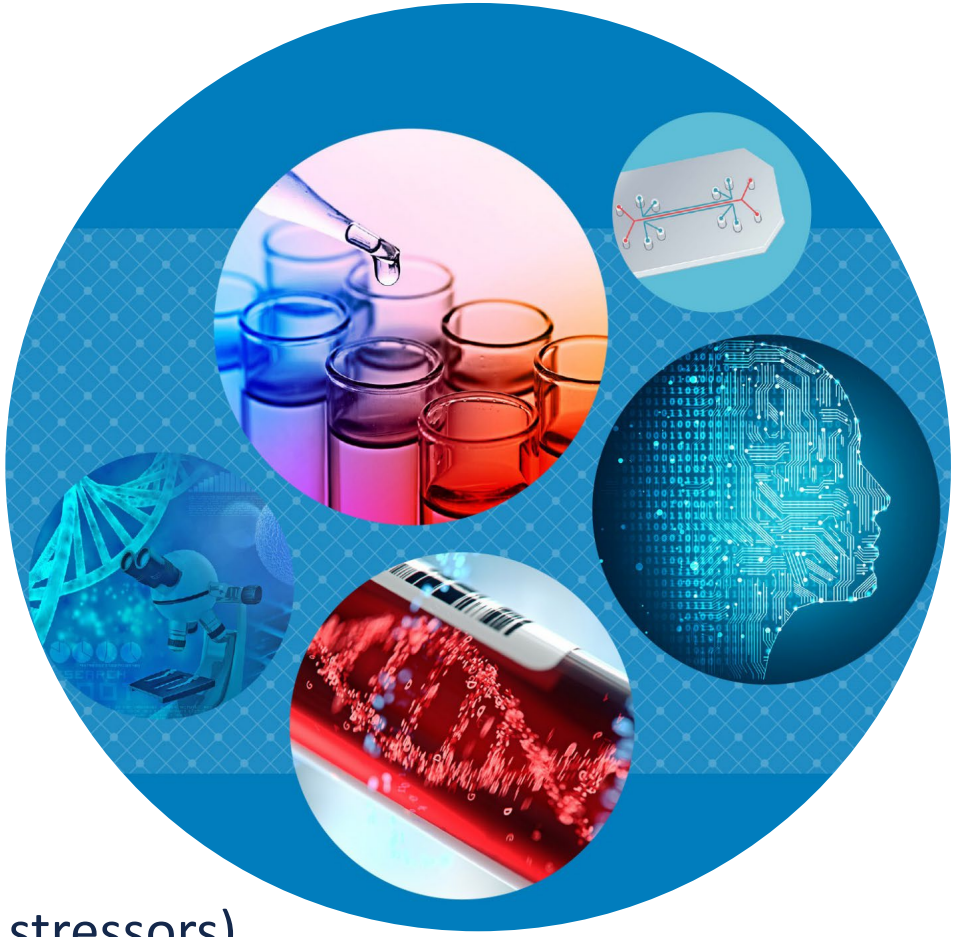


Image credit: US FDA

Next Steps: Public engagement needed. NAMs is more than a science issue

The Farmworker Association of Florida, Inc.

FARMWORKER JUSTICE

coming clean

ENVIRONMENTAL JUSTICE FOR ALL
Where we live, learn, work, play, and pray.

"Environmental justice communities and farmworkers already suffer disproportionate harms from the manufacturing, use, and disposal of chemicals that were inadequately reviewed or approved despite their known risks. EPA must not allow the development or use of NAMs to perpetuate or worsen these unequal and harmful impacts."

We requested that EPA to commit to an open process that includes fenceline communities, farmworkers, unions, environmental groups, consumer groups, and other impacted stakeholders in the development of policies surrounding the regulatory use of NAMs.

Letter to EPA Administrator Michael Regan from 38 environmental, health, and justice organizations. March 15, 2023. https://www.nrdc.org/sites/default/files/2023-03/epa-letter-tsca-nams-20230315.pdf

1. How will the EPA's use of NAMs avoid misclassifying PFAS and other toxic chemicals as safe, given the expansive data gaps and lack of consistent regulatory framework?

2. What is EPA's plan for aligning with the recommendations of the 2023 National Academies report on, 'Building Confidence in New Streams for Human Health Risk Assessment'?

3. When will EPA offer for public comment a clear set of proposed policies on how NAMs will be used for various purposes, tethered to its statutory standards that apply and that dictate the level of protection and how to address uncertainties?