In Vitro Lecture and Luncheon for Students

Making New Approach Methods Work for Regulation: Linking Validation and Biological Relevance Through Adverse Outcome Pathways

Helena Hogberg,
PhD
NIEHS/NICEATM

SOT MARCH 22-25 SAN DIEGO

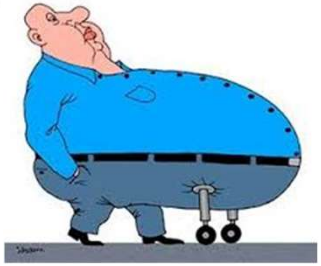
My Career Path

BSc, MSc

Stockholm University

biovitrum.

≠



PhD

European Commission

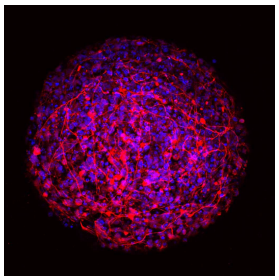
Developed methods using cells as alternatives to animals

CAAT

Johns Hopkins Bloomberg School of Public Health

Developed more complex cell models

Post Doc → Faculty → Deputy Director

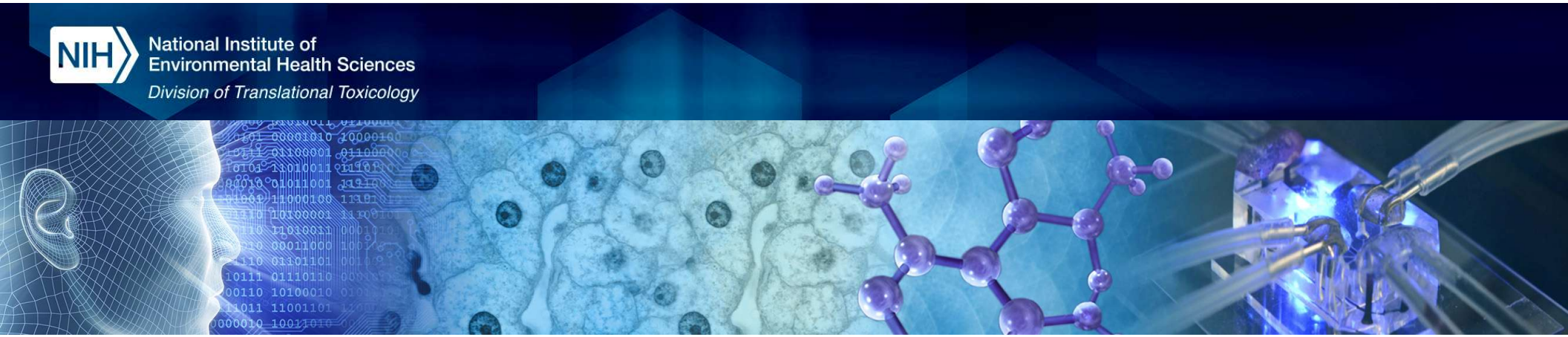


The National Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

NIH NIEHS

Acting Deputy Director → Acting Director

Make use of these methods to keep humans and the environment safe



Making New Approach Methods (NAMs) Work for Regulation: Linking Validation and Biological Relevance Through Adverse Outcome Pathways (AOPs)

Helena Hogberg

Acting Director, NTP Interagency Center for the Evaluation of Alternative Toxicological Methods

National Institutes of Health • U.S. Department of Health and Human Services

Learning Objectives

After this lecture you will be able to answer:

- What is a New Approach Method (NAM)?
- What is an Adverse Outcome Pathway (AOP)?
- How can New Approach Methods (NAMs) be used in Adverse Outcome Pathways (AOPs)?

The author declares no conflict of interest.
The views expressed in this presentation are my own and do not necessarily represent the views or policies of the NIH or the U.S. Department of Health and Human Services.

Why Do We Need New Approach Methods (NAMs)?

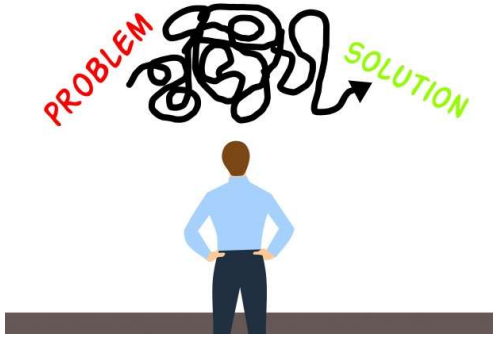
Chemicals on the market need to be safe to humans and the environment

Traditionally this was done using animal tests

We have too many chemicals and too little toxicity data

Challenges with using animal tests

- Costly and time consuming
- Provide limited mechanisms on <u>how</u> a chemical might be harmful
- Outcomes in animals may differ from outcomes in humans



New Approach Methods (NAMs) can overcome several challenges of animal tests as they often are:

faster, cheaper, provide mechanistic information on how a chemical might be harmful to humans

What are New Approach Methods (NAMs)?

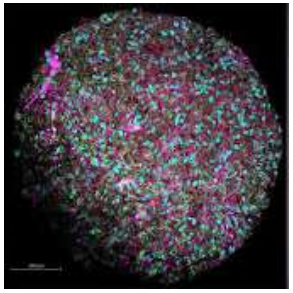
Any tool or method that helps understand the hazard and risk of chemicals while replacing, reducing or refining traditional animal tests (3Rs)

Computational Approaches/AI

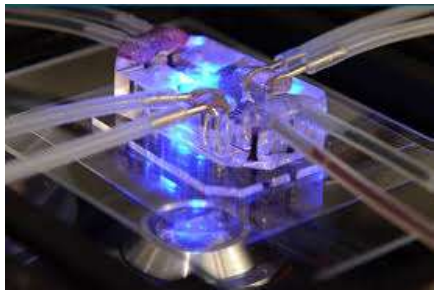
High Throughput Cell Assays

Small Model Organisms

3D Cell Models



Organs on Chips



Shorter or Less Invasive Animal Studies

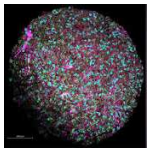
What Can We Measure with New Approach Methods (NAMs)?

Computational Approaches/AI

Computer models can use known information to predict unknown outcomes, such as whether a **chemical** will **bind to a receptor or DNA**

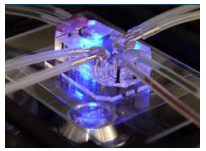
High Throughput Cell Assays

Simpler cell models and basic measurements, such as **number of cells**, how many are **alive or dead**, and the cell's **shape or size**



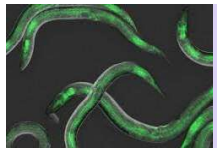
3D Cell Models

Advanced cell models often with multiple cell types that can capture **cell-cell communication** and **function** more accurately



Organs on Chips

Advanced cell models that use small **chips** to record cell functions in **real time**. Models of different organs are sometimes connected to reflect **organ-organ interactions**



Small Model Organisms

Whole animals that can be used to measure adverse outcomes such as **disease** or **behavior changes**

New Approach Methods (NAMs) for Risk Assessment

NAMs are developed in the lab to answer scientific questions →

To use NAMs in risk assessment requires a much higher standard

Validation

1) Reproducibility:
Do we obtain the same result each time the test is performed?

The experimental protocol should include clear, standardized instructions to ensure others can perform the test and obtain consistent results

2) Biological Relevance:
Is the NAM relevant to the human toxicity outcome?

How can we show human biological relevance?

Through the Adverse Outcome Pathway (AOP)

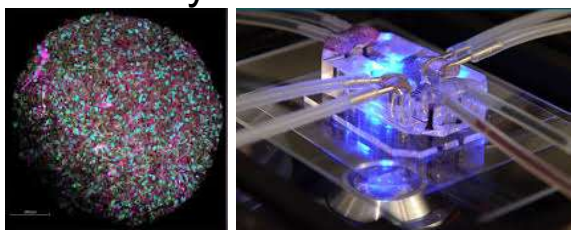
An AOP explains step by step how a chemical can cause harm, from first interaction in a cell to the final health effect in an organism

Molecular Initiating Event | **Key Events** | **Adverse Outcome**

To understand the full AOP, we need information from different kind of methods

Computational Approaches/AI

Cell Assays



Animal Tests/Human Data

Simple → Complex

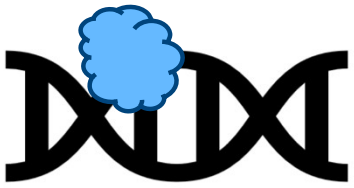
Examples of Adverse Outcome Pathways (AOPs)

Molecular Initiating Event **Key Events** **Adverse Outcome**

Molecular Cellular Tissue Organ Organism

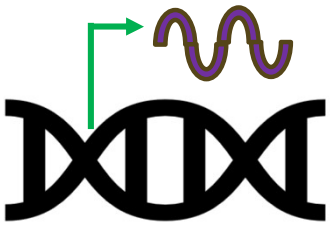
Direct interaction of the chemical

Binding to DNA



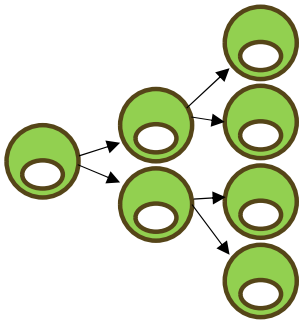
Altered cellular function

Expression of oncogenes



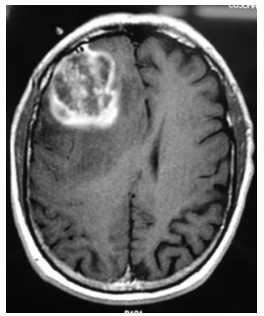
Altered physiology and/or function

Uncontrolled cell proliferation



Adverse Outcome

Cancer



Examples of Adverse Outcome Pathways (AOPs)

Molecular Initiating Event **Key Events** **Adverse Outcome**

MIE → KE_1 → KE_2 → KE_3 → AO

Molecular | Cellular | Tissue | Organ | Organism

Direct interaction of the chemical

Altered cellular function

Altered physiology and/or function

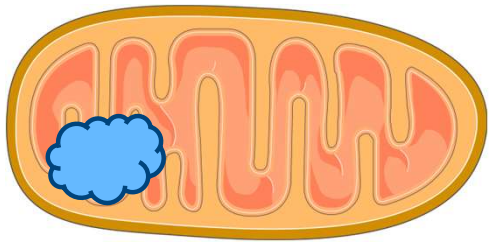
Adverse Outcome

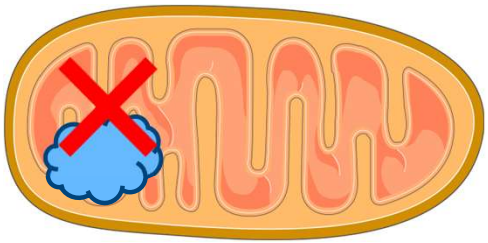
Binding to enzyme in the mitochondria

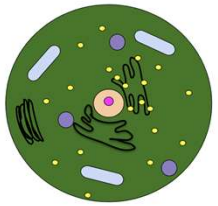
Mitochondrial dysfunction

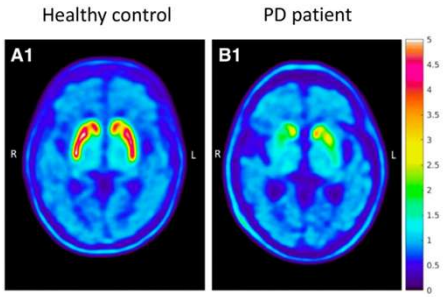
Apoptosis/ Cell death

Parkinson's Disease









Examples of Adverse Outcome Pathways (AOPs)

Molecular Initiating Event **Key Events** **Adverse Outcome**

MIE → KE_1 → KE_2 → KE_3 → AO

Molecular | Cellular | Tissue | Organ | Organism

Direct interaction of the chemical

Altered cellular function

Altered physiology and/or function

Adverse Outcome

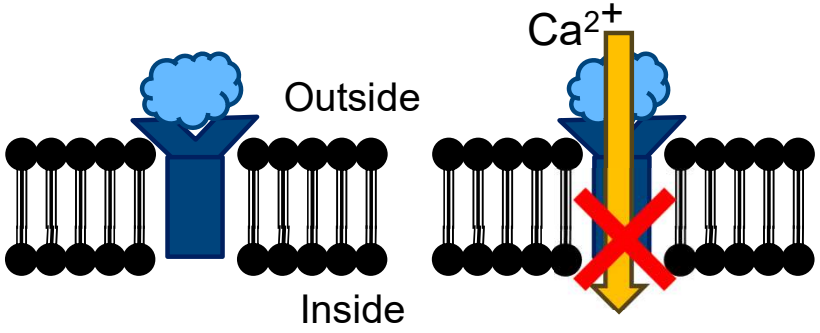
Binding to NMDA receptor

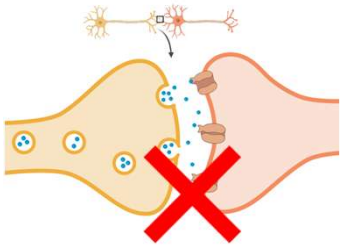
Reduced calcium influx

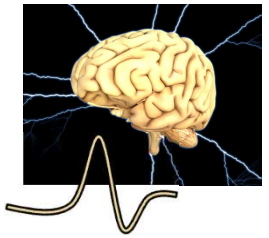
Decreased synapse formation

Decreased brain function

Impaired learning and memory



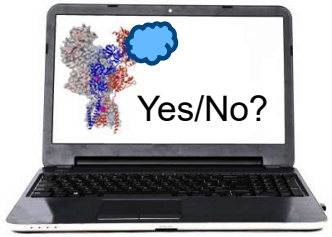
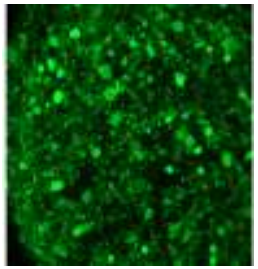
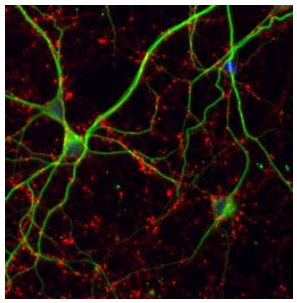
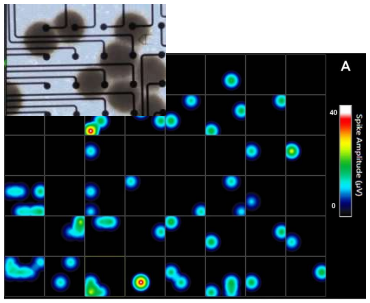
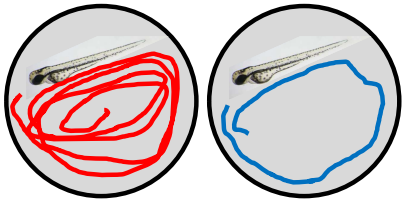




How Can We Measure Key Events with NAMs?

Molecular Initiating Event | **Key Events** | **Adverse Outcome**

MIE → KE_1 → KE_2 → KE_3 → AO

MIE	KE_1	KE_2	KE_3	AO
Binding to NMDA receptor	Reduced calcium influx	Decreased synapse formation	Decreased brain function	Impaired learning and memory
				
Computer model calculates if a chemical will bind to the NMDA receptor	High-throughput cell assay measures fluorescent dye	High-throughput cell assay counting synapses (red) and length of the neuron (green)	3D cell model on chip recording electrical activity of neurons (function)	Recording swimming behavior (distance and speed) of Zebrafish embryos

Summary

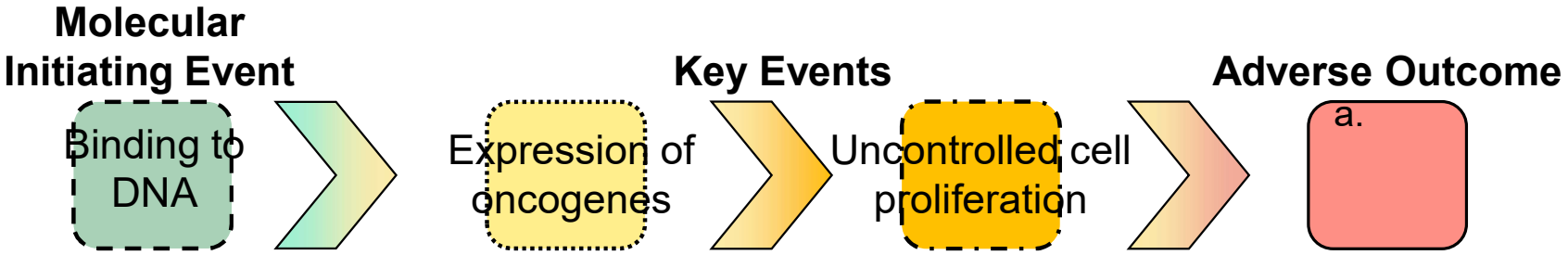
- New Approach Methods (NAMs) are often faster and cheaper than traditional animal tests
- New Approach Methods (NAMs) measure mechanistic effects
- Combining information from different methods through an Adverse Outcome Pathway (AOP) can help us understand how a chemical may lead to adverse effects in humans

Exercise 1

Match one of the six items on the right to each gap (a-f) in the three AOPs.

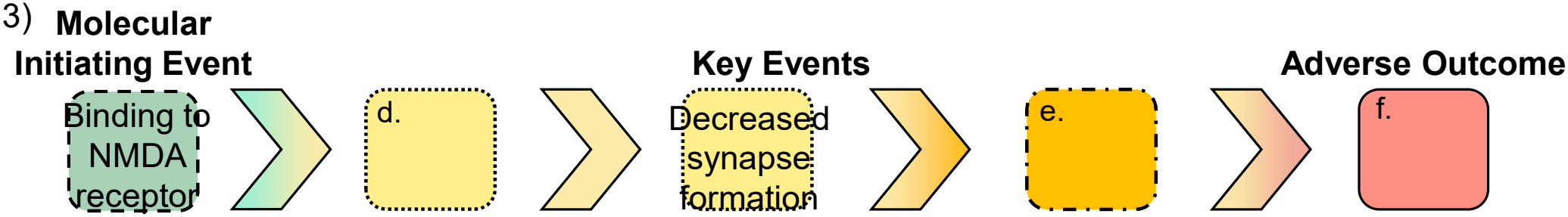
Apoptosis/ cell death	Binding to enzyme in the mitochondria	Cancer
Decreased neuronal function	Impaired learning and memory	Reduced calcium influx

1)



2)

3)

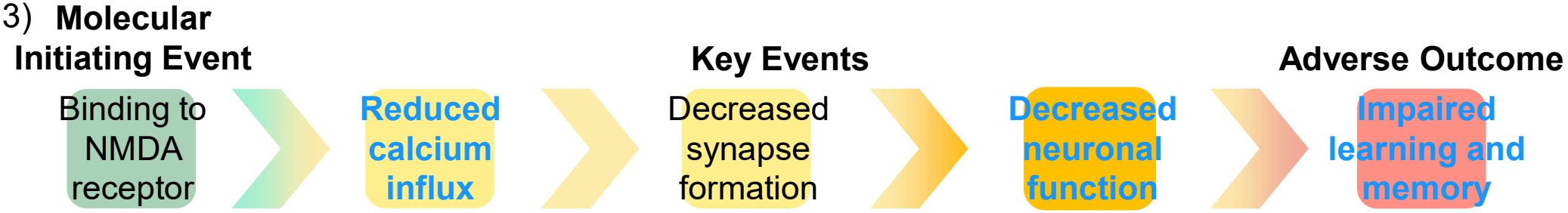


Exercise 1: Answers

1)

2)

3)

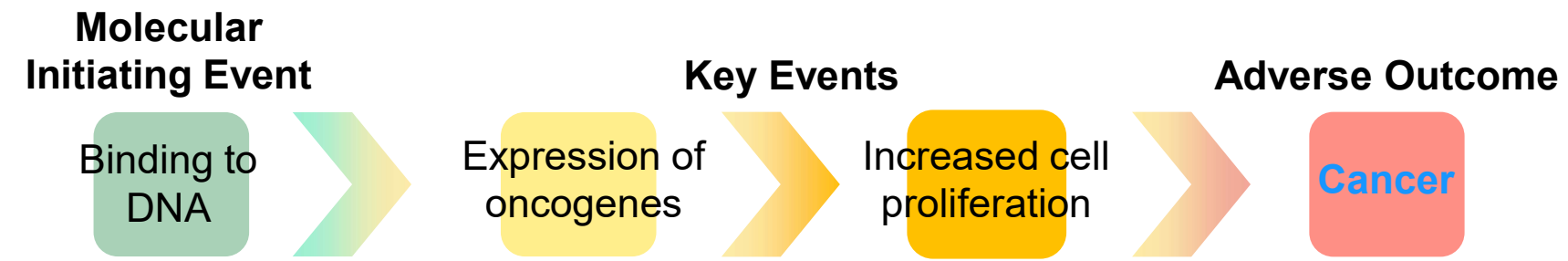


Exercise 2

Select one of the three AOPs in exercise 1, discuss and suggest types of NAMs (computational approaches, high throughput cell assays, 3D cell models, organs on chips, small model organisms), and what you could measure for the different events.

Exercise 2: Answers

1)



	Molecular Initiating Event	Key Events	Key Events	Adverse Outcome
Example NAM	Computer calculating if a chemical binds to DNA	High-throughput cell assay measuring expression of oncogenes	High-throughput cell assay counting number of cells	Cancer e.g., tumor formation in small model organism such as Zebrafish

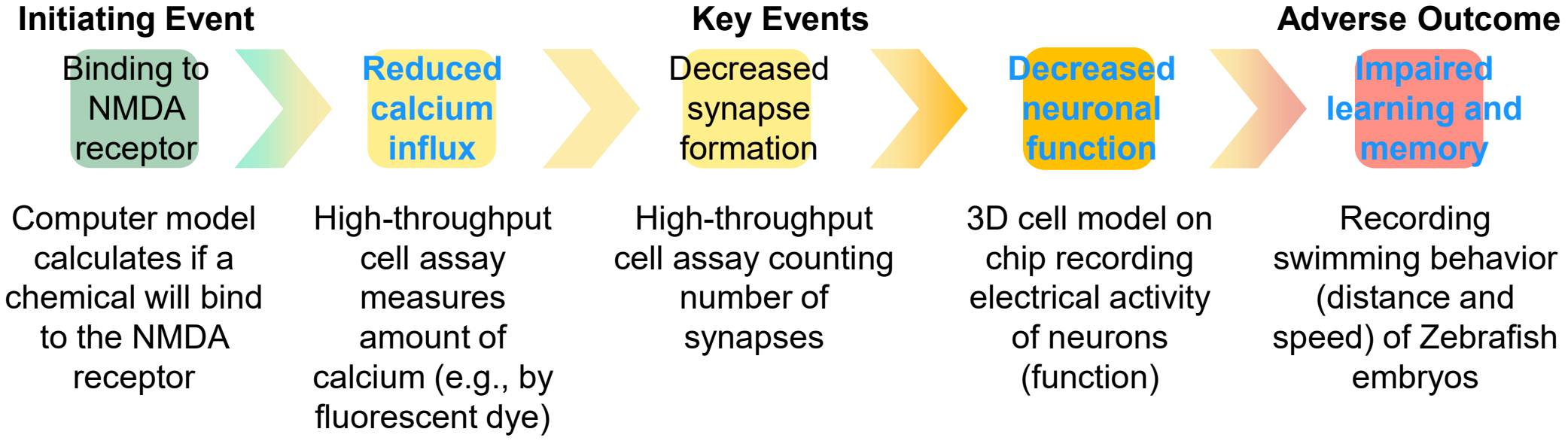
Exercise 2: Answers

2)

Example NAM	Computer calculating if a chemical binds to enzyme in mitochondria	High-throughput cell assay measuring mitochondrial function (e.g., ATP)	High-throughput cell assay counting number of alive and dead cells	Measuring number of neurons (dopaminergic) in small model organism such as Zebrafish or nematode

Exercise 2: Answers

3) **Molecular**



Summary

- New Approach Methods (NAMs) are often faster and cheaper than traditional animal tests
- New Approach Methods (NAMs) measure mechanistic effects
- Combining information from different methods through an Adverse Outcome Pathway (AOP) can help us understand how a chemical may lead to adverse effects in humans