Organizing mechanism-related information on chemical interactions using a framework based on the Aggregate Exposure and Adverse Outcome Pathways

Paul S. Price, PhD
U.S. EPA (retired)
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Risk Assessment and Mixtures Specialty Sections
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A proposal for creating a taxonomy of chemical interactions using concepts from the aggregate exposure and adverse outcome pathways

Paul Price and Jeremy Leonard

Abstract

Currently, there is no single taxonomy for organizing data on the various types of chemical interactions that may affect cells from combined exposures. A taxonomy of chemical interactions is proposed that is based on a combination of the aggregate exposure pathways (AEPs) and adverse outcome pathways (AOPs). The AEP–AOP framework organizes data on the causal events that occur over the entire source–exposure–response continuum of a chemical's release. The proposed taxonomy uses this framework in two ways. First, four top-level categories are established based on the location in the continuous source–chemical–response continuum that occurs. Second, each top-level category has two or more subcategories that are based on concepts taken from AEPs and AOPs. The categories and subcategories are potentially useful in developing standardized definitions for interaction terms and in improving our understanding of the impacts of chemical interactions on risk to human health and the environment.

Keywords

Toxicology, Chemical interactions, Aggregate exposure pathway, AOP, Adverse outcome pathway

1. Introduction

Currently, there is no single framework for categorizing the diverse types of chemical interactions that affect the adverse human and environmental outcomes from chemicals. While multiple individuals and organizations have proposed methods of organizing information on the effects of combined exposures to chemicals [1–7], these approaches have not considered the entire source–exposure–response continuum and the nature of the impact as used in the framework for toxicology. This paper offers an approach that has the potential to fill this gap.

In this paper, we propose a taxonomy that is based on the aggregate exposure pathway (AEP) framework [8–10] and the adverse outcome pathway (AOP) framework [11–13]. In this article, we argue that the combination of AEPs and AOPs provides a useful framework for organizing the diverse types of chemical interactions into a hierarchical system of mutually exclusive categories. These categories can provide a more detailed organization of interactions that have been only broadly characterized in earlier approaches. In addition, the categories organize interactions into groups with common affordances. As a result, the taxonomy can aid in the understanding and management of impacts of chemical interactions on human health and the environment.

The proposed taxonomy is presented as an initial work. The concepts in this article are offered as a discussion starter, and we welcome additional ideas, modifications, and suggestions. This article begins with a brief review of relevant components of AEPs and AOPs, followed by a description of the taxonomy and a brief discussion.

2. The combined AEP–AOP framework

The AEP–AOP framework is an object-oriented system for organizing events occurring along the source–exposure–response continuum (Figure 1A), using concepts from graph theory. In an AEP, chemical exposure is defined in terms of one or more chemical sources. AOPs provide a framework for organizing the events that occur from chemical exposure.
Contributors

• Justin Teeguarden, Yu-Maei Tan, Steven Edwards (and others) for developing the Aggregate Exposure Pathway that made this possible

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• All errors and flaws are mine
Background
Challenge of chemical interactions, mixture toxicity, and the exposome

• Mixture toxicity is a function of the combinations of chemicals involved in the interaction
• The number of combinations are far larger than the number of chemicals
• Humans and ecological receptors are exposed to millions of complex mixtures
• Exposures need not be concurrent. Chemical X’s effects may persist and affect the impacts of future exposures to chemical Y
• The combination of all exposure sources forms the exposome that has been shown to have significant impacts human health
Historical approaches to assessing chemical interactions in animal models

Defined by response data for groups of chemicals measured separately and together

Such data provides the basis for categories of interaction:

- Dose additivity
- Response additivity
- Synergy
- Antagonism
Chemical risk assessment in the 21st century and the New Assessment Methodologies

- Movement to *in vitro* and *in chemico* models of toxicity from *in vivo* models
- Leveraging *in vivo* and *in vitro* data to make *in silico* predictions
- Movement from empirical to mechanistic-based findings for toxicity, exposure, and risk analyses
- Building pipelines for high-throughput analyses
- These tools give insights on the mechanisms of toxicity but not necessarily a finding of toxicity
Adverse Outcome and Aggregate Exposure Pathways (AEP and AOP)

Created to meet the need for flexible frameworks to organize, hold, and make use of data from existing toxicity studies, new findings, and survey results.

Based on concepts from graph theory and Resource Description Framework (RDF) approaches.

Together they cover the entire source-to-outcome continuum.
Aggregate Exposure Pathway

1st KES in Aggregate Exposure Pathway for transformation product

Emission source (1st KES) → 2nd KES → 3rd KES → Target site exposure (last KES)

Relationship of target site exposure and molecular initiating event determined empirically using in vivo and in vitro data

Adverse Outcome Pathway

Molecular initiating event (first KE) → 2nd KE → 3rd KE → Adverse outcome individual receptor → Adverse outcome population receptor
AEPs differ from AOPs

• AOPs are chemically agnostic, deal in data from multiple levels of biological organization, are time and location independent, and focus on measurable effects
• The AOPs relevant to a chemical are determined by the specific MIEs triggered by a chemical and the chemical-specific relationships between the relevant TSEs and MIEs
• AEPs are chemical-specific, deal only with mass transport and chemical reactions, and are usually time and location dependent
Dividing up the source-to-response continuum

**Historical division of events by discipline**

- **Fate and transport**
  - Emission source
  - Fate and transport

- **Exposure**
  - Exposure

- **Animal based toxicology**
  - Dosimetry
  - Toxicodynamics

**Events in a combined AEP-AOP framework**

**AEP**
- Emission source
- Fate and transport
- Exposure
- Dosimetry

**AOP**
- Toxicodynamics
- Population and ecosystem dynamics
The framework
Scope of the framework

• Started with chemical interactions in *in vivo* toxicology and the AOP
• The advent of the AEP allowed the separation of toxicokinetics and toxicodynamics
• The definitions of the AEP and AOP provided the opportunity to consider interactions that occur upstream and downstream of *in vivo* toxicology
  • Release
  • Fate and transport
  • Exposure events
  • Population level and
  • Ecosystem level
Principles used in designing framework

• Start with binary interactions
• Recognize that a response in a study of combined toxicity of two chemicals can reflect multiple interactions
• Not important what the chemicals do separately
• Framework is aspirational
  • Most mixture toxicity studies do not generate the necessary mechanism data to use the framework
  • Data are not available for most chemicals
• Begin with a clear definition of what is a chemical interaction
The terms interaction and noninteraction are already defined in mixture toxicology

• Existing definitions derived from empirical data on dose and response
  • Interaction: The combined dose response cannot be explained by response addition or dose addition
  • Non interaction: The combined dose response can be explained by response addition or dose addition

• New definitions derived from mechanism
  • Interaction: The ability of one chemical (X) to cause a change in the source-to-outcome continuum of a second chemical (Y) for a defined AO
  • Non-interaction: The lack of the ability of X to cause a change the source to-outcome of Y at any dose of X below the maximum tolerated dose of X (similar to the definition of “no apparent influence”)
Interactions have direction

*In vivo* and *in vitro* models do not indicate what chemical X is doing to the toxicity of chemical Y or what Y is doing to the toxicity X.

\[ X \rightarrow \text{Response}_X \]
\[ Y \rightarrow \text{Response}_Y \]
\[ X + Y \rightarrow \text{Response}_{X+Y} \]

But mechanistic findings are directed - X changes the toxicity of Y by a specific mechanism

\[ X \]
\[ \downarrow \]
\[ Y \rightarrow \text{Response}_Y \]
Toxicological effects of a chemical
(National Academy of Sciences, 2011)

Source of Y
Fate and Transport
Exposure
Tissue dose
Biological Interaction
Perturbation

Source of X
Fate and Transport
Exposure
Tissue dose
Biological Interaction
Perturbation

Source to outcome continuum for chemical Y
Composed of AEP and AOP

Interaction based on additive dose response
Interaction based on effects of X

Mechanism of a directed chemical interaction
Modeling chemicals interactions in both directions

When two chemicals cause a common AO

\[ X \rightarrow \text{Response}_X \]
\[ Y \rightarrow \text{Response}_Y \]

It may be useful to model how chemical X changes the toxicity of chemical Y

\[ X \downarrow \]
\[ Y \rightarrow \text{Response}_Y \]

and how chemical Y changes the toxicity of chemical X

\[ Y \downarrow \]
\[ X \rightarrow \text{Response}_Y \]
A taxonomy of chemical interactions
Taxonomy is offered as a useful framework for organizing findings on chemical interactions

• Covers all interactions that occur over the source-to-outcome continuum

• The system of categories are:
  • Exhaustive – all interactions fall into one of the categories
  • Mutually exclusive (an interaction will fall into only one category)

• Binary interactions
Aggregate Exposure Pathway

1st KES in Aggregate Exposure Pathway for transformation product

Emission source (1st KES) → Movement KTR → 2nd KES → Movement KTR → 3rd KES → Movement KTR → Target site exposure (last KES)

Conversion KTR

Molecular initiating event (first KE) → KER → 2nd KE → KER → 3rd KE → KER → Adverse outcome individual receptor → KER → Adverse outcome population receptor

Adverse Outcome Pathway
Top tier of taxonomy of interactions is based on location of the interaction in the continuum.

**Category 1: Fate and Transport**
- Emission source
- Interim environmental KES
- External dose (or exposure)

**Category 2: Toxicokinetics**
- Interim internal KES
- Target site exposures
  - Movement KTRs: Inspiration, ingestion, dermal absorption

**Category 3: Toxicodynamics**
- Molecular initiating event
- Interim KEs
- Organism level adverse outcomes

**Category 4: Population and Ecosystem**
- Population level adverse outcomes
Second tier of taxonomy of interactions is based on characteristics of AEP and AOP

Category 1. Interactions in release, fate, transport and exposure processes of Y
   - Category 1A. Change in the movement of Y in the environment
   - Category 1B. Change in the conversion of Y to Y’ in the environment
   - Category 1C. Chemical reactions between X and Y in the environment

Category 2. Interactions that change the toxicokinetics of Y
   - Category 2A. Change in the movement of Y in an organism
   - Category 2B. Change in the conversion of Y to Y’ in an organism
   - Category 2C. Chemical reactions between X and Y in an organism

Category 3. Chemical Interactions that involve chemicals with a common AO
   - Category 3A. Interactions involving a common MIE(s)
   - Category 3B. Interactions involving separate MIEs but with one or more common KEs in an AOP network
   - Category 3C. Interactions involving separate MIEs that converge to a common AO but have no other common KEs

Category 4. Interactions leading to an adverse outcome in a population-based AO
   - Category 4A. Separate adverse effects affecting a common population
   - Category 4B. Chemicals that impact a population directly and indirectly by affecting another species
**Category 1A: Interactions involving a movement KTR**

- Emission source of X
- Emission source of Y
- 2nd KES of Y
- 3rd KES of Y
- External exposure to Y

**Category 1B: Interactions involving a conversion KTR**

- Emission source of X
- Emission source of Y
- 2nd KES of Y
- 3rd KES of Y
- External exposure to Y

**Category 1C: Interactions involving a chemical reaction**

- Emission source of X
- Emission source of Y
- 2nd KES of Y
- 3rd KES of Y
- External Exposure to Y
Category 2A: Interactions involving a movement KTR

Category 2B: Interactions involving a conversion KTR

Category 2C: Interactions involving a chemical reaction
Category 3A: Interactions at a common MIE

TSE X → MIE → 2nd KE → 3rd KE → AOs in individual organisms → Population level AOs

TSE Y → MIE → 2nd KE → 3rd KE → AOs in individual organisms → Population level AOs

Category 3B: Interactions at a common KE

MIE X → 2nd KE of X → 3rd KE → AOs in individual organisms → Population level AOs

MIE Y → 2nd KE of Y → 3rd KE → AOs in individual organisms → Population level AOs

Category 3C: Interactions at a common AO

MIE X → 2nd KE of X → 3rd KE of X → AOs in individual organisms → Population level AOs

MIE Y → 2nd KE of Y → 3rd KE of Y → AOs in individual organisms → Population level AOs
Category 4A: Population-based interactions

- **MIE X**
  - 2nd KE of X
  - 3rd KE of X
  - Effect 1 in individual organisms

- **MIE Y**
  - 2nd KE of Y
  - 3rd KE of Y
  - Effect 2 in individual organisms

AOP – Population level

Category 4B: Ecosystem-based interactions

- **MIE X**
  - 2nd KE of X
  - 3rd KE of X
  - Effect 1 in individual organisms in secondary population

- **MIE Y**
  - 2nd KE of Y
  - 3rd KE of Y
  - Effect 2 in individual organisms in primary population

Primary Population AO
The proposed taxonomy as a straw person

- Does it work? Can it be improved?
- Is it exhaustive? Is it exclusive?
- The relationship between tier 2 and tier 1 differ across the four categories. Is this ok?
- What would a third tier of the taxonomy look like?
  - Type of mechanism?
  - How does would it vary across categories and subcategories?
- Does location need another tier?
  - Should tier 1 of toxicokinetics be further divided into absorption, distribution, and excretion?
The framework and informatics
Directed interaction forms the basis for a semantic triple

Subject
Chemical X

Predicate
Has impact

Object
A causal event in the source-to-outcome continuum of chemical Y
Objects: Events in source-to-outcome continuum of chemical Y

Defined in terms of Y’s AEP-AOP framework

AEP for Y and Y’s transformational products
- Emission source
- Fate and transport
- Exposure
- Dosimetry

AOP (based on Y’s MIEs)
- Toxicodynamics
- Population and ecosystem dynamics

Object
- A causal event in the source-to-outcome continuum of chemical Y

Predicate
- Has impact

Subject
- Chemical X

Predicate
- A causal event in the source-to-outcome continuum of chemical Y
Top tier of taxonomy of interactions based on location in the continuum

Category 1: Fate and Transport
- Emission source
- Interim environmental KES
- External dose (or exposure)

Category 2: Toxicokinetics
- Interim internal KES
- Target site exposures
  - Movement KTRs: Inspiration, Ingestion, dermal absorption

Category 3: Toxicodynamics
- Molecular initiating event
- Interim KEs
- Organism level adverse outcomes

Category 4: Pop. and Ecosystem
- Population level adverse outcomes

Predicate
Has impact

Subject
Chemical X

Object
A causal event in the source-to-outcome continuum of chemical Y
Predicate: Impact of X on the event in the source-to-outcome continuum of Y

• The nature of the impact can be diverse:
  • Increase or decrease the TSE associated with a source
  • Increase or decrease the response associated with a specific intensity and duration of an MIE by triggering MIEs for AOP that interact with Y’s AOPs.
  • Create reaction products for chemical Y or Y’s metabolites (XY)
  • Create new key events and AOs
• Impacts are categorized differently for events in the AEP and AOP
Subject: Chemical X

• Chemical X is defined as the “acting” agent
• Chemical X, or its effects, must share the environment/organism during the time of the release-exposure-response events of chemical Y
• The ability of chemical X to act are due to its physical, chemical, or toxicological properties
• Chemical X has its own AEP and AOP separate from chemical Y’s
• Such data are metadata for chemical X in the semantic triple
Storing data as triples

• For some pairs of chemicals data are only tracked in one direction
  The ability of X to affect and event on the source-to-outcome continuum of Y

• For other pairs of chemical data are tracked in both directions
  The ability of X to affect an event on the source-to-outcome continuum of Y
  The ability of Y to affect an event on the source-to-outcome continuum of X

• The decision is a function of the AO of interest and the characteristics of chemicals X and Y
AEP-AOP networks
AOP networks have been proposed to address toxicodynamic interactions resulting from chemicals triggering different MIEs.

Villeneuve et al. 2017
Combined AEP-AOP networks are required to describe toxicokinetic interactions

Category 2B interaction Grapefruit juice and drug metabolism:
A KE in the AOP of a chemical in grapefruit juice affects the KTR in a drugs’ AEP (detoxification) leading to potentiation of the drug.
Building bridges between mixture toxicology and AOP and AEP by redefining the terms and concepts of mixture toxicology
Unlike the new definitions for interaction and non-interaction presented above, these definitions do not seek to change the existing meanings of the terms.

Rather they are meant to be bridges between definitions based on empirical *in vivo* toxicity data and the mechanism data generated by NAMs and organized in terms of AOPs and AEPs.
Historical approaches to assessing chemical interactions in *in vivo* models

Defined by response data for groups of chemicals measured separately and together

Such data provides the basis for categories of interaction:

- Dose and response additivity,
- Synergy/antagonism,
- Potentiation/inhibition, and
- Initiation and promotion
Interaction thresholds: when chemical X has a specific type of interaction with Y at one dose of X but not at a lower dose

Thresholds of interactions have been observed in empirical measurements of joint response. One of the mechanisms by which such interaction thresholds occur is when chemical X causes its impact by means of its toxicological effects.

- Several of the interaction categories are based on the impact of the toxicological properties of chemical X on the source-to-outcome sequence of chemical Y. These include interactions in Subcategories 1A, 1B, 2A, and 2B and all interactions in Subcategories 3B, 3C, 4A, and 4B. In these interactions, chemical X must reach an organism and cause a MIE leading to KES and AO(s) in its own AOP.
Most interactions are expected to have thresholds!

Interactions in categories 1B, 1C, 2B, 2C, 3B, 3C, 4A, and 4B will have thresholds.
Dose addition

Dose addition occurs between two chemicals (X and Y) when a prior, or concurrent, exposure to chemical X causes an increase in the intensity or duration of the MIE that occurs in response to Y by acting as if it was a concurrent toxicity weighted TSE of Y.

Dose addition has no interaction threshold.

Dose addition only occurs between chemicals when they have common MIEs (Category 3A). Having common KEs or common AOs is required but is not sufficient for demonstrating that dose addition occurs.
Response addition

Response addition occurs between two chemicals (X and Y) when a prior, or concurrent, exposure to chemical X causes an AO in an exposed population and changes the response to a dose of Y by reducing the number of individuals where the AO has not occurred.

Response addition occurs between chemicals that do not share a common MIE or a common KE but have a common AO in an AOP network (Category 3C).
Category 3A: Interactions at a common MIE

TSE X

TSE Y

Dose Additivity

Category 3B: Interactions at a common KE

MIE X

MIE Y

Response Additivity

Category 3C: Interactions at a common AO

MIE X

MIE Y

AOP – Organism level

Dose Additivity

Response Additivity

2nd KE of Y

AOP – Organism level

Dose Additivity

Response Additivity

Population level AOs
Subcategory 3B

- AOP networks that have one or more common KEs and no common:
  - MIEs, or
  - AOs
- Can cause a range of responses
  - Partial dose additivity
  - Antagonism
  - Synergy
  - Response additivity
- Requires construction of a qAOP network for the two chemicals
- One constant characteristic: all 3B interaction will have thresholds. The presence of X would modify to the effects of Y only when the TSE of X was sufficiently large to cause the MEI (and certain other KEs) that are prior to the KE that interacts with a KE on chemical Y’s AOP.
Synergy

Synergy occurs between two chemicals, X and Y, when a prior, or concurrent, exposure to chemical X causes an increase in the response to a release of Y from a source by:

1) increasing the ratio of the amount of Y released by a source and the TSE for Y, or its active metabolite (kinetic synergy), or

2) increasing the probability that a MIE of given intensity and duration will result in the AO (dynamic synergy).
Antagonism

Antagonism occurs between two chemicals, X and Y, when a prior or concurrent exposure to chemical X causes a decrease in the response to a release of Y from a source by:

(1) decreasing the ratio of the amount of Y released by a source and the TSE for Y, or its active metabolite (kinetic antagonism), or

(2) decreasing the probability that an MIE of a given intensity and duration will result in the adverse outcome (dynamic antagonism).
Neither chemical causes an AO independently but do so together

• Categories 1C and 2C: creation of a new chemical
• Categories 2A and 2B: increases the TSE for Y or its metabolite to exceed the threshold of the MIE.
• Category 3B: Y causes one or more KEs that allow a KE of Y to trigger the AO (initiation and promotion)
• Categories 3A and 3C: cannot cause this behavior
Future steps
Apply the taxonomy and semantic triple to actual studies

• Semantic triple
  • Subject (Chemical X)
    • Name, ability to cause the interaction (physical, chemical, or biological)
  • Predicate (interaction)
    • Description of the interaction
    • Colocation of X, or its effects, and events in source-to-outcome continuum of chemical Y
  • Object (Event in source-to-outcome continuum of chemical Y)
    • First level category of taxonomy

• Taxonomy
  • Decompose study results into findings on one or more mechanisms
  • Assign mechanisms to categories and subcategories
  • Develop additional tiers of categories

• Suggest revisions to the taxonomy and semantic triple based on the experience
Using groupings of interactions to direct research

• The ability of X to cause a change in the source-to-response continuums of other chemicals is a function of the physical, chemical, and toxicological properties of X

• This suggests that the potential to cause a specific type of interaction could be predicted based upon the chemical structure of X. Projects could be created to:
  • Identify chemicals known to affect other chemicals by a common mechanism (i.e. all chemicals that affect a common KTR, MIE, KER, or AO)
  • Development of QSARs to predict the potential to cause the interaction
  • Determination of threshold TSE for the ability to cause the interaction
Conclusions

• Advances in characterizing the risk implications of combined exposures requires an understanding of the mechanisms of chemical interactions

• Data on the mechanisms of chemical interactions need to be organized in ways that:
  • Apply to all portions of the source-to-outcome continuum
  • Facilitate the modeling of combined effects
  • Allow extrapolation to untested chemicals

The ideas presented here are offered as an initial step in this organization
A proposal for creating a taxonomy of chemical interactions using concepts from the aggregate exposure and adverse outcome pathways

Paul Price 1 and Jeremy Leonard 2

Abstract

Currently, there is no single taxonomy for organizing data on the various types of chemical interactions that may affect risks from combined exposures. A framework of chemical interactions is proposed that is based on a combination of the aggregate exposure pathways (AEPs) and adverse outcome pathways (AOPs) (AEP–AOP framework). The AEP–AOP framework organizes data on the causal events that occur over the entire exposure–response continuum of a chemical’s release. The proposed taxonomy uses this framework in three principal ways: (1) Subcategories of chemical interactions are established based on the location in the continuum where a chemical interaction occurs. Second, each top-level category has two or more subcategories that are based on concepts taken from AEPs and AOPs. The categories and subcategories are potentially useful in developing standards, guidelines, and procedures to improve our understanding of the impacts of chemical interactions on risk to human health and the environment.

1. Introduction

Toxology, exposure science, and chemical risk assessment are in the midst of a transformation. Assessors are moving towards the use of in vitro assays and in vivo predictions that provide insights into how chemicals that cause adverse outcomes (AOs) (HEM, 2007). The methodologies driving this transformation have been referred to as “New Approaches Methodologies or NAMs” (Phans et al., 2019; Wambach et al., 2019). These new approaches are necessary due to the large number of substances and the limitations in current methods. A new approach to toxicology is to evaluate the chemical and environmental context of exposure as the causal factor for the effect of chemicals on biological systems and the environment. This helps to illuminate the mechanisms that drive the causal events in the external exposure – chemical interaction continuum that describes the ability of a chemical to pose risks to human health and the environment (Cohrssen-Habib et al., 2010; Hines et al., 2014).

1. Introduction

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