



Research Article

A Modular Approach for Assembly of Quantitative Adverse Outcome Pathways

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Abstract

The adverse outcome pathway (AOP) framework is a conceptual construct that mechanistically links molecular initiating events to adverse biological outcomes through a series of causal key events (KEs) that represent the perturbation of the biological system. Quantitative, predictive AOPs are necessary for screening emerging contaminants and potential substitutes to inform their prioritization for testing. In practice, they are not widely used because they can be costly to develop and validate. A modular approach for assembly of quantitative AOPs, based on existing knowledge, would allow for rapid development of biological pathway models to screen contaminants for potential hazards and prioritize them for subsequent testing and modeling. For each pair of KEs, a quantitative KE relationship (KER) can be derived as a response-response function or a conditional probability matrix describing the anticipated change in a KE based on the response of the prior KE. This transfer of response across KERs can be used to assemble a quantitative AOP. Here we demonstrate the use of the proposed approach in two cases: inhibition of cytochrome P450 aromatase leading to reduced fecundity in fathead minnows and ionic glutamate receptor mediated excitotoxicity leading to memory impairment in humans. The models created from these chains have value in characterizing the pathway and the relative level of toxicological effect anticipated. This approach to simplistic, modular AOP models has wide applicability for rapid development of biological pathway models.

1 Introduction

Adverse outcome pathways, or AOPs, are pragmatic descriptions of a biological pathway leading to an outcome of regulatory concern that have the potential to inform chemical risk management decisions (Ankley and Giesy, 1998; OECD, 2017, 2018; Perkins et al., 2019). An AOP characterizes the biological impact of a stressor across multiple levels of organization by describing how the stressor triggers a molecular initiating event (MIE) at the protein, DNA, or other molecular level that causes subsequent measurable key events (KE) resulting in an adverse outcome (AO) of regulatory concern. A number of efforts have developed quantitative, predictive AOPs; these efforts include Bayesian network models (Jaworska et al., 2013; Pirone et al., 2014) and mechanistic models that represent the complexity of a biological pathway (Shoemaker et al., 2010; Nichols et al., 2011; Breen et al., 2013). Recent work coupled hypothalamic-pituitary-gonadal-liver, oocyte growth, and population models into a quantitative, predic-

tive AOP (Conolly et al., 2017). Development and validation of these models generally require extensive data and labor.

Given the potentially large number of AOPs needed to describe known toxicological pathways, the limited availability of relevant mechanistic data, and the extensive time required to develop mechanistic AOP models, a complementary approach is needed to utilize existing data to develop easy-to-assemble, modular, quantitative AOPs (qAOPs). Such an approach could be developed from a current understanding of the responses of biological pathways that are perturbed, and relate the level of “activation” quantitatively with the anticipated degree or probability of the adverse outcome. The ideal approach would allow construction of different AOPs from an assortment of modules describing response relationships between KEs (Fig. 1). This modular approach, although coarser and more uncertain (Fig. 1), would facilitate rapid prototyping and updating of both modules and complete AOP models, making it better-suited for screening and prioritization than the more detailed and resource-intensive

Received October 18, 2018; Accepted January 13, 2019;
Epub January 13, 2019; © The Authors, 2019.

ALTEX 36(3), 353-362. doi:10.14573/altex.1810181

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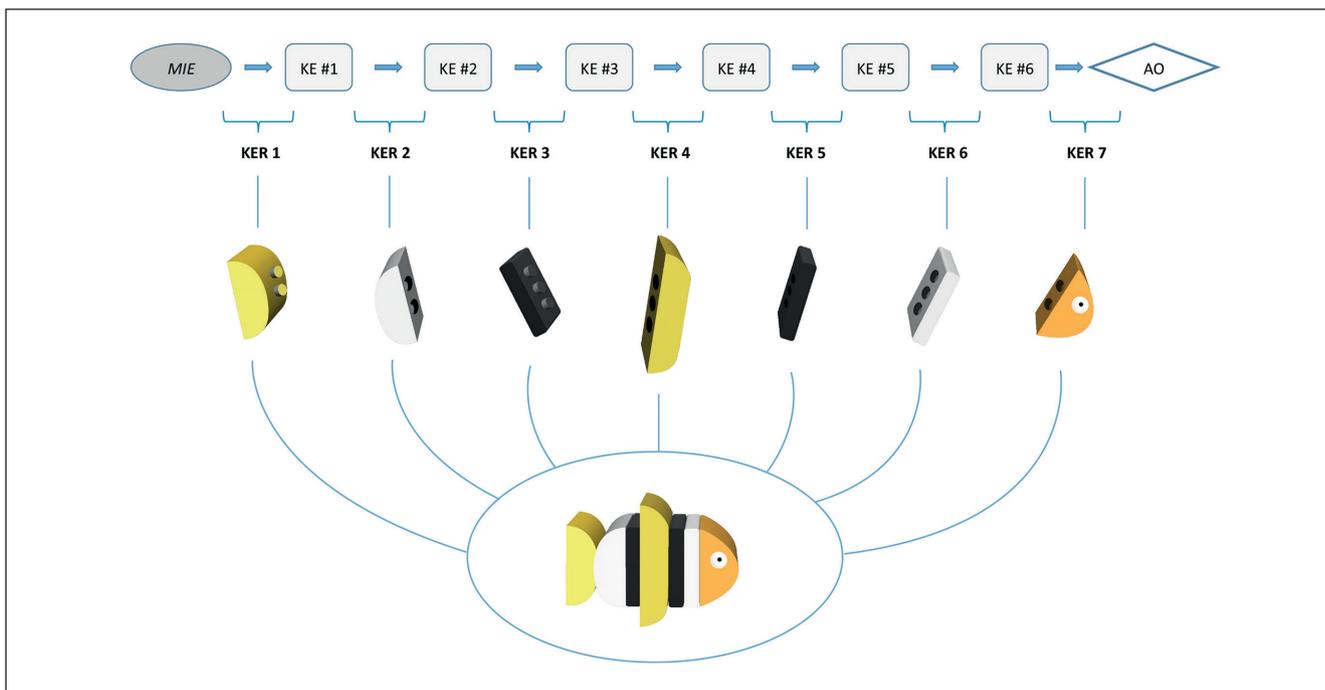


Fig. 1: The structure of an AOP displays the biological activity from a molecular initiating event (MIE), to subsequent key events (KE) derived from MIE activation, and a resulting adverse outcome (AO)

Development of quantitative relationships for each key event relationship (KER) allows the rapid prototyping of predictive AOP models. A modular approach allows for development of simple qAOP models using modular, quantitative KERs (qKERs) as pieces. However, the resulting models would be coarse, screening-level representations of more complex relationships and processes.

mechanistic models referenced above. Additional modeling and testing could augment screening-level models derived using this modular approach in order to fulfill regulatory needs as described by Wittwehr et al. (2017).

One difficulty in developing quantitative biological models is the simulation of complex, adaptive, dynamic, and interactive mechanisms underlying responses that can be expected at every level of the system. Unfortunately, the extensive datasets and mechanistic understanding required to develop models with high biological fidelity are often limited to a few responses, making highly quantitative modeling challenging. In the absence of large datasets and mechanistic understanding, we can utilize the existing information to develop correlational or probabilistic relationships between the key events in a specific biological pathway. Conolly et al. (2017) describe a process for developing a response-response (R-R) relationship linking KEs. Combining these R-R relationships or KER in a chain represents an explicit hypothesis about the biological pathway that leads to an endpoint of concern, specifically an adverse outcome at the organ, individual, or population level (Fig. 2). Development of a quantitative KER can be based on empirical and literature-derived values, which are necessarily a simplification of the systems they describe.

To support qAOP development, modules are needed that quantify KER R-R functions. The condition or state of a KE, as well as the uncertainty surrounding that measurement, must be calculated and propagated along the qAOP. The key question then

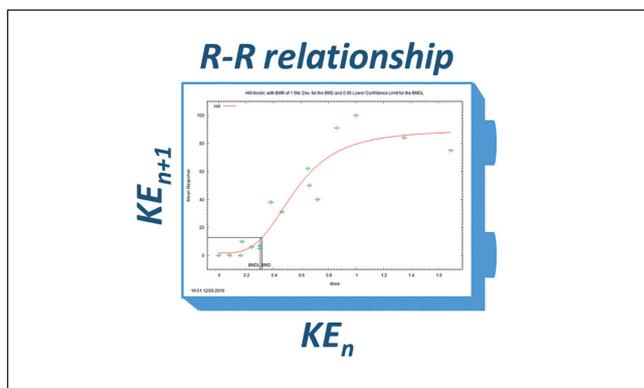


Fig. 2: A quantitative relationship between key events (KE), such as a response-response (R-R) function relating one key event (KE_n) to the subsequent one (KE_{n+1}) can be used as a piece of a model to chain together the activity of a biological pathway into a simple qAOP model

becomes whether a set of baseline data relevant to an AOP can be identified to allow one to derive quantitative KERs (qKERs) between subsequent KEs using published literature. Here we describe an approach to develop qKER modules that can be chained together to develop qAOP models that can be used in a screening or prioritization context. One benefit of this approach is that it allows for development of many simple qAOP models

from the existing literature (OECD, 2018), enabling the prioritization of pathways for future development of highly predictive, representative and mechanistic, quantitative models.

2 Methods

Target AOPs for demonstration

The first step in the development of a modular qAOP model is the same as in the development of any AOP, namely the robust identification and documentation of the MIE, KE, and AO nodes that will comprise the model, independent of a specific chemical(s) of interest (OECD, 2017, 2018). Current efforts are underway to standardize the development of AOPs in a way that would support the development of modular nodes and inter-nodal relationships (OECD, 2018). Villeneuve et al. (2014a,b) suggest that AOPs be developed such that each KE is a module that can be used in many pathways, and that the pathways can be combined to form a network. Characterization of KER provides the basis for modules that can be used together to form a qAOP.

Here we consider two AOPs for demonstration of this modular, quantitative approach (Fig. 3). First is the AOP linking the inhibition of cytochrome P450 aromatase (“aromatase inhibition,” the MIE) to reproductive dysfunction in fish (the AO) as reported in the AOP-wiki (Villeneuve, 2016). In this pathway (Fig. 3A), a key enzyme is blocked, resulting in reduced hepatic production of proteins critical to the proper development of oocytes. Impairment of oocytes leads to reduced fecundity, which

can lead to population decline. The seven KERs that connect the MIE to the AO are well-supported and thoroughly described in the literature (Becker et al., 2015), so they will only be summarized here, as follows:

- KER #1: Inhibition of aromatase activity results in decreased ovarian production of 17 β -estradiol (E2)
- KER #2: Decreased ovarian production of E2 results in reduced plasma concentration of E2
- KER #3: Reduced plasma concentration of E2 results in depressed vitellogenin (Vtg) production in the liver
- KER #4: Depressed Vtg production in the liver results in decreased plasma Vtg concentrations
- KER #5: Decreased plasma Vtg concentrations results in impaired oocyte development
- KER #6: Impaired oocyte development results in reduced fecundity
- KER #7: Reduced fecundity leads to decrease in population of fish species

The second AOP describes “binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment” (Sachana et al., 2016). As shown in Figure 3B, binding of glutamate agonists results in over-activation of the N-methyl-D-aspartate receptor (NMDAR), and subsequently results in an influx of calcium into neurons. Intracellular calcium causes mitochondrial dysfunction and leads to excitotoxicity. Excitotoxic neuronal cell death from over-activation refers to the hippocampus and cortex. The resulting damage can impair mem-

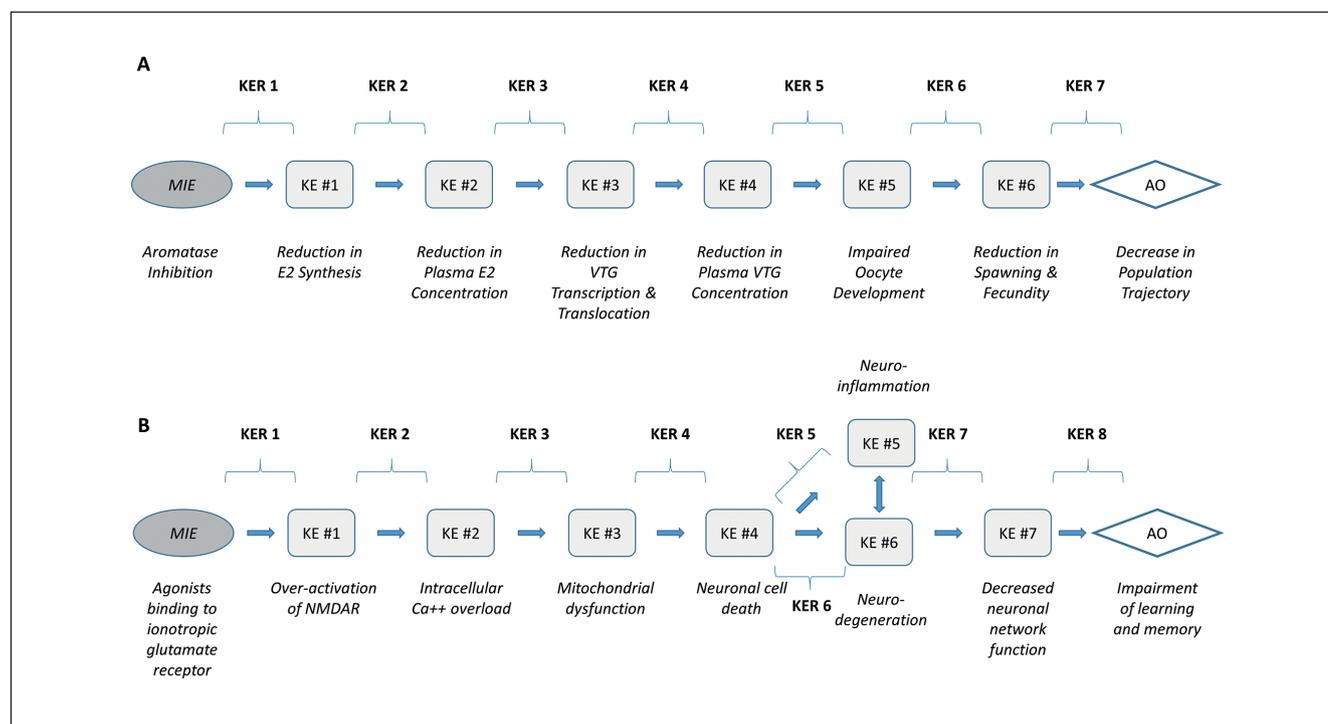


Fig. 3: Two established AOPs for demonstration

(A) The pathway for aromatase inhibition leading to reproductive dysfunction in fish (from Villeneuve, 2016) and (B) the pathway for excitation of NMDA receptors leading to impairment of memory (from Sachana et al., 2016).



ory function. The eight KERs that connect the MIE to the AO are described in the AOP-wiki. They are as follows:

- KER #1: Binding of agonists to ionotropic glutamate receptors results in the over-activation of the NMDA receptor
- KER #2: Over-activation of the NMDA receptor causes an overload of intracellular calcium
- KER #3: Intracellular calcium overload leads to mitochondrial dysfunction
- KER #4: Mitochondrial dysfunction leads to neuronal cell death
- KER #5 and #6: Neuronal cell death results in both neuro-inflammation and neurodegeneration
- KER #7: Neurodegeneration causes a decrease in the functional network in the cortex and hippocampus
- KER #8: Decreased functionality of the hippocampal and cortical neural network leads to impairment of memory and learning

Characterization of qKERs

The approach we are proposing requires the characterization of an R-R function at each KER (Fig. 2). When a change of a certain amount is detected in the MIE or any KE in the pathway, the anticipated change in the subsequent KE needs to be estimated. In this approach it is necessary to develop KERs for each pair of KEs that capture the current understanding of how one event leads to the next and the full range of potential responses. The response transfer is represented by the successful transition from one KE to the next KE. In their approach, Conolly et al. (2017) linked together models through an R-R function at the interaction points of the three models they used. They suggest that a regression could be used where information is limited, but that qKERs should reflect dose-response and time course dynamics.

In order to develop qKERs for use in a modular qAOP, literature can be surveyed for data that illustrate a relationship between two KEs. Where quantitative data are discovered, R-R relationships can be established to depict the influence that each KE in the series has on its dependent KE. For example, in order to develop a simplistic qAOP for the aromatase AOP (Fig. 3A), a series of R-R functions were derived from the models used by Conolly et al. (2017) in the development of their qAOP approach. As they suggest, a simple regression analysis could be used to correlate existing data for each of the KEs over the range that has been documented. Estimating and aligning exposures using reference doses or reference concentrations could improve the quality of the R-R curve calculations. Miller et al. (2007) documented a linear relationship between Vtg and fecundity in fathead minnows over a variety of exposures. A representation of the confidence in the relationship developed for each pair of KEs can be included as either a confidence interval or a probability distribution, data permitting. This relationship can alternatively be characterized as a transition matrix, such as in a Bayesian network. The matrix would demonstrate the probability of each of the possible states of the successor KE (conditional on reaching any of the possible states of the predecessor KE). These functions or matrices would be derived from the literature and the parameters documented. Where data for qKERs do not exist, R-R data linking non-adjacent KEs could be used. Alternatively, expert judgment could be

used as a surrogate in the absence of empirical data (Keeney and Raiffa, 1976; Belton and Stewart, 2002; Wood et al., 2012). The application of any approach to derive a qKER should involve a comprehensive review and a transparent documentation of the information that contributed to its development.

The AOP for aromatase inhibition was utilized to develop an example of how qKERs could be developed, following the same pathway utilized by Conolly et al. (2017). Data utilized for this example comes from studies in fathead minnows (*Pimephales promelas*); however, these KERs have a relatively broad biological domain of applicability that extends beyond fish to other oviparous vertebrates. Data linking non-adjacent events was used for this example because analogous data, resulting from similar exposure, species and tissues, for each key event could not be found. All R-R relationships and best-fit functions were determined using the U.S. Environmental Protection Agency's (EPA) Benchmark Dose Software (BMDS) version 2.6.0 and Microsoft Excel 2013 for simplicity. Select studies were utilized in the development of these qKERs and therefore no attempt was made to quantify the uncertainty in the relationships. Data linking the MIE to KE #2 (non-adjacent events) were utilized from a study observing the effects of aromatase inhibiting chemicals on female fathead minnows (Ankley et al., 2002). The data relating plasma E2 concentration to aromatase activity was limited to two data points, which is insufficient to plot an R-R relationship. The representation in Figure 4A, therefore, serves only as an example relationship. The best fit R-R curve was plotted in Excel and determined to be quadratic (Equation 1).

Best-fit quadratic function for qKER: MIE → KE #2

$$y = 1.06x^2 - 0.06x \quad \text{Equation 1}$$

Quantitative R-R data linking KE #2 to KE #4 (non-adjacent events) were utilized from a study observing the impacts of sex steroid status on the reproductive success of female fathead minnows (Ankley et al., 2008). The data relating plasma Vtg concentration to plasma E2 concentration was sufficient to plot an R-R relationship using BMDS (Fig. 4B). Plasma Vtg concentration and plasma E2 concentration are shown on a relative basis to depict changes relative to the experimental control. The curve with the lowest Akaike Information Criterion (AIC) value was accepted as the best fit and is represented by the Hill model (Equation 2). The best-fit curve illustrates that a given reduction in plasma E2 concentration leads to a non-linear reduction in plasma Vtg concentration that follows a sigmoidal trend.

Best-fit Hill model for qKER: KE #2 → KE #4

$$y = 1.68 + \frac{87.96x^{3.44}}{0.13 + x^{3.44}} \quad \text{Equation 2}$$

Data linking KE #4 to KE #6 (non-adjacent events) were utilized from the same study used for the previous relationship (Ankley et al., 2008) because quantitative data characterizing KER #5 and KER #6 were unavailable. The data relating fecundity to plasma Vtg concentration was sufficient to plot an R-R relation-

ship using BMDS (Fig. 4C). Fecundity (measured in eggs/female/day) and plasma Vtg concentration are shown on a relative basis to depict changes relative to the experimental control. The curve with the lowest AIC value was accepted as the best fit and is represented by the Hill model (Equation 3). The curve illustrates that a given reduction in plasma Vtg concentration leads to a nearly-linear reduction in fecundity.

Best-fit Hill model for qKER: KE #4 → KE #6

$$y = -4.09 + \frac{393.77x^{1.13}}{3.09 + x^{1.13}} \quad \text{Equation 3}$$

Empirical data characterizing KER #7 were unavailable, however *in silico* data were available from a study that developed a predictive model to translate changes in fecundity of the fathead minnow to alterations in population growth rate (Miller et al., 2007). These modeled data depict relative population size as a proportion of carrying capacity over time under five scenarios (A-E) in which plasma Vtg concentrations of female minnows is altered (Fig. 4D). Note that alterations in plasma Vtg concentrations from Miller et al. (2007) were translated to alterations in fecundity here:

- Scenario A: 9% reduction in fecundity
- Scenario B: 33% reduction in fecundity
- Scenario C: 57% reduction in fecundity
- Scenario D: 80% reduction in fecundity
- Scenario E: 100% reduction in fecundity

Combining the four qKERS developed for aromatase inhibition as depicted in Figure 5, the level of “activation” can be tracked for hypothetical exposure scenarios, which can be used to estimate declines in a fathead minnow population.

The AOP for glutamate receptor agonism, described in “Binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment,” was also used to develop simplistic qKERS that could be linked together to estimate relative memory impairment. For this case, select mammalian studies centered on the hippocampus and the cortex were utilized to develop R-R functions along the AOP. When data from different species was utilized, the responses were normalized from highest (1) to lowest (0) in order to chain the R-R function. Data linking non-adjacent events were also used for this example, and R-R relationships and best-fit functions were similarly determined. The relationship between NMDA (MIE) and the influx of calcium (KE#2; non-adjacent events) was captured from Alano et al. (2002). Data from this paper on the influx of calcium in striatal neurons incubated with NMDA was fitted using the Hill model in BMDS (Fig. 6A).

Best-fit Hill model for qKER: MIE → KE #2

$$y = 0.10 + \frac{x^{4.35}}{157.56x^{4.35} + x^{4.35}} \quad \text{Equation 4}$$

Data linking the MIE to KE #4 (non-adjacent events) was found in a study on the efficacy of NMDA antagonists in

blocking the loss of neurons in the cortex in response to NMDA (Chen et al., 1992). The data relating NMDA exposure with the loss of cortical neurons is limited to two control points (solvent and 50 μM NMDA) and therefore insufficient to plot an R-R relationship. The representation in Figure 6B is only an example of this relationship. The best fit R-R line was plotted in Excel (Equation 5).

Best-fit line for qKER: MIE → KE #4

$$y = -0.0083x + 1 \quad \text{Equation 5}$$

Quantitative R-R data linking KE #4 to the AO (non-adjacent events) were utilized from a study of brain injury from mild lateral fluid percussion on memory in rats (Hicks et al., 1993). The performance of rats in a Morris water maze test was measured 42 h after injury and compared to a sham treatment group. Following testing, the loss of neurons in specific regions of the hippocampus was quantified. The relationship between memory score and neuronal loss was captured from these data, and normalized between 0 (lowest) and 1 (highest; Fig. 6C). The data relating normalized memory score and the loss of hilar neurons is limited to three points and therefore insufficient to plot a complete R-R relationship. An example of this relationship is represented in Figure 6C. The best fit R-R line was plotted in Excel (Equation 6).

Best-fit line for qKER: KE #4 → AO

$$y = 1.14x - 0.14 \quad \text{Equation 6}$$

In order to examine the predictive capacity of this chained model, an additional relationship was needed. A study was utilized that compiles observations on amnesiac shellfish poisoning from human ingestion of shellfish containing domoic acid, which acts as a glutamate agonist (Lefebvre and Robertson, 2010). Data linking the MIE to KE #2 (non-adjacent events) were utilized to develop an R-R function between domoic acid and calcium influx in neurons (Berman et al., 2002). The monitored influx of calcium in cerebellar granule neurons upon exposure to domoic acid was reported across a range of concentrations. From these data, a best fit curve was calculated using the exponential continuous model (Equation 7). The best-fit curve illustrates the influx of calcium in response to a specified exposure to domoic acid (Fig. 6D).

Best-fit exponential continuous model for qKER: MIE → KE #2

$$y = 0.35(30.40 - 29.40e^{-53800x}) \quad \text{Equation 7}$$

Domoic acid can be related to calcium influx through this equation. Based on the calcium influx, we can estimate the NMDA “equivalents” of that exposure. The impact of the estimated NMDA “equivalents” on neuronal cell death can then be derived from the R-R function represented by Equation 5. The level of neuronal cell death can then be used to predict the relative memory impairment. The chain of these estimations is depicted in Figure 7.

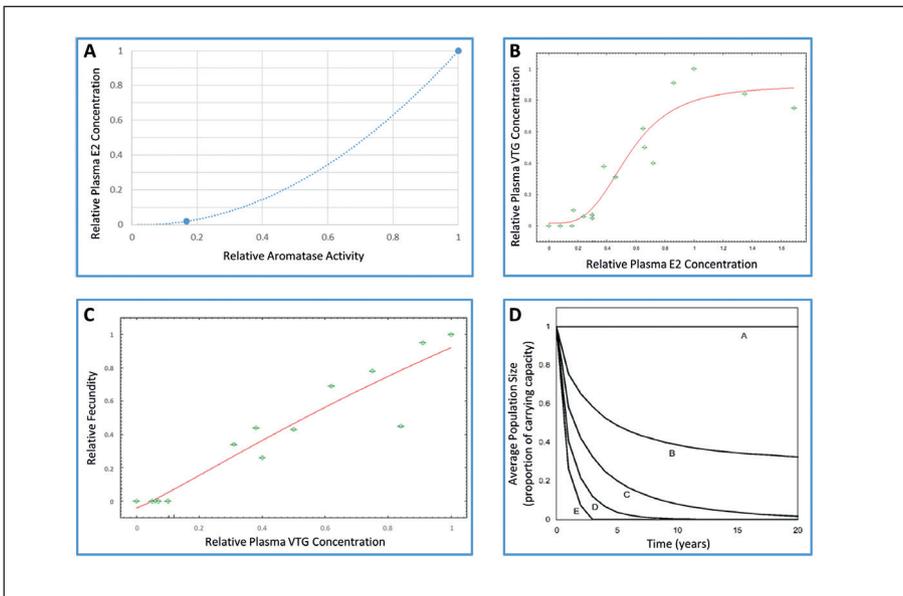


Fig. 4: Response-response (R-R) relationships used to establish qKERS describing the influence that each KE has on its dependent KE for the pathway from aromatase inhibition to the decline in fathead minnow population (A) R-R relationship between relative plasma E2 concentration and relative aromatase activity in female fathead minnows. (B) R-R relationship between relative plasma VTG concentration and relative plasma E2 concentration in female fathead minnows. (C) R-R relationship between relative fecundity and relative plasma VTG concentration in female fathead minnows. (D) Population trajectories as a function of changes in fecundity of female fathead minnows (from Miller et al., 2007).

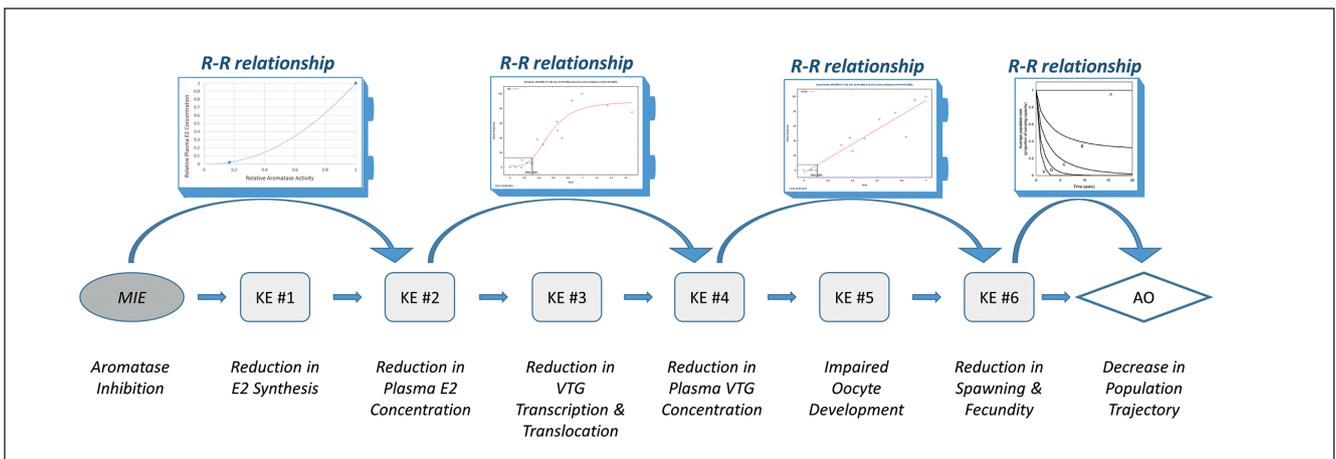


Fig. 5: Four modular response-response (R-R) relationships shown in Fig. 4 were used to transfer “activation” along the AOP for aromatase inhibition leading to reproductive dysfunction in fish, resulting in a prediction of the change in fish population The order of the calculations corresponds to the schematics of functions shown above the AOP; the R-R relationship from Fig. 4A was used to estimate the change in plasma E2 concentration from the reported aromatase inhibition. That level of E2 was used with the function in Fig. 4B to estimate plasma VTG concentration. Plasma VTG was related to change in fecundity through the R-R relationship in Fig. 4C. Using the curves in Fig. 4D, the change in fecundity was used to estimate the change in population.

3 Results

The utility of these simplistic, chained models for quantitatively estimating adverse outcomes is considered using these two AOPs. By combining the four qKERS developed for aromatase inhibition as depicted in Figure 5, the level of activation of specific nodes can be tracked for a hypothetical scenario in which a fathead minnow population is exposed to a chemical that reduces aromatase activity by 25%. From the R-R functions, it can be determined that such an exposure would lead to a 44% reduction in plasma E2 concentration. It can then be verified that a 44% re-

duction in plasma E2 concentration will lead to a 53% reduction in plasma Vtg concentration, which will, in turn, lead to a 57% reduction in fecundity. According to the qKER in Figure 4D, a 57% reduction in fecundity (curve C) is projected to result in an 80% reduction in population size after 5 years. The outcomes calculated from this simple chain of modular qKERS are similar to the results from the linked systemic models developed by Conolly et al. (2017). Estimating the outcome of a 25% reduction in aromatase activity from the Conolly model results in a calculated plasma Vtg level of 70 μM and average fecundity of 15 eggs/f/d; the estimated population size would be approximately 50%

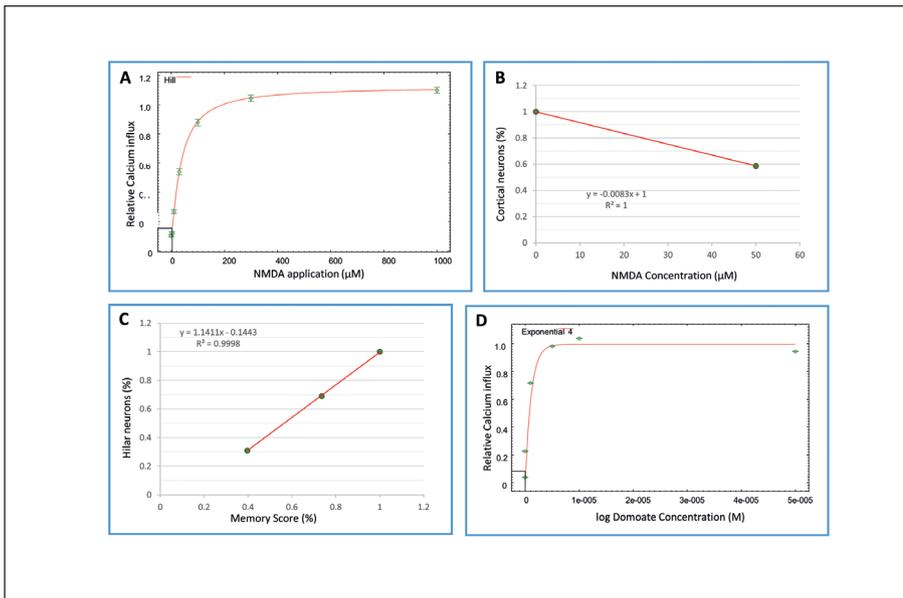


Fig. 6: Response-response (R-R) relationships estimating qKERS that reflect the influence that a KE has on its dependent KE for the pathway from glutamate agonism to impaired memory

(A) R-R relationship between relative NMDA concentration and calcium influx in neurons. (B) R-R relationship between NMDA concentration and the loss of cortical neurons. (C) R-R relationship between the loss of hilar neurons and relative memory score. (D) The relationship between domoic acid concentration and calcium influx in neurons in culture.

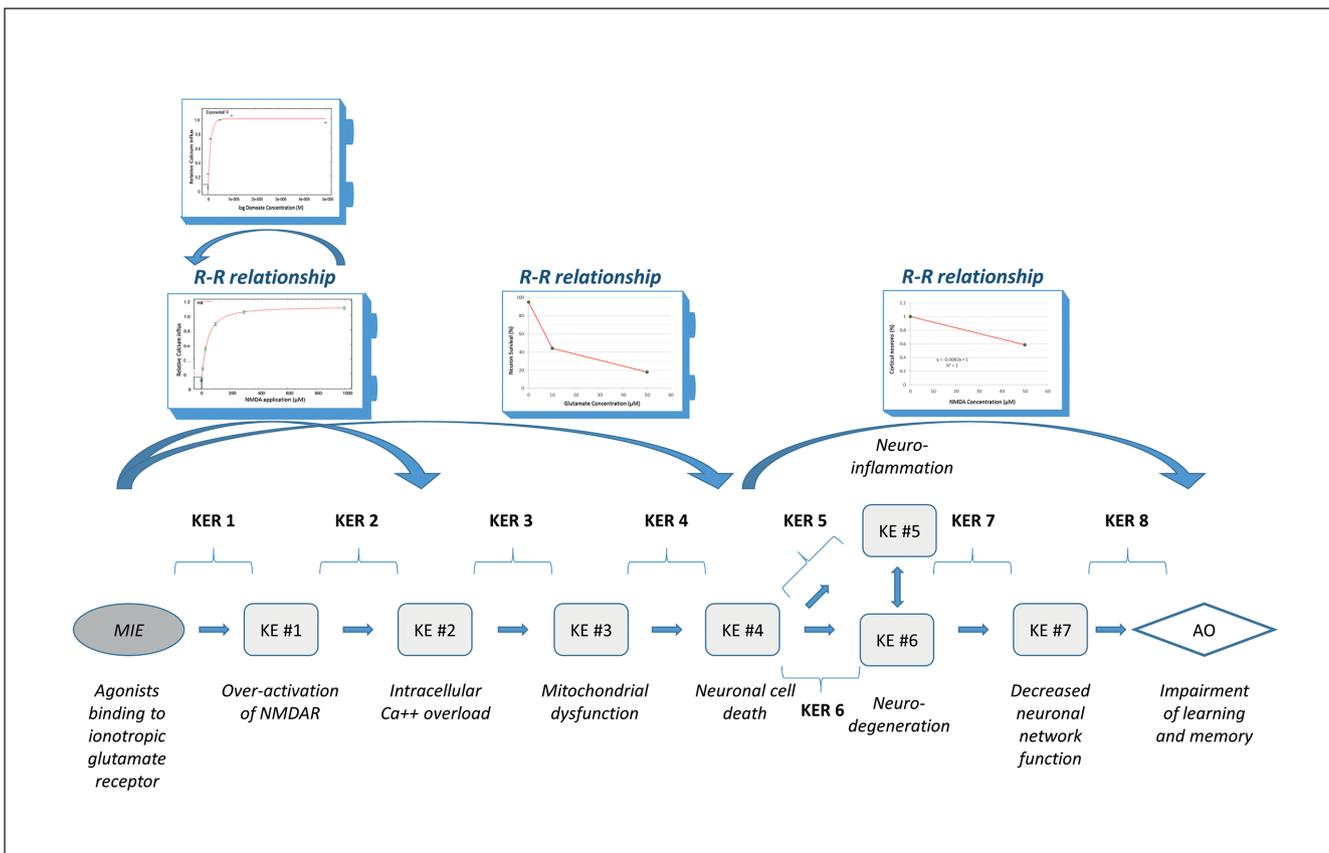


Fig. 7: The modular R-R relationships shown in Fig. 6 were used to transfer “activation” along the AOP for glutamate agonism through activation of NMDAR, resulting in a loss of neurons and a prediction of relative memory impairment

The use of these functions corresponds to the schematics of functions shown above the AOP. The R-R relationship from Fig. 6A was used to estimate the calcium overload from glutamate binding. The loss of neurons from NMDA exposure was estimated from the R-R relationship in Fig. 6B. The relationship between neuron loss and memory was based on the function in Fig. 6C. In order to consider the impact of domoic acid, the R-R relationship in Fig. 6D was used to estimate the calcium overload associated with the reported human intake.



of carrying capacity. The qKER approach foregoes the representations of biological complexity and dynamisms in the systemic models in exchange for utilization of empirical data sets to derive single node transitions. For example, the HPGL model predicts feedback responses that compensate for aromatase inhibition (Connolly et al., 2017). In this approach, the reduction in overall aromatase activity resulting from exposure must be estimated for use as a starting point for the calculations.

The qAOP represented in Figure 7 can be used to estimate the potential memory loss from domoic acid exposure in people as reviewed by Lefebvre and Robertson (2010). A reported oral dose between 0.2 and 0.3 mg domoic acid/kg body weight had no observable effect in humans. Using empirical observations of domoic acid reported by Preston and Hynie (1991), we can estimate the exposure of the hippocampus from this oral dose (approximately 0.5 μ M). This neuronal dose corresponds to a level of 0.22 for relative calcium influx, which is the equivalent of 25 μ M NMDA (Fig. 6). This level is associated with retention of 80% of cortical neurons, which corresponds to a memory score of 80%. An oral dose associated with disorientation was reported to be 2.0 mg domoic acid/kg body weight (Lefebvre and Robertson, 2010). This corresponds to a hippocampal exposure of approximately 6 μ M, following the conversion derived from Preston and Hynie (1991). This neuronal dose corresponds to a level of 0.90 for relative calcium influx, which is the equivalent of 100 μ M NMDA (Fig. 6). This NMDA level is associated with retention of 17% of cortical neurons. The loss of 83% of neurons exceeds the reported range for hilar neurons and memory score and can be considered, therefore, to be predicted to result in substantial memory impairment; however, if we extend the relationship in Figure 6C, the neuronal loss corresponds to a memory score of approximately 20%. For the example of amnesiac shellfish poisoning from human ingestion of shellfish containing domoic acid, the simplistic model chaining qKER reflects the relative severity of the adverse outcome.

The qKERs depicted in these cases are intended to serve as examples and do not represent scientific consensus of the quantitative relationships between the KEs of the selected AOPs. A more robust analysis would require an uncertainty determination for each best-fit R-R curve in order to establish 95% confidence intervals; statistical methods such as bootstrapping may aid in this requirement. Additionally, the BMDS utilized here is specifically intended for dose-response modeling in order to estimate reference doses and reference concentrations, which are used by the EPA along with other scientific information to set standards for non-cancer human health effects (U.S. EPA, 2015). The BMDS was used here because of its ease-of-use; however, more advanced statistical programming software may be preferable and better-suited for probabilistic AOP development. Lastly, empirical data pooled from a comprehensive literature review would make the derived R-R function more representative of the relationship between the KEs.

An important step in this process is the documentation of the data and literature that formed the basis of each qKER, which can be facilitated by archiving the sources in AOP-wiki pages describ-

ing individual KER¹. The qKERs could represent a range of taxa or species. Since KERs depict relationships between biological events, they are chemical agnostic and could be used in the context of many different chemicals (Villeneuve et al., 2014a,b). The analysis conducted in this case example can be applied to any AOP that has quantitative information associated with its KERs. Information may only be available for specific species (e.g., rat or zebrafish), so qKER would need to be extrapolated for species-specific applications and the appropriate error factors applied.

4 Discussion

Tens of thousands of chemicals are in use in the commercial and industrial markets today, yet for many we have little information on the potential hazards that they may cause. Here we describe a simple and rapid approach for predicting chemical hazards for screening and prioritization based on creation of qAOPs using qKERs derived from the literature. The approach differs from that proposed by others in that it requires less time and data and focuses on developing R-R relationships between KEs. Wittwehr et al. (2017) describe ambitious plans to engage the modeling community to advance the development of qAOP models in support of rapid characterization of chemical effects across a wide range of adverse outcomes and species using *in vitro* assays (Yoon and Clewell, 2016). However, the approaches described by Wittwehr et al. (2017) and Conolly et al. (2017) often require significant investment in data generation and model development. This effort would benefit from further efforts to archive qKERs in the existing section of the AOP-wiki to support input of R-R functions or the underlying empirical data.

Effectopedia² is an “*open-knowledge and structured platform able to display quantitative information*” on AOPs. It is being designed and released in part to allow for the input and sharing of data that support the generation of quantitative relationships between KEs. Effectopedia provides a platform on which these modular qAOPs can be applied and disseminated. On the one hand, the ease of creation and use of qAOPs made from a simple linking of qKERs could lead to greater use and adoption by the larger toxicological community. However, this is balanced by coarse modeling, resulting in a lower degree of biological fidelity, which must be considered when used for different applications. This approach does not aim to represent the complex and dynamic response of a KE with high biological fidelity, but does attempt to provide a rapid approach for developing qAOPs that can be used in situations where the certainty surrounding a model is sufficient to support decisions in areas such as prioritization. Various efforts have been made to develop methods to transparently and reliably assess when there is sufficient weight of evidence to support an AOP or a specific KER (Becker et al., 2015; Collier et al., 2016; Rycroft et al., 2018).

The approach demonstrated here meets the practical considerations for qAOP construction outlined in Conolly et al. (2017). The

¹ OECD Series on Adverse Outcome Pathways. doi:10.1787/2415170X

² Effectopedia (2018). <https://www.effectopedia.org> (accessed 10.17.2018).

state of upstream KE activation can be derived from the previous node and utilized to derive the amount or level of the downstream KE in our example. In conjunction with the level of MIE activation estimated from high throughput screening, a structured set of qKERs allows the transfer of response to response through the AOP (Fig. 5, 7). The MIE is predicted at a specific level and the qKER function is used to determine the corresponding level of activation for the first KE. A series of qKERs linked into a qAOP is chemically agnostic. The specification of a qKER is transparent and can be iterative when new experimental data is available. The development of qAOP using this method is practical and accessible.

This approach can add value in screening or prioritization by informing the potential or relative risk of an adverse outcome occurring. The qAOP models developed using this modular approach can be refined for predictability but their use is anticipated to be limited to screening-level decisions; mechanistic models derived from systemic data analysis are needed in applications where high biological fidelity is required. One strength of this probabilistic approach is that uncertainty can be captured in the qKERs. This uncertainty is useful not only in the comparison of different classes of compounds or different taxa but also as an indication of the value of more information in the refinement of the calculated relationship between the KEs. When multiple studies are available, the R-R function in a qKER can be expressed as a 95% confidence interval capturing the uncertainty between studies. The “level” of the input KE_n would be estimated from the MIE, and be used to simulate the range and probability of potential responses at KE_{n+1} . The development of sets of probabilistic qKERs across key biological events within an AOP can be used in a Markov chain model to develop models that capture the known uncertainty (Kim et al., 2002).

The chained approach described here utilizes existing literature and can capture the uncertainty associated with poorly characterized AOPs. It provides a characterization of the current understanding of responses at each level of biological organization. Each qKER that is derived would need to be documented and the literature archived. Therefore, these models would support the living library of AOPs represented in the AOP-wiki (OECD, 2018). The existing data associated with each AOP on AOP-wiki can be used to support the development of a qAOP. Note, however, supporting studies may not represent the relevant tissue (i.e., necrosis in liver versus central nervous system) or relevant taxa or species. Development of these models necessarily identifies missing information needed to complete a qAOP, which suggests high value experiments for characterization of a specific pathway. Because the assessment is at the level of the KER and the calculation uses R-R functions, there can be continual improvement as more information becomes available. In specific situations, these relationships can be used in many different AOPs. When specific qKERs are refined experimentally, many related AOPs would benefit. When uncertainty is captured in the KERs, the probabilistic outcomes generated would support drawing appropriate conclusions about the level of risk.

Necessarily, future research would be required to provide best practices for the development of R-R relationships that inform qKERs. The fidelity and predictions of qAOPs developed using this approach should be compared to measured adverse outcomes,

Bayesian network analysis of systemic observations, and ongoing weight of evidence analyses in order to determine the utility of models developed using this methodology. Describing the transfer of response to response at each KE in the chain leading to an adverse outcome can provide for the rapid development of screening-level qAOPs.

References

- Alano, C. C., Beutner, G., Dirksen, R. T. et al. (2002). Mitochondrial permeability transition and calcium dynamics in striatal neurons upon intense NMDA receptor activation. *J Neurochem* 80, 531-538. doi:10.1046/j.0022-3042.2001.00738.x
- Ankley, G. T. and Giesy, J. P. (1998). Endocrine disruptors in wildlife: A weight of evidence perspective. In R. Kendall, R. Dickerson, W. Suk and J. P. Giesy (eds.), *Principles and Processes for Assessing Endocrine Disruption in Wildlife* (349-368). Pensacola, FL, USA: SETAC Press.
- Ankley, G. T., Kahl, M. D., Jensen, K. M. et al. (2002). Evaluation of the aromatase inhibitor fadrozole in a short-term reproduction assay with the fathead minnow (*Pimephales promelas*). *Toxicol Sci* 67, 121-130. doi:10.1093/toxsci/67.1.121
- Ankley, G. T., Miller, D. H., Jensen, K. M. et al. (2008). Relationship of plasma sex steroid concentrations in female fathead minnows to reproductive success and population status. *Aquat Toxicol* 88, 69-74. doi:10.1016/j.aquatox.2008.03.005
- Becker, R. A., Ankley, G. T., Edwards, S. W. et al. (2015). Increasing scientific confidence in adverse outcome pathways: Application of tailored Bradford-Hill considerations for evaluating weight of evidence. *Regul Toxicol Pharmacol* 72, 514-537. doi:10.1016/j.yrtph.2015.04.004
- Belton, V. and Stewart, T. (2002). *Multiple Criteria Decision Analysis: An Integrated Approach*. Norwell, MA, USA: Kluwer Academic Publishers. doi:10.1007/978-1-4615-1495-4
- Berman, F. W., LePage, K. T. and Murray, T. F. (2002). Domoic acid neurotoxicity in cultured cerebellar granule neurons is controlled preferentially by the NMDA receptor Ca^{2+} influx pathway. *Brain Res* 924, 20-29. doi:10.1016/S0006-8993(01)03221-8
- Breen, M., Villeneuve, D. L., Ankley, G. T. et al. (2013). Developing predictive approaches to characterize adaptive responses of the reproductive endocrine axis to aromatase inhibition: II. Computational modeling. *Toxicol Sci* 133, 234-247. doi:10.1093/toxsci/kft067
- Chen, H. S., Pellegrini, J. W., Aggarwal, S. K. et al. (1992). Open-channel block of N-methyl-D-aspartate (NMDA) responses by memantine: Therapeutic advantage against NMDA receptor-mediated neurotoxicity. *J Neurosci* 12, 4427-4436. doi:10.1523/JNEUROSCI.12-11-04427.1992
- Collier, Z. A., Gust, K. A., Gonzalez-Morales, B. et al. (2016). A weight of evidence assessment approach for adverse outcome pathways. *Regul Toxicol Pharmacol* 75, 46-57. doi:10.1016/j.yrtph.2015.12.014
- Conolly, R. B., Ankley, G. T., Cheng, W. et al. (2017). Quantitative adverse outcome pathways and their application to predictive toxicology. *Environ Sci Technol* 51, 4661-4672. doi:10.1021/acs.est.6b06230



- Hicks, R. R., Smith, D. H., Lowenstein, D. H. et al. (1993). Mild experimental brain injury in the rat induces cognitive deficits associated with regional neuronal loss in the hippocampus. *J Neurotrauma* 10, 405-414. doi:10.1089/neu.1993.10.405
- Jaworska, J., Dancik, Y., Kern, P. et al. (2013). Bayesian integrated testing strategy to assess skin sensitization potency: From theory to practice. *J Appl Toxicol* 33, 1353-1364. doi:10.1002/jat.2869
- Keeney, R. L. and Raiffa, H. (1976). *Decisions with Multiple Objectives: Preferences and Value Tradeoffs*. New York, USA: Wiley.
- Kim, S., Li, H., Dougherty, E. R. et al. (2002). Can Markov chain models mimic biological regulation? *J Biol Syst* 10, 337-357. doi:10.1142/S0218339002000676
- Lefebvre, K. A. and Robertson, A. (2010). Domoic acid and human exposure risks: A review. *Toxicol* 56, 218-230. doi:10.1016/j.toxicol.2009.05.034
- Miller, D. H., Jensen, K. M., Villeneuve, D. L. et al. (2007). Linkage of biochemical responses to population-level effects: A case study with vitellogenin in the fathead minnow (*Pimephales promelas*). *Environ Toxicol Chem* 26, 521-527. doi:10.1897/06-318R.1
- Nichols, J. W., Breen, M., Denver, R. J. et al. (2011). Predicting chemical impacts on vertebrate endocrine systems. *Environ Toxicol Chem* 30, 39-51. doi:10.1002/etc.376
- OECD (2017). Revised Guidance Document on Developing and Assessing Adverse Outcome Pathways. *OECD Environment, Health and Safety Publications, Series on Testing and Assessment No. 184*. <https://bit.ly/1RJXOEx>
- OECD (2018). Users' Handbook Supplement to the Guidance Document for Developing and Assessing AOPs. *OECD Series on Adverse Outcome Pathways No.1, OECD Publishing, Paris*. doi:10.1787/5jlv1m9d1g32-en
- Perkins, E. J., Gayen, K., Shoemaker, J. E. et al. (2019). Chemical hazard prediction and hypothesis testing using quantitative adverse outcome pathways. *ALTEX* 36, 91-102. doi:10.14573/altex.1808241
- Pirone, J. R., Smith, M., Kleinstreuer, N. C. et al. (2014). Open source software implementation of an integrated testing strategy for skin sensitization potency based on a Bayesian network. *ALTEX* 31, 336-340. doi:10.14573/altex.1310151
- Preston, E. and Hynie, I. (1991). Transfer constants for blood-brain barrier permeation of the neuroexcitatory shellfish toxin, domoic acid. *Can J Neurol Sci* 18, 39-44. doi:10.1017/S0317167100031279
- Rycroft, T., Massey, O., Foran, C. M. and Linkov, I. (2018). Weight of Evidence Frameworks in Evaluation of Adverse Outcome Pathways. In N. Garcia-Reyero and C. Murphy (eds.), *A Systems Biology Approach to Advancing Adverse Outcome Pathways for Risk Assessment* (303-316). Cham, Switzerland: Springer. doi:10.1007/978-3-319-66084-4_15
- Sachana, M., Munn, S. and Bal-Price, A. (2016). Binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment. AOP-Wiki. <https://aopwiki.org/aops/48> (accessed 28.06.2018).
- Shoemaker, J. E., Gayen, K., Garcia-Reyero, N. et al. (2010). Fathead minnow steroidogenesis: In silico analyses reveals tradeoffs between nominal target efficacy and robustness to cross-talk. *BMC Syst Biol* 4, 89. doi:10.1186/1752-0509-4-89
- U.S. EPA (2015). BMDs 2.6.0 User Manual. National Center for Environmental Assessment (NCEA), US Environmental Protection Agency, Arlington, Virginia. https://www.epa.gov/sites/production/files/2015-11/documents/bmds_manual.pdf (accessed 12.27.2016).
- Villeneuve, D. L., Crump, D., Garcia-Reyero, N. et al. (2014a). Adverse outcome pathway (AOP) development I: Strategies and principles. *Toxicol Sci* 142, 312-320. doi:10.1093/toxsci/kfu199
- Villeneuve, D. L., Crump, D., Garcia-Reyero, N. et al. (2014b). Adverse outcome pathway development II: Best practices. *Toxicol Sci* 142, 321-330. doi:10.1093/toxsci/kfu200
- Villeneuve, D. L. (2016). Aromatase inhibition leading to reproductive dysfunction. AOP-Wiki. <https://aopwiki.org/wiki/index.php/Aop:25> (accessed 15.03.2017).
- Wittwehr, C., Aladjov, H., Ankley, G. et al. (2017). How adverse outcome pathways can aid the development and use of computational prediction models for regulatory toxicology. *Toxicol Sci* 155, 326-336. doi:10.1093/toxsci/kfw207
- Wood, M. D., Bostrom, A., Bridges, T. and Linkov, I. (2012). Cognitive mapping tools: Review and risk management needs. *Risk Anal* 32, 1333-1348. doi:10.1111/j.1539-6924.2011.01767.x
- Yoon, M. and Clewell, H. J., 3rd (2016). Addressing early life sensitivity using physiologically based pharmacokinetic modeling and in vitro to in vivo extrapolation. *Toxicol Res* 32, 15. doi:10.5487/TR.2016.32.1.015

Competing financial interests

The authors declare they have no actual or potential competing financial interests.

Acknowledgement

Permission was granted by the Chief of Engineers to publish this material. The views and opinions expressed in this paper are those of the individual authors and not those of the U.S. Army, or other sponsor organizations. This work was supported by the project "NexGen predictive ecotoxicology toolbox for assessment of military compounds," funded by the US Army Environmental Quality Research Program.