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LINKING MITOCHONDRIAL DYSFUNCTION TO ORGANISMAL AND POPULATION HEALTH IN CONTEXT OF ENVIRONMENTAL POLLUTANTS: PROGRESS AND CONSIDERATIONS FOR MITOCHONDRIAL ADVERSE OUTCOME PATHWAYS

David A. Dreier¹, Danielle Mello², Joel Meyer², Christopher J. Martyniuk^{1,3,*}

¹Center for Environmental and Human Toxicology, Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, 32611 USA

²Nicholas School of the Environment, Duke University, Durham, NC, 27708-0328 USA

³University of Florida Genetics Institute, Interdisciplinary Program in Biomedical Sciences Neuroscience, University of Florida, Gainesville, FL, 32611 USA

Abstract

Mitochondria are key targets of many environmental contaminants, as specific chemicals can interact directly with mitochondrial proteins, lipids, and ribonucleic acids. These direct interactions serve as molecular initiating events (MIEs) that impede ATP production and other critical functions that mitochondria serve within the cell (e.g., calcium and metal homeostasis, apoptosis, immune signaling, redox balance). A limited but growing number of adverse outcome pathways (AOPs) have been proposed to associate mitochondrial dysfunction with effects at organismal and population levels. These pathways involve key events (KEs), such as altered membrane potential, mitochondrial fission/fusion, and mtDNA damage, among others. This critical review and analysis reveal current progress on AOPs involving mitochondrial dysfunction and identifies, using a network-based computational approach, the localization of mitochondrial MIEs and KEs within multiple existing AOPs. We also present two case studies: one examining the interaction between mitochondria and immunotoxicity, and a second case study examining the role of early mitochondrial dysfunction in the context of behavior (i.e., locomotor activity). We discuss limitations in current mitochondrial AOPs and highlight opportunities for improving their clarity and detail. Advancing our knowledge regarding KE relationships within the AOP framework will require high-throughput datasets that permit the development and testing of chemical-agnostic AOPs, and high-resolution research that will enhance mechanistic testing and validation of these KE relationships. Given the wide range of chemicals that affect mitochondria, and the centrality of energy production and signaling to ecologically important outcomes such as pathogen defense, homeostasis, growth, and reproduction, mitochondrial AOPs are expected to play a significant, if not central, role in environmental toxicology.

*Address correspondence to cmartyn@ufl.edu.

Keywords

Mitochondria; Adverse outcome pathways; High-throughput screening; Immune toxicity; Locomotion; Weight of evidence

1. MITOCHONDRIA ARE MOLECULAR TARGETS OF ENVIRONMENTAL CONTAMINANTS

Mitochondria are organelles responsible for the majority of energy production within the cell. Within the mitochondria, hundreds of proteins participate in oxidative phosphorylation (OXPHOS), beta-oxidation, the citric acid cycle, and other metabolic pathways, all of which are required to ensure the cell has sufficient ATP and metabolic intermediates to support diverse cellular functions. The generation of ATP is arguably the primary function of the mitochondria, but the roles of this organelle are diverse and include calcium regulation, detoxification, regulation of apoptosis, and steroidogenesis, among others. Mitochondria also act as a signaling platform for the immune system (West et al. 2011), redox homeostasis (Lee et al. 2012), and cellular regeneration (Vendemiale et al. 1995), and acts much like a control center for the cell to ensure proliferation and survival (Mandal et al. 2011). The integrity of mitochondrial function is imperative, as demonstrated by the multitude of human diseases resulting from mitochondrial dysfunction and oxidative damage (Chan 2006; Gorman et al. 2015).

The mitochondrial electron transport chain (ETC) is responsible for integrating signals and creating a proton gradient across the inner mitochondrial membrane, which is essential to ensure adequate energy production for the cell. The ETC comprises several mitochondrial-membrane associated protein complexes. These are referred to as Complex I (NADH:ubiquinone oxidoreductase), Complex II (succinate dehydrogenase), Complex III (cytochrome bc1 complex), and Complex IV (cytochrome c oxidase) as well as proteins, such as ubiquinone and cytochrome C, that carry or shuttle electrons between complexes. ATP synthase, sometimes referred to as Complex V, uses the electrochemical gradient created by the ETC for ATP production via OXPHOS. This system of electron transport must remain intact for energy homeostasis, and perturbations in the process by chemicals can affect mitochondrial bioenergetics, leading to many changes including altered redox imbalance, mitochondrial membrane potential, ATP/ADP ratio, and reactive oxygen species (ROS) production. Known environmental contaminants and pharmaceutical compounds can block each of the five mitochondrial complexes. To take just a few examples, pesticides that affect Complex I include Rotenone and Fenazaquin (Hollingworth et al. 1994), while the mycotoxin beta-Nitropropionic acid is an inhibitor of Complex II (Huang et al. 2006). Environmental pollutants that affect Complex III include dieldrin (Bergen 1971) and pyraclostrobin (Luz et al. 2018; Nicodemo et al. 2018), while chemicals that affect Complex IV include carbon monoxide and cyanide (Cooper and Brown 2008). Lastly, Complex V or ATP synthase can be affected by pharmacological inhibitors such as oligomycin (Symersky et al. 2012) and dicyclohexylcarbodiimide or DCCD (Penefsky 1985). Thus, there is a diverse group of chemicals that interact with specific proteins of the ETC and ATP synthase.

Other mitochondrial macromolecules, processes, and structures can also be targeted by chemicals, such as mitochondrial DNA, citric acid cycle, ribosomal and other non-ETC proteins, and the mitochondrial membrane itself, among others. For example, arsenic inhibits many mitochondrial (as well as extra-mitochondrial) proteins, including pyruvate dehydrogenase (Luz et al. 2016). Triclosan uncouples mitochondria (Weatherly et al. 2016), and lipophilic chemicals such as chloroanilines can impair membrane integrity via polar narcosis (Argese et al. 2001). Similarly, mitochondria are important for maintaining calcium homeostasis in the cell, and narcotic chemicals can affect membrane integrity and calcium transport, leading to basal toxicity (Antczak et al. 2015). In addition, paraquat and related bipyridyls enhance generation of ROS by redox cycling at several sites within the ETC (Drechsel and Patel 2009). Emerging chemicals, including phthalates (Rosado-Berrios et al. 2011), fungicides (Olsvik et al. 2010), and flame retardants (Pereira et al. 2014), among others, continue to be characterized as mitochondrial toxicants, although their mechanisms of action are still unclear and require investigation. Indeed, there has been rapid growth recently in our understanding of the importance of mitochondria as targets of environmental contaminants (Meyer and Chan 2017; Meyer et al. 2018), including pharmaceuticals (Nadanaciva and Will 2011), to which aquatic species in particular are often exposed at relatively high levels.

2. ADVERSE OUTCOME PATHWAYS FOR MITOCHONDRIAL DYSFUNCTION: CURRENT STATUS

Adverse outcome pathways (AOPs) offer a useful framework to better conceptualize and characterize toxicity pathways involving mitochondria, and to connect these effects to organism or population-level impacts. These pathways involve one or more key events (KE) linking a molecular initiating event (MIE) to an adverse outcome (AO) through a series of causal key-event relationships (KERs) (Villeneuve et al. 2014a). Here, we note that MIEs and AOs are specialized KEs and may be classified accordingly. Based on these structural features, AOPs are modular and can be used to link KEs into larger AOP networks (Knapen et al. 2018). To illustrate the current state of the science for AOPs involving mitochondria (mtAOPs), we identified AOPs involving KEs for various hallmarks of mitochondrial dysfunction (we use the phrase “mitochondrial dysfunction” broadly here, to encompass mtDNA damage, altered OXPHOS, altered signaling, etc.) from the AOPWiki (Table 1). Furthermore, we used these pathways to build an exploratory mtAOP network (Figure 1). Methods for AOP extraction and network construction are provided in the supplemental information.

There are numerous KEs related to mitochondrial dysfunction, which were represented in various AOP constructs. Among 224 AOPs in the wiki, 29 included KEs related to mitochondrial dysfunction, which were combined to build a concise network structure (Figure 1). In this mtAOP network, there were several cases where KEs for some form of mitochondrial interference or dysfunction also represented a MIE (e.g., KE 1260 “Direct mitochondrial inhibition”; KE 1446 “Increase, Uncoupling of oxidative phosphorylation”; KE 1481 “Inhibition of mitochondrial DNA polymerase gamma”), thus signifying direct mitochondrial toxicity. However, in most of the current mitochondrial AOPs, mitochondrial

dysfunction was an intermediate KE linked to both upstream and downstream KEs. For example, KE 40 “Decrease, Mitochondrial ATP production” was a network hub associated with numerous pathways. In addition, the network was used to identify potential downstream consequences of mitochondrial dysfunction. For example, “parkinsonian motor deficits”, “growth inhibition”, “kidney toxicity”, “mortality”, and “reproduction decline” were AOs for specific AOPs including mitochondria dysfunction as a KE (Table 1). At a larger scale, existing KEs can be combined to develop novel pathways between MIEs and AOs (Knapen et al. 2018). For example, the mitochondrial “hub” represented by KE 40 linked several MIEs (e.g., MIE 51, 244, 257, 1446, 1471, 1481, 1516) to AOs (e.g., AO 328, 350, 814, 1480, 1521), thereby elucidating multiple putative toxicity pathways. Although these events are potentially linked, here we note it is important to examine causality and define critical paths for toxicity (Villeneuve et al. 2018). Taken together, this organization indicates that the current state of knowledge identifies mitochondria as involved with multiple AOPs, primarily as an intermediate KE.

While AOPs offer a useful framework to explore mitochondrial toxicity, there are several limitations with the current status of mtAOPs. First, we note that KEs for mitochondrial dysfunction can be highly redundant. For example, several KEs in the network were described as “N/A, Mitochondrial dysfunction 1”, “Damaging, Mitochondria”, “Injury, Mitochondria”, “Mitochondrial impairment”, and “Dysfunction, Mitochondria”, which may represent a similar response. In the network structure, this led to segregation of AOPs that may in fact be linked. Thus, a common syntax should be applied for mitochondrial dysfunction in the AOPWiki to consolidate these events and develop novel pathways. However, to prevent information loss, the domain of applicability for these consolidated KEs (i.e., tissue, taxonomic-specific effects) should also be annotated to aid with network refinement and weight of evidence evaluations. Furthermore, while the literature demonstrates that there are many potential MIEs for mitochondrial dysfunction conceptually, this level of resolution was not reflected in the AOPWiki. For example, KE 1261 “Mitochondrial impairment” was a component of AOP 205 “AOP from chemical insult to cell death”; while MIE 1260 “Direct mitochondrial inhibition” was offered in the AOP, additional mechanistic detail would refine this pathway. Thus, mtAOP development should include specific MIEs in order to identify direct mitochondrial toxicity. In addition, current pathways in the AOPWiki did not include several common hallmarks for mitochondrial dysfunction, such as altered mitochondrial membrane potential (MMP) or oxygen consumption rate (OCR). Finally, while mitochondrial dysfunction has been linked to several AOs in the wiki, it will be important to validate and expand these relationships to better understand downstream organismal and population responses. Thus, various approaches will be required to improve mtAOPs, discussed further below.

3. IDENTIFYING AND DEFINING DIRECT MITOCHONDRIAL TOXICITY

It is not always clear whether mitochondrial changes reflect a direct or indirect effect of a toxicant exposure—that is whether mitochondrial alterations are MIEs or KEs, respectively. Mitochondria are essential to numerous cellular processes, and altered function may result indirectly from perturbations of these other processes. For example, nuclear DNA damage can lead to apoptosis, which involves significant mitochondrial dysfunction. It is important

to distinguish these non-specific effects from direct mitochondrial toxicity. Fortunately, AOPs provide a useful way to conceptualize mode of action and develop testable hypotheses, especially when numerous pathways are possible. In this framework, direct mitochondrial toxicity would include mitochondrial MIEs, such as mtDNA damage, uncoupling, redox cycling, or inhibition of specific protein complexes (Figure 2). Ultimately, these MIEs affect the function of mitochondria, which can be reflected in parameters such as membrane potential, oxygen consumption rate, mitochondrial fission/fusion, mitochondrial biogenesis and removal, and ROS production (Figure 2). These processes are dynamic and highly integrated in order to preserve mitochondrial function, and in many cases show non-monotonic responses to stressors (Meyer et al. 2017). Thus, while alterations in these endpoints may be measured as KEs, the directionality of a change in, for example, mitochondrial content (which integrates mitochondrial biogenesis and removal) must be interpreted carefully. For example, in cardiomyocytes, the chemotherapeutic doxorubicin causes an increase in mitochondrial content at lower levels of exposure, and a decrease at higher levels (Yuan et al. 2016). A final caveat is that mitochondrial dysfunction itself may result in damage to mitochondrial molecules in a “feed-forward cycle”. For example, oxidative damage to DNA and proteins may result in additional dysfunction, leading to greater production of ROS, and so on. In this case, events we identify as MIEs may also serve as KEs; such effects may be illustrated as feedback arrows in KERs (Figure 2). Likewise, the possibility of a feed-forward loop involving neuroinflammation and dopaminergic neurodegeneration was identified in a mtAOP developed by Terron et al. (2018).

As mitochondria are indirectly affected by non-mitochondrial MIEs and KEs, it is important to include these targets in mtAOPs. Indeed, in our mtAOP network (Figure 1), it was evident there were many upstream events for mitochondrial dysfunction. Based on our analysis, these events included Ca^{2+} -ATPase inhibition, ionotropic glutamate receptor activation, nicotinic acetylcholine receptor activation, phospholipase A inhibition, endocytic lysosomal uptake, ROS generation, NADPH oxidase activation, protein alkylation, D1 protein blockage, and activation of several nuclear receptors (e.g., AhR, ER, PPAR α , LXR, CAR, PXR, FXR). Since AOPs are living documents, over time, additional KEs and MIEs will be associated with mitochondrial dysfunction. As specific MIEs are further defined for mitochondrial dysfunction, this information can be used to examine mode of action. An important question that remains unanswered is whether all mitochondrial MIEs ultimately converge on common KEs. For example: (1) a mitochondrial uncoupler may decrease MMP, decrease ROS, and decrease ATP:ADP ratio; (2) an ATP synthase inhibitor may increase MMP, increase ROS, but decrease ATP:ADP ratio; (3) and an ETC inhibitor may decrease MMP slightly, increase ROS, and still decrease ATP:ADP ratio. In these three scenarios, decreased ATP:ADP ratio is a common KE, but decreased MMP or increased ROS are not. Answering this question will help clarify whether there can be “a” mtAOP, or whether multiple pathways are needed.

Ultimately, a weight of evidence approach will be required to examine the contributions of specific toxicity pathways (e.g., ARE/NRF2) for mitochondrial dysfunction. Several criteria have been established to compare mode of action (i.e., contribution of different MIE/KEs for an AO) within the AOP framework (Mihaich et al. 2017), which can also be employed to

identify mitochondrial toxicants. Specifically, modified Bradford-Hill criteria can be used to examine the strength of KERs in terms of biological plausibility, essentiality, and empirical evidence (Becker et al. 2015). Similarly, AOPs can be used to integrate multiple lines of evidence across different levels of biological organization (Ankley et al. 2010). Thus, AOPs offer a useful framework to describe both specific and non-specific effects on mitochondria.

4. INCORPORATING HIGH-THROUGHPUT SCREENING DATA

Several high-throughput screening assays have examined mitochondrial dysfunction, and these data may be useful to identify mitochondrial toxicants. High-throughput screening assays have improved our understanding of molecular and cellular responses to environmental chemicals, and these assays can be integrated in the AOP framework to better characterize MIEs and KEs for specific toxicity pathways (Tollefsen et al. 2014). While many of these assays have focused on endocrine-mediated events, there are a growing number of assays examining other toxicity pathways, such as mitochondrial dysfunction. For example, the ToxCast and Tox21 programs include several assays examining various aspects of mitochondrial bioenergetics and function, including mitochondrial content, MMP, and oxidative stress (Wills 2017). In ToxCast, high-content imaging has been used to characterize changes in mitochondrial content and membrane potential from chemical exposures (Shah et al. 2016). Similarly, high-throughput cellular respirometry has enabled screening of ToxCast chemicals for changes in mitochondrial-linked OCR (Wills et al. 2015), and an MMP assay has been deployed in Tox21 (Attene-Ramos et al. 2015). Put together, these assays offer an increasingly integrative perspective on mitochondrial responses to environmental chemicals, including in some cases sophisticated dose-responses capable of distinguishing adaptive from toxic responses. Accordingly, identifying alterations in these parameters can then direct the experimentalist to more mechanistic analysis (discussed below).

However, while these types of assays have been leveraged in tiered testing approaches (Xia et al. 2018), they have not been used in AOP development. Based on our compilation of mtAOPs, it is evident many responses examined in such assays (e.g., altered MMP, mitochondrial content, OCR) were not included in many pathways. In Figure 2, we highlight where current ToxCast assays can be incorporated in the AOP framework for mitochondria. Because these assays have screened large chemical libraries, this information is useful to validate AOPs across multiple compounds with various levels of specific pathway activity. In addition, it is important to point out that while these assays provide a rich source of information on chemicals that interact with mtAOPs, one must recognize these assays have a specific taxonomic domain of applicability. Ecotoxicology considers a broad range of wildlife, including aquatic and terrestrial invertebrates and vertebrates. Mitochondrial assays in the Tox21 and ToxCast programs are mammalian based and predominantly focused on humans and rodents; thus caution must be taken when extrapolating to non-model species (LaLone et al. 2018). For example, a chemical that antagonizes human ETC proteins may not do so with the same potency in another species. In these cases, advances in comparative bioinformatics, such as the Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool, may help refine the domain of applicability for MIEs involving conserved molecular targets (e.g., mitochondrial protein complexes) (LaLone et al. 2016). Despite

limitations, these cell-based assays provide data that can be reasonably used to narrow down MIEs for chemicals in non-model species.

5. LINKING MITOCHONDRIAL DYSFUNCTION TO DOWNSTREAM EVENTS

As mitochondria are central to cellular physiology and energetics, there are many KEs and AOs affected by mitochondrial dysfunction, and it is intuitive that energetic defects are likely to impact many organismal functions including growth, reproduction, and survival (Sokolova 2013). Studies are beginning to describe AOs in non-mammalian species for mitochondrial bioenergetic perturbations and apical endpoints, such as development (Cao et al. 2019), growth (Bolser et al. 2018), and locomotor activity (Zhang et al. 2017). Other apical endpoints, such as reproduction, are also expected to be affected by mitochondrial dysfunction and should be included in the construction of mtAOPs (Figure 2). In many cases, it can be challenging to integrate endpoints from various levels of biological organization, as illustrated in the examples described below.

Recent studies have pointed to mitochondria as crucial regulators of several components of the immune system (Lartigue and Faustin 2013; Monlun et al. 2017). Mitochondrial metabolic reprogramming and molecular signals are needed to support immune responses during bacterial and viral infections, as well as sterile cellular insults. Although this link is well established in mammalian models, studies directly connecting toxicant-induced mitochondrial dysfunction and disturbances in immune function are lacking. Nevertheless, there is a growing number of studies in mammalian and non-mammalian species demonstrating that immunotoxicity and inflammation are important outcomes of exposure to several pollutants, many of which are associated with mitochondrial dysfunction (e.g. heavy metals, pesticides, polycyclic aromatic hydrocarbons, nanoparticles, and others) (Renault 2015; West 2017). For example, arsenic is known to elicit mitochondrial ROS production, mtDNA damage, and loss of MMP, among other effects (Prakash et al. 2016; Luz et al. 2017); it is also associated with several immune-related disorders in organisms from different taxa (Lage et al. 2006). In zebrafish, low levels of arsenic decreased the ability of embryos to clear bacterial and viral pathogens (Nayak et al. 2007). Despite evidence that different mitochondrial toxicants may lead to aberrant immune responses, it is challenging to define clear KERs between mitochondrial dysfunction and immune system AOs, given the current level of mechanistic understanding. The immune system is composed of several components and pathways that, together with mitochondria, can be direct targets of pollutants. Moving forward, there is a significant need for knowledge regarding the direct targets of contaminants, as well as the pathways involved in the cross-talk between mitochondria and the immune system.

In a second example, pesticides have been screened for mitochondrial dysfunction in early embryonic zebrafish to determine how OXPHOS is related to developmental programming and larval behavior. Locomotor activity in fish larvae can be important for predator avoidance and for early feeding, and is intimately related to individual fitness. Herbicides (Wang et al. 2018a), fungicides (Cao et al. 2018; Perez-Rodriguez et al. 2018; Wang et al. 2018b), and biocides can affect mitochondrial bioenergetics in developing embryos, in particular basal respiration. This can be associated with changes in the prevalence of

deformities and hypoactivity in larvae, and one hypothesis for these AOs is lower energy availability as the process of development and locomotion require ATP. However, it is challenging to draw a direct link between mitochondrial dysfunction and deformities or impaired locomotor activity, as experimentally testing these KEs can be confounded by other factors (i.e., genetics, general chemical toxicity), as well as temporal separation in the endpoints measured (i.e., early assessments of OXPHOS in embryos being extrapolated to growth or behavior). Nevertheless, it is increasingly important to strengthen knowledge as to the relationships between mitochondrial dysfunction, ATP levels, and ecologically relevant endpoints (e.g., developmental trajectories, growth, and behavior). AOPs therefore must accommodate the effects of genetic differences among individuals (Luz et al. 2017), and in long-term, incorporate epigenetic effects for mitochondrial toxicity (Ditzel et al. 2016; Weinhouse 2017; Cheikhi et al. 2018).

6. MECHANISTIC APPROACHES TO CONSTRUCT AOPS FOR MITOCHONDRIAL DYSFUNCTION

Moving forward, experimental validation of mtAOPs will be essential. Mechanistic data are necessary to strengthen or refute mitochondrial dysfunction as MIEs or KEs in AOPs, and to resolve many of the questions addressed above (e.g., Is the mitochondrial effect direct or indirect? Does inhibition of a specific protein decrease or increase MMP? Is there a non-monotonic dose-response? How does this mitochondrial-induced event relate to those observed in other cases of mitochondrial toxicity?). One experimental approach is to utilize multiple chemicals that impair the same mitochondria-associated protein and measure the same phenotypic response. For example, Rotenone and Fenazaquin are both inhibitors of Complex I in the ETC. Convergence of these and other Complex I inhibitors on an AO such as dopaminergic neurodegeneration (Terron et al. 2018) would strengthen the AOP. Genetic knockdown or overexpression, pharmacological rescue or exacerbation, and detailed analysis of (dys)function of specific mitochondrial proteins or processes, and many other techniques (reviewed in detail: (Will and Dykens 2018)) can all be helpful in determining causality. Transcriptomic profiling (Pearson et al. 2016) and *in silico* methods, such as quantitative structure-activity relationship modeling (Dreier et al. 2019), may also be useful to identify novel compounds affecting specific mitochondrial targets. On the same note, mechanistic studies comparing organisms from different taxa would also offer important contributions, not only for strengthening mtAOPs, but also in identifying species-specific effects. Ideally, these specific effects would build on a “Core AOP” that reflects the highly conserved structure and function of mitochondria between species.

CONCLUSIONS

It is increasingly apparent that mitochondria are a key target for environmental contaminants, and mitochondrial function is unmistakably critical for many ecologically-relevant outcomes. This highlights that the AOP framework may be valuable for understanding important ecological impacts of many chemicals. Thus, we encourage environmental toxicologists to explore the strategies, principles, and best practices for AOP development (Villeneuve et al. 2014a; Villeneuve et al. 2014b), create and refine mtAOPs,

and incorporate them into public domains, such as the AOPWiki (<https://aopwiki.org/>). Moving forward, future research for mtAOPs should be guided by the following principles:

- i. Greater mechanistic detail is required to identify and define direct mitochondrial toxicity in the AOP framework;
- ii. High-throughput screening assays offer useful information to improve AOPs for mitochondrial toxicity;
- iii. A weight of evidence approach should be used to evaluate critical paths in AOP networks involving mitochondria;
- iv. Mitochondrial KEs require consolidation and refinement to resolve conserved AOPs from those with a specific domain of applicability;
- v. Species comparisons in mitochondrial biology and toxicology should be explicitly researched experimentally.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1: Adverse outcome pathway network involving key events related to mitochondrial dysfunction. Key events (KE) and key event relationships (KER) from the AOPWiki were used to construct the network (methods in supplemental information). KEs classified as molecular initiating events (MIE), KEs, or adverse outcomes (AO) are colored in green, orange, and red, respectively. The level of KER evidence (gray arrows) is indicated by edge weight, and mitochondrial KEs are highlighted by squares in the network. Node size is proportional to the number of upstream KERs.

Figure 2:

Conceptual adverse outcome pathway network for mitochondrial dysfunction. There are several molecular initiating events (MIEs) that affect hallmarks of mitochondrial dysfunction, which can lead to adverse outcomes for ecological endpoints. Additional MIEs, including but not limited to those mentioned in the text, such as citric acid cycle or beta-oxidation inhibition, may also be important in some circumstances. Gray arrows represent key event relationships, and dashed boxes highlight key events measured in ToxCast assays. Note that mitochondrial dysfunction may promote damage to its own molecules in a “feed-forward cycle” (large gray arrow).

Table 1:

List of key events (KEs), molecular initiating events (MIEs), and associated adverse outcome pathways (AOPs) related to mitochondrial dysfunction from the AOPWiki.

KE	KE Type	KE Name	AOP	AOP Name
40	KE	Decrease, Mitochondrial ATP production	26	Calcium-mediated neuronal ROS production and energy imbalance
40	KE	Decrease, Mitochondrial ATP production	238	Excessive reactive oxygen species production leading to reduced ATP production-associated reproduction decline
40	KE	Decrease, Mitochondrial ATP production	245	Uncoupling of photophosphorylation leading to reduced ATP production associated growth inhibition
40	KE	Decrease, Mitochondrial ATP production	258	Renal protein alkylation leading to kidney toxicity
40	KE	Decrease, Mitochondrial ATP production	265	D1 protein blockage leading to photosystem II (PSII)-inhibition associated growth reduction
40	KE	Decrease, Mitochondrial ATP production	267	Uncoupling of oxidative phosphorylation leading to mortality
40	KE	Decrease, Mitochondrial ATP production	268	Uncoupling of oxidative phosphorylation leading to reproduction decline
176	KE	Damaging, Mitochondria	34	LXR activation leading to hepatic steatosis
176	KE	Damaging, Mitochondria	207	NADPH oxidase and P38 MAPK activation leading to reproductive failure in <i>Caenorhabditis elegans</i>
177	KE	N/A, Mitochondrial dysfunction 1	3	Inhibition of the mitochondrial complex I of nigro-striatal neurons leads to parkinsonian motor deficits
177	KE	N/A, Mitochondrial dysfunction 1	48	Binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment.
177	KE	N/A, Mitochondrial dysfunction 1	77	Nicotinic acetylcholine receptor activation contributes to abnormal foraging and leads to colony death/failure 1
177	KE	N/A, Mitochondrial dysfunction 1	78	Nicotinic acetylcholine receptor activation contributes to abnormal role change within the worker bee caste leading to colony death failure 1
177	KE	N/A, Mitochondrial dysfunction 1	79	Nicotinic acetylcholine receptor activation contributes to impaired hive thermoregulation and leads to colony loss/failure
177	KE	N/A, Mitochondrial dysfunction 1	80	Nicotinic acetylcholine receptor activation contributes to accumulation of damaged mitochondrial DNA and leads to colony loss/failure
177	KE	N/A, Mitochondrial dysfunction 1	87	Nicotinic acetylcholine receptor activation contributes to abnormal foraging and leads to colony loss/failure
177	KE	N/A, Mitochondrial dysfunction 1	144	Endocytic lysosomal uptake leading to liver fibrosis
177	KE	N/A, Mitochondrial dysfunction 1	178	Nicotinic acetylcholine receptor activation contributes to mitochondrial dysfunction and leads to colony loss/failure
177	KE	N/A, Mitochondrial dysfunction 1	200	Estrogen receptor activation leading to breast cancer
178	KE	Disruption, Mitochondrial electron transport chain	26	Calcium-mediated neuronal ROS production and energy imbalance
179	KE	Decreased, Mitochondrial fatty acid beta-oxidation	36	Peroxisomal Fatty Acid Beta-Oxidation Inhibition Leading to Steatosis
179	KE	Decreased, Mitochondrial fatty acid beta-oxidation	60	NR1H2 (Pregnane X Receptor, PXR) activation leading to hepatic steatosis
447	KE	Reduction, Cholesterol transport in mitochondria	18	PPARalpha activation in utero leading to impaired fertility in males
447	KE	Reduction, Cholesterol transport in mitochondria	51	PPARalpha activation leading to impaired fertility in adult male rodents
451	KE	Inhibition, Mitochondrial fatty acid beta-oxidation	57	AhR activation leading to hepatic steatosis

KE	KE Type	KE Name	AOP	AOP Name
451	KE	Inhibition, Mitochondrial fatty acid beta-oxidation	58	NR1I3 (CAR) suppression leading to hepatic steatosis
451	KE	Inhibition, Mitochondrial fatty acid beta-oxidation	61	NFE2L2/FXR activation leading to hepatic steatosis
570	KE	Accumulation, Damaged mitochondrial DNA	80	Nicotinic acetylcholine receptor activation contributes to accumulation of damaged mitochondrial DNA and leads to colony loss/failure
664	KE	Overwhelmed, Mitochondrial DNA repair mechanisms	80	Nicotinic acetylcholine receptor activation contributes to accumulation of damaged mitochondrial DNA and leads to colony loss/failure
832	KE	Injury, Mitochondria	130	Phospholipase A inhibitors lead to hepatotoxicity
1260	MIE	Direct mitochondrial inhibition	205	AOP from chemical insult to cell death
1261	KE	Mitochondrial impairment	205	AOP from chemical insult to cell death
1446	KE	Increase, Uncoupling of oxidative phosphorylation	238	Excessive reactive oxygen species production leading to reduced ATP production-associated reproduction decline
1446	MIE	Increase, Uncoupling of oxidative phosphorylation	267	Uncoupling of oxidative phosphorylation leading to mortality
1446	MIE	Increase, Uncoupling of oxidative phosphorylation	268	Uncoupling of oxidative phosphorylation leading to reproduction decline
1477	KE	Decrease, Oxidative phosphorylation	245	Uncoupling of photophosphorylation leading to reduced ATP production associated growth inhibition
1481	MIE	Inhibition of mitochondrial DNA polymerase gamma (Pol gamma)	256	Inhibition of mitochondrial DNA polymerase gamma leading to kidney toxicity
1482	KE	Depletion, mtDNA	256	Inhibition of mitochondrial DNA polymerase gamma leading to kidney toxicity
1483	KE	Dysfunction, Mitochondria	256	Inhibition of mitochondrial DNA polymerase gamma leading to kidney toxicity
1483	KE	Dysfunction, Mitochondria	258	Renal protein alkylation leading to kidney toxicity