This review responds to revisions undertaken by the authors of the AOP263 "Uncoupling of oxidative phosphorylation leading to growth inhibition" and the accompanying ET&C manuscript in response to the initial review of the original submission that we have provided in May 2021. The four reviewers David Dreier, Ksenia Groh, Joel Meyer and Terry Schultz have jointly discussed, prepared, and approved the final review text below.

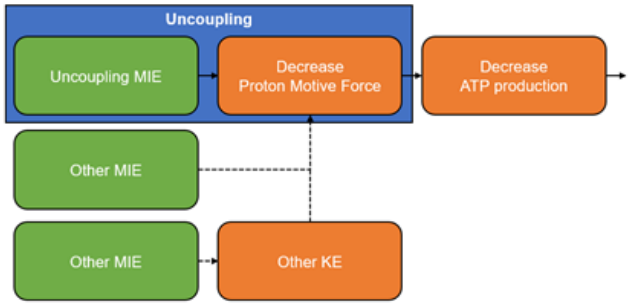
The reviewers feel that the authors have provided convincing explanations and/or revisions in response to most of the points raised in the initial review. We have only one major concern remaining, along with two other minor comments and a few suggestions for minor edits that we would like to share below.

Our major remaining concern is about the authors' argument that uncoupling should remain grouped ("lumped together") with decrease of proton motive force (PMF). We still think that dissipation of PMF should be included as a separate KE, as we recommended previously. We emphasize that decrease of PMF should be included as a KE, and not as an MIE—we cannot quite follow the authors' idea that loss of PMF leads to uncoupling, as we think it is actually the other way around, at least usually. With the latter point we specifically refer to the authors' statement "What really matters for downstream biological processes, in most cases, is not dissipation of the PMF itself, but dissociation of oxidation with phosphorylation *as the consequence* of PMF dissipation" (emphasis ours). We actually do not think there is clear evidence available to say that this is true.

In fact, it is the PMF itself (and not the downstream phosphorylation of ADP) that is critical for many biological processes (e.g., import of mitochondrial proteins; mitophagy; ion exchange; etc). In addition, a very strong evidence for the importance of maintaining membrane potential per se is the evolution of the possibility of cells burning ATP to run ATP synthase in reverse in order to maintain membrane potential.

We believe that some of this confusion may have derived from somewhat different literature definitions of the term "uncoupling"—e.g., in the Arnould review that the authors cite, it is defined as "a dissociation between mitochondrial membrane potential generation and its use for mitochondria-dependent ATP synthesis". However, more commonly, "uncoupling" is defined as uncoupling oxygen consumption from ATP production—which may or may not actually result in PMF loss. We were also wondering if another source of confusion regarding the sequence of events could stem from AOP311 (which is also being developed by one of the authors). Though that AOP is dealing with a slightly different mechanisms (and it is currently not open for review), we politely observe that there is certainly an opportunity to harmonize e.g. the names of some KEs there and we hope that our review for AOP263 could set a precedent for the required clarity of definitions.

Based on the considerations above, we maintain that uncoupling and PMF loss should not be "lumped" into one event. Our specific suggestion, depicted in the diagram below, would be to split the broader process of uncoupling (in blue) into a specific MIE and KE, with uncoupling (green MIE) leading to a decrease in the PMF (orange KE).



The authors said that they want to group this into a broader process, because "uncoupling of OXPHOS is normally not directly measurable." However, we believe that having PMF decrease listed as a separate KE would not preclude using the respective measurements to inform this particular AOP. Hence, we still think it would be valuable to have the PMF dissipation as a separate KE, as this would allow accommodating other upstream MIEs/KEs (uncoupling or otherwise) and provide an opportunity for future research instead of artificially limiting the possibilities to link additional MIEs/KEs to this particular AOP. Again, we do understand the desire to group these events for the sake of simplicity, but hope nonetheless that the authors will also see this is as an opportunity to "future-proof" their AOP.

Provided the authors agree to follow the suggestions above, sentence on page 3, lines 109-110 of the revised manuscript would also need to be revised. Namely, from "partitioning of protonophores (uncouplers) into the inner mitochondrial membrane is known to uncouple OXPHOS by dissipating proton motive force, leading to reduced ATP synthesis" to "partitioning of protonophores (uncouplers) into the inner mitochondrial membrane is known to uncouple OXPHOS, leading to dissipation of proton motive force and subsequent reduction in ATP synthesis."

Lastly to the above-discussed topic, we invite the authors to consider whether it is justified to postulate the "coupling of OXPHOS, decrease" as an MIE, although it is in fact preceded by another—truly initiating—event, which authors have also identified themselves, namely the "partitioning of protonophores (uncouplers) into the inner mitochondrial membrane"? Categorizing the uncoupling of OXPHOS as a KE instead of an MIE would align with the fact that it can be caused by several different mechanisms apart from the protonophores-dependent one. Hence, this could essentially create "space" for other MIEs to feed into the same KE "decreased coupling of OXPHOS".

Next, with regard to the authors' response to our comment number 16 in the initial review, we must say that we still do not completely understand the reasons for the authors' decision to not include population decline as a second AO in their AOP, despite the fact that, as they state themselves, "that particular linkage has been long accepted within the field of ecotoxicology to the point where it is accepted as canonical knowledge." If this is "canonical knowledge" indeed, then why wouldn't the authors acknowledge this and add the second AO? In other words, we were wondering if there are no other AOPs in the AOPwiki currently that have already sufficiently characterized the link from the AO "growth, decrease" to the AO "population, decline"? And if yes, could the authors "reuse" this particular relationship in their own AOP? After all, the possibility to "reuse" the already-existing KEs and KERs is one of the main advantages offered by the AOPwiki, hence we feel that the authors could have made a conscious effort to promote this practice.

Lastly, with regard to the authors' response to our comment number 15 in the initial review, we would like to share that we still feel that the descriptions accompanying this AOP continue to have a strong environmental bias, while human health applications are less visible. This is okay in the end, as this simply reflects the authors' main expertise. We, however, suggest that the authors consider adding a clear upfront statement acknowledging this and explicitly postulating that this AOP does have both the environmental and human health application.

Minor edits suggested:

Page 3, line 102: insert "on" before "growth" to have "focuses on growth inhibition"

Page 6, line 221: delete "that" before "not every"; should become "There can also be large tissue-specific effects and not every cell type is equally susceptible […]"