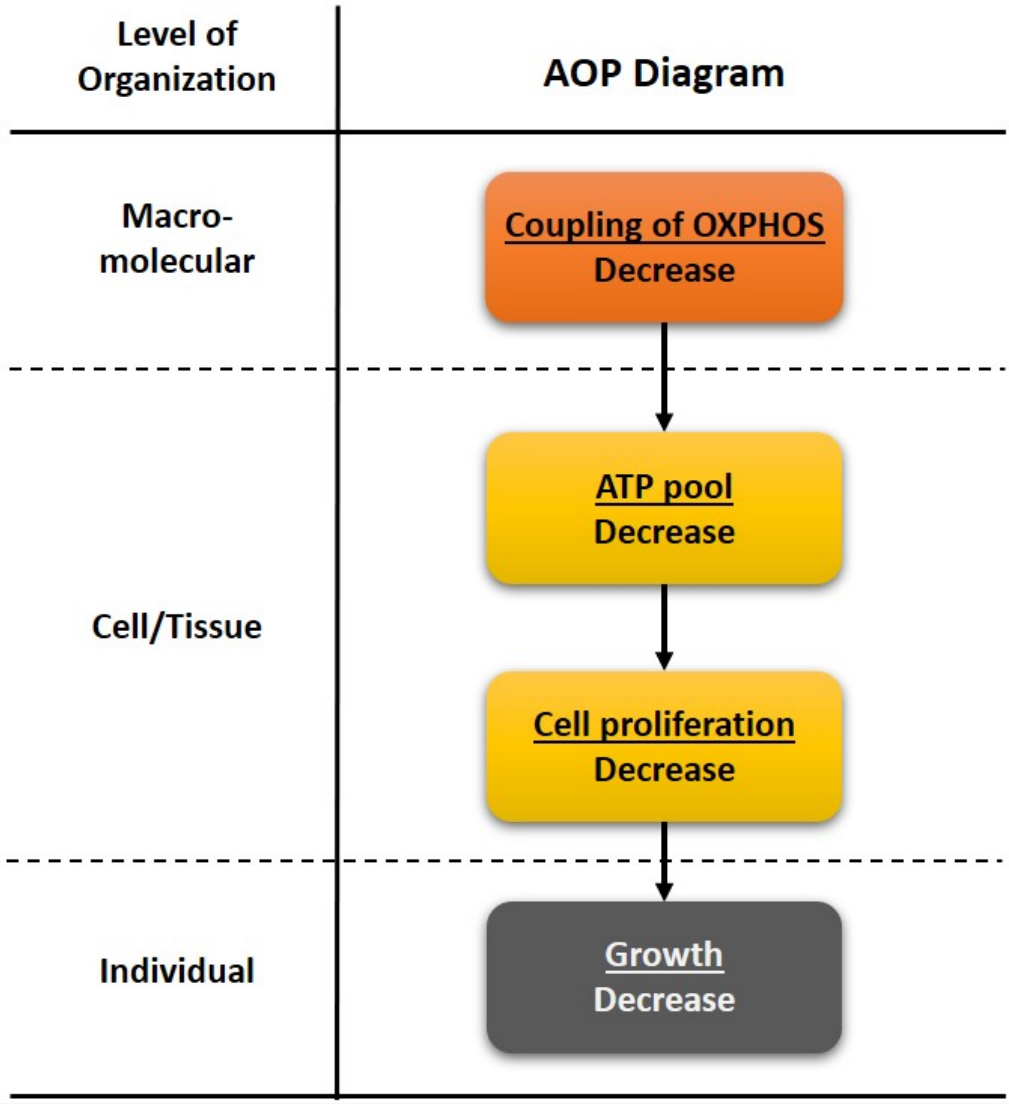
AOP ID and Title:

AOP 263: Uncoupling of oxidative phosphorylation leading to growth inhibition via decreased cell proliferation
Short Title: Uncoupling of OXPHOS leading to growth inhibition 1

Graphical Representation



Authors

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Status

Author status	OECD status	OECD project	SAAOP status
Open for citation & comment	WPHA/WNT Endorsed	1.92	Included in OECD Work Plan

Abstract

Uncoupling of oxidative phosphorylation (OXPHOS) is a well-known mechanism of action of many chemicals. Mitochondrial uncoupler-mediated energetic dysfunction is known to affect growth, a critical process in most organisms and a chronic toxicity endpoint included in many OECD test guidelines. This adverse outcome pathway (AOP) causally links uncoupling of OXPHOS to growth inhibition, through ATP depletion and reduced cell proliferation as the intermediate key events (KEs), with strong weight of evidence support. The AOP is generalized to reflect its expected applicability to a broad range of taxa, ranging from microalga to human. Three out of four KEs included can be quantified using high-throughput methods, making this AOP particularly useful for screening, prioritization and hazard assessment of mitochondrial uncouplers as potential growth inhibiting chemicals. This AOP is of high regulatory relevance, as it is considered applicable to both human health and ecological risk assessments. The AOP also forms the core of a larger AOP network addressing uncoupling of OXPHOS mediated growth inhibition (AOP 263-268).

AOP Development Strategy

Context

The mitochondrial OXPHOS machinery is a key physiological process responsible for producing the primary cellular energy, adenosine triphosphate (ATP). During OXPHOS, a series of redox reactions (oxidation) are mediated by protein complexes in an electron transport chain to create a protonmotive force (PMF) across the inner mitochondrial membrane (Liberman 1969). The PMF acts as a driving force of ATP synthesis through phosphorylation of adenosine diphosphate (ADP). Mitochondrial oxidation and phosphorylation are coupled to ensure continuous ATP supply for various physiological processes. A number of chemicals can bind to the inner mitochondrial membrane and dissipate the PMF, thus leading to uncoupling of OXPHOS and reduction in ATP synthetic efficiency. Classical “uncouplers” are normally protonophores with major characteristics of bulky hydrophobic moiety, an acid dissociable group and a strong electron-withdrawing group (Terada 1990). With the rapid development of in silico (Russom 1997; Schultz 1997; Naven 2012; Dreier 2019; Troger 2020) and in vitro (Escher 2002; Attene-Ramos 2013; Attene-Ramos 2015; Xia 2018) approaches, more and more uncouplers have been identified. However, their hazards to biota remain to be assessed. Uncoupling of OXPHOS can affect many ATP-dependent biological functions. In particular, cell proliferation as a major process to achieve organismal growth is positively correlated with the cellular ATP level and highly susceptible to energy depletion (Ramaiah 1964; Bonora 2012). Therefore, a link between uncoupling of OXPHOS and growth inhibition can be established with ATP depletion and reduced cell proliferation as the intermediate steps.

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
	MIE	1446	Decrease, Coupling of oxidative phosphorylation	Decrease, Coupling of OXPHOS
	KE	1771	Decrease, Adenosine triphosphate pool	Decrease, ATP pool
	KE	1821	Decrease, Cell proliferation	Decrease, Cell proliferation
	AO	1521	Decrease, Growth	Decrease, Growth

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Decrease, Coupling of oxidative phosphorylation	adjacent	Decrease, Adenosine triphosphate pool	High	High
Decrease, Adenosine triphosphate pool	adjacent	Decrease, Cell proliferation	Moderate	Moderate
Decrease, Cell proliferation	adjacent	Decrease, Growth	Moderate	Moderate

Stressors

Name	Evidence

Name	Evidence
2,4-Dinitrophenol	High
Pentachlorophenol	High
Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone	High
Carbonyl cyanide m-chlorophenyl hydrazone	High
Triclosan	High
Dinoseb	Moderate
3,5-Dichlorophenol	Moderate
Emodin	High
Arsenite	High
Niclosamide ethanolamine	Moderate
Oxyclozanide	Moderate

Overall Assessment of the AOP

The weight of evidence (WoE) assessment of the AOP was conducted based on the evolved Bradford-Hill considerations (Becker 2015) and according to the criteria in OECD's Guidance Document for Developing and Assessing AOPs (OECD 2018). In terms of evidence for the essentiality of the key events, the MIE (Event 1446) and KE1 (Event 1771) were scored as high, whereas KE2 (Event 1821) was scored as moderate due to a lack of solid evidence to support its essentiality. The overall WoE of KER1 (Relationship 2203) is considered high, as strong biological plausibility, empirical evidence and fairly good quantitative understanding were evidenced from multiple studies. The overall WoE of KER2 (Relationship 2204) is considered moderate, due to high biological plausibility, acceptable empirical concordance and some biological understanding. The overall WoE of KER3 (Relationship 2205) is scored as moderate, mainly due to biological plausibility, but there is presently a lack of empirical evidence and quantitative understanding to further support causality. The AOP is considered applicable to a wide range of species as well as a broad domain of chemicals. The rationales for making these judgements will be discussed in detail in the following sections.

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Embryo	High
Juvenile	High
Adult	High

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI
Lemna minor	Lemna minor	Moderate	NCBI
human	Homo sapiens	Moderate	NCBI
mouse	Mus musculus	High	NCBI
rat	Rattus norvegicus	High	NCBI
Caenorhabditis elegans	Caenorhabditis elegans	Moderate	NCBI

Sex Applicability

Sex	Evidence
Unspecific	High

The taxonomic application domain of the AOP potential covers all animals, plants and some microorganisms such as fungus and protists, as mitochondrial OXPHOS is highly conserved in eukaryotes (Roger 2017).

The life stage applicability domain of the AOP mainly contains embryos and juveniles, as growth is more relevant to developing organisms. It should be noted that fully grown adults are also susceptible to uncouplers, as tissue/organ (e.g., adipose tissue) growth and regeneration still occur in adults (Yun 2015; Demine 2019). Classical uncouplers such as 2,4-DNP have been reported to cause weight loss in adult humans (Grundlingh 2011). In fact, 2,4-DNP was sold for weight loss until its legal sale was banned over toxicity and abuse concerns (Baker 2020). These suggest that

adults are in the applicability domain of this AOP.

The sex applicability domain of the AOP is unspecific, as the AOP is mainly targeting growth effects in sexually immature organisms and the KEs are therefore harmonized between male and females. However, male and females may have different sensitivities to OXPHOS uncoupling, as strategies for allocating energy for developmental processes may be gender specific (Demarest 2015).

The chemical applicability domain of the AOP mainly includes weak acids, such as phenols, benzimidazoles, N-phenylanthranilates, salicylanilides, phenylhydrazones, salicylic acids, acyldithiocarbazates, cumarines, and aromatic amines, which are well-known protonophoric uncouplers. Uncouplers typically have properties as both weak acids and hydrophobic substances. As weak acids, they are capable of gaining and losing an electron. As hydrophobic substances, they are capable of distributing a negative charge over a number of atoms (often by π-orbitals which delocalize a proton's charge when it attaches to the molecule), so that they can diffuse back and forth across the inner mitochondrial membrane in either the charged or uncharged state, thus moving protons back across the concentration gradient generated by the electron transport chain. Classical uncouplers, such as 2,4-dinitrophenol (2,4-DNP), carbonyl cyanide-p-trifluoromethoxyphenyl hydrazone (FCCP), carbonyl cyanide m-chlorophenyl hydrazone (CCCP), pentachlorophenol (PCP), 3,5-dichlorophenol (3,5-DCP), 6-sec-butyl-2,4-dinitrophenol (dinoseb), SF 6847 (3,5-di-t-butyl-4-hydroxybenzylidinemalononitrile) have been widely used as positive controls in (eco)toxicological tests, whereas the hazards of “new” uncouplers, such as triclosan, emodin and metabolites of polybrominated diphenyl ethers (PBDEs) are also under extensive assessments. Other types of uncouplers that are SH-reactive chemicals or hydrophobic ions may also be in the applicability domain of this AOP. A number of potential uncouplers have been identified by in silico (Russom 1997; Schultz 1997; Naven 2012; Dreier 2019; Troger 2020) and in vitro (Escher 2002; Attene-Ramos 2013; Attene-Ramos 2015; Xia 2018) approaches, and are considered in the chemical applicability domain of the AOP.

Essentiality of the Key Events

<table>
<tr><td rowspan="4">Support for Essentiality of KEs</td><td>Defining Question</td><td>What is the impact on downstream KEs and/or the AO if an upstream KE is modified or prevented?</td></tr>
<tr><td>High</td><td>Direct evidence from specifically designed experimental studies illustrating prevention or impact on downstream KEs and/or the AO if upstream KEs are blocked or modified.</td></tr>
<tr><td>Moderate</td><td>Indirect evidence that modification of one or more upstream KEs is associated with a corresponding (increase or decrease) in the magnitude or frequency of downstream KEs.</td></tr>
<tr><td>Low</td><td>No or contradictory experimental evidence of the essentiality of any of the KEs.</td></tr>
<tr><td>Event 1446:

(Decrease, Coupling of OXPHOS)</td><td colspan="2">Essentiality of Event 1446 is high.

Rationale: There is direct evidence from several specifically designed studies showing that removal of an uncoupler from exposure, or addition of a “recoupler” can lead to recovery of the mitochondrial membrane potential (MMP) and total ATP caused by the uncoupler.

Evidence:
• Removal of the classical uncoupler carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) led to recovery of both MMP and ATP in rat cerebellar granule cells (Weisová 2012).
• In the red abalone (Haliotis rufescens) larvae, removal of the uncoupler pentachlorophenol also led to recovery of the ATP level (Shofer 2002).
• Addition of the recoupler GDP led to a rapid increase in ATP/ADP ratio in isolated guinea pig brown-adipose-tissue mitochondria where high activities of natural coupling by the UCPs were expected (Rafael 1976).
• Addition of octanoate to 2,4-DNP exposed rat hepatocytes mitigated the uncoupling effect and partial restored the ATP/ADP ratio (Sibille 1995).
• Removal of FCCP led to recovery from FCCP-mediated MMP and ATP reduction in Swiss mouse embryos (Zander-Fox 2015).</td></tr>
</table>

Event 1771: **(Decrease, ATP pool)**	**Essentiality of Event 1771 is** moderate. **Rationale:** There is limited direct evidence from specifically designed studies. However, multiple lines of indirect evidence show that modulation of ATP levels by uncouplers can also lead to corresponding changes in cell proliferation. **Evidence**: • Addition of emodin blunted ATP-induced cell proliferation in a concentration-dependent manner in human lung adenocarconoma (A549) cells (Wang 2017), hence providing direct evidence to support the essentiality of this KE. • Positive relationships between uncoupler-mediated ATP depletion and reduced cell proliferation have been documented by multiple studies(Sweet 1999; Fine 2009; Guimarães 2012; Sugiyama 2019).
Event 1821: **(Decrease, Cell proliferation)**	**Essentiality of Event 1821 is** moderate. **Rationale:** There is no direct evidence from specifically designed studies to support this KE. However, there are multiple lines of indirect evidence showing positive relationships between cell proliferation and growth. **Evidence**: • Indirect evidence can be obtained from a limited number of relevant studies showing a positive role of cell proliferation in mammalian tumor (Figarola 2018) zebrafish embryo growth(Bestman 2015). • Indirect evidence showing that the mitochondrial uncouplers niclosamide ethanolamine and oxyclozanide either completely prevented or drastically reduced hepatic metastasis of colon cancer cells from spleen (Alasadi et al., 2018).
Inconsistencies & uncertainties	There is an uncertainty related to KE1446 that mild uncoupling of OXPHOS may also increase the ATP pool in some cases (Desquiret 2006), possibly as a compensatory response. The underlying mechanism remains to be further elucidated.

Weight of Evidence Summary

Biological plausibility

Support for Biological Plausibility of KERs	**Defining Question**	**Is there a mechanistic (i.e., structural or functional) relationship between KE_{up} and KE_{down} consistent with established biological knowledge?**
	High	**Extensive understanding based on extensive previous documentation and broad acceptance -Established mechanistic basis.**
	Moderate	**The KER is plausible based on analogy to accepted biological relationships but scientific understanding is not completely established.**
	Low	**There is empirical support for a statistical association between KEs, but the structural or functional relationship between them is not understood.**
Relationship 2203: **(Decrease, Coupling of OXPHOS leads to Decrease, ATP pool)**	**Biological Plausibility of Relationship 2203 is** high. **Rationale:** In eukaryotic cells, the major metabolic pathways responsible for ATP production are OXPHOS, citric acid (TCA) cycle, glycolysis and photosynthesis. Oxidative phosphorylation is much (theoretically 15-18 times) more efficient than the rest due to high energy derived from oxygen during aerobic respiration (Schmidt-Rohr 2020). As the ATP level is relatively balanced between production and consumption (Bonora 2012), ATP depletion is a plausible consequence of reduced ATP synthetic efficiency following uncoupling of OXPHOS.	
Relationship 2204: **(Decrease, ATP pool leads to Decrease, Cell proliferation)**	**Biological Plausibility of Relationship 2204 is** high. **Rationale:** Cell proliferation is a well-known ATP-dependent process. Cell division processes, such as the mitotic cell cycle uses ATP for chromosome movements and DNA replication (Kingston 1999). The synthetic processes of major cellular components that are necessary for cell structure and growth, such as proteins and lipids, also require sufficient ATP supply (Bonora 2012). Depletion of ATP therefore has a negative impact on these processes.	

Relationship 2205: **(Decrease, Cell proliferation leads to Decrease, Growth)**	**Biological Plausibility of Relationship 2205 is** high. **Rationale:** The biological causality between cell proliferation and growth has also been well established. It is commonly accepted that the size of an organism, organ or tissue is dependent on the total number and volume of the cells it contains, and the amount of extracellular matrix and fluids (Conlon 1999). Impairment to cell proliferation can logically affect tissue and organismal growth.
Inconsistencies & uncertainties	There are currently no inconsistencies and uncertainties identified by the authors.

Empirical support

Empirical Support for KERs	**Defining Question**	**Does KE_{up} occur at lower doses and earlier time points than KE down and at the same dose of stressor, is the incidence of KE_{up} >than that for KE_{down}? Are there inconsistencies in empirical support across taxa, species and stressors that don't align with expected pattern for hypothesized AOP?**
	High	**Multiple studies showing dependent change in both events following exposure to a wide range of specific stressors. (Extensive evidence for temporal, dose- response and incidence concordance) and no or few critical data gaps or conflicting data.**
	Moderate	**Demonstrated dependent change in both events following exposure to a small number of specific stressors and some evidence inconsistent with expected pattern that can be explained by factors such as experimental design, technical considerations, differences among laboratories, etc.**
	Low	**Limited or no studies reporting dependent change in both events following exposure to a specific stressor (i.e., endpoints never measured in the same study or not at all); and/or significant inconsistencies in empirical support across taxa and species that don't align with expected pattern for hypothesised AOP.**
Relationship 2203: **(Decrease, Coupling of OXPHOS leads to Decrease, ATP pool)**	**Empirical support of Relationship 2203 is** high. **Rationale:** The majority of relevant studies show good incidence, temporal and/or dose concordance in different organisms and cell types after exposure to known uncouplers, with relatively few exceptions (see the Relationship 2203 page and concordance table for detailed evidence).	
Relationship 2204: **(Decrease, ATP pool leads to Decrease, Cell proliferation)**	**Empirical support of Relationship 2204 is** moderate. **Rationale:** Although only a few studies were found to be relevant, incidence concordance was found for mammalian cells (see the Relationship 2204 page and concordance table for detailed evidence).	
Relationship 2205: **(Decrease, Cell proliferation leads to Decrease, Growth)**	**Empirical support of Relationship 2205 is** low. **Rationale:** This KER was included in a very limited number of studies, as it addresses effects occurring at the apical level that *in vitro* studies cannot cover. There is one zebrafish study reporting concordant relationship between reduced cell proliferation and embryo growth with some inconsistencies (see the Relationship 2205 page and concordance table for detailed evidence).	
Inconsistencies & uncertainties	There are some inconsistencies regarding temporal and dose concordance: • A significant decrease followed by a significant increase of total ATP (KE1) was observed in human RD cells during a 48h exposure to the uncoupler FCCP(Kuruvilla 2003), possibly due to the enhancement of other ATP synthetic pathways (e.g., glycolysis) as a compensatory action to impaired OXPHOS (Jose 2011). • In zebrafish embryos exposed to 2,4-DNP, significant growth inhibition (AO) was identified after 21h, whereas non-significant reductions in ATP (KE1) and cell proliferation (KE2) were reported(Bestman 2015).	

Quantitative Consideration

Quantitative understanding of the KERs	**High**	**Change in $KE_{downstream}$ can be precisely predicted based on a relevant measure of $KE_{upstream}$. Uncertainty in the quantitative prediction can be precisely estimated from the variability in the relevant measure of $KE_{upstream}$. Known modulating factors and feedback/feedforward mechanisms are accounted for in the quantitative description. There is evidence that the quantitative relationship between the KEs generalises across the relevant applicability domain of the KER.**
	Moderate	**Change in $KE_{downstream}$ can be precisely predicted based on a relevant measure of $KE_{upstream}$. Uncertainty in the quantitative prediction is influenced by factors other than the variability in the relevant measure of $KE_{upstream}$. Quantitative description does not account for all known modulating factors and/or known feedback/feedforward mechanisms. The quantitative relationship has only been demonstrated for a subset of the overall applicability domain of the KER (e.g., based on a single species).**
	Low	**Only a qualitative or semi-quantitative prediction of the change in $KE_{downstream}$ can be determined from a measure of $KE_{upstream}$. Known modulating factors and/or known feedback/feedforward mechanisms are not accounted for. The quantitative relationship has only been demonstrated for a narrow subset of the overall applicability domain of the KER (e.g., based on a single species).**
Relationship 2203: **(Decrease, Coupling of OXPHOS leads to Decrease, ATP pool)**	**Quantitative understanding of Relationship 2203 is** high. **Rationale:** The theoretical quantitative relationship between OXPHOS and ATP yield has been well established. There are also published computational/mathematical models in which modulating factors known to affect OXPHOS and ATP synthesis are considered. **Evidence:** • A biophysical computational model developed for mitochondrial respiration and OXPHOS (Beard 2005). • Continuous development of the mitochondrial energy transduction models since 1967 (Schmitz 2011). • A comprehensive mathematical model developed for OXPHOS and ATP production under different physiological and pathological conditions (Heiske 2017). • A comprehensive analysis of the quantitative relationships between protonmotive force, ATP synthase rotation, ATP synthesis and hydrolysis (Kubo 2020). • A regression based response-response relationship for uncoupling of OXPHOS and ATP depletion (Song 2020).	
Relationship 2204: **(Decrease, ATP pool leads to Decrease, Cell proliferation)**	**Quantitative understanding of Relationship 2204 is** moderate. **Rationale:** The total ATP level has been used as an indicator of cell proliferation. Several studies have reported the quantitative relationships between the two events, as well as a threshold value for KE1 to trigger KE2. However, not all modulating factors have been accounted and no well-established computational/mathematical models are found. **Evidence**: • Quantitative understanding of ATP level, cell viability and colony growth (Ahmann 1987). • Quantitative relationship between ATP level and cell proliferation (Crouch 1993). • Thresholds for ATP depletion (85-90% reduction) to determine cell cycle arrest (<85-90%) or cell death (>85-90%) (Nieminen 1994).	
Relationship 2205: **(Decrease, Cell proliferation leads to Decrease, Growth)**	**Quantitative understanding of Relationship 2205 is** moderate. **Rationale:** Multiple mathematical models describing the quantitative relationships between cell proliferation and tissue growth exist for both animals (Binder 2008) and plants (Mosca 2018). There are also numerous models that are specifically developed for predicting tumor growth based on the proliferation rate (Jarrett 2018). However, there is currently a lack of quantitative model to link cell proliferation and individual growth in the presence of uncouplers. Evidence: • A mathematical model developed for describing the quantitative relationship between cell proliferation and tissue growth (Binder 2008). • A mathematical model developed for cell division and plant tissue growth (Mosca 2018). • Multiple mathematical models developed for cell proliferation and tumor growth (Jarrett 2018).	

Considerations for Potential Applications of the AOP (optional)

The present AOP has several potential applications. First, the AOP anchors a recognized endpoint of regulatory concern (i.e., growth), at least in OECD member countries, and is directly relevant for a number of OECD test guidelines (e.g., TG206, 208, 201, 210, 211, 212, 215, 221, 228, 241, 407, 408, 416, 422, 443 and 453). These guidelines cover a diversity of taxonomic groups including mammals, birds, fish, amphibians, terrestrial plants, aquatic plants and algae, and various invertebrates. Second, the AOP anchors an important molecular initiating event (e.g., uncoupling of oxidative phosphorylation) and can be used to support several initiatives (e.g., Tox21 and ToxCast) for identification of mitochondrial toxicants. The present AOP helps establish the utility of such assays for identifying chemicals with potential to cause growth impacts. Third, three out of four key events in this AOP can be measured using high-throughput *in vitro* assays, hence offering a tiered testing strategy (i.e.,*in silico→in vitro→in vivo*) or integrated approaches to testing and assessment (IATA) for efficient screening, classification and assessment of potential mitochondrial uncouplers and growth-regulating chemicals. The key events can be considered as useful biomarkers in (eco)toxicological studies. However, it is not recommended to use a single key event (e.g., ATP level alone) as a biomarker for classification and hazard assessment of chemicals, as key events such as decreased ATP pool and cell proliferation can also be the consequences of other biological processes. A combined measurement of 2-3 key events can normally yield more reliable results. Finally, the quantitative relationships of the key events in this AOP have been relatively well defined, allowing it to be further developed into quantitative prediction models for higher tier assessments. This is a range of potential applications that were conceived during the development of the present AOP. However, it is neither an exhaustive list of potential applications, nor can explicit examples of these applications in practice be cited at this time.

We invite users of this AOP to share their applications of this AOP via the Discussion so that practical examples of use can be added.

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Appendix 1

List of MIEs in this AOP

Event: 1446: Decrease, Coupling of oxidative phosphorylation

Short Name: Decrease, Coupling of OXPHOS

Event Component

Process	Object	Action
proton binding	mitochondrion	increased
oxidative phosphorylation uncoupler activity	mitochondrion	increased
regulation of mitochondrial membrane potential	mitochondrion	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:267 - Uncoupling of oxidative phosphorylation leading to growth inhibition via glucose depletion	MolecularInitiatingEvent
Aop:263 - Uncoupling of oxidative phosphorylation leading to growth inhibition via decreased cell proliferation	MolecularInitiatingEvent
Aop:264 - Uncoupling of oxidative phosphorylation leading to growth inhibition via ATP depletion associated cell death	MolecularInitiatingEvent
Aop:265 - Uncoupling of oxidative phosphorylation leading to growth inhibition via increased cytosolic calcium	MolecularInitiatingEvent
Aop:266 - Uncoupling of oxidative phosphorylation leading to growth inhibition via decreased Na-K ATPase activity	MolecularInitiatingEvent
Aop:268 - Uncoupling of oxidative phosphorylation leading to growth inhibition via mitochondrial swelling	MolecularInitiatingEvent
Aop:534 - Succinate dehydrogenase (SDH) inhibition leads to oxidative stress	KeyEvent
Aop:331 - Increase in reactive oxygen species leading to growth inhibition via lipid peroxidation and cell death	KeyEvent
Aop:596 - Excessive reactive oxygen species leading to growth inhibition via protein oxidation and cell injury/death	KeyEvent
Aop:598 - Excessive reactive oxygen species leading to growth inhibition via protein oxidation and reduced cell proliferation	KeyEvent
Aop:612 - Peroxisome proliferator-activated receptor alpha activation leading to early life stage mortality via reduced adenosine triphosphate	KeyEvent
Aop:613 - Peroxisome proliferator-activated receptor alpha activation leading to early life stage mortality via increased reactive oxygen species production	KeyEvent
Aop:326 - Increase in reactive oxygen species leading to growth inhibition via lipid peroxidation and decreased cell proliferation	KeyEvent
Aop:332 - Increase in reactive oxygen species leading to growth inhibition via protein oxidation and decreased cell proliferation	KeyEvent
Aop:333 - Increase in reactive oxygen species leading to growth inhibition via protein oxidation and cell death	KeyEvent

Stressors

Name
2,4-Dinitrophenol
Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone
Carbonyl cyanide m-chlorophenyl hydrazone
Pentachlorophenol
Triclosan
Emodin
Malonoben

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
cell

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI
human	Homo sapiens	High	NCBI
mouse	Mus musculus	High	NCBI
rat	Rattus norvegicus	High	NCBI
Lemna minor	Lemna minor	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Embryo	High
Juvenile	High
Adult, reproductively mature	Moderate

Sex Applicability

Sex	Evidence
Unspecific	High

Taxonomic applicability domain

This key event is in general considered applicable to most eukaryotes, as the mitochondrion and oxidative phosphorylation are highly conserved (Roger 2017).

Life stage applicability domain

This key event is considered applicable to all life stages, as ATP synthesis by oxidative phosphorylation is an essential biological process for most living organisms.

Sex applicability domain

This key event is considered sex-unspecific, as both males and females use oxidative phosphorylation as a main process to generate ATP.

Key Event Description

Decreased coupling of oxidative phosphorylation (OXPHOS), or uncoupling of OXPHOS, describes dissipation of protonmotive force (PMF) across the inner mitochondrial membrane (IMM) by environmental stressors. In eukaryotes, the mitochondrial electron transport chain mediates a series of redox reactions to create a PMF across the IMM. The PMF is used as energy to drive adenosine triphosphate (ATP) synthesis through phosphorylation of adenosine diphosphate (ADP). These processes are coupled and referred to as OXPHOS. A number of chemicals can dissipate the PMF, leading to uncoupling of OXPHOS. This key event describes the main outcome of the interactions between an uncoupler and the transmembrane PMF. An uncoupler can bind to a proton in the mitochondrial inter membrane space, transport the proton to the matrix side of the IMM, release the proton and move back to the inter membrane space. These processes are repeated until the transmembrane PMF is dissipated. This KE is therefore a lumped term of these processes and represents the final consequence of the interactions.

How it is Measured or Detected

Uncoupling of oxidative phosphorylation can be indicated by reduced mitochondrial membrane potential, increased proton leak and/or increased oxygen consumption rate.

- Mitochondrial membrane potential can be determined using ToxCast high-throughput screening bioassays such as “APR_HepG2_MitoMembPot”, “APR_Hepat_MitoFxnI”, and “APR_Mitochondrial_membrane_potential”, and the Tox21 high-throughput screening assay “tox21-mitotox-p1”.
- Mitochondrial membrane potential can also be measured using commercially available fluorescent probes such

as TMRM (tetramethylrhodamine, methyl ester, perchlorate), TMRE (tetramethylrhodamine, ethyl ester, perchlorate) and JC-1 (Perry 2011).
- Proton leak and oxygen consumption rate can be measured using a high-resolution respirometry (Affourtit 2018) or a Seahorse XF analyzer (Divakaruni 2014).

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List of Key Events in the AOP

Event: 1771: Decrease, Adenosine triphosphate pool

Short Name: Decrease, ATP pool

Event Component

Process	Object	Action
ATP biosynthetic process	ATP	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:328 - Excessive reactive oxygen species production leading to mortality (2)	KeyEvent
Aop:329 - Excessive reactive oxygen species production leading to mortality (3)	KeyEvent
Aop:264 - Uncoupling of oxidative phosphorylation leading to growth inhibition via ATP depletion associated cell death	KeyEvent
Aop:263 - Uncoupling of oxidative phosphorylation leading to growth inhibition via decreased cell proliferation	KeyEvent
Aop:290 - Mitochondrial ATP synthase antagonism leading to growth inhibition (1)	KeyEvent
Aop:291 - Mitochondrial ATP synthase antagonism leading to growth inhibition (2)	KeyEvent
Aop:286 - Mitochondrial complex III antagonism leading to growth inhibition (1)	KeyEvent
Aop:287 - Mitochondrial complex III antagonism leading to growth inhibition (2)	KeyEvent
Aop:266 - Uncoupling of oxidative phosphorylation leading to growth inhibition via decreased Na-K ATPase activity	KeyEvent
Aop:331 - Increase in reactive oxygen species leading to growth inhibition via lipid peroxidation and cell death	KeyEvent
Aop:596 - Excessive reactive oxygen species leading to growth inhibition via protein oxidation and cell injury/death	KeyEvent
Aop:598 - Excessive reactive oxygen species leading to growth inhibition via protein oxidation and reduced cell proliferation	KeyEvent
Aop:599 - Excessive reactive oxygen species leading to growth inhibition via fatty acid oxidation and cell injury/death	KeyEvent
Aop:600 - Excessive reactive oxygen species leading to growth inhibition via fatty acid oxidation and reduced cell growth	KeyEvent
Aop:601 - Excessive reactive oxygen species leading to growth inhibition via fatty acid oxidation and reduced cell proliferation	KeyEvent
Aop:612 - Peroxisome proliferator-activated receptor alpha activation leading to early life stage mortality via reduced adenosine triphosphate	KeyEvent
Aop:326 - Increase in reactive oxygen species leading to growth inhibition via lipid peroxidation and decreased cell proliferation	KeyEvent
Aop:332 - Increase in reactive oxygen species leading to growth inhibition via protein oxidation and decreased cell proliferation	KeyEvent
Aop:333 - Increase in reactive oxygen species leading to growth inhibition via protein oxidation and cell death	KeyEvent

Stressors

Name
Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone
Carbonyl cyanide m-chlorophenyl hydrazone
2,4-Dinitrophenol

Name
Malonoben
Pentachlorophenol
Triclosan
Emodin

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
cell

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI
human	Homo sapiens	High	NCBI
rat	Rattus norvegicus	High	NCBI
mouse	Mus musculus	High	NCBI
Lemna minor	Lemna minor	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Embryo	High
Juvenile	High
Adult, reproductively mature	Moderate

Sex Applicability

Sex	Evidence
Unspecific	High

Taxonomic applicability domain

This key event is in general considered applicable to all eukaryotes utilizing ATP as a direct source of energy and signaling molecule.

Life stage applicability domain

This key event is considered applicable to all life stages, as all developmental stages require energy supply to maintain necessary physiological processes.

Sex applicability domain

This key event is considered sex-unspecific, as both males and females use ATP as an essential energy molecule.

Key Event Description

Decreased adenosine triphosphate (ATP) pool describes the loss of balance between ATP synthesis and ATP consumption, leading to reduced total ATP. As a primary form of biological energy, ATP is used by many biological

processes (Bonora 2012). Decrease in ATP level normally attributes to metabolic disorders in major ATP synthetic pathways, such as mitochondrial oxidative phosphorylation, fatty acid β-oxidation, glycolysis and plant photophosphorylation.

How it is Measured or Detected

-The ATP pool in cells or tissue can be quantified using a well-established ATP bioluminescent assay (Lemasters 1978; Wibom 1990). Assay principles: ATP can react with luciferase and luciferin from firefly and the luminescence emitted from the reaction is proportional to the ATP concentration:

ATP + D-Luciferin + O_2 è Oxyluciferin + AMP + PPi + CO_2 + Light

-ToxCast high-throughput screening bioassays, such as “NCCT_HEK293T_CellTiterGLO” and “NIS_HEK293T_CTG_Cytotoxicity” can be used to measure this KE.

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Event: 1821: Decrease, Cell proliferation

Short Name: Decrease, Cell proliferation

Event Component

Process	Object	Action
cell proliferation	cell	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:263 - Uncoupling of oxidative phosphorylation leading to growth inhibition via decreased cell proliferation	KeyEvent
Aop:290 - Mitochondrial ATP synthase antagonism leading to growth inhibition (1)	KeyEvent
Aop:286 - Mitochondrial complex III antagonism leading to growth inhibition (1)	KeyEvent
Aop:399 - Inhibition of Fyna leading to increased mortality via decreased eye size (Microphthalmos)	KeyEvent
Aop:460 - Antagonism of Smoothened receptor leading to orofacial clefting	KeyEvent
Aop:267 - Uncoupling of oxidative phosphorylation leading to growth inhibition via glucose depletion	KeyEvent
Aop:491 - Decrease, GLI1/2 target gene expression leads to orofacial clefting	KeyEvent
Aop:502 - Decrease, cholesterol synthesis leads to orofacial clefting	KeyEvent
Aop:591 - DBDPE-induced DNA damage increase in liver leading to Non-alcoholic fatty liver disease via liver steatosis and inhibition of regeneration	KeyEvent
Aop:598 - Excessive reactive oxygen species leading to growth inhibition via protein oxidation and reduced cell proliferation	KeyEvent
Aop:602 - Excessive reactive oxygen species leading to growth inhibition via oxidative DNA damage	KeyEvent
Aop:603 - Excessive reactive oxygen species leading to growth inhibition via protein oxidation and cell cycle disruption	KeyEvent

AOP ID and Name	Event Type
Aop:601 - Excessive reactive oxygen species leading to growth inhibition via fatty acid oxidation and reduced cell proliferation	KeyEvent
Aop:324 - Increase in reactive oxygen species leading to growth inhibition via oxidative DNA damage and cell cycle disruption	KeyEvent
Aop:326 - Increase in reactive oxygen species leading to growth inhibition via lipid peroxidation and decreased cell proliferation	KeyEvent
Aop:332 - Increase in reactive oxygen species leading to growth inhibition via protein oxidation and decreased cell proliferation	KeyEvent

Stressors

Name
2,4-Dinitrophenol
Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone
Carbonyl cyanide m-chlorophenyl hydrazone
Pentachlorophenol
Triclosan
Emodin
Malonoben

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
cell

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI
human	Homo sapiens	High	NCBI
rat	Rattus norvegicus	High	NCBI
mouse	Mus musculus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Embryo	High
Juvenile	High

Sex Applicability

Sex	Evidence
Unspecific	High

Taxonomic applicability domain

This key event is in general applicable to all eukaryotes, as most organisms are known to use cell proliferation to achieve growth.

Life stage applicability domain

This key event is in general applicable to all life stages. As cell proliferation not only occurs in developing organisms, but also in adults.

Sex applicability domain

This key event is sex-unspecific, as both genders use the same cell proliferation mechanisms.

Key Event Description

Decreased cell proliferation describes the outcome of reduced cell division and cell growth. Cell proliferation is considered the main mechanism of tissue and organismal growth (Conlon 1999). Decreased cell proliferation has been associated with abnormal growth-factor signaling and cellular energy depletion (DeBerardinis 2008).

How it is Measured or Detected

Multiple types of *in vitro* bioassays can be used to measure this key event:

- ToxCast high-throughput screening bioassays such as “BSK_3C_Proliferation”, “BSK_CASM3C_Proliferation” and “BSK_SAg_Proliferation” can be used to measure cell proliferation status.
- Commercially available methods such as the well-established 5-bromo-2’-deoxyuridine (BrdU) (Raza 1985; Muir 1990) or 5-ethynyl-2’-deoxyuridine (EdU) assay. Both assays measure DNA synthesis in dividing cells to indicate proliferation status.

Update - non-endorsed (by You Song, 02/09/2026)

ToxCast/Tox21 assays (invitrodb v4.3): APR_HepG2_CellCycleArrest_*; APR_HepG2_CellLoss_*; IUF_NPC1b_proliferation_BrdU_*; BSK_3C_Proliferation

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List of Adverse Outcomes in this AOP

Event: 1521: Decrease, Growth

Short Name: Decrease, Growth

Event Component

Process	Object	Action
growth	multicellular organism	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:263 - Uncoupling of oxidative phosphorylation leading to growth inhibition via decreased cell proliferation	AdverseOutcome
Aop:290 - Mitochondrial ATP synthase antagonism leading to growth inhibition (1)	AdverseOutcome

AOP ID and Name	Event Type
Aop:291 - Mitochondrial ATP synthase antagonism leading to growth inhibition (2)	AdverseOutcome
Aop:286 - Mitochondrial complex III antagonism leading to growth inhibition (1)	AdverseOutcome
Aop:287 - Mitochondrial complex III antagonism leading to growth inhibition (2)	AdverseOutcome
Aop:245 - Reduction in photophosphorylation leading to growth inhibition in aquatic plants	AdverseOutcome
Aop:265 - Uncoupling of oxidative phosphorylation leading to growth inhibition via increased cytosolic calcium	AdverseOutcome
Aop:264 - Uncoupling of oxidative phosphorylation leading to growth inhibition via ATP depletion associated cell death	AdverseOutcome
Aop:266 - Uncoupling of oxidative phosphorylation leading to growth inhibition via decreased Na-K ATPase activity	AdverseOutcome
Aop:267 - Uncoupling of oxidative phosphorylation leading to growth inhibition via glucose depletion	AdverseOutcome
Aop:268 - Uncoupling of oxidative phosphorylation leading to growth inhibition via mitochondrial swelling	AdverseOutcome
Aop:473 - Energy deposition from internalized Ra-226 decay lower oxygen binding capacity of hemocyanin	AdverseOutcome
Aop:331 - Increase in reactive oxygen species leading to growth inhibition via lipid peroxidation and cell death	AdverseOutcome
Aop:596 - Excessive reactive oxygen species leading to growth inhibition via protein oxidation and cell injury/death	AdverseOutcome
Aop:598 - Excessive reactive oxygen species leading to growth inhibition via protein oxidation and reduced cell proliferation	AdverseOutcome
Aop:599 - Excessive reactive oxygen species leading to growth inhibition via fatty acid oxidation and cell injury/death	AdverseOutcome
Aop:600 - Excessive reactive oxygen species leading to growth inhibition via fatty acid oxidation and reduced cell growth	AdverseOutcome
Aop:602 - Excessive reactive oxygen species leading to growth inhibition via oxidative DNA damage	AdverseOutcome
Aop:603 - Excessive reactive oxygen species leading to growth inhibition via protein oxidation and cell cycle disruption	AdverseOutcome
Aop:601 - Excessive reactive oxygen species leading to growth inhibition via fatty acid oxidation and reduced cell proliferation	AdverseOutcome
Aop:567 - Binding to plastoquinone B site leading to decreased population growth rate via photosystem II inhibition	AdverseOutcome
Aop:324 - Increase in reactive oxygen species leading to growth inhibition via oxidative DNA damage and cell cycle disruption	AdverseOutcome
Aop:325 - Increase in reactive oxygen species leading to growth inhibition via oxidative DNA damage and cell death	AdverseOutcome
Aop:326 - Increase in reactive oxygen species leading to growth inhibition via lipid peroxidation and decreased cell proliferation	AdverseOutcome
Aop:332 - Increase in reactive oxygen species leading to growth inhibition via protein oxidation and decreased cell proliferation	AdverseOutcome
Aop:333 - Increase in reactive oxygen species leading to growth inhibition via protein oxidation and cell death	AdverseOutcome
Aop:647 - Increase in reactive oxygen species leading to growth inhibition via increased caspase activation	AdverseOutcome

Stressors

Name
2,4-Dinitrophenol
Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone

Name
Carbonyl cyanide m-chlorophenyl hydrazone
Pentachlorophenol
Triclosan
Emodin
Malonoben

Biological Context

Level of Biological Organization
Individual

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	NCBI
rat	Rattus norvegicus	Moderate	NCBI
mouse	Mus musculus	Moderate	NCBI
zebrafish	Danio rerio	High	NCBI
fathead minnow	Pimephales promelas	High	NCBI
Lemna minor	Lemna minor	High	NCBI
Daphnia magna	Daphnia magna	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
Embryo	High
Juvenile	High

Sex Applicability

Sex	Evidence
Unspecific	High

Taxonomic applicability domain

This key event is in general applicable to all eukaryotes.

Life stage applicability domain

This key event is applicable to early life stages such as embryo and juvenile.

Sex applicability domain

This key event is sex-unspecific.

Key Event Description

Decreased growth refers to a reduction in size and/or weight of a tissue, organ or individual organism. Growth is normally controlled by growth factors and mainly achieved through cell proliferation (Conlon 1999).

How it is Measured or Detected

Growth can be indicated by measuring weight, length, total volume, and/or total area of a tissue, organ or individual organism.

Revised version (non-endorsed, added by You Song, 02/09/2026)

Growth can be measured as a change in size, mass, biomass, length, area, volume, or growth rate of a tissue, organ, or whole organism over a defined exposure period. The appropriate measurement depends on the biological system, life stage, and test species.

At the organism level, growth is commonly assessed by measuring body weight, wet weight, dry weight, body length, standard length, total length, shell length, shoot height, root length, frond number, frond area, cell density, biomass, or relative growth rate. Growth inhibition is typically expressed as a statistically significant reduction relative to the control, or as an effect concentration causing a defined percentage reduction in growth, such as ECx, ErCx, EyCx, LOEC, NOEC, or benchmark concentration/dose values.

For unicellular algae and cyanobacteria, growth is commonly quantified from changes in biomass over time. Biomass may be estimated using direct cell counts, electronic particle counters, microscopy, optical density, fluorescence, chlorophyll-related measurements, or other validated biomass proxies. Growth inhibition is commonly calculated from average specific growth rate and yield.

For aquatic macrophytes such as Lemna spp., growth is typically quantified using frond number and at least one additional growth-related variable, such as total frond area, dry weight, fresh weight, or image-based area measurements. Digital imaging, flatbed scanning, stereomicroscopy, or automated image-analysis tools can be used to quantify frond area and related morphological endpoints.

For terrestrial plants, growth can be measured using seedling emergence, survival, shoot height, root length, fresh or dry biomass, leaf area, and visual signs of phytotoxicity such as chlorosis, necrosis, deformation, or delayed development. Measurements may be made using rulers or calipers, analytical balances, scanners, digital imaging, or plant image-analysis software.

For aquatic invertebrates, growth may be assessed using body length, body area, wet weight, dry weight, developmental stage, or size at defined time points. In small organisms such as Daphnia, body length is often measured from microscope or stereomicroscope images using calibrated image-analysis software.

For fish and amphibians, growth is commonly assessed using wet weight, dry weight, standard length, total length, snout–vent length, condition factor, developmental stage, and specific growth rate. Measurements may be obtained using balances, calipers, digital imaging, or stereomicroscopy, depending on organism size and life stage.

For mammals and other vertebrates in repeated-dose or reproductive/developmental toxicity studies, growth-related endpoints are typically evaluated using body weight, body-weight gain, food and water consumption, pup or offspring weight, litter growth, organ weight, and developmental landmarks. These endpoints are usually interpreted together with clinical observations, survival, pathology, and reproductive or developmental parameters.

Because growth is an integrative apical endpoint, measurement should be performed over a biologically appropriate time window and interpreted in relation to survival, developmental delay, nutritional status, reproductive output, and general toxicity. Direct measurements of organismal size, biomass, or growth rate provide the strongest evidence for this key event, whereas indirect indicators such as altered morphology, reduced condition factor, or reduced organ weight may provide supporting evidence depending on biological context.

Relevant OECD Test Guidelines include, but are not limited to:

- OECD TG 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. This guideline directly assesses inhibition of algal or cyanobacterial growth, typically using average specific growth rate and yield as response variables.
- OECD TG 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test. This guideline assesses effects on seedling emergence and early growth of terrestrial plants, including biomass, shoot height, and visible phytotoxic effects.
- OECD TG 211: Daphnia magna Reproduction Test. Although the primary endpoint is reproductive output, the test can provide chronic organism-level information relevant to population performance, and growth-related observations such as body size may be reported as supporting endpoints when included in the study design.
- OECD TG 212: Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages. This guideline assesses toxicity during early fish development from fertilized egg to the end of the sac-fry stage. Growth-related developmental observations may support interpretation of early-life-stage impairment.
- OECD TG 215: Fish, Juvenile Growth Test. This guideline directly evaluates effects on juvenile fish growth. Effects are expressed using growth rate, and concentration-response analysis can be used to estimate concentrations causing a defined percentage change in growth rate.
- OECD TG 221: Lemna sp. Growth Inhibition Test. This guideline directly assesses effects on vegetative growth of Lemna spp. using average specific growth rate and yield, commonly based on frond number and additional variables such as frond area or biomass.
- OECD TG 228: Determination of Developmental Toxicity to Dipteran Dung Flies. This guideline is relevant where chemical exposure affects development and growth-related performance in terrestrial invertebrates.

- OECD TG 241: Larval Amphibian Growth and Development Assay. This guideline evaluates growth and development in amphibians from fertilization through the early juvenile period.
- OECD TG 407: Repeated Dose 28-day Oral Toxicity Study in Rodents. This guideline includes body weight and food/water consumption measurements as part of the assessment of repeated-dose systemic toxicity.
- OECD TG 408: Repeated Dose 90-day Oral Toxicity Study in Rodents. This guideline includes body weight and related clinical observations as part of subchronic systemic toxicity assessment.
- OECD TG 416: Two-Generation Reproduction Toxicity. This guideline evaluates effects on reproduction as well as growth and development of offspring across generations.
- OECD TG 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test. This guideline includes body weight, food/water consumption, offspring observations and measurements, and developmental parameters.
- OECD TG 443: Extended One-Generation Reproductive Toxicity Study. This guideline evaluates reproductive and developmental effects, including health, growth, development, and function of offspring following pre- and postnatal exposure.
- OECD TG 453: Combined Chronic Toxicity/Carcinogenicity Studies. This guideline includes body weight and body-weight gain as part of the evaluation of chronic systemic toxicity and carcinogenicity.

Regulatory Significance of the AO

Growth is a regulatory relevant chronic toxicity endpoint for almost all organisms. Multiple OECD test guidelines have included growth either as a main endpoint of concern, or as an additional endpoint to be considered in the toxicity assessments. Relevant test guidelines include, but not only limited to:

-Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test

-Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test

-Test No. 211: Daphnia magna Reproduction Test

-Test No. 212: Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages

-Test No. 215: Fish, Juvenile Growth Test

-Test No. 221: Lemna sp. Growth Inhibition Test

-Test No. 228: Determination of Developmental Toxicity to Dipteran Dung Flies (Scathophaga stercoraria L. (Scathophagidae), Musca autumnalis De Geer (Muscidae))

-Test No. 241: The Larval Amphibian Growth and Development Assay (LAGDA)

-Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents

-Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents

-Test No. 416: Two-Generation Reproduction Toxicity

-Test No. 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test

-Test No. 443: Extended One-Generation Reproductive Toxicity Study

-Test No. 453: Combined Chronic Toxicity/Carcinogenicity Studies

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Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 2203: Decrease, Coupling of OXPHOS leads to Decrease, ATP pool

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Uncoupling of oxidative phosphorylation leading to growth inhibition via decreased cell proliferation	adjacent	High	High
Uncoupling of oxidative phosphorylation leading to growth inhibition via ATP depletion associated cell death	adjacent	Moderate	Not Specified
Uncoupling of oxidative phosphorylation leading to growth inhibition via decreased Na-K ATPase activity	adjacent		
Increase in reactive oxygen species leading to growth inhibition via lipid peroxidation and cell death	adjacent	High	High
Excessive reactive oxygen species leading to growth inhibition via protein oxidation and cell injury/death	adjacent		
Peroxisome proliferator-activated receptor alpha activation leading to early life stage mortality via reduced adenosine triphosphate	adjacent		
Increase in reactive oxygen species leading to growth inhibition via lipid peroxidation and decreased cell proliferation	adjacent	High	High
Increase in reactive oxygen species leading to growth inhibition via protein oxidation and decreased cell proliferation	adjacent	High	High
Increase in reactive oxygen species leading to growth inhibition via protein oxidation and cell death	adjacent	High	High

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI
human	Homo sapiens	High	NCBI
rat	Rattus norvegicus	High	NCBI
mouse	Mus musculus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Embryo	High
Juvenile	High

Sex Applicability

Sex	Evidence
Unspecific	High

Taxonomic applicability

Relationship 2203 is considered applicable to eukaryotes, as mitochondrial oxidative phosphorylation and ATP synthesis are highly conserved in these organisms. Uncoupling of oxidative phosphorylation leading to ATP depletion is a well-documented relationship in many taxa, such as human, rodents and fish.

Sex applicability

Relationship 2203 is considered applicable to all genders, as mitochondrial oxidative phosphorylation and ATP synthesis are fundamental biological processes and are not sex-pecific.

Life-stage applicability

Relationship 2203 is considered applicable to all life-stages, as mitochondrial oxidative phosphorylation and ATP synthesis are essential energy production processes for maintaining basic biological activities.

Key Event Relationship Description

This key event relationship describes the dissipation of protonmotive force across the inner mitochondrial membrane by uncouplers (uncoupling of oxidative phosphorylation), leading to reduced total adenosine triphosphate (ATP) pool in cells or organisms.

Evidence Supporting this KER

The overall evidence supporting Relationship 2203 is considered high.

Biological Plausibility

The biological plausibility of Relationship 2203 is considered high.

Rationale: In eukaryotic cells, the major metabolic pathways responsible for ATP production are OXPHOS, citric acid (TCA) cycle, glycolysis and photosynthesis. Oxidative phosphorylation is much (theoretically 15-18 times) more efficient than the rest due to high energy derived from oxygen during aerobic respiration (Schmidt-Rohr 2020). As the ATP level is relatively balanced between production and consumption (Bonora 2012), ATP depletion is a plausible consequence of reduced ATP synthetic efficiency following uncoupling of OXPHOS.

Empirical Evidence

The empirical support of Relationship 2203 is considered high.

Rationale: The majority of relevant studies show good incidence, temporal and/or dose concordance in different organisms and cell types after exposure to known uncouplers, with relatively few exceptions.

Evidence:

- ***Temporal concordance***: Exposure of zebrafish embryos to 0.5 µM of the classical uncoupler 2,4-DNP led to significantly uncoupling of OXPHOS after 21h, whereas significant reduction in ATP was only observed after 45h (Bestman 2015).
- ***Dose concordance:*** The uncoupler triclosan induced significant uncoupling of OXPHOS in zebrafish embryos at 15 µM, whereas higher (30 µM) concentration was required to caused significant ATP depletion (Shim 2016).
- ***Dose concordance:*** Exposure to 1 µM of of the uncoupler CCCP led to 40% uncoupling of OXPHOS in rat RBL-2H3 cells, whereas the same magnitude of effect for ATP reduction required 1.6 µM of CCCP (Weatherly 2016).
- ***Dose concordance:*** Exposure to 10 µM of the uncoupler triclosan caused significant uncoupling of OXPHOS in rat RBL-2H3 cells, whereas significant reduction in ATP was observed at a higher concentration (30 µM) (Weatherly 2018).
- ***Dose concordance:*** Significant effect on uncoupling of OXPHOS required 2 µM FCCP, whereas a significant reduction in ATP required 20 µM FCCP in human RD cells (Kuruvilla 2003).
- ***Incidence concordance***: In human colon cancer cells (SW480), exposure to 150 µM of the uncoupler flavanoid morin caused 60% reduction in MMP, whereas only around 35% decrease in ATP (Sithara 2017).
- ***Incidence concordance:*** Exposure of rat RBL-2H3 cells to 10 µM of the uncoupler triclosan led to 50% uncoupling of OXPHOS, whereas only 40% reduction in ATP (Weatherly 2016).
- ***Incidence concordance:*** Exposure to 5 µM of the uncoupler CCCP caused 71% uncoupling of OXPHOS, whereas only 64% reduction of ATP in human HL-60 cells (Sweet 1999).
- ***Incidence concordance:*** Exposure of human HeLa cells to 50 µM of the uncoupler CCCP for 1h led to 77% uncoupling of OXPHOS and 25% reduction in ATP (Koczor 2009).
- ***Incidence concordance***: Exposure of the nematode Caenorhabditis elegans to 50 µM Arsenite for 1h led to approximately 45% uncoupling of OXPHOS and 20% reduction in ATP (Luz 2016).

Uncertainties and Inconsistencies

- A significant decrease followed by a significant increase in total ATP was observed in human RD cells during a 48h exposure to the uncoupler FCCP (Kuruvilla 2003), possibly due to the enhancement of other ATP synthetic pathways (e.g., glycolysis) as a compensatory action to impaired OXPHOS (Jose 2011).

Quantitative Understanding of the Linkage

The quantitative understanding of Relationship 2203 is high.

Rationale: Multiple mathematical models have been developed for describing the quantitative relationships between uncoupling of OXPHOS and ATP synthesis in vertebrates (Beard 2005; Schmitz 2011; Heiske 2017; Kubo 2020). These models, however, are highly complex metabolic or systems biological models and warrant further simplification to be used for this AOP.

Response-response relationship

A regression based quantitative response-response relationship between uncoupling of OXPHOS and ATP depletion was proposed for the crustacean *Daphnia magna* under UVB stress (Song 2020).

Update - non-endorsed (by You Song, 06/10/2026)

Across the other studies, ATP decreased in the same direction as uncoupling but usually by a smaller amount at the same exposure. For example, 50 µM CCCP for 1 h caused 77% uncoupling and a 25% ATP reduction in human HeLa

cells (Koczor et al., 2009), 50 µM arsenite for 1 h caused about 45% uncoupling and a 20% ATP reduction in *Caenorhabditis elegans* (Luz et al., 2016), and 5 µM CCCP caused 71% uncoupling and a 64% ATP reduction in human HL-60 cells (Sweet and Singh, 1999). Likewise, higher concentrations were needed to reduce ATP than to uncouple OXPHOS, such as 15 versus 30 µM triclosan in zebrafish embryos (Shim et al., 2016), 10 versus 30 µM triclosan in rat RBL-2H3 cells (Weatherly et al., 2018), and 2 versus 20 µM FCCP in human RD cells (Kuruvilla et al., 2003). These data come from different uncouplers, cell types, and exposure durations, so they cannot be combined into a single function, and the general form of the relationship and the precision with which ATP depletion can be predicted from a measured degree of uncoupling have not been defined.

Time-scale

Update - non-endorsed (by You Song, 06/10/2026)

The time-scale varies with the system. Reductions in ATP were observed within 1 h of exposure to uncouplers in human HeLa cells and *C. elegans* (Koczor et al., 2009; Luz et al., 2016). In zebrafish embryos exposed to 0.5 µM 2,4-DNP, uncoupling of OXPHOS was significant after 21 h, whereas a significant reduction in ATP was observed only after 45 h (Bestman et al., 2015). In human RD cells exposed to FCCP, ATP decreased and then increased again within 48 h (Kuruvilla et al., 2003). The lag between uncoupling and ATP depletion can therefore range from less than an hour to about a day, depending on the organism, the stressor, and the compensatory capacity.

Known modulating factors

Update - non-endorsed (by You Song, 06/10/2026)

No modulating factors with quantified effects on this KER have been identified. The factors below are indicated by the studies cited in this KER.

Modulating factor	Details	Effects on the KER	References
Compensatory ATP production	Increased activity of the electron transport chain and activation of other ATP-synthesizing pathways (e.g., glycolysis)	Mild uncoupling can be compensated, so ATP decreases less than the degree of uncoupling would suggest. In human RD cells exposed to FCCP for 48 h, a significant decrease in ATP was followed by a significant increase.	Jose et al., 2011; Kuruvilla et al., 2003
Metabolic phenotype of the cell or tissue	Relative reliance on glycolysis versus oxidative phosphorylation for ATP production	Systems with higher glycolytic capacity are expected to maintain ATP better when OXPHOS coupling decreases.	Jose et al., 2011

Known Feedforward/Feedback loops influencing this KER

- It is known that mild uncoupling of oxidative phosphorylation can enhance the activity of the mitochondrial electron transport chain to produce more ATP, and/or activate other ATP synthetic pathways (e.g., glycolysis) as a compensatory action to impaired OXPHOS (Jose 2011)

Update - non-endorsed (by You Song, 06/10/2026)

Compensatory mechanisms act as negative feedback on this KER. ATP levels are normally balanced between production and consumption (Bonora et al., 2012), and mild uncoupling of oxidative phosphorylation can increase the activity of the electron transport chain to produce more ATP and activate other ATP-synthesizing pathways such as glycolysis (Jose et al., 2011). This compensation explains the transient decrease in ATP in RD cells (Kuruvilla et al., 2003). Its extent and homeostatic limits have not been quantified for this KER, but it is expected to be exceeded when uncoupling is strong or sustained. These mechanisms mainly shape the magnitude and time course of the ATP response and are therefore described here and not represented as separate KEs. No positive feedback or feedforward loops have been identified for this KER.

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Relationship: 2204: Decrease, ATP pool leads to Decrease, Cell proliferation

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Uncoupling of oxidative phosphorylation leading to growth inhibition via decreased cell proliferation	adjacent	Moderate	Moderate
Mitochondrial ATP synthase antagonism leading to growth inhibition (1)	adjacent		
Mitochondrial complex III antagonism leading to growth inhibition (1)	adjacent		
Excessive reactive oxygen species leading to growth inhibition via fatty acid oxidation and reduced cell proliferation	adjacent		
Increase in reactive oxygen species leading to growth inhibition via lipid peroxidation and decreased cell proliferation	adjacent	High	Moderate
Increase in reactive oxygen species leading to growth inhibition via protein oxidation and decreased cell proliferation	adjacent	High	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Embryo	High

Sex Applicability

Sex	Evidence
Unspecific	High

Taxonomic applicability

Relationship 2204 is considered applicable to all eukaryotes, as ATP and cell proliferation are known to be tightly coupled in animals, plants and some microorganisms.

Sex applicability

Relationship 2204 is considered applicable to all sexes, as ATP-dependent cell proliferation are used by both males and females in eukaryotes.

Life-stage applicability

Relationship 2204 is considered applicable to all life stages, as ATP-dependent cell proliferation is an essential process for an organism throughout the entire life.

Key Event Relationship Description

This key event relationship describes reduced adenosine triphosphate (ATP) supply leading to reduced cell proliferation (cell growth, division or a combination of these).

Evidence Supporting this KER

The overall evidence supporting Relationship 2204 is considered moderate.

Biological Plausibility

The biological plausibility of Relationship 2204 is considered high.

Rationale: Cell proliferation is a well-known ATP-dependent process. Cell division processes, such as the mitotic cell cycle uses ATP for chromosome movements and DNA replication (Kingston 1999). The synthetic processes of major cellular components that are necessary for cell structure and growth, such as proteins and lipids, also require sufficient ATP supply (Bonora 2012). Depletion of ATP therefore has a negative impact on these processes.

Empirical Evidence

The empirical support of Relationship 2204 is considered moderate.

Evidence:

- ***Incidence concordance***: Exposure of human HeLa cells to 50 µM of the uncoupler CCCP for 1h led to 25% reduction in ATP, whereas a non-significant reduction in cell proliferation (Koczor 2009).
- I***ncidence concordance***: Exposure of human RD cells to 20 µM of the uncoupler CCCP for 2h led to 20% ATP depletion, whereas a non-significant decrease in cell proliferation (Kuruvilla 2003).
- ***Incidence concordance***: Exposure of human SE480 cells to 150 µM of the uncoupler flavanoid morin for 48h led to 35% ATP depletion and 35% reduction in cell proliferation (Sithara 2017).

Uncertainties and Inconsistencies

There are currently no inconsistencies based on the supporting literature.

Quantitative Understanding of the Linkage

The quantitative understanding of Relationship 2204 is moderate.

Rationale: Quantitative relationships between total ATP and cell proliferation have been extensively

investigated (Ahmann 1987; Crouch 1993). In general, a monotonic positive relationship can be assumed for the two events, albeit the actual quantitative relationship can vary across biological systems (e.g. cell types and species). It has also been suggested that a threshold of ATP depletion (85-90% compared to normal status) may exist to determine whether proliferation arrest (<85-90%) or cell death (>85-90%) will be triggered in mammals (Nieminen 1994).

Response-response relationship

Update - non-endorsed (by You Song, 06/10/2026)

A monotonic positive relationship between total cellular ATP and cell proliferation can be assumed (Ahmann et al., 1987; Crouch et al., 1993). The available studies that measured both events in the same experiment suggest that small decreases in ATP are not sufficient to reduce proliferation. In human HeLa and RD cells, ATP reductions of 25% (50 µM CCCP, 1 h) and 20% (20 µM CCCP, 2 h) were accompanied by non-significant decreases in proliferation (Koczor et al., 2009; Kuruvilla et al., 2003). In human SW480 cells, a 35% ATP depletion after 48 h of exposure to 150 µM morin was accompanied by a 35% reduction in proliferation (Sithara et al., 2017). These data points come from different stressors, cell types, and exposure durations, so they cannot be combined into a single response-response function, and the general form of the relationship (linear, threshold, or sigmoidal) has not been defined. In mammalian cells, a threshold of ATP depletion of about 85–90% relative to normal has been suggested to separate proliferation arrest (depletion below the threshold) from cell death (depletion above it) (Nieminen et al., 1994). The precision with which a measured decrease in ATP predicts a decrease in proliferation has not been quantified.

Time-scale

Update - non-endorsed (by You Song, 06/10/2026)

The time-scale of this KER has not been quantified. In the available studies, ATP was reduced by 20–25% after 1–2 h of exposure to CCCP without a significant decrease in proliferation, whereas a 35% reduction in ATP after 48 h of exposure to morin was accompanied by a 35% reduction in proliferation (Koczor et al., 2009; Kuruvilla et al., 2003; Sithara et al., 2017). This is consistent with a lag between ATP depletion and measurable effects on proliferation, but because the studies used different stressors and cell types, the length of the lag cannot be estimated.

Known modulating factors

Update - non-endorsed (by You Song, 06/10/2026)

No modulating factors with quantified effects on this KER have been identified. The factors below are indicated by the literature cited in this KER.

Modulating factor	Details	Effects on the KER	References
Cell type and species	Cell types and species in which ATP and proliferation are measured	The quantitative relationship between cellular ATP and cell proliferation varies across biological systems.	Ahmann et al., 1987; Crouch et al., 1993
Severity of ATP depletion	Degree of ATP depletion relative to the normal ATP level	In mammalian cells, ATP depletion beyond a threshold of about 85–90% of normal is associated with cell death rather than proliferation arrest.	Nieminen et al., 1994

Known Feedforward/Feedback loops influencing this KER

Update - non-endorsed (by You Song, 06/10/2026)

No feedforward or feedback loops have been characterized for this KER in the studies cited here. Cells can maintain ATP by increasing ATP production or by reducing ATP consumption, which would influence how much ATP depletion is needed to reduce proliferation, but the extent, time course, and homeostatic limits of these compensatory responses have not been quantified for this KER. They are therefore described here and not represented as separate KEs.

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Relationship: 2205: Decrease, Cell proliferation leads to Decrease, Growth

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Uncoupling of oxidative phosphorylation leading to growth inhibition via decreased cell proliferation	adjacent	Moderate	Moderate
Mitochondrial ATP synthase antagonism leading to growth inhibition (1)	adjacent		
Mitochondrial complex III antagonism leading to growth inhibition (1)	adjacent		
Uncoupling of oxidative phosphorylation leading to growth inhibition via glucose depletion	adjacent		
Excessive reactive oxygen species leading to growth inhibition via oxidative DNA damage	adjacent		
Excessive reactive oxygen species leading to growth inhibition via protein oxidation and cell cycle disruption	adjacent		
Excessive reactive oxygen species leading to growth inhibition via fatty acid oxidation and reduced cell proliferation	adjacent		
Increase in reactive oxygen species leading to growth inhibition via oxidative DNA damage and cell cycle disruption	adjacent	High	Moderate
Increase in reactive oxygen species leading to growth inhibition via lipid peroxidation and decreased cell proliferation	adjacent	High	Moderate
Increase in reactive oxygen species leading to growth inhibition via protein oxidation and decreased cell proliferation	adjacent	High	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Embryo	High

Sex Applicability

Sex	Evidence
Unspecific	High

Taxonomic applicability

Relationship 2205 is considered applicable to all eukaryotes (both unicellular and multicellular), as growth (or population growth of alga) is well known to be achieved through cell proliferation in animals, plants and some microorganisms.

Sex applicability

Relationship 2205 is considered applicable to both all sexes, as cell proliferation leading to growth is a fundamental process and not sex-specific.

Life-stage applicability

Relationship 2205 is considered applicable to all life stages, as cell proliferation leading to growth is essential for maintaining basic biological processes throughout an organism's life.

Key Event Relationship Description

This key event relationship describes reduced cell proliferation (cell growth, division or a combination of these) leading to reduced tissue, organ or individual growth.

Evidence Supporting this KER

The overall evidence supporting Relationship 2205 is considered moderate.

Biological Plausibility

The biological plausibility of Relationship 2205 is considered high.

Rationale: The biological structural and functional relationship between cell proliferation and growth is well established. It is commonly accepted that the size of an organism, organ or tissue is dependent on the total number and volume of the cells it contains, and the amount of extracellular matrix and fluids (Conlon 1999). Impairment to cell proliferation can logically affect tissue and organismal growth.

Empirical Evidence

The empirical support of Relationship 2205 is considered low.

Rationale: Because cell proliferation is typically measured in vitro, while growth of an organism is measured in vivo, few studies have measured both in the same experiment. There is one zebrafish study reporting concordant relationship between reduced cell proliferation and embryo growth with some inconsistencies (Bestman 2015).

Uncertainties and Inconsistencies

- In zebrafish embryos exposed to 2,4-DNP, significant growth inhibition (AO), as indicated by whole embryo length, caudal primary (CaP) motor neuron axons and otic vesicle length (OVL) ratio after 21h, somite width and eye diameter after 45h exposure was identified, after 21h, whereas a non- significant reduction in cell proliferation was observed (Bestman 2015).

Quantitative Understanding of the Linkage

The quantitative understanding of Relationship 2205 is moderate.

Rationale: Multiple mathematical models describing the quantitative relationships between cell proliferation and tissue growth exist for both animals (Binder 2008) and plants (Mosca 2018). There are also numerous models that are specifically developed for predicting tumor growth based on the proliferation rate (Jarrett 2018).

Response-response relationship

Update - non-endorsed (by You Song, 05/10/2026)

Tissue, organ, and organism size depend on the total number and volume of cells and on the amount of extracellular matrix and fluids (Conlon and Raff, 1999). A decrease in proliferation therefore reduces growth in proportion to the resulting loss of cell number, modified by changes in cell size and matrix content. This relationship has been formalized in mathematical models of proliferative tissue growth in animals (Binder et al., 2008) and of tissue growth and cell division in plants (Mosca et al., 2018), and numerous models predict tumor growth from the rate of proliferation (Jarrett et al., 2018). These models describe growth as a function of proliferation rate, but they have not been fitted to data on stressor-induced decreases in proliferation, so the precision with which a measured decrease in proliferation predicts a decrease in growth has not been quantified. The only study that measured both events in the same experiment, in zebrafish embryos exposed to 2,4-DNP, showed that growth was significantly inhibited after 21 h while the reduction in cell proliferation was not significant (Bestman et al., 2015), so the relationship cannot be quantified from that study.

Time-scale

Update - non-endorsed (by You Song, 05/10/2026)

The time-scale of this KER has not been quantified. Growth is the cumulative result of cell divisions, so effects on

growth are expected to lag behind effects on proliferation, with the lag depending on the cell-cycle length and growth rate of the system. In the zebrafish study, growth inhibition was detected after 21 h, whereas the reduction in cell proliferation was not yet significant (Bestman et al., 2015). Studies that measure both events at a single time point cannot resolve their temporal order.

Known modulating factors

Update - non-endorsed (by You Song, 05/10/2026)

Modulating factor	Details	Effects on the KER	References
Cell size	Cell volume, which can change independently of cell number	An increase in cell size can partly compensate for fewer cells, so a given decrease in proliferation produces a smaller decrease in tissue or organ growth. A decrease in cell size adds to the effect.	Conlon and Raff, 1999
Extracellular matrix and fluid content	Amount of extracellular matrix and fluids in the tissue	Tissue and organ size depend on these components as well as on cell number, so growth can change independently of proliferation.	Conlon and Raff, 1999
Cell death	Rate of cell loss in the tissue	Net cell number depends on the balance between proliferation and cell loss. Increased cell death adds to the effect of reduced proliferation, and decreased cell death can offset it.	Conlon and Raff, 1999
Growth mode of the organism	Animals and plants: growth by cell division and by cell expansion. Unicellular algae: population growth by cell division	In plants, tissue growth results from both cell division and cell expansion, so reduced division can be partly offset by expansion. In unicellular algae, population growth is achieved through cell proliferation, so the relationship is direct.	Mosca et al., 2018 (plants)

Known Feedforward/Feedback loops influencing this KER

Update - non-endorsed (by You Song, 05/10/2026)

Modulating factor	Details	Effects on the KER	References
Cell size	Cell volume, which can change independently of cell number	An increase in cell size can partly compensate for fewer cells, so a given decrease in proliferation produces a smaller decrease in tissue or organ growth; a decrease in cell size adds to the effect.	Conlon and Raff, 1999
Extracellular matrix and fluid content	Amount of extracellular matrix and fluids in the tissue	Tissue and organ size depend on these components as well as on cell number, so growth can change independently of proliferation.	Conlon and Raff, 1999
Cell death	Rate of cell loss in the tissue	Net cell number depends on the balance between proliferation and cell loss; increased cell death adds to the effect of reduced proliferation, and decreased cell death can offset it.	Conlon and Raff, 1999
Growth mode of the organism	Growth by cell division and by cell expansion (plants); population growth by cell division (unicellular algae)	In plants, reduced division can be partly offset by cell expansion; in unicellular algae, population growth is achieved through cell proliferation, so the relationship is direct.	Mosca et al., 2018

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