**Integration of** **Advanced Large Language Models into Adverse Outcome Pathways Construction: Opportunities and Challenges**

Haochun Shi,† and Yanbin Zhao†,\*

† State Environmental Protection Key Laboratory of Environmental Health Impact Assessment of Emerging Contaminants, School of Environmental Science and Engineering, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China.

\*Corresponding author:

Dr. Yanbin Zhao (Tel.: +86 188 1820 7732; E-mail: [zhaoyanbin@sjtu.edu.cn](mailto:zhaoyanbin@sjtu.edu.cn)).

AOP Reconstruction Process:

To evaluate the effectiveness of large language models (LLMs) in Adverse Outcome Pathway (AOP) construction, we used GPT-4 for automated annotation to reconstruct five well-documented AOPs described in the AOPwiki. These AOPs are:

1. (AOP38): Protein Alkylation leading to Liver Fibrosis
2. (AOP25): Aromatase inhibition leading to reproductive dysfunction
3. (AOP54): Inhibition of Na+/I- symporter leads to learning and memory impairment
4. (AOP155): Deiodinase 2 inhibition leading to increased mortality
5. (AOP220): Cyp2E1 Activation Leading to Liver Cancer

The general procedure for AOPs reconstruction and verification is outlined below:

**a. AOP Reconstruction:**

1. Identify MIE and AO: Determine the Molecular Initiating Event (MIE) and the Adverse Outcome (AO) of interest, using the AOPwiki or other relevant data sources.
2. Input and refine specific questions: Provide GPT-4 with detailed questions about the MIE and AO. Ask GPT-4 to describe the key events (KEs) that connect the MIE to the AO based on available data. Additionally, take into account the Life Stage Applicability, Taxonomic Applicability, and Sex Applicability during this process.
3. Integrate annotations: Incorporate the GPT-4-generated annotations into a comprehensive AOP repository, ensuring each component is correctly linked to its corresponding MIE, KE, and AO.
4. Visualization: Use the annotated data to develop a visual representation of the AOP, illustrating the sequence of events from the MIE through KEs to the AO.

**b. AOP Verification:**

1. Retrieve AOP information: Retrieve expert-derived AOP information from AOPwiki, including clear descriptions of the MIE, KEs, AO and associated detailed annotations and references.
2. AOPs comparison: Compare the GPT-4 reconstructed AOP with the expert-derived AOP to check if all components and relationships were accurately identified by GPT-4. Determine if any key component or relationship was omitted or supplemented.
3. Qualitative analysis: Assess GPT-4's ability to replicate the expert-derived AOP structure, evaluating the clarity, depth, and relevance of the annotations. Consider how well GPT-4 captures the biological complexity and the evidence supporting each step in the pathway.

* Detailed procedure for AOPwiki: AOP38 (Protein Alkylation leading to Liver Fibrosis) reconstruction is outlined below:

1. Refine the specific question:

“Construct the potential Adverse Outcome Pathways (AOPs) using the following keywords: Protein alkylation leading to liver fibrosis in animals. Meanwhile, provide detailed descriptions for each step.”

1. GPT-4's annotations:
2. Molecular Initiating Event (MIE): Protein Alkylation

Protein alkylation is the process by which an alkyl group covalently binds to a protein, often as a result of exposure to alkylating agents such as certain chemicals, drugs, or environmental toxins. This chemical modification can alter the structural integrity or interactions of the protein, leading to a cascade of downstream effects that disrupt normal cellular functions. Protein alkylation typically affects critical cellular proteins, including enzymes and signaling molecules, initiating a sequence of adverse biological responses.

1. KE1: Oxidative Stress

Following protein alkylation, oxidative stress can occur due to an imbalance between the production of reactive oxygen species (ROS) and the cell’s ability to detoxify these reactive intermediates. Oxidative stress damages cellular components, including lipids, proteins, and DNA, leading to further cellular dysfunction and signaling disruptions. ROS are highly reactive molecules that exacerbate cellular injury, especially in the liver, which is a major site of metabolism and detoxification.

1. KE2: Activation of Pro-inflammatory Pathways

The damage caused by oxidative stress can activate pro-inflammatory pathways, such as NF-κB and MAPK signaling cascades. This activation leads to the production and release of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukins (e.g., IL-6, IL-1β), and chemokines. These cytokines create a pro-inflammatory environment within the liver, promoting the recruitment of immune cells (e.g., neutrophils, macrophages) and sustaining the inflammatory response, which contributes to further tissue damage.

1. KE3: Hepatic Stellate Cell Activation

Chronic oxidative stress and inflammation lead to hepatocyte injury. Hepatocyte injury and the ensuing inflammatory response activate hepatic stellate cells (HSCs), which are the primary cells responsible for producing extracellular matrix (ECM) components in the liver. When activated, HSCs transform into myofibroblast-like cells, proliferating and secreting large amounts of ECM proteins, including collagen. This activation is driven by factors such as transforming growth factor-beta (TGF-β), which is released by injured hepatocytes and immune cells. Activated HSCs play a central role in the development of fibrosis by depositing excessive ECM.

1. KE4: Excessive Extracellular Matrix (ECM) Deposition

As hepatic stellate cells become activated, they produce and secrete excessive amounts of ECM proteins, leading to the accumulation of fibrotic tissue in the liver. This excessive ECM disrupts the normal architecture of the liver, leading to the formation of fibrous scar tissue. The accumulation of ECM components such as collagen interferes with liver function, impairs tissue regeneration, and increases tissue stiffness, contributing to the progressive nature of liver fibrosis.

1. KE5: Accumulation of Scar Tissue and Formation of Fibrotic Lesions

As hepatic stellate cells continue to deposit collagen and other ECM components, there is a progressive accumulation of scar tissue in the liver. This scar tissue disrupts the normal architecture of the liver, leading to the formation of fibrotic lesions. These lesions are areas of dense fibrous tissue that replace normal liver tissue, impeding the liver's ability to function properly. Over time, the formation of these lesions can contribute to the development of cirrhosis, where the liver becomes extensively scarred and its function severely compromised.

1. Adverse Outcome (AO): Liver Fibrosis

Liver fibrosis is the result of the chronic and excessive deposition of ECM, particularly collagen, which leads to the thickening and scarring of liver tissue. Over time, fibrosis can progress to cirrhosis, characterized by severe scarring that disrupts liver function and increases the risk of liver failure and hepatocellular carcinoma (liver cancer). The fibrotic tissue impairs the liver’s ability to perform vital functions such as detoxification, metabolism, and production of essential proteins, ultimately leading to a decline in overall health.

Recommended AOP framework:

Protein Alkylation (MIE) → 2. Oxidative Stress (KE) → 3. Activation of Pro-inflammatory Pathways (KE) → 4. Hepatic Stellate Cell Activation (KE) → 5. Excessive ECM Deposition (KE) → 6. Accumulation of Scar Tissue and Formation of Fibrotic Lesions (KE) → 7. Liver Fibrosis (AO)

1. Integration of the annotations and visualization: Based on the GPT-4 annotations and the recommended AOP framework described above, we create a visual representation of the AOP to depict the sequence of key events from the MIE through KEs to the AO:
2. Retrieve the expert-derived AOP information from the AOPwiki database:

AOP introduction: This AOP describes the linkage between hepatic injury caused by protein alkylation and the formation of liver fibrosis. The molecular initiating event (MIE) is protein alkylation, leading to structural and functional cell injury and further to cell death, the first KE. Apoptotic hepatocytes undergo genomic DNA fragmentation and formation of apoptotic bodies. Upon engulfment of apoptotic bodies Kupffer cells (KCs) are activated, the next KE along the pathway. Activated KCs are the main source of TGF-β1, the most potent profibrogenic cytokine. TGF-β1 expression therefore is considered a KE that causes the next KE, hepatic stellate cell (HSCs) activation, meaning the transdifferentiation from a quiescent vitamin A–storing cell to a proliferative and contractile myofibroblast, the central effector in hepatic fibrosis. Activated HSCs cause progressive collagen accumulation, which together with changes in ECM composition signifies the KE on tissue level. The excessive accumulation of extracellular matrix proteins progressively affects the whole organ and alters its normal functioning, which corresponds to liver fibrosis, the adverse outcome (AO).

1. AOPs comparison: Compare the GPT-4 reconstructed AOP with the expert-derived AOP from AOPwiki. Green indicates the identical MIEs and KEs, red indicates mismatched or missed KEs in the GPT-4 annotations, and blue color indicates KEs that is present in GPT-4 reconstructed AOP but missing in the AOPwiki framework.

* Detailed procedure for AOPwiki: AOP25 (Aromatase inhibition leading to reproductive dysfunction) reconstruction is outlined below:

1. Refine the specific question:

“Construct the potential Adverse Outcome Pathways (AOPs) using the following keywords: Aromatase inhibition leading to reproductive dysfunction in female fish. Meanwhile, provide detailed descriptions for each step.”

1. GPT-4's annotations:
2. Molecular Initiating Event (MIE): Aromatase Inhibition

Aromatase is an enzyme that plays a crucial role in converting androgens (such as testosterone) into estrogens (such as estradiol). In female fish, this enzyme is vital for maintaining normal levels of estrogen, which is essential for various reproductive processes. Aromatase inhibition can occur due to exposure to certain environmental chemicals, including some pesticides, industrial byproducts, or pharmaceuticals. Inhibiting aromatase leads to a reduction in estrogen levels, disrupting the endocrine balance necessary for proper reproductive function in female fish.

1. KE1: Reduction in Estrogen Synthesis

The inhibition of aromatase leads directly to a reduction in the synthesis of estrogens, particularly estradiol. Estrogens are essential hormones that regulate many reproductive processes in female fish, including the development of oocytes (eggs), vitellogenesis (yolk formation), and the regulation of spawning cycles. A decrease in estrogen levels can have wide-ranging effects on reproductive health.

1. KE2: Impaired Vitellogenesis

Vitellogenesis is the process by which yolk proteins are synthesized in the liver and deposited in developing oocytes. This process is primarily driven by estradiol. When estrogen levels drop due to aromatase inhibition, the production of vitellogenin (the yolk precursor protein) in the liver is significantly reduced. This impairment leads to poor yolk formation, resulting in oocytes that are underdeveloped or deficient in nutrients necessary for embryo development. Consequently, egg quality and quantity are diminished, negatively affecting the reproductive potential of female fish.

1. KE3: Disruption of Ovarian Follicle Development

Estrogens play a key role in regulating the growth and maturation of ovarian follicles, which are the structures that contain and nurture developing oocytes. With reduced estrogen levels, the normal progression of follicular development is disrupted, leading to delayed or arrested follicle maturation. This can result in a higher incidence of atresia (degeneration of oocytes) and a decrease in the number of mature eggs available for spawning. In severe cases, it can lead to complete reproductive failure.

1. KE4: Altered Reproductive Behavior

In many fish species, reproductive behavior is strongly influenced by estrogen levels. These behaviors include courtship displays, nest building, and spawning rituals, all of which are crucial for successful reproduction. A reduction in estrogen levels due to aromatase inhibition can lead to altered or diminished reproductive behaviors, such as reduced spawning activity, failure to engage in mating displays, or a lack of response to male courtship. These behavioral changes further decrease the likelihood of successful fertilization and reproduction.

1. Adverse Outcome (AO): Reproductive Dysfunction in Female Fish

The final outcome of this AOP is reproductive dysfunction, which encompasses a range of potential reproductive failures, including reduced egg production, poor egg quality, unsuccessful spawning, and reduced offspring survival. This dysfunction can lead to population-level effects if the reproductive success of female fish is significantly compromised over time. In ecologically sensitive environments, such disruptions can have cascading effects on the ecosystem, particularly if the affected fish species play a critical role in the food web.

Recommended AOP framework:

**Aromatase Inhibition** (MIE) **→** 2. **Reduction in Estrogen Synthesis** (KE) → 3. **Impaired Vitellogenesis** (KE) **→** 4. **Disruption of Ovarian Follicle Development** (KE) → 5. **Altered Reproductive Behavior** (KE) → 6. **Reproductive Dysfunction in Female Fish** (AO)

1. Integration of the annotations and visualization: Based on the GPT-4 annotations and the recommended AOP framework described above, a visual representation of the AOP to depict the sequence of key events from the MIE through KEs to the AO is created:
2. Retrieve the expert-derived AOP information from the AOPwiki database:

AOP introduction: This adverse outcome pathway details the linkage between inhibition of gonadal aromatase activity in females and reproductive dysfunction, as measured through the adverse effect of reduced cumulative fecundity and spawning. Initial development of this AOP draws heavily on evidence collected using repeat-spawning fish species. Cumulative fecundity is the most apical endpoint considered in the OECD 229 Fish Short Term Reproduction Assay. The OECD 229 assay serves as screening assay for endocrine disruption and associated reproductive impairment (OECD 2012). Cumulative fecundity is one of several variables known to be of demographic significance in forecasting fish population trends. Therefore, this AOP has utility in supporting the application of measures of aromatase, or in silico predictions of the ability to inhibit aromatase, as a means to identify chemicals with known potential to adversely affect fish populations and potentially other oviparous vertebrates.



1. AOPs comparison: Compare the GPT-4 reconstructed AOP with the expert-derived AOP from AOPwiki. Green indicates the identical MIEs and KEs, red indicates mismatched or missed KEs in the GPT-4 annotations, and blue color indicates KEs that is present in GPT-4 reconstructed AOP but missing in the AOPwiki framework.

* Detailed procedure for AOPwiki: AOP54 (Inhibition of Na+/I- symporter leads to learning and memory impairment) reconstruction is outlined below:

1. Input the specific question:

“Construct the potential Adverse Outcome Pathways (AOPs) using the following keywords: Inhibition of Na+/I- symporter leads to learning and memory impairment during brain development in animals. Meanwhile, provide detailed descriptions for each step.”

1. GPT-4's annotations:
2. Molecular Initiating Event (MIE): Inhibition of Na+/I- Symporter

The Na+/I- symporter (NIS) is responsible for the active transport of iodide ions into thyroid follicular cells, a critical step in the synthesis of thyroid hormones (THs), particularly thyroxine (T4) and triiodothyronine (T3). Environmental contaminants like perchlorate, thiocyanate, or nitrate can inhibit NIS function, leading to a decreased iodide uptake by the thyroid gland. This reduced uptake impairs the thyroid’s ability to produce sufficient levels of thyroid hormones, initiating a cascade of downstream effects.

1. KE1: Reduced Synthesis of Thyroid Hormones (T3 and T4)

The inhibition of the NIS decreases the availability of iodide, a necessary substrate for thyroid hormone synthesis. Without adequate iodide, the thyroid gland’s production of T4 and T3 is reduced. These hormones are essential for regulating metabolism and are particularly important during developmental stages for brain development and function.

1. KE2: Decreased Circulating Levels of Thyroid Hormones

The reduction in thyroid hormone synthesis leads to decreased levels of T3 and T4 in the bloodstream. These hormones are critical for the regulation of gene expression in various tissues, including the brain. In developing animals, thyroid hormone levels are tightly regulated, and even small disruptions can have significant impacts on brain development.

1. KE3: Altered Thyroid Hormone-Dependent Gene Expression

Thyroid hormones regulate the expression of a variety of genes involved in brain development, including those that control neuronal migration, differentiation, and synaptic formation. Decreased levels of circulating thyroid hormones can lead to altered expression of these genes, disrupting normal developmental processes in the brain.

1. KE4: Impaired Synaptogenesis and Myelination

Synaptogenesis (the formation of synapses) and myelination (the formation of the myelin sheath around axons) are essential for establishing functional neural circuits. Thyroid hormones are crucial for these processes. Disruption in thyroid hormone levels can lead to impaired synapse formation and defective myelination, which are key to learning and memory functions.

1. KE5: Impaired Synaptic Plasticity

Synaptic plasticity, including long-term potentiation (LTP), is essential for learning and memory. It refers to the ability of synapses to strengthen or weaken over time in response to increases or decreases in their activity. Reduced thyroid hormone levels can impair synaptic plasticity, which directly affects cognitive functions such as learning and memory.

1. Adverse Outcome (AO): Learning and Memory Impairment During Brain Development

The final adverse outcome is a significant impairment in learning and memory, which is particularly critical during the developmental stages of the brain. In animals, this may manifest as difficulties in acquiring, retaining, and utilizing information, leading to long-lasting cognitive and behavioral abnormalities.

Recommended AOP framework:

Inhibition of Na+/I- Symporter (MIE) → 2. Reduced Synthesis of Thyroid Hormones (T3 and T4) (KE) → 3. Decreased Circulating Levels of Thyroid Hormones (KE) → 4. Altered Thyroid Hormone-Dependent Gene Expression (KE) → 5. Impaired Synaptogenesis and Myelination (KE) → 6. Impaired Synaptic Plasticity (KE) → 7. Learning and Memory Impairment During Brain Development (AO)

1. Integration of the annotations and visualization: Based on the GPT-4 annotations and the recommended AOP framework described above, a visual representation of the AOP to depict the sequence of key events from the MIE through KEs to the AO is created:
2. Retrieve the expert-derived AOP information from the AOPwiki database:

AOP introduction: The thyroid hormones (TH) are essential for brain development, maturation, and function as they regulate the early key developmental processes such as neurogenesis, cell migration, proliferation, myelination and neuronal and glial differentiation. Normal human brain development and cognitive function relays on sufficient production of TH during the perinatal period. The function of Na+/I- symporter (NIS) is critical for the physiological production of TH levels in the serum, as it is a membrane bound glycoprotein that mediates the transport of iodide form the bloodstream into the thyroid cells, and this constitutes the initial step for TH synthesis. NIS is a well-studied target of chemicals, and its inhibition results in decreased TH synthesis and its secretion into blood leading to subsequent TH insufficiency in the brain with detrimental effects in neurocognitive function in children. The present AOP describes causative links between inhibition of NIS function (the molecular initiating event) leading to the decreased levels of TH in the blood and consequently in the brain, causing learning and memory deficit in children (Adverse outcome). Three key events of this AOP (decrease of TH synthesis; T4 in serum and T4 in neuronal tissue) are common with AOP 42. Learning and memory depend upon the coordinated action of different brain regions and neurotransmitter systems creating functionally integrated neural networks. Hippocampus and cortex are the most critical brain structures involved in the process of cognitive functions (also learning and memory) in rodents and primates, including man. The overall weight of evidence for this AOP is strong. The function of NIS and its essentiality for TH synthesis is well known across species, however, quantitative information of KERs is limited.

1. AOPs comparison: Compare the GPT-4 reconstructed AOP with the expert-derived AOP from AOPwiki. Green indicates the identical MIEs and KEs, red indicates mismatched or missed KEs in the GPT-4 annotations, and blue color indicates KEs that is present in GPT-4 reconstructed AOP but missing in the AOPwiki framework.

* Detailed procedure for AOPwiki: AOP155 (Deiodinase 2 inhibition leading to increased mortality) reconstruction is outlined below:

1. Input the specific question:

“Construct the potential Adverse Outcome Pathways (AOPs) using the following keywords: Deiodinase 2 inhibition leading to increased mortality via reduced posterior swim bladder inflation in fish. Meanwhile, provide detailed descriptions for each step.”

1. GPT-4's annotations:
2. Molecular Initiating Event (MIE): Deiodinase 2 (DIO2) Inhibition

Deiodinase 2 (DIO2) is an enzyme crucial for converting the thyroid hormone thyroxine (T4) into its more active form, triiodothyronine (T3). T3 plays a vital role in regulating various developmental processes in fish, including swim bladder formation. Inhibition of DIO2 reduces the levels of T3, disrupting normal thyroid hormone signaling pathways essential for proper development.

1. KE1: Reduced Thyroid Hormone (T3) Levels

The inhibition of DIO2 leads to a significant reduction in T3 levels. T3 is essential for activating gene expression involved in key developmental processes. A decrease in T3 disrupts these processes, particularly those related to the proper development of the swim bladder in fish.

1. KE2: Impaired Posterior Swim Bladder Development and Inflation

The swim bladder, a gas-filled organ, allows fish to control buoyancy and maintain their position in the water. The swim bladder has two chambers: the anterior and posterior. Proper inflation of the posterior chamber is critical for its function, and this process is dependent on adequate T3 levels. Reduced T3 disrupts the development and inflation of the posterior swim bladder, leading to underinflation or non-inflation.

1. KE3: Reduced Swimming Performance

Fish with impaired posterior swim bladder inflation face significant challenges in maintaining buoyancy. This impairment affects their swimming performance, making it difficult to swim efficiently, maintain position in the water column, and respond quickly to environmental stimuli. Poor swimming performance can result in reduced ability to forage for food, escape predators, and perform other essential behaviors.

1. Adverse Outcome (AO): Increased Mortality

The combination of impaired swim bladder function, reduced swimming performance, and increased energy expenditure significantly reduces the survival prospects of the fish. These factors contribute to higher mortality rates, particularly during early developmental stages when proper swim bladder function is critical for survival.

Recommended AOP framework:

Deiodinase 2 (DIO2) Inhibition (MIE) → 2. Reduced Thyroid Hormone (T3) Levels (KE) → 3. Impaired Posterior Swim Bladder Development and Inflation (KE) → 4. Reduced Swimming Performance (KE) → 5. Increased Mortality (AO)

1. Integration of the annotations and visualization: Based on the GPT-4 annotations and the recommended AOP framework described above, a visual representation of the AOP to depict the sequence of key events from the MIE through KEs to the AO is created:
2. Retrieve the expert-derived AOP information from the AOPwiki database:

AOP introduction: This AOP describes the sequence of events leading from deiodinase inhibition to increased mortality via reduced posterior swim bladder inflation. Disruption of the thyroid hormone system is increasingly being recognized as an important toxicity pathway that can cause many adverse outcomes, including developmental abnormalities. Three types of iodothyronine deiodinases (DIO1-3) have been described in vertebrates that activate or inactivate THs and are therefore important mediators of thyroid hormone (TH) action. Type II deiodinase (DIO2) has thyroxine (T4) as a preferred substrate and is mostly important for converting T4 to the more biologically active triiodothyronine (T3). Inhibition of DIO2 therefore reduces T3 levels. As in amphibians, the transition between the different developmental phases in fish, including maturation and inflation of the swim bladder, is mediated by THs (Brown et al., 1988; Liu and Chan, 2002). The swim bladder is a gas-filled organ that typically consists of two chambers (Robertson et al., 2007). The posterior chamber inflates during early development in the embryonic phase, while the anterior chamber inflates during late development in the larval phase. This AOP describes how DIO2 inhibition results in reduced T3 levels, which prohibit normal inflation of the posterior chamber of the swim bladder in the embryonic phase. The posterior chamber is important for regulating buoyancy and thus for swimming performance (Robertson et al., 2007). Reduced swimming performance reduces chances of survival due to a decreased ability to forage and avoid predators. The final adverse outcome is a decrease of the population growth rate. Since many AOPs eventually lead to this more general adverse outcome at the population level, the more specific and informative adverse outcome at the organismal level, increased mortality, is used in the AOP title. Support for this AOP is mainly based on chemical exposures in zebrafish and fathead minnows (Jomaa et al., 2014; Cavallin et al., 2017; Stinckens et al., 2018) and on knockdown/knockout and TH supplementation studies in zebrafish embryos where the DIO2 gene is inactivated (Walpita et al., 2009, 2010; Heijlen et al., 2014; Bagci et al., 2015; Houbrechts et al., 2016).

1. AOPs comparison: Compare the GPT-4 reconstructed AOP with the expert-derived AOP from AOPwiki. Green indicates the identical MIEs and KEs, red indicates mismatched or missed KEs in the GPT-4 annotations, and blue color indicates KEs that is present in GPT-4 reconstructed AOP but missing in the AOPwiki framework.

* Detailed procedure for AOPwiki: AOP220 (Cyp2E1 Activation Leading to Liver Cancer) reconstruction is outlined below:

1. Input the specific question:

“Construct the potential Adverse Outcome Pathways (AOPs) using the following keywords: Cyp2E1 activation leading to liver cancer. Meanwhile, provide detailed descriptions for each step.”

1. GPT-4's annotations:
2. Molecular Initiating Event (MIE): Cyp2E1 Activation

Cytochrome P450 2E1 (Cyp2E1) is an enzyme that metabolizes various endogenous and exogenous substances, including alcohol, drugs, and environmental toxins. Activation of Cyp2E1 results in the oxidation of these substances, which leads to the production of reactive oxygen species (ROS) and reactive metabolites. These reactive species are capable of inducing oxidative stress and DNA damage, which can contribute to the initiation of carcinogenesis in the liver.

1. KE1: Increased Production of Reactive Oxygen Species (ROS)

The activation of Cyp2E1 enhances the production of ROS, such as superoxide anions, hydrogen peroxide, and hydroxyl radicals. These ROS can damage cellular components, including lipids, proteins, and DNA. The accumulation of ROS leads to oxidative stress, which overwhelms the cell's antioxidant defense mechanisms and causes further cellular damage.

1. Oxidative DNA Damage

The ROS generated as a result of Cyp2E1 activation can cause oxidative modifications to DNA, including base damage, strand breaks, and cross-linking. These oxidative lesions, if not adequately repaired, can lead to mutations. Oxidative stress induces cytotoxicity, where excessive DNA damage overwhelms the repair mechanisms, leading to cell death (necrosis or apoptosis). This cytotoxicity not only contributes to tissue injury but also triggers regenerative proliferation, increasing the risk of acquiring further mutations during cell division.

1. Chronic Inflammation

Persistent oxidative stress and tissue damage can trigger an inflammatory response in the liver. Chronic inflammation is characterized by the continuous release of pro-inflammatory cytokines, chemokines, and other signaling molecules. This pro-inflammatory environment promotes further cellular damage, increases ROS production, and enhances the proliferation of hepatocytes, contributing to the carcinogenic process.

1. Hepatocyte Proliferation and Regeneration

In response to the damage induced by ROS and inflammation, hepatocytes (liver cells) undergo increased proliferation and regeneration as a compensatory mechanism. However, this regenerative process is accompanied by an increased risk of acquiring mutations due to the ongoing oxidative stress. These mutations can accumulate in genes that regulate cell growth and apoptosis, increasing the likelihood of malignant transformation.

1. Dysregulation of Apoptosis

Apoptosis, or programmed cell death, is a critical mechanism that eliminates damaged or potentially cancerous cells. However, oxidative stress and inflammation can disrupt the regulation of apoptosis, allowing damaged cells to survive and proliferate. The inhibition of apoptosis, coupled with continued cell proliferation, creates an environment conducive to cancer development.

1. Adverse Outcome (AO): Liver Cancer (Hepatocellular Carcinoma)

The combination of oxidative DNA damage, chronic inflammation, and dysregulated cell proliferation and apoptosis culminates in the development of liver cancer, specifically hepatocellular carcinoma (HCC). HCC is a primary malignancy of the liver and is strongly associated with chronic liver injury, oxidative stress, and inflammation. The transformation of normal hepatocytes into cancerous cells is driven by the accumulation of genetic mutations and the tumor-promoting environment created by chronic oxidative stress and inflammation.

Recommended AOP framework:

Cyp2E1 Activation (MIE) → 2. Increased Production of Reactive Oxygen Species (ROS) (KE) → 3. Oxidative DNA Damage (KE) → 4. Chronic Inflammation (KE) → 5. Hepatocyte Proliferation and Regeneration (KE) → 6. Dysregulation of Apoptosis (KE) → 7. Liver Cancer (Hepatocellular Carcinoma) (AO)

1. Integration of the annotations and visualization: Based on the GPT-4 annotations and the recommended AOP framework described above, a visual representation of the AOP to depict the sequence of key events from the MIE through KEs to the AO is created:
2. Retrieve the expert-derived AOP information from the AOPwiki database:

AOP introduction: Cyp2E1 is a cytochrome P450 mono-oxygenase that bioactivates over 85 substrates, thereby creating electrophilic metabolites and oxidative stress. Substrates are low molecular weight compounds that include acetone, acetaminophen, ethanol, chloroform, carbon tetrachloride, furan and molecular oxygen. Mono-oxygenation of these substrates to their reactive metabolites, and the accompanying oxidative stress produced during metabolism, pose health risks because they lead to hepatotoxicity and, often, to liver cancer. Here we describe the AOP for the prolonged activation of Cyp2E1 (MIE) leading to liver cancer (AO). The intervening KEs are oxidative stress (KE1), hepatocytotoxicity (KE2), and sustained/persistent cellular proliferation (KE3). These events occur in the liver, which is the primary site of xenobiotic metabolism in the body. Briefly, the MIE occurs when Cyp2E1 binds a substrate. The Cyp2E1 catalytic cycle is prone to decoupling (adjacent KER1, non-adjacent KER1), which produces oxidative stress (KE1), and mono-oxidation of substrates produces reactive metabolites. Both reactive oxygen species and metabolites cause cytotoxicity (KE2). However, following injury, the liver is able to regenerate itself through an increase in cellular proliferation (KE3). Under conditions of chronic activation of Cyp2E1, excessive chronic increases in levels of reactive oxygen species and cell death, and subsequent dysregulated cellular proliferation, leads to tumour formation (AO). We evaluate the essentiality of the KEs and the biological plausibility of and empirical support for the KERs and report that most are well supported by a large body of scientific literature. Here, we’ve focused on data generated in rodent studies using the Cyp2E1 substrates carbon tetrachloride, chloroform, ethanol and furan. These compounds are all liver carcinogens, but generate negative or equivocal results in short-term genotoxicity tests. In fact, they are widely thought to cause cancer through a cytotoxicity and sustained/persistent proliferation mode of action. We expect that the data and information summarized here will be useful to scientists and regulators that are investigating chemical carcinogens that act through this mechanism. Given the importance of oxidative stress and cytotoxicity in a broad array of toxicological effects, the KE(R)s described should be broadly useful for development of other AOPs. Finally, this AOP describes an important widely acknowledged pathway to toxicity and thus should have many regulatory applications. Further development of the quantitative aspects of this AOP will enable the development of more predictive models of effects resulting from oxidative stress.

1. AOPs comparison: Compare the GPT-4 reconstructed AOP with the expert-derived AOP from AOPwiki. Green indicates the identical MIEs and KEs, red indicates mismatched or missed KEs in the GPT-4 annotations, and blue color indicates KEs that is present in GPT-4 reconstructed AOP but missing in the AOPwiki framework.