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SYMPOSIUM

Example of Adverse Outcome Pathway Concept Enabling Genome-to-Phenome Discovery in Toxicology

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Synopsis The following article represents a mini-review of an intensive 10-year progression of genome-to-phenome (G2P) discovery guided by the adverse outcome pathway (AOP) concept. This example is presented as a means to stimulate crossover of this toxicological concept to enhance G2P discovery within the broader biological sciences community. The case study demonstrates the benefits of the AOP approach for establishing causal linkages across multiple levels of biological organization ultimately linking molecular initiation (often at the genomic scale) to organism-level phenotypes of interest. The case study summarizes a US military effort to identify the mechanism(s) underlying toxicological phenotypes of lethargy and weight loss in response to nitroaromatic munitions exposures, such as 2,4,6-trinitrotoluene. Initial key discoveries are described including the toxicogenomic results that nitrotoluene exposures inhibited expression within the peroxisome proliferator activated receptor α (PPARα) pathway. We channeled the AOP concept to test the hypothesis that inhibition of PPARα signaling in nitrotoluene exposures impacted lipid metabolic processes, thus affecting systemic energy budgets, ultimately resulting in body weight loss. Results from a series of transcriptomic, proteomic, lipidomic, *in vitro* PPARα nuclear signaling, and PPARα knock-out investigations ultimately supported various facets of this hypothesis. Given these results, we next proceeded to develop a formalized AOP description of PPARα antagonism leading to body weight loss. This AOP was refined through intensive literature review and polished through multiple rounds of peer-review leading to final international acceptance as an Organisation for Economic Cooperation and Development-approved AOP. Briefly, that AOP identifies PPARα antagonist binding as the molecular initiating event (MIE) leading to a series of key events including inhibition of nuclear transactivation for genes controlling lipid metabolism and ketogenesis, inhibition of fatty acid beta-oxidation and ketogenesis dynamics, negative energy budget, and ultimately the adverse outcome (AO) of body-weight loss. Given that the PPARα antagonism MIE represented a reliable indicator of AO progression within the pathway, a phylogenetic analysis was conducted which indicated that PPARα amino acid relatedness generally tracked species relatedness. Additionally, PPARα amino acid relatedness analysis using the Sequence Alignment to Predict Across Species Susceptibility predicted susceptibility to the MIE across vertebrates providing context for AOP extrapolation across species. Overall, we hope this illustrative example of how the AOP concept has benefited toxicology sows a seed within the broader biological sciences community to repurpose the concept to facilitate enhanced G2P discovery in biology.

Introduction

The present article provides a case-study overview of a decade-longeffort to functionally integrate genomic responses directly to toxicological/ecotoxicological phenotypes of regulatory concern. Specifically, the adverse outcome pathway (AOP) concept developed by Ankley et al. (2010) served as the organizing principle for conducting a sequential series of studies to enable functional integration of causal data linked through increasing levels of biological organization and ultimately culminating with a whole-organism-level toxicological phenotype of concern, the adverse outcome (AO). In addition to the review of efforts leading to development of an internationally-

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accepted AOP, we provide a new phylogenetic analysis for a critical gene involved in the progression of the AOP to provide context for species-to-species extrapolation (Guindon et al. 2010, LaLone et al. 2016). The AOP concept has been highly influential in leading rapid mechanistically-based genome-to-phenome (G2P) discoveries in the toxicological sciences (Hecker and LaLone 2019, Pollesch et al. 2019). The purpose of the case-study provided herein is to illustrate how the AOP concept has been used within the toxicological sciences with the intent that the concept be repurposed to support G2P discoveries within the general biological sciences.

Background/problem identification

The case-study described herein was initiated based on the need for the US military to understand the environmental impacts of munitions compounds on military ranges. The US Department of Defense (DOD) maintains stewardship of 26.1 million acres of land worldwide (Hardy et al. 2017) where sustainable management is necessary to provide continued access for warfighter training and readiness. The US military has utilized nitro-aromatics, such as 2,4,6-trinitrotoluene (TNT), in munitions for over 100 years (Steen 2006) where various toxicological impacts of nitrotoluenes have been identified in occupational exposures (ATSDR 1995) and in ecotoxicology (Sunahara et al. 2009). The mechanism(s) underlying toxicological phenotypes of lethargy (Dilley et al. 1982) and bodyweight loss (Harrington 1917) in chronic nitrotoluene exposures had remained unexplained in the scientific literature. Further, the body weight loss phenotype has been observed across diverse phylogenetic lineages including humans (Harrington 1917), rats (Deng et al. 2011), Northern bobwhite quail (Quinn et al. 2007), Japanese quail (Quinn et al. 2013), and Western fence lizards (McFarland et al. 2008) suggesting a common mechanism of action (MOA).

Initiation of G2P discovery

As a means to initiate MOA discovery for this bodyweight loss phenotype, we first employed toxicogenomics investigations to assist with MOA hypothesis generation by testing the effects of nitrotoluene exposures on global transcript expression in the Northern bobwhite quail to identify molecular pathways having plausible connections to the phenotype (Rawat et al. 2010). In this investigation, multiple Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways had significant enrichment of differentially expressed transcripts in liver tissues of Northern bobwhite exposed to the nitrotoluene, 2,6-dinitrotoluene (2,6-DNT), in 60d subchronic exposures. In concordance with the body-weight loss phenotype, significant enrichment of the bioenergetics-related KEGG pathway, glycolysis, and gluconeogenesis were observed (Rawat et al. 2010). Several gene transcripts were observed to be differentially expressed within this pathway wherein the directional expression (increased vs. decreased) for all genes indicated inertia toward glycolysis versus gluconeogenesis within that equilibrium pathway. This inertia toward glycolysis suggested that glucose was being used to produce cellular energy from glucose versus being committed to energy storage via gluconeogenesis. Additionally, the peroxisome proliferator activated receptor (PPAR) pathway was significantly enriched in response to the 2,6-DNT exposure (Rawat et al. 2010). The PPAR pathway serves as a master regulatory network for controlling energy metabolism in higher vertebrates (Kersten 2014). In our investigation with Northern bobwhite, transcriptional expression within the PPAR pathway was suggestive of inhibited PPAR signaling, especially within the PPARα branch of the pathway where several transcripts of coding for genes involved in lipid catabolism showed markedly decreased expression (Rawat et al. 2010). At this time, we also discovered that Wintz et al. (2006) observed impaired lipid metabolism and lipid inundation in livers of fathead minnows exposed to a structurally analogous nitrotoluene, 2,4-DNT. Given the sum of these observations, we hypothesized that nitrotoluenes caused body weight loss by impairing PPARα signaling which impaired the ability of the individual to utilize lipid to sustain a positive systemic energy budget (Rawat et al. 2010).

Nitrotoluenes as a class, do they cause similar effects?

Given the observations that 2,6-DNT and 2,4-DNT exposures showed the potential to inhibit PPAR signaling and lipid metabolism (Wintz et al. 2006; Rawat et al. 2010), Deng et al. (2011) sought to determine if nitroaromatics as a chemical class had similar effects in rodent exposures. In that study, rats were exposed to individual dose series of TNT, 2,6-DNT, 2,4-DNT, 2-amino-dinitrotoluene (2A-DNT), and 4-amino-dinitrotoluene (4A-DNT) where transcriptomic investigation of liver tissues indicated significant enrichment of lipid metabolic processes for the three non-amino-substituted nitroaromatics and all five nitroaromatics regarding lipid

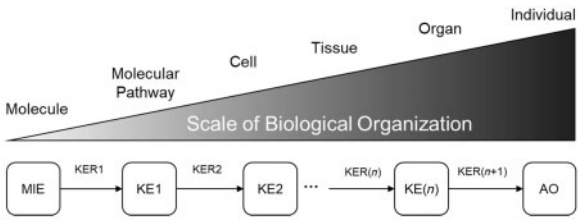

Fig. 1. Conceptual overview of the AOP. *n* = next number in a sequence.

biosynthetic processes where expression was significantly decreased. In concert with the transcriptomics investigation, Deng et al. (2011) also investigated lipidomics profiles in liver which confirmed impaired lipid metabolism in response to nitroaromatics exposures, especially for the non-amino-substituted chemical structures. These observations provided evidence that nitroaromatics, as a chemical class, indeed affected lipid metabolism, supporting our initial hypothesis.

Enter the AOP, and with it, intensive G2P investigation

Around the same time that we had published our genomics-based hypothesis connecting nitrotoluene-induced PPARα inhibition to the phenotype of body weight loss (Rawat et al. 2010), the inception of the AOP concept (Ankley et al. 2010) was forcing the toxicological and ecotoxicological communities of practice to be more thorough in establishing causality among molecular responses and adverse outcome phenotypes. Specifically, the AOP concept challenged scientists to connect the dots between the molecular initiating event (MIE), the AO, and the essential key events (KEs) in between (Fig. 1). To establish causality, key event relationships (KERs) among the MIE, the KEs, and the AO were required to functionally integrate and validate all connections within the AOP. With this concept in mind, we began to brainstorm development of an AOP linking inhibition of PPARα signaling with the AO of body weight loss to help test the hypothesis that nitoaromatic compounds elicit weight loss via this mechanism. As a means to functionally connect the effect of nitroaromatics exposure on PPARα signaling and body weight loss, we utilized experiments comparing responses among PPARα gene knock-out (K/O) versus wild type (WT) mice in 2-week 2,4-DNT dosing experiments (Wilbanks et al. 2014). In that study, WT mice exposed to 2,4-DNT showed significant body-weight loss relative to PPARα K/Os exposed to 2,4-DNT and unexposed controls. Further, in the 2,4-DNT exposures, the PPARα K/O mice performed significantly better than WT mice in a swim endurance challenge experiment. These results confirmed that the nitroaromatic compound 2,4-DNT interfered with PPARα signaling which resulted in decreased exercise performance and body weight loss (Wilbanks et al. 2014).

Connecting the dots, first draft AOP for body weight loss

With a confirmed connection between PPARα and body weight loss in response to nitrotoluenes (Wilbanks et al. 2014), we mined deeper into the molecular processes underlying this observation. In the Wilbanks et al. (2014) study, we further investigated transcriptional expression in livers of mice exposed to 2,4-DNT, which recapitulated the previously observed results of decreased transcriptional expression for genes involved in lipid metabolic processes in the PPAR signaling pathway for WTs. Further, the PPARα K/O mice demonstrated dramatically increased expression of PPARγ, where PPARγ has overlapping coverage with PPARα regarding transcriptional control of several lipid metabolic genes (KEGG pathway: mmu03320). To supplement the PPARα K/O results, we conducted *in vitro* assessment of 2,4-DNT dosing in human PPAR nuclear signaling assays where the nitroaromatic caused significant inhibition of PPARα and PPARδ nuclear signaling, whereas PPARγ signaling was potentiated (Wilbanks et al. 2014). Given the transcript expression and nuclear signaling observations, we hypothesized that potentiation of PPARγ provided alternative control of systemic energy maintenance in PPARα K/O mice that was unperturbed by the 2,4-DNT exposure, thus mitigating the negative phenotypes of reduced exercise performance and body weight loss. Rolling up all of the results described thus far, the nitroaromatics caused (1) inhibition of PPARα nuclear signaling, (2) decreased transcriptional expression of PPARα-regulated genes that control lipid metabolism, (3) interference with lipidomic profiles in the liver, (4) induced transcriptional expression profiles indicative of increased glucose catabolism to produce cellular energy, (5) impaired exercise performance, and (6) decreased body weight. With this body of evidence in place, a plausible skeleton of an AOP was present, thus we published the first draft AOP for PPARα inhibition resulting in body weight loss in Wilbanks et al. (2014).

AOP refinement, literature review, and weight of evidence

The evidence that nitroaromatics elicited the body-weight loss phenotype via this newly developed AOP

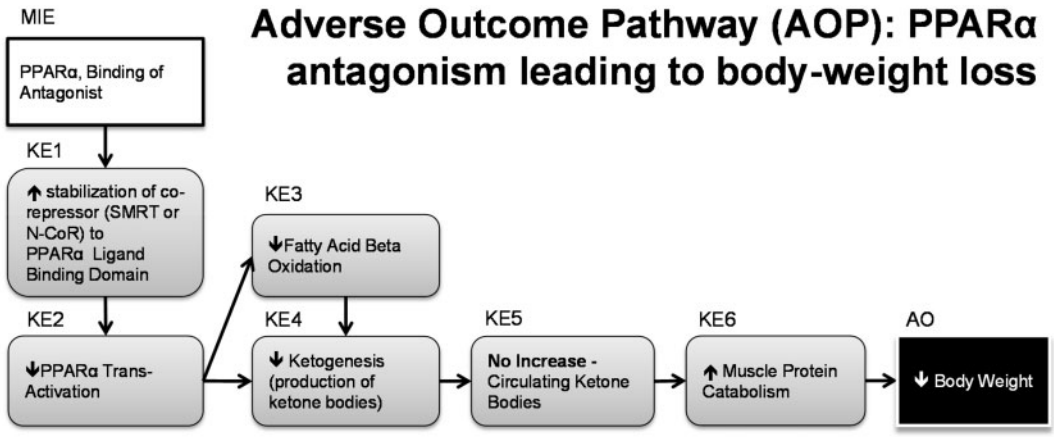

Fig. 2. An AOP describing PPARα antagonism leading to body-weight loss. This AOP is adapted from Gust et al. (2019) which has been reviewed and approved under OECD, AOP Development Programme.

continued to mount with subsequent experimental investigations. For example, in Gust et al. (2015), combined global transcriptomic and proteomic expression in liver tissue of Northern bobwhite quail exposed to 2A-DNT again implicated nitroaromatic interference with PPARα signaling and expression of PPARα-regulated genes involved in lipid metabolism (Gust et al. 2015). The experimental results generated to this point provided a strong impetus to develop a more formalized AOP for consideration within the AOP community of practice for expanded utility and use. By definition, AOPs are chemical-agnostic constructs (Ankley et al. 2010); however, our AOP development was exclusively driven by observations from nitroaromatic exposures. In the next phase of AOP development, we utilized a literature review to test, refine, and strengthen the MIE, KEs, AO, and all KERs within the AOP. Google Scholar was utilized to search a variety of term sets relevant to each component of the AOP, where for example PPARα, lipid metabolism, bioenergetics, etc. were searched. A wealth of literature from diabetes therapeutics research provided key insights into PPARα antagonist binding (Xu et al. 2002) and the molecular mechanisms involved in subsequent inhibition of nuclear transactivation for genes controlling lipid metabolism and ketogenesis (Xu et al. 2002, Desvergne and Wahli 1999). Additionally, observations describing the effects of inhibited PPARα-regulated gene expression on fatty acid beta-oxidation and ketogenesis dynamics (Evans et al. 2004, Badman et al. 2007) provided context for understanding negative energy budget processes and body-weight loss tradeoffs during the onset and progression of starvation (Cahill 2006). The literature review provided a refined structural architecture of the AOP; robust characterization of the MIE, each KE, and the AO; and delivered literature-supported causal relationships for all KERs, ultimately culminating in the second-generation AOP build presented in Collier et al. (2016). As a means to establish the weight of evidence supporting the AOP, we developed a method to evaluate the quality of each published study contributing to the AOP and the strength of causal linkages established for AOP components by employing US Environmental Protection Agency (USEPA) general assessment factors and Bradford Hill criteria, respectively (Collier et al. 2016). These approaches provided insights into aspects of the AOP that had strong literature support versus weaker links to help understand sources of uncertainty and provide guidance for future research efforts.

International review and the resulting finalized AOP

As a means to seek consideration of our AOP within the international community, we submitted our second-generation AOP for review with the Organisation for Economic Cooperation and Development (OECD), AOP Development Programme. The AOP was harmonized with the requirements of the OECD AOP development handbook (OECD 2018) and received rigorous peer-review, undergoing four total rounds of review and revision. The AOP received final acceptance by the Working Group of the National Coordinators for the Test Guidelines Programme and Working Party on Hazard Assessment (WPHA) in May of 2019 (Gust et al. 2019, https://aopwiki.org/aops/6). The structure of the accepted AOP is provided in Fig. 2, which is adapted from Gust et al. (2019).

Although we recommend consulting the Gust et al. (2019) paper directly for a detailed description of the AOP structure/function, a brief overview is provided in the following text to give context (Fig. 2). The MIE for this AOP involves antagonistic chemical binding to PPARα resulting in KE1, increased stabilization of the antagonistic co-repressors SMRT or N-CoR to the PPARα ligand binding domain, thus suppressing PPARα nuclear signaling. As a result, decreased nuclear transactivation of PPARα-regulated genes occurs (KE2), which decreases expression of the genes which catalyze fatty acid beta-oxidation and ketogenesis. Given KE2, decreased fatty-acid beta oxidation (KE3) limiting utilization of lipids for energy substrate production occurs in concert with decreased ketogenic repackaging of lipid-based energy substrates (KE4), the combination of which result in diminished capacity for maintaining the systemic energy budget of the organism. Under this energy-budget short-fall, ketone body production would typically be induced to provide cellular energy for sensitive organ systems, such as the central nervous system. However, sustained absence of supplemental ketone bodies in circulation (KE5) necessitate catabolism of structural proteins including muscle protein (KE6) to enable glutamine and alanine recycling for gluconeogenesis to meet basic systemic energy needs. Under this continued negative systemic energy budget, the AO of whole-body weight loss occurs. The AO has implications in occupational exposures as well as in ecotoxicological exposures where decreased energy allocations to organismal maturation and reproduction (Nisbet et al. 2000) can affect ecological fitness (Martin 1987).

Moving forward—AOP utilities in development

Having an internationally validated AOP provides a platform for continued toxicological investigation, regulatory interpretation, and utilities development. We recently conducted screening of several nitroaromatic compounds against alternative high-nitrogen molecular structures using the *in vitro* human nuclear-signaling activation and inhibition assays described above. The results indicated that various nitrotoluene and related nitroaromatic structures inhibited PPARα nuclear signaling whereas, the non-nitroaromatic high-nitrogen structures did not. Further, the various nitroaromatic structures displayed a broad dynamic range in the antagonism of PPARα nuclear signaling suggesting the potential to identify differential potency using the *in vitro* nuclear signaling assays. This work is currently being developed for publication; however, the results have sparked interest in understanding the progression of the AOP based on this *in vitro* data to provide context for the MIE through KE2 and how this might be used to assess/predict onset of the AO.

Evidence for AOP relevance across phylogenetic lineages

The research in nitroaromatics toxicology which sparked the development of the present AOP utilized results from Northern bobwhite quail, rats, mice, fathead minnows, and *in vitro* human assays, indicating commonalities in toxicological responses across species and a basal connection to PPARα inhibition. In subsequent investigations of TNT exposures in Western fence lizards (Gust et al. 2018a) and exposures to the new insensitive munitions compound, 2,4-dinitroanisole, in fathead minnows (Gust et al. 2018b), transcriptional and phenotypic responses congruent with the PPARα antagonism to body weight loss AOP have been observed. Overall, these results indicate that the AOP may be applicable across broad phylogenetic lineages. Given that PPARα antagonism represents a critical component in the progression of the AOP (Fig. 2), understanding the phylogenetics of PPARα has potential utility for understanding the taxonomic domain of applicability for the AOP.

Phylogenetic analysis of PPARα in vertebrates

We conducted a broad-scale phylogenetic analysis for vertebrate species based on PPARα amino acid sequence to provide evolutionary context for considering AOP conservation across species. Protein sequences for PPARα were identified in a total of 261 vertebrate species within the National Center for Biotechnology Information (NCBI) Genbank repository (https://www.ncbi.nlm.nih.gov/gene/5465/ortholog/? scope=7776). The amino acid sequence data were downloaded and a first-phase alignment was conducted using CLUSTALW by MegAlign Pro™ (DNASTAR, Inc.). The evolutionary relationship distance was calculated using uncorrected pairwise distance metrics based on the amino acid sequence alignment. These results were then used to construct phylogenetic trees with the BioNJ neighbor-joining algorithm (Gascuel 1997) where species identities were then sorted into taxonomic groups to aid evaluation of PPARα relatedness among vertebrates. The results of this first-phase analysis (Supplementary Fig. S1) were next utilized to conduct a higher-fidelity phylogenetic analysis in

a constrained set of 71 species selected to represent the overall group. The selections were made to represent the overall phylogenetic diversity of the greater sequence group, relative abundance of species within each taxonomic grouping, and breadth of phylogenetic distance scores measured among species. The second-phase analysis utilized MUltiple Sequence Comparison by Log-Expectation (MUSCLE, http://www.trex.uqam.ca/index.php?action=muscle) for protein sequence alignment. Evolutionary relationship distance and the phylogenetic tree were established using PhyML v3.0 (http://www.atgc-montpellier.fr/phyml) to calculate maximum likelihood relationships (Guindon et al. 2010). The phylogenetic tree was visualized using Interactive Tree of Life (ITOL) software (https://itol.embl.de/). Overall, the results indicated that closely related species groups tended to also have closely related PPARα amino acid sequences, and thus cluster together in the phylogenetic tree (Fig. 3). Concordantly, the majority of species segregated by ancestral lineages which is showcased by separation of the major sub-trees into discreet taxonomic groups with increasing phylogenetic distance among mammals → birds → reptiles → amphibians → fishes. Overall, the results show predictable PPARα amino acid sequence similarity among species based on species relatedness.

Implications of PPARα phylogenetics in AOP cross-species extrapolation

Given the observation that PPARα amino acid sequence relatedness among species generally tracked species relatedness, we sought to investigate how this phylogenetic relatedness translated to potential conservation of the present MIE (PPARα antagonism), and the overall AOP, across species. To provide this context, we utilized the Sequence Alignment to Predict Across Species Susceptibility(SeqAPASS) software tool (https://seqapass.epa.gov/) which provides amino acid similarity analyses for the complete protein sequence ("level 1"), specific functional domains ("level 2"), and specific amino acid loci ("level 3") for user-defined proteins of importance within AOPs (LaLone et al. 2016). We utilized human PPARα as the target sequence for comparative analysis of PPARα and PPARα orthologs across eukaryotes. The results of the primary amino acid sequence analysis (level 1) indicated a high degree of similarity among humans and most vertebrate species with decreased similarity against invertebrate orthologs (Fig. 4A). The level 2 analysis indicated even greater sequence similarity between human and vertebrate species when focusing on the PPARα ligand-binding domain (Fig. 4B), the specific location of chemical interaction that triggers the MIE. Based on these level 2 sequence similarity results, all vertebrates were predicted to be susceptible to this MIE, whereas the majority of invertebrates were not (Supplementary Table S1). Zhou et al. (2015) also demonstrated that the ligand binding site for PPARα was conserved across humans, mice, birds, amphibians, and fish, indicating that the MIE for our AOP (Fig. 2) is likely to be conserved across the majority of vertebrates. Focusing on the species investigated in our AOP case study (highlighted in Fig. 3), amino acid sequence similarity to humans for the PPARα ligand-binding domain was >95% for all species except zebrafish, which shared 86.3% similarity, and all species were predicted to be susceptible (Table 1). This susceptibility information ultimately provides context for species-to-species extrapolation of the AOP where conservation of the MIE (Fig. 2) is likely to be conserved among species having closely related PPARα ligand binding domains (Table 1). Although the MIE might be conserved, differences in PPARα signaling networks can also affect the progression of the AOP. For example, different responses to PPARα signaling among human and mouse have been identified, however, for the purposes of the present AOP, PPARα regulation of genes involved in lipid metabolic processes (Rakhshandehroo et al. 2009), metabolic outcomes, and adverse outcomes tended to be largely conserved (Gust et al. 2019). These observations suggest potentially broad applicability of the AOP for vertebrate species.

Conclusions

The case-study for AOP development provided herein represents an illustrative example of rigorous G2P discovery in the toxicological sciences. Utilizing the AOP framework focused our research effort in nitroaromatic munitions toxicology to enable MIE discovery, identification of critical KEs, all causally-linked through KERs to the phenotype of interest, the AO (Fig. 1). Specifically, the MIE of PPARα antagonism was causally linked to a progression of inhibited expression for genes involved in lipid catabolism and ketogenesis leading to a negative energy budget and ultimately the AO of body-weight loss (Fig. 2). Given that PPARα antagonism represented a critical response in the AOP, we conducted a phylogenetic analysis across vertebrates which indicated that PPARα amino acid relatedness generally tracked species relatedness. Additionally, amino acid sequence similarity for the PPARα ligand binding

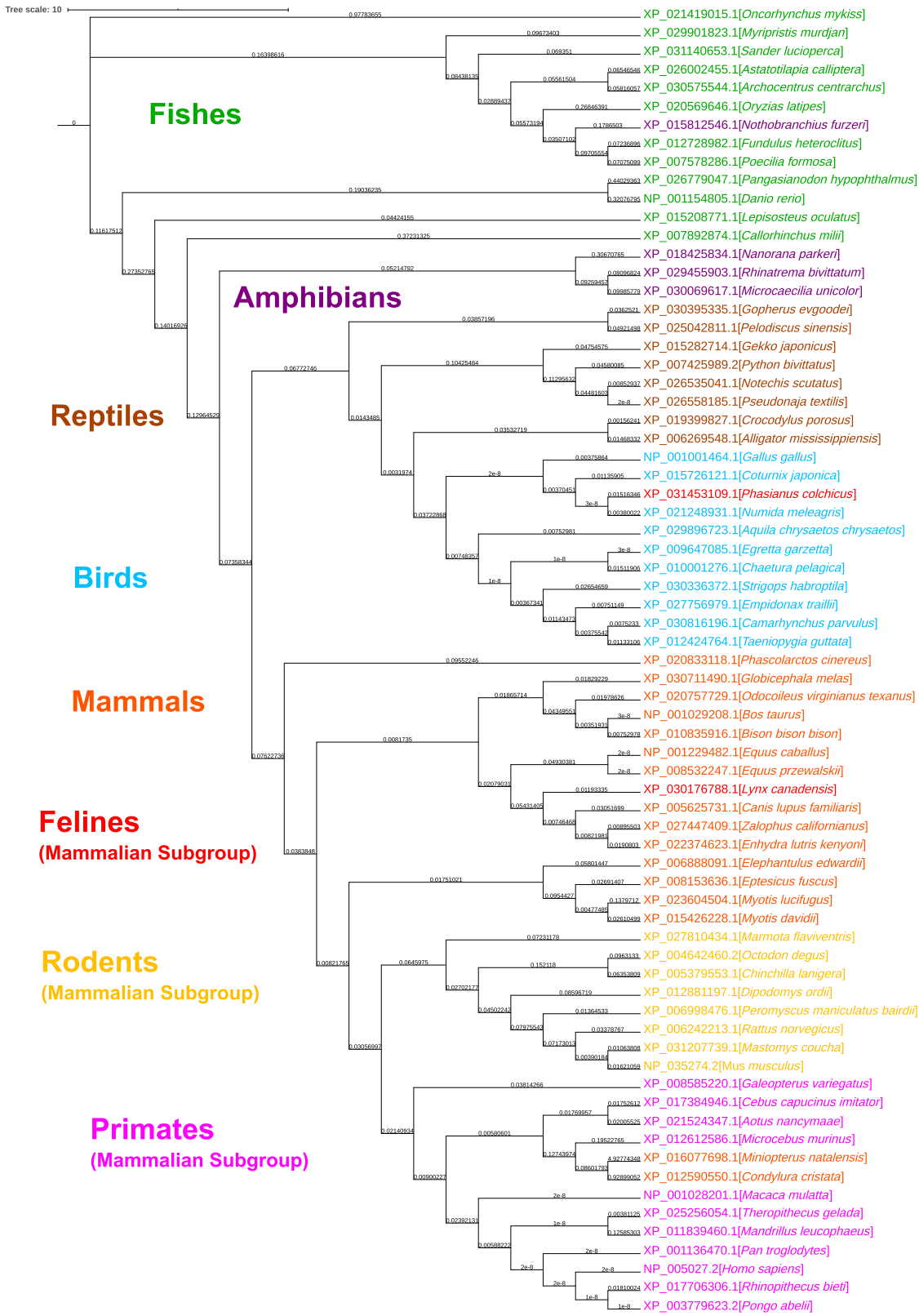

Fig. 3. Phylogenetic analysis of PPARα protein sequence in 71 vertebrate species subsampled from the 261 total species displayed in Supplementary Fig. S1. Species names (and RefSeq Protein IDs) have been color-coded into taxonomic groupings to aid results interpretation. The evolutionary relationship distance scores represent maximum likelihood metrics based on the sequence alignment. Highlighted species were investigated in nitroaromatic exposures relevant to the PPARα antagonism AOP.

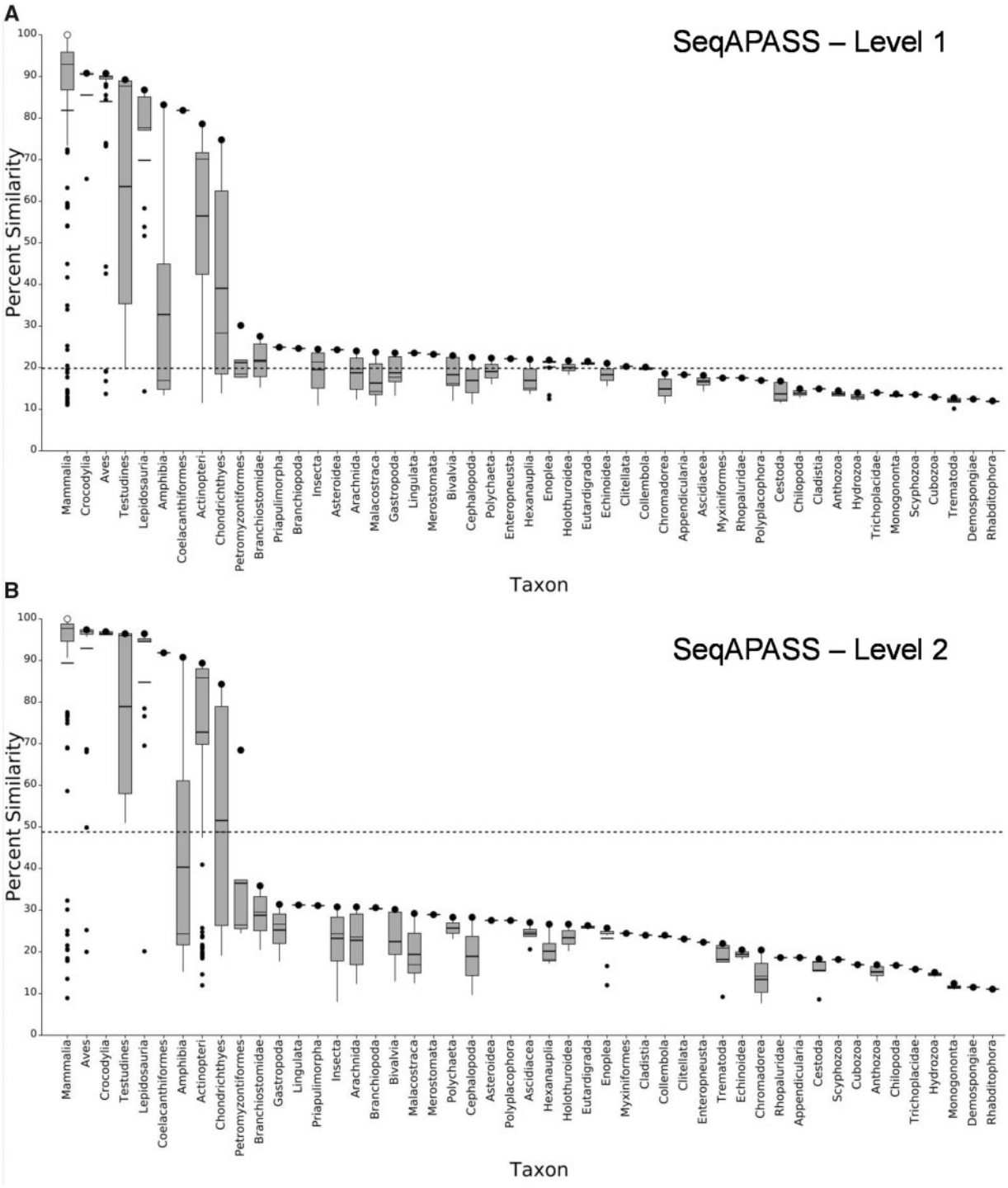

Fig. 4. A boxplot of the protein sequence similarity across phylogenetic groups for PPARα and PPARα orthologs as calculated by the SeqAPASS software tool (https://seqapass.epa.gov/). (**A**) Plot represents a "Level 1" analysis which establishes similarity based on the entire PPARα amino acid sequence. (**B**) Plot provides a "Level 2" analysis which establishes the similarity based on the amino acids represented within the PPARα ligand binding domain.

domain suggested conservation of susceptibility across vertebrates for the MIE (Fig. 4, Table 1), providing context for species-to-species extrapolation of the overall AOP (Fig. 3). We hope the description of this case study showcases the scientific advances that can be achieved when using the AOP concept to focus on scientific investigations. Now, we challenge the greater biological sciences community of practice to repurpose the AOP concept to advance G2P discovery across the board.

Table 1. Results for PPARα "Level 2" ligand-binding domain amino acid sequence similarity analysis comparing human against species investigated during the AOP development described in this case study

NCBI accession	Taxonomic group	Scientific name	Common name	Protein name	Domain name	Percentage similarity	Susceptibility prediction
NP_005027.2	Mammalia	*Homo sapiens*	Human	peroxisome proliferator-activated receptor alpha	NR_LBD	100.0	Y
XP_015145415.1	Aves	*Gallus gallus*	Chicken	peroxisome proliferator-activated receptor alpha isoform X1	NR_LBD	97.4	Y
XP_015726121.1	Aves	*Coturnix japonica*	Japanese quail	PREDICTED: peroxisome proliferator-activated receptor alpha	NR_LBD	96.0	Y
EDL04428.1	Mammalia	*Mus musculus*	House mouse	peroxisome proliferator activated receptor alpha, isoform CRA_b, partial	NR_LBD	95.2	Y
XP_006242213.1	Mammalia	*Rattus norvegicus*	Norway rat	PREDICTED: peroxisome proliferator-activated receptor alpha isoform X1	NR_LBD	95.2	Y
XP_009295973.1	Actinopteri	*Danio rerio*	Zebrafish	peroxisome proliferator-activated receptor alpha isoform X1	NR_LBD	86.3	Y

Note: The analysis was conducted using the SeqAPASS software tool (https://seqapass.epa.gov/).

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Supplementary data

Supplementary data available at *ICB* online.

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