

Table 1. List of Key Events and Associative Events in AOP 107 and Typical Data Generated to Demonstrate Them

Key Events	Associative Events	Typical Data to Demonstrate the AOP
MIE: CAR Activation	AE1: Increased CYP2B, CYP3A activity and/or protein	<p>Typical data generation for MIE and KE1 will include one of more of the following:</p> <ul style="list-style-type: none"> • Show ↑ <i>Cyp2b</i> mRNA (KE1) • Show ↑ CYP2B activity and/or protein (AE1) • Show ↑ pro-proliferative genes responsive to CAR (e.g ↑ <i>Gadd45b</i>) (KE1) • Show ↑ hepatocellular hypertrophy (AE2) • Show ↑ liver wt. (AE3) <p>Other useful data: show CAR activation in species-specific reporter assay (MIE) (e.g. Omiecinski <i>et al.</i>, 2011); show altered gene pathways indicative of CAR activation via microarrays (KE1) (e.g. Oshida <i>et al.</i>, 2015a). Investigate other CYP enzymes (e.g. CYP3A, CYP1A, CYP4A isoforms) via mRNA or enzyme activity measurements, to help exclude alternative modes of action. Note: some CAR activators have been shown to inhibit CYP enzymes that they induce (e.g. pronamide, LeBaron <i>et al.</i>, 2014), so both mRNA changes and enzyme activities may need to be examined if this inhibition is suspected.</p>
KE1: (Hepatocytes) Altered gene expression secondary to CAR activation	AE2: Hepatocellular hypertrophy AE3: Increased liver weight	
KE2: (Hepatocytes) Increased mitogenic proliferation		<p>Typical data generation for KE2 will include:</p> <ul style="list-style-type: none"> • Show ↑ cell proliferation via BrdU (5-bromo-2'-deoxyuridine), EdU (5-ethynyl-2'-deoxyuridine), ³H-thymidine labeling index, or • Show ↑ cell proliferation via Ki67 labelling index <p>Other useful data: show markers of ↑ cell proliferation via gene expression changes (KE1), or by histopathology changes such as increased mitotic figures.</p>
KE3: Increased preneoplastic foci (hepatocytes)		<p>May or may not be observed in long-term study, depending on timing of sacrifices.</p> <p>Typical data for KE3: ↑ altered foci via histopathology</p>

AO: Increased hepatocellular adenomas, carcinomas		Typical data for AO: ↑ hepatocellular adenomas and/or carcinomas via histopathology
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A more thorough review of current methods and emerging methods for demonstrating a CAR mode of action is available in recent published literature (Peffer et al., 2017 in press).