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

Key Events	Associative Events (AE's)	Typical Data to Demonstrate the AOP
MIE: CAR Activation KE1: (Hepatocytes) Altered gene expression specific to CAR activation	Increased CYP2B, CYP3A activity and/or protein Hepatocellular hypertrophy Increased liver weight	Typical data generation for MIE and KE1 will include one of more of the following: • Show ↑ *Cyp2b* mRNA (KE1) • Show ↑ CYP2B activity and/or protein (AE) • Show ↑ pro-proliferative genes responsive to CAR (e.g ↑*Gadd45b*) (KE1) • Show ↑ hepatocellular hypertrophy (AE) • Show ↑ liver wt. (AE) Other useful data: show CAR activation in species-specific reporter assay (MIE) (e.g. Omiecinski et al., 2011b); show altered gene pathways indicative of CAR activation via microarrays (KE1) (e.g. Oshida et al., 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 et al., 2014), so both mRNA changes and enzyme activities may need to be examined if this inhibition is suspected.
KE2: (Hepatocytes) Increased mitogenic proliferation		Typical data generation for KE2 will include: • Show ↑ cell proliferation via BrdU (5-bromo-2'-deoxyuridine), EdU (5-ethynyl-2'-deoxyuridine), ^{3}H-thymidine labeling index, or • Show ↑ cell proliferation via Ki67 labelling index Other useful data: show markers of ↑ cell proliferation via gene expression changes (KE1), or by histopathology changes such as increased mitotic figures.
KE3: Increased preneoplastic foci (hepatocytes)		May or may not be observed in long-term study, depending on timing of sacrifices. Typical data for KE3: ↑ altered foci via histopathology
AO: Increased hepatocellular adenomas, carcinomas		Typical data for AO: ↑ hepatocellular adenomas and/or carcinomas via histopathology

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., 2018b).

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