AOP ID and Title:

SNAPSHOT

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AOP 117: Androgen receptor activation leading to hepatocellular adenomas and carcinomas (in mouse and rat)

Short Title: AR- HCC

Authors

Cancer AOP Workgroup. National Health and Environmental Effects Research Laboratory, Office of Research and Development, Integrated Systems Toxicology Division, US Environmental Protection Agency, Research Triangle Park, NC. Corresponding author for wiki entry (wood.charles@epa.gov)

Status

Author status	OECD status	OECD project	SAAOP status
Under development: Not open for comment. Do not cite	Under Development	1.26	Included in OECD Work Plan

Abstract

This putative adverse outcome pathway (AOP) outlines potential key events leading to a tumor outcome in standard carcinogenicity models. This information is based largely on modes of action described previously in cited literature sources and is intended as a resource template for AOP development and data organization. Presentation in this Wiki does not indicate EPA acceptance of a particular pathway for a given reference agent, only that the information has been proposed in some manner. In addition, this putative AOP relates to the model species indicated and does not directly address issues of human relevance.

Summary of the AOP

Events

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

Sequence	Type	Event ID	Title	Short name
1	MIE	785	Activation, Androgen receptor (https://aopwiki.org/events/785)	Activation, Androgen receptor
2	KE	716	Increase, Mitogenic cell proliferation (hepatocytes) (https://aopwiki.org/events/716)	Increase, Mitogenic cell proliferation (hepatocytes)
3	KE	774	Increase, Preneoplastic foci (hepatocytes) (https://aopwiki.org/events/774)	Increase, Preneoplastic foci (hepatocytes)
4	AO	719	Increase, Adenomas/carcinomas (hepatocellular) (https://aopwiki.org/events/719)	Increase, Adenomas/carcinomas (hepatocellular)

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Activation, Androgen receptor (https://aopwiki.org/relationships/787)	adjacent	Increase, Mitogenic cell proliferation (hepatocytes)		
Increase, Mitogenic cell proliferation (hepatocytes) (https://aopwiki.org/relationships/773)	adjacent	Increase, Preneoplastic foci (hepatocytes)		
Increase, Preneoplastic foci (hepatocytes) (https://aopwiki.org/relationships/774)	adjacent	Increase, Adenomas/carcinomas (hepatocellular)		

Overall Assessment of the AOP

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Mus musculus	Mus musculus		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
Rattus norvegicus	Rattus norvegicus		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

Sex Applicability

Sex	Evidence
Male	
Female	

References

NTP (National Toxicology Program). (September 2010). Toxicology and Carcinogenesis Studies of Androstenedione in F344/N Rats and B6C3F1 Mice (Vol. NTP TR 560).

Appendix 1

List of MIEs in this AOP

Event: 785: Activation, Androgen receptor (https://aopwiki.org/events/785)

Short Name: Activation, Androgen receptor

Key Event Component

Process	Object	Action
androgen receptor activity	androgen receptor	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:117 - Androgen receptor activation leading to hepatocellular adenomas and carcinomas (in mouse and rat) (https://aopwiki.org/aops/117)	MolecularInitiatingEvent

Stressors

Name
Androstenedione

Biological Context

Level of Biological Organization
Molecular

List of Key Events in the AOP

Event: 716: Increase, Mitogenic cell proliferation (hepatocytes) (https://aopwiki.org/events/716)

Short Name: Increase, Mitogenic cell proliferation (hepatocytes)

Key Event Component

Process	Object	Action
cell proliferation	mitogenic signaling cell	increased
hepatocyte proliferation	hepatocyte	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:107 - Constitutive androstane receptor activation leading to hepatocellular adenomas and carcinomas in the mouse and the rat (https://aopwiki.org/aops/107)	KeyEvent
Aop:117 - Androgen receptor activation leading to hepatocellular adenomas and carcinomas (in mouse and rat) (https://aopwiki.org/aops/117)	KeyEvent
Aop:37 - PPARalpha-dependent liver cancer (https://aopwiki.org/aops/37)	KeyEvent

Stressors

Name
Phenobarbital
Epidermal growth factor
pirinixic acid
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
hepatocyte

Organ term

Organ term
liver

Evidence for Perturbation by Stressor

Phenobarbital

Phenobarbital

1. NaPB treatment has been shown to increase replicative DNA synthesis in cultured mouse (Haines et al., 2018c) and rat hepatocytes (Haines et al., 2018c; Hirose et al., 2009).

2. NaPB treatment (1 week 500-2500 ppm in the diet) was shown to significantly increase the BrdU labeling index in the livers of male CD-1 mice and male Wistar rats compared to their respective vehicle-treated controls (Yamada et al., 2014).

3. An increase in replicative DNA synthesis was observed in male and female mice administered 1000 ppm NaPB in the diet for 1 month (Jones et al., 2009).

4. PB at 0, 10, 50, 100 and 500 mg/kg (ppm) in the diet was administered to 8 week old male rats and male mice for 90 days. A significant induction of hepatic replicative DNA synthesis (as determined by [3H]-thymidine incorporation) was observed in the rat liver at 7 days, but had returned to control levels by 14 days. In mice, there was a significant increase in hepatic replicative DNA synthesis throughout treatment (Kolaja et al., 1996a). In both species, the most pronounced effect was observed in the centrilobular region.

Epidermal growth factor

Epidermal growth factor

1. Human epidermal growth factor (hEGF) treatment was shown to significantly increase replicative DNA synthesis, and Ki-67 mRNA levels in human hepatocytes of chimeric mice with humanized livers (human hepatocyte chimeric livers) (Yamada et al., 2014).

2. EGF has been shown to increase the proliferation of mouse (Bowen et al., 2014; Haines et al., 2018c), rat (Bowen et al., 2014; Haines et al., 2018c; Hodges et al., 2000), and human (Haines et al., 2018c; Parzefall et al., 1991) hepatocyte cultures as determined by increase in replicative DNA synthesis compared to appropriate controls.

pirinixic acid

WY-14,643 (pirinixic acid)

1. WY-14,643 (pirinixic acid) is a potent PPARα activator, and its ability to stimulate cell proliferation has been reviewed in Corton et al. (2018).

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)

1. TCDD is a potent AhR activator, and its ability to stimulate cell proliferation has been reviewed in Becker et al. (2015).

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
human	Homo sapiens		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Hamster	Hamster		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=0)

Term	Scientific Term	Evidence	Links
dog	Canis lupus familiaris		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9615)

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Epidermal growth factor (EGF) is one of several extracellular ligands of the epidermal growth factor receptor (EGFR). The EGFR signaling pathway is conserved in most animals, in which it controls processes such as cell proliferation, differentiation, adhesion, and migration (Barberan and Cebria, 2018).

EGFR is a transmembrane protein that is classified as a tyrosine kinase receptor. EGFR has several structural domains: 1) an N-terminal extracellular domain that binds ligands such as EGF, 2) a transmembrane domain, 3) an intracellular domain containing tyrosine kinase activity, and 4) a C-terminal region that contains tyrosine residues that are the sites of autophosphorylation. Ligand binding results in a cascade of events that include EGFR homo-or heterodimerization, activation of the tyrosine kinase domain, tyrosine autophosphorylation, and ultimately the activation of downstream signaling cascades that control various processes in the liver such as proliferation, survival, differentiation, response to injury, and repair (Berasain and Avila, 2014; Komposch and Sibilia, 2015).

EGF has been used as an agent to stimulate proliferation of rat, mouse, and human hepatic cells in culture (Bowen et al., 2014; Haines et al., 2018c; Hodges et al., 2000; Parzefall et al., 1991).

Other mitogenic agents produce a cell proliferation response in rats and mice, but not other mammalian species such as humans, hamsters or dogs. These agents include phenobarbital (a model CAR activator) (Haines et al., 2018c; Hirose et al., 2009; Parzefall et al., 1991), WY-14,643 (pirinixic acid) (a model PPARalpha activator) (Corton et al., 2018) and TCDD (a model AhR activator) (Becker et al., 2015; Budinsky et al., 2014).

Key Event Description

Key Event Description:

One of the mechanisms known to induce cell proliferation in the livers of rats and mice occurs through exposure to a mitogen. Mitogenic cell proliferation is characterized by liver enlargement without evidence of necrosis, as opposed to regenerative/compensatory proliferation, in which the liver parenchyma is restored after loss due to necrosis or hepatectomy.

In mammals that have been administered a mitogenic xenobiotic, several factors impact the nature of the hepatocyte proliferative response. These include the identity of the mitogen, the time course and dose of administration, and the species and strain of the test system. The effects on the liver may be confined to certain lobes, or may be observed throughout the organ (Columbano and Shinozuka, 1996).

How it is Measured or Detected

There are several well-characterized and well-accepted techniques that have been used to detect mitogenic proliferation in vitro and in vivo (Peffer et al., 2018b). These include the detection of labeled nucleosides or nucleoside analogs that have been incorporated into newly synthesized DNA, or the detection of endogenous markers of proliferation such as antigen Ki-67 or proliferating cell nuclear antigen (PCNA) (Kee et al., 2002; Muskhelishvili et al., 2003; Wood et al., 2015). Several of these techniques may involve immunohistochemical techniques to detect proliferating cells, thus allowing for the detection of proliferation within specific tissue sections. For each of these methods, a labeling index (fraction of labeled cell population/total number of cells in population) is calculated, and this index can be statistically compared between different groups (Wood et al., 2015).

Nucleoside and nucleoside analog labeling. Actively proliferating cells undergo DNA synthesis in a highly regulated process during the S (synthesis) phase of the cell cycle. Once the DNA of a cell is replicated during S phase, the cell undergoes mitosis. This results in two cells, each of which has a complete copy of the genome. The DNA replication that occurs in S phase may be detected by the incorporation radiolabeled (e.g., 3H-thymidine) into the newly synthesized DNA, which can be detected from isolated livers using standard autoradiographic techniques. Nucleoside analogs may also be incorporated into the newly-synthesized DNA, including 5-bromo-2-deoxyuridine (BrdU) or 5-ethyl-2'-deoxy uridine (EdU), which may be detected using standard immunohistochemical and biolabeling techniques, respectively (Cavanagh et al., 2011). Drawbacks of the use of nucleoside analogs include concerns regarding the proper administration (dose, route of administration and length of exposure) to animals that allow for adequate labeling without inducing considerable toxicity (Cavanagh et al., 2011; Cohen, 2010). In addition, nucleoside/nucleoside analog incorporation techniques are not specific for the detection of proliferation but may also identify cells that are undergoing DNA synthesis during apoptosis or DNA repair.

Endogenous markers of proliferation. Ki-67 and PCNA are endogenous proteins expressed by mammalian cells that are in active phases of the

cell cycle (G1, S, G2, M) and are not expressed in quiescent (G0) cells (Dietrich, 1993; Eldrige et al., 1993; Scholzen and Gerdes, 2000). They are detected in hepatocytes using standard immunohistochemical techniques. The advantage of using endogenous markers is that they do not require administration of exogenous markers for labeling, and they can be used for both prospective and retrospective cell proliferation analysis. A direct comparison of BrdU, Ki67 and PCNA labeling in various proliferating tissues of male Sprague-Dawley rats (Muskhelishvili et al., 2003) has indicated that Ki67 and BrdU immunohistochemistry methods gave similar labelling index results, whereas PCNA immunohistochemistry was not concordant with these methods and gave highly variable results. These authors suggested that PCNA is less accurate as a measure of cell proliferation because it has a long half-life and can be retained in cells that are not dividing, and is more involved in DNA repair mechanisms than Ki67. As a result, Ki67 has emerged as a more preferred endogenous marker for assessing cell proliferation in hepatocytes in recent years compared to PCNA.

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Event: 774: Increase, Preneoplastic foci (hepatocytes) (https://aopwiki.org/events/774)

Short Name: Increase, Preneoplastic foci (hepatocytes)

Key Event Component

Process	Object	Action
preneoplasia	abnormal cell mass	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:107 - Constitutive androstane receptor activation leading to hepatocellular adenomas and carcinomas in the mouse and the rat (https://aopwiki.org/aops/107)	KeyEvent
Aop:117 - Androgen receptor activation leading to hepatocellular adenomas and carcinomas (in mouse and rat) (https://aopwiki.org/aops/117)	KeyEvent

AOP ID and Name	Event Type
Aop:118 - Chronic cytotoxicity leading to hepatocellular adenomas and carcinomas (in mouse and rat) (https://aopwiki.org/aops/118)	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
hepatocyte

List of Adverse Outcomes in this AOP

Event: 719: Increase, Adenomas/carcinomas (hepatocellular) (https://aopwiki.org/events/719)

Short Name: Increase, Adenomas/carcinomas (hepatocellular)

Key Event Component

Process	Object	Action
	Adenoma	increased
	Carcinoma	increased
hepatocellular carcinoma	Adenoma	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:107 - Constitutive androstane receptor activation leading to hepatocellular adenomas and carcinomas in the mouse and the rat (https://aopwiki.org/aops/107)	AdverseOutcome
Aop:108 - Inhibition of pyruvate dehydrogenase kinase leading to hepatocellular adenomas and carcinomas (in mouse and rat) (https://aopwiki.org/aops/108)	AdverseOutcome
Aop:117 - Androgen receptor activation leading to hepatocellular adenomas and carcinomas (in mouse and rat) (https://aopwiki.org/aops/117)	AdverseOutcome
Aop:118 - Chronic cytotoxicity leading to hepatocellular adenomas and carcinomas (in mouse and rat) (https://aopwiki.org/aops/118)	AdverseOutcome
Aop:37 - PPARalpha-dependent liver cancer (https://aopwiki.org/aops/37)	AdverseOutcome

Biological Context

Level of Biological Organization
Tissue

Organ term

Organ term
liver

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 787: Activation, Androgen receptor leads to Increase, Mitogenic cell proliferation (hepatocytes) (https://aopwiki.org/relationships/787)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Androgen receptor activation leading to hepatocellular adenomas and carcinomas (in mouse and rat) (https://aopwiki.org/aops/117)	**adjacent**		

Relationship: 773: Increase, Mitogenic cell proliferation (hepatocytes) leads to Increase, Preneoplastic foci (hepatocytes) (https://aopwiki.org/relationships/773)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Constitutive androstane receptor activation leading to hepatocellular adenomas and carcinomas in the mouse and the rat (https://aopwiki.org/aops/107)	**adjacent**	**High**	**High**
Androgen receptor activation leading to hepatocellular adenomas and carcinomas (in mouse and rat) (https://aopwiki.org/aops/117)	**adjacent**		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Studies in various species, or in isolated hepatocytes from various mammalian species including humans, have demonstrated that CAR activators such as phenobarbital or metofluthrin produce a cell proliferation response that is seen in mice or rats, but not in hamsters, guinea pigs or humans (Hasmall and Roberts, 1999; Hirose et al., 2009; James and Roberts, 1996; Yamada et al., 2014; Yamada et al., 2009). Accordingly, phenobarbital and other CAR activators do not produce liver tumors in long term studies in hamsters (Diwan et al., 1986; Elcombe et al., 2014). Consistent with the lack of effects on proliferation, Diwan et al. (1986) also reported that in Syrian hamsters, phenobarbital treatment at 500 ppm in the drinking water did not produce any increases in preneoplastic foci of cellular alteration compared to groups that received an initiator alone. Therefore, this key event of increased foci in the liver has strong data indicating it is specific to mice and rats, the species which also develop hepatocellular tumors in response to known CAR activators.

Key Event Relationship Description

Based on altered gene expression under the influence of CAR activation, an increase in cell proliferation of hepatocytes leads to a greater chance of normal, spontaneous errors in DNA replication and thus a higher proportion of altered hepatocytes. The hepatocytes with abnormal DNA can

exhibit cell-cell communication differences from normal hepatocytes, and experience greater cell division even in the presence of contact inhibition with other hepatocytes. The islands of more actively dividing hepatocytes can be detected via histology based both on the larger numbers of cells (hyperplasia) and possibly a characteristic staining property of the clonally expanded cells (foci of cellular alteration – either eosinophilic, basophilic or clear cell). Thus, a higher rate of proliferation in the rodent liver leads to greater prevalence of altered hepatocytes, which clonally expand to generate an increase in preneoplastic foci.

Evidence Supporting this KER

Biological Plausibility

The increased cell replication rate in the liver due to CAR activation (i.e. via a mitogenic signaling) is similar to other well-understood modes of action where an increase in cell proliferation leads to an eventual increase in preneoplastic foci, such as PPARα activating ligands and AhR activating ligands, which also lead to an increase in preneoplastic foci via clonal expansion of transformed hepatocytes. In mice lacking the CAR receptor, including initiation-promotion assays, the upstream events (e.g. CAR activation, altered gene expression, and increased cell proliferation) and the downstream events (e.g. preneoplastic foci) are all blocked, providing strong support for the biological plausibility of this Key Event Relationship (Huang et al., 2005; Tamura et al., 2015; Tamura et al., 2013; Yamamoto et al., 2004).

Empirical Evidence

The observed increase in numbers of preneoplastic foci, usually with eosinophilic staining properties, is observed with great regularity in mode of action work of CAR activating xenobiotics where histopathology at later times has been examined. This increase in foci (mixed or eosinophilic) after 2 years was observed at tumorigenic dose levels with metofluthrin in male rats (Deguchi et al., 2009), and at tumorigenic dose levels in mice treated with phenobarbital (Jones et al., 2009). With TCPOBOP in mice, multiple eosinophilic foci were reported to co-occur along with an increased incidence of eosinophilic adenomas and carcinomas after 60 weeks of treatment (Diwan et al., 1992). With well-studied CAR activators such as phenobarbital and TCPOBOP, increased cell proliferation has been detected at similar dose levels where increased altered foci are seen (Geter et al., 2014; Huang et al., 2005; Kolaja et al., 1996a; Kolaja et al., 1996b) (Tables 2 and 3); therefore, there is strong support for the linkage of these earlier key events with CAR activators leading to an increase in pre-neoplastic foci.

Uncertainties and Inconsistencies

The incidence of altered foci, and their histological staining properties (e.g. eosinophilic, basophilic, clear cell, mixed) are not always reported in published studies of carcinogenicity with CAR activating compounds. In addition, the timing of interim or final sacrifices and histopathology data may possibly miss a window of time (for certain molecules) where the increase in preneoplastic foci can be quantified. However, the consistent findings with well-known CAR activating compounds and their absence in CAR knockout mouse studies provide a strong basis for their existence in the CAR AOP.

References

[see reference list at end of this AOP; it includes all cited references]

Relationship: 774: Increase, Preneoplastic foci (hepatocytes) leads to Increase, Adenomas/carcinomas (hepatocellular) (https://aopwiki.org/relationships/774)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Constitutive androstane receptor activation leading to hepatocellular adenomas and carcinomas in the mouse and the rat (https://aopwiki.org/aops/107)	**adjacent**	**High**	**High**
Androgen receptor activation leading to hepatocellular adenomas and carcinomas (in mouse and rat) (https://aopwiki.org/aops/117)	**adjacent**		
Chronic cytotoxicity leading to hepatocellular adenomas and carcinomas (in mouse and rat) (https://aopwiki.org/aops/118)	**adjacent**		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Phenobarbital and other CAR activators do not produce liver tumors in long term studies in hamsters (Diwan et al., 1986; Elcombe et al., 2014).

Consistent with the lack of effects on proliferation and on tumor development, Diwan et al. (1986) also reported that phenobarbital treatment at 500 ppm in the drinking water did not produce any increases in preneoplastic foci of cellular alteration compared to groups that received an initiator alone. Further, treatment of CAR knockout mice lacking the CAR nuclear receptor with phenobarbital or TCPOBOP produced none of the early key events (e.g. altered expression of CAR-responsive cell cycle genes, increased cell proliferation) and no increases in altered foci or tumors (Huang et al., 2005; Yamamoto et al., 2004). Therefore, the development of increased foci in the liver in response to treatment with CAR activators has strong data indicating it is specific to mice and rats, the species which also develop hepatocellular tumors in response to known CAR activators.

Key Event Relationship Description

Clonally expanded cells (foci of cellular alteration – either eosinophilic, basophilic or clear cell) have been shown to be increased at tumorigenic dose levels of CAR activators such as phenobarbital, TCPOBOP and metofluthrin. As discussed for earlier key events, the CAR-mediated events that lead to an increase in altered foci lead to a greater abundance of cells with mutations in their DNA that are less responsive to normal cell-cell signaling and control mechanisms. As a result, these foci are considered preneoplastic lesions, and can progress with time into adenomas and carcinomas. The continued CAR-mediated stimulus for increased cell proliferation within these foci (e.g. as demonstrated in studies by Kolaja et al., 1996b) will also provide an environment where the mutant cells can survive and develop into tumors.

Evidence Supporting this KER

Biological Plausibility

The development of liver tumors in rodents, whether spontaneously or induced by a non-genotoxic carcinogen, has consistently included the development of altered foci as a precursor step to hepatocellular adenomas and carcinomas (Goldsworthy and Fransson-Steen, 2002; Tamura et al., 2015). These foci are considered preneoplastic lesions, and their ability to progress to form adenomas and/or carcinomas in rodents has been previously recognized. In the case of CAR activators, an increased incidence of preneoplastic foci has been consistently shown to precede tumor development, and there is a high biological plausibility for this Key Event Relationship (Elcombe et al., 2014; Goldsworthy and Fransson-Steen, 2002; Jones et al., 2009; Lake, 2009).

Empirical Evidence

The observed increase in numbers of preneoplastic foci, usually with eosinophilic staining properties, is observed with great regularity in mode of action work of CAR activating xenobiotics where histopathology at later times has been examined. This increase in foci (mixed or eosinophilic) after 2 years was observed at tumorigenic dose levels with metofluthrin in male rats (Deguchi et al., 2009), and at tumorigenic dose levels in mice treated with phenobarbital (Jones et al., 2009). With TCPOBOP in mice, multiple eosinophilic foci were reported to co-occur along with an increased incidence of eosinophilic adenomas and carcinomas after 60 weeks of treatment (Diwan et al., 1992).

In addition, experiments where the MIE (CAR activation) is blocked have been performed with these model CAR activators. For phenobarbital and TCPOBOP in mice, the early key events and the progression to increased altered foci and hepatocellular tumors were all blocked in CAR knockout mice (Huang et al., 2005; Yamamoto et al., 2004). Foci of cellular alteration in CAR knockout mice were also prevented in an initiation-promotion model using the CAR activators cyproconazole and fluconazole (Tamura et al., 2015), and the incidence of adenomas and carcinomas was similarly decreased (Tamura et al., 2015). Thus, there is strong support for the involvement of CAR activation in these mechanisms, and that the stated sequence of key events following CAR activation leads to an increase in pre-neoplastic foci and then liver tumors in mice and rats.

Uncertainties and Inconsistencies

The incidence of altered foci, and their staining properties (e.g. eosinophilic, basophilic, clear cell, mixed) are not always reported in published studies of carcinogenicity with CAR activation compounds. However, the consistent findings with well-known CAR activating compounds and their absence in CAR knockout mouse studies provide a strong basis for their existence in the CAR AOP.

References

[see reference list at end of this AOP; it includes all cited references]