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Minimum datasets to establish a CAR-mediated mode of action for rodent liver tumors

Richard C. Peffer[a,*], Matthew J. LeBaron[b], Michael Battalora[c], Werner H. Bomann[d], Christoph Werner[e], Manoj Aggarwal[f], Rocky R. Rowe[g], Helen Tinwell[h]

[a] Syngenta Crop Protection, LLC, P.O. Box 18300, Greensboro, NC, 27419-8300, United States
[b] The Dow Chemical Company, Toxicology & Environmental Research & Consulting, Midland, MI, 48674, United States
[c] DuPont Crop Protection, 1090 Elkton Road, Newark, DE, 19711, United States
[d] Tox-Consult LLC for ADAMA MAH BV, Amsterdam NL Schaffhausen Branch, 8200, Schaffhausen, Switzerland
[e] BASF SE, FEP/PP, Z473, 67056, Ludwigshafen, Germany
[f] Dow AgroSciences Ltd, 3B Park Square, Milton Park, Abingdon, Oxfordshire, OX14 4RN, United Kingdom
[g] European Crop Protection Association (ECPA), Brussels, BE, Belgium
[h] Bayer SAS, 16 rue Jean-Marie Leclair, 69009, Lyon, France

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ABSTRACT

Methods for investigating the Mode of Action (MoA) for rodent liver tumors via constitutive androstane receptor (CAR) activation are outlined here, based on current scientific knowledge about CAR and feedback from regulatory agencies globally. The key events (i.e., CAR activation, altered gene expression, cell proliferation, altered foci and increased adenomas/carcinomas) can be demonstrated by measuring a combination of key events and associative events that are markers for the key events. For crop protection products, a primary dataset typically should include a short-term study in the species/strain that showed the tumor response at dose levels that bracket the tumorigenic and non-tumorigenic dose levels. The dataset may vary depending on the species and the test compound. As examples, Case Studies with nitrapyrin (in mice) and metofluthrin (in rats) are described. Based on qualitative differences between the species, the key events leading to tumors in mice or rats by this MoA are not operative in humans. In the future, newer approaches such as a CAR biomarker signature approach and/or *in vitro* CAR3 reporter assays for mouse, rat and human CAR may eventually be used to demonstrate a CAR MoA is operative, without the need for extensive additional studies in laboratory animals.

1. Introduction

The inclusion of information concerning the mode of action (MoA) for rodent tumor formation in the data package for crop protection active substances submitted for registration or re-registration to regulatory authorities is becoming more frequent. Such data should provide dose-response and temporal information to support the MoA (including supporting evidence for each key event) and whether or not the MoA is relevant to humans. A conceptual framework was developed by the World Health Organization – International Programme on Chemical Safety (WHO-IPCS) as described by Sonich-Mullin et al. (2001) to aid in the process of characterizing a proposed MoA and determining the human non-relevance. This framework allows the reviewer to apply a rigorous and transparent approach in the assessment of the weight of evidence for rodent tumor formation and identification of critical data needs. This framework has gone through several iterations, with the most recent in 2014 (Meek et al., 2014), and the concepts it describes have been adopted by several organizations and agencies. For example, the US Environmental Protection Agency (US EPA) has proposed that the MoA for carcinogenicity can be an integral part of its cancer risk assessment process (U.S. Environmental Protection Agency, 2005), and the European Chemicals Agency (ECHA) has provided guidance on the use of MoA data in the harmonized classification, labelling and packaging (CLP) process (ECHA, 2015). In the guidance provided by ECHA, they recommend that the IPCS framework (IPCS, 2007) be followed when evaluating MoA data for carcinogenicity findings in

Abbreviations: AOP, Adverse Outcome Pathway; AE, associative events; BrdU, 5-bromo-2′-deoxyuridine; BROD, benzyloxyresorufin-O-debenzylation; BQ, benzyloxyquinoline-O-debenzylation; CAR, constitutive androstane receptor; ECHA, European Chemicals Agency; EdU, 5-ethynyl-2′-deoxyuridine; EGF, epidermal growth factor; EPA, Environmental Protection Agency; KE, key event; KO, Knockout; MoA, mode of action; ModF, modulating factors; PB, phenobarbital; PROD, pentoxyresorufin-O-depentylation; WHO-IPCS, World Health Organization – International Programme on Chemical Safety

* Corresponding author.
E-mail address: richard.peffer@syngenta.com (R.C. Peffer).

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animals and their relevance to humans.

Although the IPCS Conceptual Framework provides a means for identification of critical data needs and subsequently organizing them, the choice of models and methods to generate the data as well as the amount of data to submit to EU authorities as part of the registration of a an active ingredient is very much up to the individual submitter. This can lead to mechanistic data packages of varying sizes generated using different approaches, which can complicate the assessment of MoA data for a particular compound, unless the reviewer is very familiar with the generally-accepted standards of Biological Plausibility and sufficient Weight of Evidence (components of the IPCS framework). One such example of a MoA where a number of plant protection products, human drugs and industrial chemicals have been shown to produce rodent liver tumors is a MoA via activation of the constitutive androstane receptor (CAR). A recent review captured the state of the science for the CAR MoA as of 2013 (Elcombe et al., 2014); it also provided a review of the evidence that mouse or rat liver tumors that occur via a CAR MoA are not relevant to humans based on qualitative differences between the species. However, newer publications continue to shed light on this MoA, and describe additional experimental models that were not reviewed at the time of the Elcombe et al. (2014) review. Therefore, the objective of the present manuscript is to define a minimum set of data that would adequately support a MoA for rodent tumor formation via CAR activation based on the current (2018) state of the science, the principles of the IPCS Framework, and experience with specific experimental models. Necessary components that are outlined in the IPCS Framework, such as disproving alternative MoAs and demonstrating data related to the human relevance of the animal MoA will also be described. It should be noted that it is not the objective of this document to provide compulsory data requirements. The means by which data are generated when embarking on a program of mechanistic studies to test a hypothesized CAR MoA should take the recommendations of this paper into consideration, but the data needs for a specific chemical will need to be sufficiently flexible to handle the unique properties of that molecule, ongoing feedback from EU or other regulatory agencies, plus the emergence of new tools and new knowledge about the CAR pathway. The content of this manuscript and the regulatory needs that it addresses were based upon experience in the EU crop protection environment; however, the content may also be useful to defining a CAR MoA within other regulatory and chemical spaces.

2. Materials and methods

2.1. Background information on CAR activation and other nuclear receptor-mediated MoAs

Hepatic tumor formation in rodents following life-time exposure to exogenous compounds is a common phenomenon that has been investigated extensively. Several MoAs have been described for liver tumor formation, including DNA reactivity or via a non-genotoxic mode of action that may be either receptor or non-receptor mediated (Cohen, 2010). A public workshop involving scientists from government, industry and academia was held at the National Institute of Environmental Health Sciences (USA) in September 2010 that explored the MoAs for chemicals causing rodent liver tumors mediated by nuclear receptors (Andersen et al., 2014). Resulting from that Workshop, a series of publications were issued that described a state-of-the-science view for MoAs via the peroxisome proliferator activated receptor alpha (PPARα) (Corton et al., 2014), the aromatic hydrocarbon receptor (AhR) (Budinsky et al., 2014) and the constitutive androstane receptor/pregnane-X receptor (CAR/PXR) (Elcombe et al., 2014). A fourth publication described the approach used in the Workshop as a whole, and defined terminology for describing the component parts in a MoA that were followed in all of these publications (Andersen et al., 2014). For these nuclear receptors, each MoA consists of a series of key event (KEs), which are integral to tumor formation, providing the dose is sufficiently high and the duration of exposure is sufficiently long. A MoA can also include associative events (AEs), which are not required for tumor development, but can be used as markers for certain required KEs. In addition, modulating factors (ModFs) may be identified that are not necessary for tumor development, but can modulate the severity or dose response kinetics of KEs leading to tumor development.

The manuscripts from this Workshop provide thorough reviews up to their date of publication on each of these nuclear receptor-mediated MoAs, but scientific publications on each MoA have continued to describe new tools for the study of the underlying biology as well as insights into the mechanisms that occur in different species. For example, Becker et al. (2015) subsequently have described an Adverse Outcome Pathway (AOP) for AhR-mediated liver tumors, building on and expanding the prior publication. The OECD has launched an international programme for development of AOPs, which attempts to capture mode of action information in a prescribed manner that emphasizes a series of readily measurable key event relationships, and encourages scientists to capture these AOPs in an online tool known as AOPwiki as part of the AOP process (Kleinstreuer et al., 2016; OECD, 2013; OECD, 2016).

In the published proceedings of the nuclear receptor workshop on the CAR/PXR MoA (Elcombe et al., 2014), the authors could not identify a suitable non-genotoxic PXR activator for which carcinogenicity data were available and hence a MoA was not developed for liver tumor formation by PXR activators. CAR and PXR are often cited together regarding potential MoAs for a specific chemical agent, because extensive cross-talk between these two nuclear receptors has been described (Stanley et al., 2006), and some agents can activate both CAR and PXR in a particular species (Elcombe et al., 2014). In fact, PXR is activated by a large array of diverse chemical substances, far more than those that activate CAR (Martin et al., 2010; Timsit and Negishi, 2007; Willson and Kliewer, 2002). Those chemicals that are pure PXR activators have been shown to increase liver weight after activation but do not increase cell proliferation in the same way that activators of CAR or PPARα do (Shizu et al., 2013; Thatcher and Caldwell, 1994). Recently, co-administration of a PXR activator along with known activators of other nuclear receptors has shown that while PXR does not produce an increase in cell proliferation on its own, it may enhance the proliferative signals of CAR or PPARα activators (Shizu et al., 2013). Given the lack of actual tumorigenic key events due to PXR activators alone, the rest of this current publication will focus on the CAR MoA by itself.

2.2. Approach of this paper

To assist in clarifying the current state of the art for the MoA via CAR activation, and to describe the experimental models available to demonstrate its key events, a consortium of scientists from the European Crop Protection Association (ECPA) was formed involving scientists actively conducting mechanistic research to demonstrate MoAs for various pesticide active ingredients. The review paper on the CAR MoA by Elcombe et al. (2014) was used as a basis for defining the key events and associative events that are part of this MoA. Building on this publication, the authors also reviewed current examples (2012–2017) where mechanistic data were submitted to EU regulatory authorities and also were published in the peer reviewed literature to establish that rodent liver tumors occurred via CAR activation. In particular, MoA data for metofluthrin and nitrapyrin (Deguchi et al., 2009; LaRocca et al., 2017; Yamada et al., 2009) were used to illustrate data that are typically generated from mechanistic studies investigating the CAR MoA. Feedback from EU regulatory authorities (or at times regulatory authorities in other geographical regions) was considered, as well as the guidance described in the AOP process (Kleinstreuer et al., 2016; OECD, 2013; OECD, 2016) that is incorporated within an AOP for CAR activation leading to hepatocellular adenomas and carcinomas in rodents (Peffer et al., 2017). Based on these experiences, the authors identified a “Primary” set of experiments and measurements that registrants typically perform to 1) establish that the CAR MoA is operative