Case Study on the Use of Integrated Approaches for Testing and Assessment (IATA) for Chronic Toxicity and Carcinogenicity of Agrichemicals with Exemplar Case Studies - Ninth Review Cycle (2023)

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Ninth Review Cycle (2023)

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The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank, Basel, Rotterdam and Stockholm Conventions and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

Foreword

OECD member countries have been making efforts to expand the use of alternative methods in assessing chemicals. The OECD has been developing guidance documents and tools for the use of alternative methods such as (Q)SAR, chemical categories and Adverse Outcome Pathways (AOPs) as a part of Integrated Approaches for Testing and Assessment (IATA). There is a need for the investigation of the practical applicability of these methods/tools for different aspects of regulatory decision-making, and to build upon case studies and assessment experience across jurisdictions.

The objective of the IATA Case Studies Project is to increase experience with the use of IATA by developing case studies, which constitute examples of predictions that are fit for regulatory use. The aim is to create common understanding of using novel methodologies and the generation of considerations/guidance stemming from these case studies.

This case study was developed by the International Council on Animal Protection in OECD Programmes (ICAPO) submitted to the 2023 review cycle of the IATA Case Studies Project. This case study was reviewed by the project team. The Working Party on Hazard Assessment approved the case study at the 8th WPHA meeting in June 2024.

This case study is an illustrative example, and its publication does not translate into direct acceptance of the methodologies for regulatory purposes across OECD countries. In addition, this case study should not be interpreted as official regulatory decisions made by the authoring member countries.

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Acknowledgements

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Abbreviations and acronyms

ADME	Absorption, Distribution, Metabolism and Excretion
APVMA	Australian Pesticides and Veterinary Medicines Authority
AUC	Concentration-Time Curve
CAS	Chemical Abstract Services
CAS RN	Chemical Abstract Services Registration Number
CFR	Code of Federal Regulations
cPAD	Chronic Population-Adjusted Dose
DERs	Data Evaluation Records
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
HED	US EPA Health Effects Division
HHRA	Human Health Risk Assessment
HRAC	Herbicide Resistance Action Committee
IATA	Integrated Approach for Testing and Assessment
ICAPO	International Council on Animal Protection in OECD Programmes
ICH	International Programme on Chemical Safety
IPCS	International Programme on Chemical Safety
IUPAC	International Union of Pure and Applied Chemistry
LOAEL	Lowest Observed Adverse Effect Levels
MoA	Mode of Action
NOAEL	No Observed Adverse Effect Levels
OCSPP	Office of Chemical Safety and Pollution Prevention
OECD	Organisation for Economic Co-operation and Development
PBPK	Physiologically Based Pharmacokinetic
pMoA	Pesticide Mode of Action
PMRA	Health Canada Pest Management Regulatory Agency
PPO	Protoporphyrinogen Oxidase
POD	Point of Departure
QSAR	Quantitative Structure-Activity Relationship
ReCAAP	Rethinking chronic toxicity and carcinogenicity assessment for agrochemicals project
TK	Toxicokinetic
US EPA	United States Environmental Protection Agency
WHO	World Health Organization
WoE	Weight of Evidence
WSSA	Weed Science Society of America

Executive summary

This document provides a framework to fulfil an Integrated Approach to Testing and Assessment (IATA) for chronic toxicity and carcinogenicity assessment through a weight of evidence (WoE)-based approach – without lifetime rodent bioassays (OECD Test Guidelines 451; 452; 453). Rodent cancer bioassays are long-required studies for regulatory assessment of human cancer hazard and risk. These studies use hundreds of animals, are resource intensive, and certain aspects of these studies have limited human relevance. In many cases, the critical, human-relevant effect(s) driving the risk assessment can be identified as precursor events to chronic toxicity, including carcinogenicity, or key events in a mode of action in studies other than the chronic toxicity and carcinogenicity tests (Felter et al, 2021; OECD, 2012). There are no specific criteria to determine when not to require chronic toxicity and/or carcinogenicity studies for agrichemicals based on toxicological and exposure data. The goal of this framework is to structure an integrated approach for assessment with illustrations of the process that could be used to help determine when sufficient information is available to identify a health protective endpoint for risk assessment for a non-genotoxic agrichemical without performing the OECD Test Guideline rodent chronic toxicity and carcinogenicity studies. The necessary information for the integrated approach for assessment includes a retrospective evaluation of use pattern(s), exposure scenario(s), pesticidal mode-of-action, physicochemical properties, metabolism, toxicokinetics, toxicological data including mechanistic data, and an evaluation of the reliability and consistency of the toxicological response(s) between the agrichemical of interest and chemical analogues used for read-across assessment. The framework and associated example case studies illustrate a WoE-based approach to identify sufficiency of data to estimate a point of departure (POD) that is human health protective against chronic risk – without performing the lifetime rodent bioassays. This framework, and its associated example case studies, can be used to inform and encourage the development of additional case studies to build confidence in the WoE approach. Further discussion is needed to explore the applicability beyond agrichemicals (e.g., industrial chemicals). Additional case studies are encouraged to investigate the use of the framework to support information needs in a WoE-based safety evaluation to fulfil requirements under a variety of regulatory legislations (e.g., Classification, Labelling and Packaging).

1 Introduction

Assessing the risks of chronic toxicity and carcinogenicity from long-term exposure to chemicals is a critical part of the regulatory approval process. To evaluate the potential for chronic toxicity and carcinogenicity resulting from exposure to chemicals, regulatory authorities typically require the conduct of long-term rodent bioassays, such as the Organisation for Economic Co-operation and Development (OECD) guideline studies for chronic toxicity (OECD Test Guideline 452), carcinogenicity (OECD Test Guideline 451), and/or combined chronic/carcinogenicity (OECD Test Guideline 453). These chronic rodent bioassays are costly, take a long time to perform, require testing on a large number of animals, and are thought to be poorly reproducible (Boobis et al., 2016; Cohen et al., 2004; Cohen et al., 2019; Corvi et al., 2017; Doe et al., 2019; Gottmann et al., 2001). New approach methodologies (NAMs) may provide a less resource intensive and more rapid means to assess chronic toxicity and carcinogenicity of chemicals with equivalent or better accuracy and resultant human health protection.

For example, as part of the registration process for agrichemicals, hundreds of studies, including at least 20 mammalian toxicology studies, are required for regulatory review of conventional food-use agrichemical active substances to assess the risk to human health. The registration data requirements for food use agrichemicals include a variety of acute, subchronic and chronic studies evaluating multiple organ systems. In addition, mechanistic studies are frequently conducted to support a proposed mode of action (MoA[1]) and used to inform the potential risk of human carcinogenicity, which is considered a key component of evaluating the need for conducting chronic toxicity and carcinogenicity studies. MoA assessments should be evaluated according to the World Health Organization (WHO) International Programme on Chemical Safety (IPCS) framework (Boobis et al., 2006; Meek et al., 2003, 2014). Traditionally, an analysis of the MoA and human relevance would commence after chronic adverse effects (e.g., tumours) were observed in a chronic toxicity and carcinogenicity study. However, a greater understanding of the mechanism and MoA, and subsequently potential targets of toxicity, can be developed proactively to increase confidence in the protection of the chronic outcome and tumour potential for a chemical (Cohen, 2017; Cohen et al., 2019; AOP-Wiki, 2023).

In some regulatory jurisdictions, regulators have the flexibility to consider WoE-based approaches that satisfy toxicological data requirements without conducting the lifetime rodent bioassays (Table 1). Depending on regional legislation, agrichemical registrants have the opportunity to submit scientific rationales addressing the toxicological data requirements for regulatory assessment, which allow for the integration of available information to make data need determinations that are health protective and scientifically sound.

[1] According to OECD, the MoA for toxicity is the description of key events and processes, starting with interaction of an agent with the cell through functional and anatomical changes, resulting in cancer or other health endpoints. MoA relates to the events including, and downstream of, the toxicity pathway, which could lead to an adverse effect. The MoA starts with the molecular initiating event. Unlike AOP, MoA does not (usually) include consideration of exposure or effects at higher levels than the individual. MoA differs from mechanism, in that the MoA requires a less detailed understanding of the molecular basis of the toxic effect. https://www.oecd.org/chemicalsafety/testing/49963576.pdf

In some cases, the critical effect(s) driving the risk assessment are not derived from chronic toxicity and carcinogenicity rodent bioassays. In other cases, precursor events to chronic toxicity and carcinogenicity or key events in the MoA can be derived from other toxicity studies and are not solely derived from the chronic toxicity and carcinogenicity rodent bioassays (Cohen, 2017; Cohen et al., 2019; Felter et al, 2021; OECD, 2012). Therefore, the following problem statement addresses the need to develop a framework that can be used to satisfy the regulatory data needs for human health risk assessment:

There are no specific criteria to determine when not to require the chronic toxicity and/or carcinogenicity studies (OECD 451; 452; 453) for agrichemicals based on toxicological and exposure data.

To address the problem formulation, a workgroup convened under the Rethinking Carcinogenicity Assessment for Agrichemicals Project (ReCAAP) to develop a framework (Hilton et al., 2022) to help structure a WoE-based estimation of a POD that would be health-protective against chronic risk for an agrichemical safety evaluation, including effects related to carcinogenicity. This framework was inspired by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) carcinogenicity testing guidelines (S1) expert working group (ICH S1 EWG) (ICH, 2021, 2019; Sistare et al., 2011) that developed an addendum to the ICH S1B guidance document to outline a WoE-based approach to predict the outcome of the 2-year rat study. The ICH S1B addendum builds on findings that non-chronic toxicological data, including evidence of endocrine perturbation and positive genetic toxicology results, could be potentially predictive indicators of carcinogenic risk (Bourcier et al., 2024; ICH, 2022). It is worth noting that while effects from non-chronic toxicological data (endocrine perturbation, immunosuppression, etc.) have been documented as potential precursors for tumorigenesis, further investigation is warranted to reduce uncertainties that reflect key events that may not be well captured in toxicological studies. Efforts within the OECD Expert Workgroup to develop an Integrated Approach to the Testing and Assessment of Non-genotoxic Carcinogens will help to identify key events related to carcinogenicity that can be used to reduce uncertainty in taking a WoE-based approach (Jacobs 2020; Louekari 2024).

Building off the ICH S1 WoE, the ReCAAP framework described herein was developed using an iterative process of 1) outlining regulatory data needs to identify information to be considered in the framework, 2) evaluating the framework by writing case study scientific rationales using published data from regulatory reviews for registered agrichemicals, and 3) reviewing these case study WoE-based rationales by regulators and other workgroup members to identify areas to address in subsequent rounds of revisions to the reporting framework. For the purposes of this framework, the focus will be on the scientific WoE and not include the regional regulations/policy specifics of selecting the uncertainty factors and conducting the risk evaluation (i.e., points of departure can be estimated, but the risk decision for selecting uncertainty factors is ultimately up to the regulatory authority). While the initial intent of this framework was to structure WoE-based carcinogenicity assessments for agrichemicals without the need for rodent cancer bioassays, the framework is also applicable for structuring WoE-based chronic toxicity assessments as well as other toxicity data requirements, and for substances beyond agrichemicals. The framework to achieve this IATA is described herein.

Table 1. Example of regulatory guidance and processes available to allow submission of WoE-based assessment for APVMA, PMRA, and US EPA (APVMA, 2017; US EPA, 2013b; Health Canada, 2021).

	Existing Guidance	Pre-submission Opportunity
Australian Pesticides and Veterinary	Agricultural data guidelines: 3.1.1. Submission (2017)	Pre-application assistance: https://apvma.gov.au/node/106

Medicines Authority (APVMA)	https://apvma.gov.au/node/1036	
Health Canada Pest Management Regulatory Agency (PMRA)	Guidance for developing datasets for conventional pest control product applications: data codes for parts 1, 2, 3, 4, 5, 6, 7 and 10 (2021) https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/policies-guidelines/guidance-developingapplications-data-codesparts-1-2-3-4-5-6-7-10.html	PMRA Presubmission Consultation Request: https://sec2.hc-sc.gc.ca/pmra6117-eng.php
United States Environmental Protection Agency (US EPA)	Guiding Principles for Data Requirements (2013) https://www.epa.gov/sites/production/files/2016-01/documents/data-require-guide-principle.pdf	Guidance for Pre-Application Meetings on New Active Ingredients, Major New Uses and Other Registration Actions: https://www.epa.gov/pesticide-registration/guidance-pre-application-meetings-new-active-ingredients-major-new-uses-and

Note: Source: Hilton et al. (2022)

2 Purpose

The purpose of this IATA is to illustrate use of the ReCAAP framework whereby a scientific WoE-based approach allows the estimation of a POD for use in agrichemical risk assessment that is health protective in preventing a chronic event, including carcinogenicity, from exposure to humans. This IATA is specific to agrichemicals as the necessary exposure, toxicological and read-across data are available to inform potential for chronic toxicity and carcinogenicity. While use of the framework may be applicable to other chemical uses (e.g., industrial chemicals) further discussion and case studies would be beneficial to evaluate the sufficiency of data to support a variety of regulatory decisions.

To illustrate the use of the ReCAAP framework, two examples are appended to this IATA (Annex A and Annex B), with a goal to demonstrate the use of a WoE-based approach to estimate a POD for food-use active ingredient agrichemicals to fulfil data needs in a human health risk assessment.

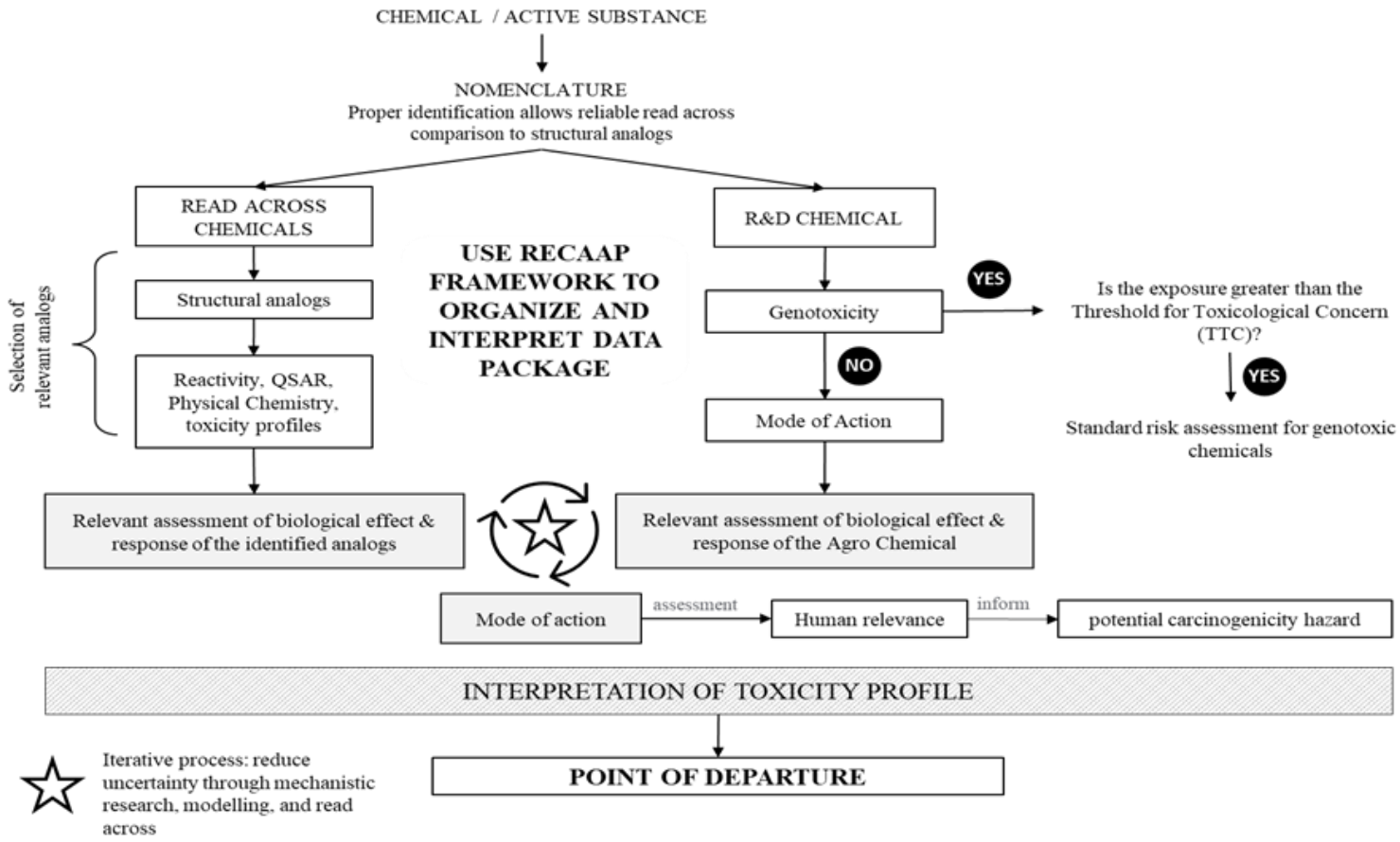
Safety studies and endpoint(s) addressed in this IATA

- 18-month mouse carcinogenicity study (OECD Test Guideline 451)
- 24-month rat combined chronic toxicity/carcinogenicity study (OECD Test Guideline 453)
- 12-month rodent chronic toxicity study (OECD Test Guideline 452).
- POD estimation that can be used for chronic and carcinogenicity risk assessment.

3 Hypothesis for performing IATA

This IATA combines the targeted toxicity data for the compound of interest with data from selected analogues for the read-across assessment to provide sufficient information to address the potential for chronic toxicity and carcinogenicity (Figure 1). The IATA also describes how to apply the ReCAAP framework to estimate a POD for use in a risk assessment that is protective for chronic exposure and to address the potential for chronic toxicity and carcinogenicity of a target compound (Figure 2). Using this framework, the chronic toxicity/carcinogenicity data requirements are addressed through a scientific WoE-based rationale, which supports that long-term bioassays in rats and mice will neither impact the chronic dietary risk assessment nor provide additional useful toxicological information (Hilton 2022).

Figure 1. Flowchart to estimate a health protective point of departure without conducting the chronic rodent bioassays



Data and sources

In practice, the data on the target agrichemical would be provided by the registrant and submitted to the regulatory authority. Before generating new study data an assessment of the knowledge base for the relevant class of chemistry may provide useful information to identify target tissues, highlight target tissue toxicity/ies, and identify the need for focused areas of safety testing, in turn, guiding a tailored data package. The data for selected analogues to be included in the read-across assessment (see Section 4 Performing the IATA) should be obtained from publicly available sources and combined with a comprehensive literature review of analogue chemicals. Information collected includes the nomenclature, pesticidal mode of action (pMoA) (i.e., the intended target mode of action), physicochemical property data, exposure information, and toxicological data (Table 2). As above, an assessment of the reliability of the data used for read-across must be conducted to ensure the information is fit for purpose.

Nomenclature includes chemical name, CAS registration number (CAS RN), synonyms, chemical structure, and chemical class. Such information is critical to the identification of potentially relevant data from different sources that may use different chemical naming conventions. Proper identification of the agrichemical of interest is necessary to allow for reliable read-across comparison to structural chemical analogues. Additionally, *in silico* prediction models, such as validated quantitative structure-activity relationship (QSAR) models, used to support a WoE assessment rely on chemical structure. Information on the chemical structure, chemical class, and pMoA are important to identify analogues to be used for the read-across assessment.

Physicochemical properties can contribute further to these similarity and reliability assessments. For example, solubility or hydrophobicity can influence bioavailability. Further, identification of chemical class, intended pMoA, and any known MoAs (i.e., off-target or unintended toxicity mode of action) allow for consideration of toxicological, including carcinogenic, effects.

Exposure data are collected to determine the extent, duration and route(s) of potential exposure. This information impacts the hazard assessment as well as the risk evaluation. These data inform the level of refinement used for the exposure estimates, which may be used in the overall WoE decision for the need of a chronic toxicity and carcinogenicity study. For instance, an unrefined low-level estimate of exposure and no toxicity concerns may contribute significantly to the WoE rationale.

Toxicological data could include genetic toxicity, toxicokinetics including absorption, distribution, metabolism and excretion (ADME), acute toxicity, subacute toxicity, subchronic toxicity, reproductive and developmental toxicity, chronic toxicity, carcinogenicity, neurotoxicity, immunotoxicity, and mechanistic toxicity studies. In the context of this IATA, toxicokinetics refers to ADME as well as the toxicokinetic parameters (C_{max}, AUC, T_{max}, $T_{1/2,}$ VD), and physiological based pharmacokinetic modelling (PBPK). Information available from NAMs such as *in vitro* assays (cell-based, tissue-based) and toxicogenomics and computational models are important to address potential MoAs to elucidate potential systemic toxicity. When available, the No Observed Adverse Effect Levels (NOAELs), Lowest Observed Adverse Effect Levels (LOAELs) and toxicological effects at the LOAEL can be used to help determine the potential for chronic effects. In the summation of available toxicological data, it is important to describe the adverse effects that occur not only at the LOAEL (in mg/kg/day) but adverse effects that occur at higher dose levels as well, where effects may progress with longer treatment or when the adverse effects more accurately define the overall toxicity for that chemical and/or the class of chemicals. It is noteworthy that toxicological data considered in a WoE will evolve with advances in technology, as well as increased regulatory acceptance of NAMs. Thus, the list of toxicological data outlined in Table 2 is not meant to be prescriptive and should considered in the context of newly available and fit-for-purpose data to fulfil the WoE for a health-protective chronic risk assessment.

It is also worth noting that although acute toxicity is unlikely to be a predictor for tumour formation, the acute effects of an agrichemical may provide context for dose selection in longer-term studies. For some chemical classes, the acute and chronic effects considered to be human-relevant are the same, and thus an understanding of the toxicokinetic and MoA(s) for a chemical can inform the need for chronic toxicity and carcinogenicity testing (EPA, 2007). Additionally, acute and subacute toxicity studies are traditionally used to provide information if the target chemical is expected to cause point-of-contact irritation.

Subchronic toxicity can be informative both to make a prediction of chronic toxicity and to estimate a POD for a protective chronic risk assessment. Although tumours are a consequence of long-term toxicity, in some cases there are observable short-term preneoplastic effects that precede tumour formation. Precursor effects often occur at the same or lower dose levels than tumours and can be detected much earlier than the conclusion of a chronic toxicity and carcinogenicity study (Allen et al., 2004; Boobis et al., 2009; Mistry et al., 2021). For instance, hepatocellular hypertrophy, cytomegaly, and hepatocyte necrosis in short-term studies can help inform the potential for liver carcinogenicity in chronic studies (Allen et al., 2004). The absence of pre-neoplastic lesions in a subchronic study, for a few tissues such as the liver, has been shown to reliably predict the absence of tumours in a chronic study in rats (Boobis et al., 2009; Reddy et al., 2010). Data informing the need for chronic toxicity and carcinogenicity studies can also come from non-guideline studies, including short-term studies integrating toxicogenomics data (Yauk et al., 2020) *in vitro* assays as well as computational models.

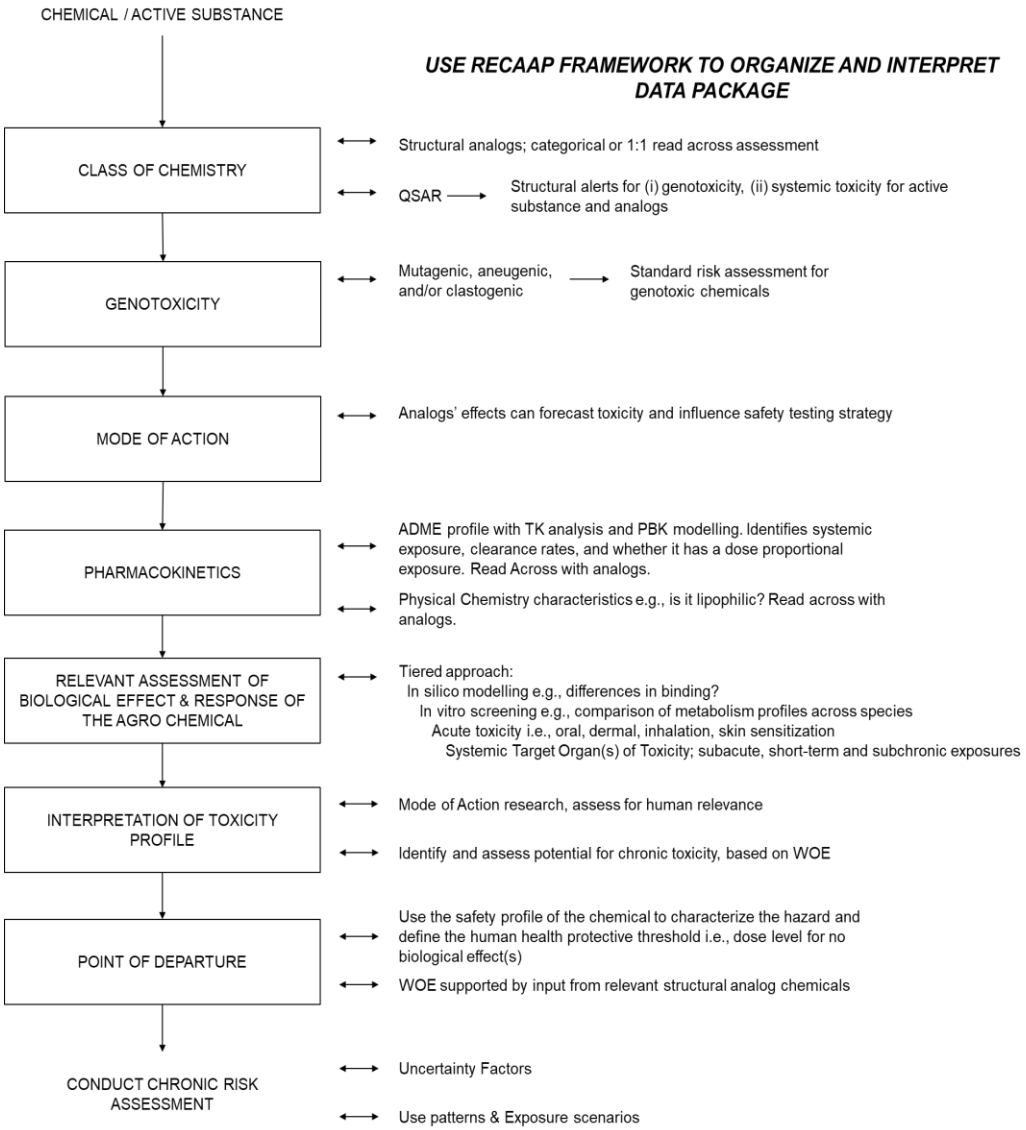
Mechanistic studies should be considered to support a proposed MoA. The MoA assessment serves to inform the potential risk of human carcinogenicity, which is considered a key component of evaluating the need for conducting chronic toxicity and carcinogenicity studies. A greater understanding of the MoA, and subsequently potential targets of toxicity, can be developed proactively to increase confidence in the prediction of the chronic outcome and tumour potential for a chemical (Cohen, 2017; Cohen et al., 2019).

Table 2. Data and sources

Data	Endpoint	Sources
Nomenclature	Common Name	https://regulations.gov https://pubchem.ncbi.nlm.nih.gov https://comptox.epa.gov/dashboard/ https://hracglobal.com/ https://irac-online.org/ https://www.frac.info/ https://echa.europa.eu/information-on-chemicals
	IUPAC Chemical Name	
	Chemical Class	
	CAS Registry Number	
	Synonyms	
	Structural Identifiers	
	2D Structure	
Pesticidal MoA	XRAC and WSSA groups	https://hracglobal.com/ https://irac-online.org/ https://www.frac.info/ https://wssa.net/
Physiochemical properties	Molecular weight (g/mole)	https://regulations.gov https://pubchem.ncbi.nlm.nih.gov https://comptox.epa.gov/dashboard/ https://www.bcpc.org/open-access/pest-crop-databases https://hracglobal.com/ https://irac-online.org/ https://www.frac.info/ https://wssa.net/ https://ice.ntp.niehs.nih.gov/ https://echa.europa.eu/information-on-chemicals
	Physical state at room temperature	
	Melting point	
	Density at 20 °C.	
	Water solubility	
	Solubility, organic solvents	
	Vapor pressure	
	Henry's law constant	
	Octanol/water partition coefficient Log (P_{ow})	
	Dissociation constant in water, pKa	
	pH	
Exposure	Routes and duration of exposure	https://regulations.gov https://comptox.epa.gov/dashboard/ https://www.fao.org/agriculture/crops/thematic-sitemap/thaem/pests/lpe/en/ https://publications.gc.ca/site/eng/home.html https://www.efsa.europa.eu/en/publications
Toxicological data	Genetic toxicity	https://regulations.gov https://www.foia.gov/ https://publications.gc.ca/site/eng/home.html https://www.efsa.europa.eu/en/publications https://www.fao.org/agriculture/crops/thematic-sitemap/thaem/pests/lpe/en/ https://ice.ntp.niehs.nih.gov/ https://echa.europa.eu/information-on-chemicals
	Toxicokinetics/ADME	
	Acute toxicity	
	Subchronic toxicity	
	Developmental toxicity	
	Reproductive toxicity	
	Chronic toxicity	
	Carcinogenicity	
	Neurotoxicity	
	Immunotoxicity	
	Mechanistic toxicity	

4 Performing the IATA

Figure 2. ReCAAP framework for performing the IATA.



The boxes denote the steps in the WoE assessment as adapted by the ReCAAP framework. Following the collection of data on the active substance and structural analogues and the reliability assessments, this

workflow is structured to assess the information in a prioritised manner. Each chemical is assessed for its potential to cause chronic and/or carcinogenic effects following long-term exposure, using the available data. This workflow serves to identify the point of departure for use in chronic risk assessment and whether there is a need to conduct chronic toxicity testing.

Gather Information: Once the available information is gathered and/or generated as detailed in Section 3.1. Data and sources, the data can be organized and interpreted as depicted in Figure 2 and explained, as detailed in the example case studies (Annex A and Annex B).

Assess Reliability/Acceptability of Data: An assessment of the reliability of data must be conducted to ensure the information is fit for purpose. Typical quality measures include Klimisch scores and studies conducted in accordance with current OECD test guidelines. All studies should be conducted studies conducted in accordance with Good Laboratory Practice (GLP). A comprehensive review of the literature is also necessary.

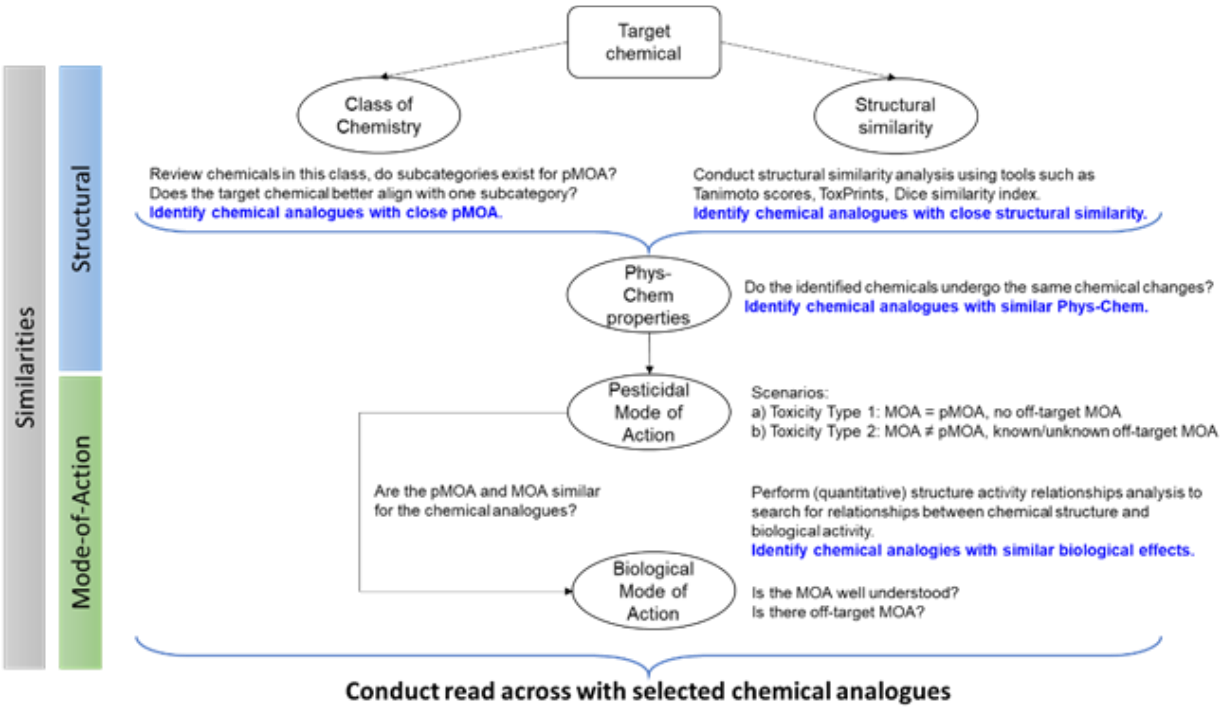
Application of *in silico* prediction models, such as validated quantitative structure-activity relationship (QSAR) models are recommended as necessary depending on the amount and types of data available (OECD, 2014a; OECD, 2007). For the target compound, QSAR analysis can supplement the available experimental data.

Class of Chemistry: The class of chemistry informs the pMoA and may inform the MoA. The class of chemistry provides information for the selection of surrogate compounds for the read-across assessment.

Evaluate Potential for Genotoxicity: From these data, it should be determined if the targeted compound has the potential for genotoxicity. Based on the framework presented in Figure 1, if the compound is non-genotoxic, then further evaluation of the database of toxicological studies with the target compound and identification of analogues for read-across assessment can be conducted. It should be noted there are unique regional regulatory requirements for assessing risk to compounds found to be genotoxic, which may affect the overall WoE.

Read-across Assessment: Agrichemicals including non-food use pesticides are designed to have a specific effect on the target pest species. Unless the agrichemical is novel, it is likely to belong to a known chemical and/or biological class of compounds grouped together based on the common pMoA. These groupings may or may not be structurally similar but share a common pMoA. In some cases, the pMoA (e.g., chemical class) may be the same or similar to the off-target MoA (e.g., toxicity) that leads to an adverse outcome, or the pMoA may have a different but distinct MoA. For agrichemicals, the selection of appropriate analogues to be used in a read-across assessment could be based on a combination of factors including the pMoA, and/or MoA, and then further filtered based on structural similarity (Figure 3). Therefore, structural similarity, physical chemical properties, and toxicokinetics should not be the only metrics used to justify appropriate analogue selection for read-across.

Figure 3. Proposed workflow to identify relevant biological and structurally similar analogues to be considered in an agrichemical-based read-across assessment.



One of the goals in selecting analogues for a read-across assessment is to identify those that have, or are expected to have, similar biological effects or MoA to assist in the evaluation of the target compound. That is, if the MoA is the same or if the key toxicological response is the same for those chemicals, the structural similarity and physical chemical properties may be instructive but are not necessarily key to the selection and evaluation of the appropriate analogues for read-across. For analogue compounds with a similar chemical class but no similar biological effects, the structural similarities and physical chemical properties become more important because the biological response to those chemicals are not known or well-understood. Justification for the inclusion or exclusion of chemicals into this group should be provided. For example, structural similarity measures (e.g., Jaccard, Tanimoto, and Dice similarity index) can also be used to further refine the grouping or to expand the group size if needed (ECHA, 2018; Escher et al., 2019; Helman et al., 2019; Schultz et al., 2015). Gathering information, such as physicochemical properties, toxicokinetics, toxicity profiles, and MoA, for relevant analogues will strengthen the justification for grouping, and increase confidence in the predictions made for the chemical of interest (Patlewicz, 2014; Schultz et al., 2015). Likewise, dissimilarities and differences within a class of chemistry can be used to refine the selection of analogues and exclude the non-relevant chemistries in the read across to narrow the focus on biological effects. For adequate comparison, the analogues must have reliable, high-quality data overall such that they will provide useful information to the WoE assessment. Data on the analogue chemicals used for the read-across assessment should be obtained and recorded. There are many software programs available for a chemical structurally based read-across assessment, some of which are publicly available (e.g., Morgan fingerprints, OECD QSAR Toolbox, ToxPrints, US EPA's GenRA). As such, a comprehensive read-across assessment is available for determining appropriate analogue inclusion/exclusion based on structural similarities where needed.

The data gathered on the analogue chemicals used in the read-across assessment should be compared to the target compound to determine consistency of response between compounds, identify toxicological effects of potential concern, and support the read-across potential, including the proposed MoA, if applicable. Evaluation of the read-across data can support the assessment of potential for increased toxicity with longer exposure durations (subchronic to chronic duration studies). The read-across data can also supplement the available data on the target compounds and be used to provide additional data to address the potential for chronic toxicity and carcinogenicity. The reliability and acceptability of the data supporting analogue inclusion should be assessed as a part of the read-across evaluation.

Evaluate Toxicity Mode of Action: The MoA of the targeted data and analogues used in the read-across assessment should be evaluated. If the MoA is described in the peer-reviewed public literature and/or an adverse outcome pathway (AOP) is available for the pesticidal class and/or chemical family, the data should be evaluated in accordance with the key and associated events. It is not necessary to have experimental data for every key and associated event for the compounds, but the WoE should support a consistent MoA across the compounds. Mechanistic studies should be evaluated to support a proposed toxicological MoA. MoA assessments should be evaluated according to the WHO IPCS framework (Boobis et al., 2006; Meek et al., 2003, 2014). As stated previously, a greater understanding of MoA, and subsequently potential targets of toxicity, can be developed proactively to increase confidence in the prediction of the chronic outcome and tumour potential for a chemical (Bourcier et al., 2024; Cohen, 2017; Cohen et al., 2019).

Evaluate Toxicokinetics: The toxicokinetics of the target and analogue compounds for read-across should be reviewed and evaluated. Toxicokinetic information could be collected from *in vitro* assays, *in vivo* studies, and *in silico* models which contribute to the WoE for assessing the need for additional chronic toxicity/carcinogenicity information. These data can aid in understanding the clearance rate or the potential for bioaccumulation with chronic exposure, and identify species, sex, or age differences that may influence the extrapolation of data from animal studies to human safety assessment. Human risk can be better estimated when internal, systemic, concentrations in animals are understood and can be directly compared to human exposure using physiologically based kinetic (PBK) modelling. Toxicokinetic data can help inform dose-response relationships and MoA (Barton et al., 2006; Carmichael et al., 2008), which may improve the read-across of data from chemical analogues or understanding of toxicological effects. Further, knowledge of toxicokinetic species differences may provide justification for waiving the chronic toxicity and carcinogenicity studies in one or both species, depending on relevance to humans.

Toxicological Profile of the Target Compound: Where available, the toxicological data should be reviewed to identify the key toxicological effects, target organs, the most sensitive species, and to determine if there is any increase in toxicity from short to longer durations of exposure. Identification of the most sensitive adverse effect, in the most sensitive species and the most sensitive sex accounts for any species or sex pharmacokinetic or pharmacodynamic differences. The toxicological database should identify any precursor (hypertrophy, cytomegaly, hyperplasia, neoplasia, necrosis, fibrosis) or potential carcinogenic effects.

The toxicological database should be evaluated for hormone perturbation and immune suppression, as these effects impact human risk for carcinogenicity (Cohen et al., 2004; Cohen et al., 2019). In a retrospective review of carcinogenicity results with pharmaceutical compounds, the definition of hormone perturbation was expanded to include hormones and tissues outside of those that are oestrogen sensitive. In an analysis with pharmaceuticals, it was concluded that evidence for lack of hormone perturbation was an important criterion (along with lack of genotoxicity and lack of histopathologic risk factors for rat neoplasia) to identify compounds where little value was gained by conducting rodent carcinogenicity studies (Sistare et al., 2011). Furthermore, the toxicological database should be evaluated for evidence of immune suppression. Immunosuppression increases the risk of cancer in humans, as demonstrated by

increased risk of certain cancers following the use of immunosuppressive drugs (Bugelski et al., 2010; Hasserjian et al., 2009; Kubica and Sangle, 2016; LaCasce, 2006; Peters et al., 2018; Simon et al., 2015). Therefore, agrichemicals that specifically cause suppression of immune system function should be considered a human-relevant risk factor for the development of cancer (Cohen, 2004).

Estimation of POD for Chronic Exposure: Characterisation of the dose-response of toxicological effects can be used to determine a reasonable estimate for a chronic POD in the absence of a chronic toxicity study (Cohen et al., 2019). In some cases, mechanistic information can inform the MoA of key toxicological events to define the POD or effects that could progress with time. Default selection of the lowest NOAEL should be avoided. Based on the principles of dose-response assessment the LOAELs and treatment related effects should be evaluated across studies when available. The lowest biologically relevant LOAEL is selected and the overall NOAEL should be protective of that LOAEL as well as potential effects in the full toxicological database. This NOAEL should be the POD. Benchmark dose (BMD) analysis, where applicable, may also be used to evaluate dose-response effects and estimate the POD (US EPA, 2012; EFSA, 2022).The analogue chemicals used in a read-across assessment can also be used to derive the POD. Inconsistency in subchronic or chronic effects (including tumours) between chemicals in the read-across grouping, and lack of MoA data, such that there was high uncertainty in predicting chronic effects for the unknown molecule, may indicate the need for further testing or the development of additional mechanistic data to support POD selection (Hilton et al., 2022). The resultant POD from read-across must be protective for the target chemicals.

Basis for WoE: The assessment should include a section summarizing and justifying the proposed conclusions, specifically a proposal to estimate a POD that would be protective against chronic risk, as well as an indication if one or both mouse and rat chronic toxicity and carcinogenicity studies are not necessary to develop a scientifically defensible health protective risk assessment. This proposed conclusion should include a discussion of uncertainties remaining in the analysis, as well as qualitative and quantitative strengths of the evaluation. There may also be uncertainties around the sources of data. Full disclosure of uncertainties is important to increase confidence in the associated conclusions. A conclusion to waive the chronic toxicity and/or carcinogenicity study is possible, for example, if data indicate the carcinogenicity risk is highly likely to be tumorigenic in rats and mice, but not in humans through prior established and well recognized mechanisms known to be non-human relevant (e.g., thyroid follicular cell hypertrophy and hyperplasia), or if data indicate the carcinogenicity risk is highly likely not to be tumorigenic, such that a carcinogenicity study would not add value (Bourcier et al., 2024, Huisinga et al., 2020, Vansell, 2022). Waiving the chronic toxicity and/or carcinogenicity study could also be possible if the estimated POD demonstrates health protection such that the chronic studies would not increase the risk determination past the level of concern for sensitive populations.

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5 Introduction to the Annexes

The IATA has appended two exemplar case studies (Annex A and Annex B) to illustrate how one could use the ReCAAP framework to structure a WoE to estimate a POD to fulfil a health-protective chronic risk assessment. Although the types of information, level of detail, and data interpretations will likely vary for different chemicals and different regulatory jurisdictions, the examples presented herein are provided to familiarise the reader with the format, and information content that could be considered in a WoE assessment within the IATA.

The saflufenacil example (Annex A) was a retrospective approach conducted in 2019 to evaluate publicly available risk assessments and supporting data evaluation records (DER) from the US EPA while excluding the chronic and carcinogenicity studies from the WoE development. The spiropidion example (Annex B) was conducted in 2019-2020, prior to the conclusion of the chronic and carcinogenicity studies, while the chemical was still in development. Both case studies were used as part of the testing and development activities of the ReCAAP framework (Hilton 2022).

Following the example case studies, Annex C provides the 'Lessons Learned' throughout the development and revisions of the case studies through the OECD IATA Case Study Project, as well as proposed 'Next Steps' for continued use and evaluation of the ReCAAP framework in subsequent IATA case studies. Additional case studies are encouraged to investigate the use of the framework to support information needs in a WoE-based safety evaluation to fulfil requirements under a variety of regulatory legislations (e.g., Classification, Labelling and Packaging).

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Annex A. Example 1 – Saflufenacil

The purpose of this example is to evaluate the toxicological data that was publicly available from risk assessments and data evaluation records (DERs) generated by the United States Environmental Protection Agency (US EPA) for saflufenacil and selected analogue compounds for the read-across assessment to determine if a health-protective POD can be established without the need for rodent chronic toxicity and carcinogenicity studies (OECD TG 451, 452, 453). Specifically, the case study presented herein provides an example of a WoE-based assessment to fulfil regulatory carcinogenicity data needs stipulated by the United States United States Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Title 40 Part 158, as regulated by the US EPA (Code of Federal Regulations, CFR, 2012). Other regulatory legal statutes may require different types of information, level of detail, or toxicity assessment that is not similar in regulation to FIFRA, and thus may result in data requirements that are unique by regulatory region (e.g., Commission Regulation (EU) No 283/2013; Canada's Pest Control Products Act).

The case study was developed within the ReCAAP initiative, which was supported by the United States EPA's Guiding Principles for Data Requirements that outlines their availability to accept a WoE-based safety assessment for agrichemicals (US EPA 2013; Craig et al., 2019). Given the available landscape to develop a WoE-based safety assessment, in this case, for estimating a POD that is protective against chronic outcomes, the data requirements under the US EPA were the starting point to explore the sufficiency of data needed to achieve a health-protective outcome. While the focus of this case study was fulfilling agrichemical data requirements for the United States, additional case studies are encouraged to help identify the sufficiency of data in other regions that fulfil different regulatory statutes in addressing chronic toxicity and carcinogenicity.

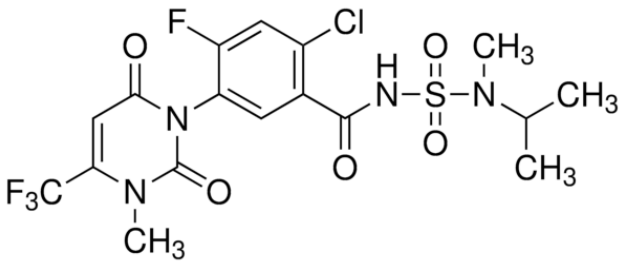
It should be noted that the case study on saflufenacil was developed in 2019, the contents of which are reported in this IATA. The 'uncertainties section' of this IATA case study explores data gaps, as well as the learnings acquired from the OECD case study project review that are important to consider in the present day (2024) and beyond. The idea is to clearly identify gaps and uncertainties in this case to inform the development of subsequent case studies on carcinogenicity.

1. Data

For this example case study, relevant data were used to obtain the necessary information on the target compound, saflufenacil. The data were obtained from publicly available risk assessments and DERs generated by the United States EPA. The supporting study data were considered reliable and acceptable as per the US EPA use of the studies to support the registration of saflufenacil in fulfilment of the United States FIFRA. OECD Test Guidelines were used for the following studies evaluated in this WoE: 408, 409, 414, 416, 417, 424, 452, 453. The immunotoxicity and genotoxicity studies followed the US EPA guidelines available at the time the studies were conducted (EPA guideline: OPPTS 870.7800 (1998); EPA guidelines: OPPTS 870.5100, 870.5300, 870.5375, 870.5395, 870.5550 (1998)). Due to the retrospective nature of this case study (e.g., no new data was generated), the currently available revisions of the OECD Test Guidelines for immunotoxicity and genotoxicity were not taken into account to determine the reliability of

the available studies beyond what the United States EPA classifies as acceptable.[2] The nomenclature and chemical structure of saflufenacil are detailed in Table A A.1. Saflufenacil contains both a 6-membered heterocyclic ring derived from uracil and a phenyl ring (CompTox 2023; PubChem, 2021a).

Table A A.1. Nomenclature for saflufenacilt

Parameter	Result
Common Name	Saflufenacil
IUPAC Chemical Name	2-Chloro-4-fluoro-5-[3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]-N-[methyl(propan-2-yl)sulfamoyl]benzamide
CAS Registry Number	372137-35-4
Synonyms	Benzamide N'-[2-Chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)-pyrimidinyl)benzoyl]-N-isopropyl-N-methylsulfamide
Chemical Class	N-Phenylimides
Molecular Formula:	$C_{17}H_{17}ClF_4N_4O_5S$
2D Structure	

The intended pMoA of saflufenacil is inhibition of the plant enzyme protoporphyrinogen oxidase (PPO), which places it in Herbicide Resistance Action Committee (HRAC) group E (HRAC, 2019) and Weed Science Society of America (WSSA) group 14 (WSSA, 2019) for resistance management considerations.

2. Evaluation of genotoxic potential

A full set of genotoxicity studies that were classified as acceptable by US EPA in 2005 are available for saflufenacil, as shown in Table A A.7. Saflufenacil was not mutagenic in bacterial cells or in Chinese Hamster Ovary cells with or without metabolic activation. Saflufenacil was weakly clastogenic in the *in vitro* chromosomal aberration assay in V79 cells in the presence of S9 activation, but the response was not

[2] All studies were conducted in accordance with the latest version of the OECD Test Guidelines at that time. The studies for saflufenacil were conducted before 2010 and subsequent revisions to OECD guidelines may affect the results. While new studies should not be initiated using old versions of the OECD TG, results of studies conducted of an older test guideline (that was current at the time the study was initiated) are still accepted under the agreement on Mutual Acceptance of Data (OECD; Section 4 – Health effects: replaced and cancelled Test Guidelines).

evident in the absence of S9 activation. It was not clastogenic in rodents in an *in vivo* cytogenetics study, even when dosed up to a limit dose (2000 mg/kg/day) for this study. Since a higher-tier *in vivo* study did not show any evidence of clastogenicity, the overall data indicate that saflufenacil is not clastogenic. An *in vivo* unscheduled DNA synthesis (UDS) study in rats showed no evidence of genotoxicity (US EPA, 2014a; US EPA, 2015). Therefore, there is no indication that saflufenacil has the potential for a genotoxic-based carcinogenic effect. It should be noted that due to the low sensitivity of the Unscheduled DNA Synthesis (UDS) assay in detecting *in vivo* genotoxicants, the negative result of the UDS is of lower predictive value, and thus, not acceptable to follow-up positive results in the *in vitro* gene mutation tests (EFSA Scientific Opinion (Clarification of some aspects related to genotoxicity assessment, EFSA Journal 2017;15(12):5113).

3. Data review of target chemical

All data generated for the saflufenacil reported herein was conducted in accordance to Good Laboratory Practice following OECD guidelines as well as US EPA Office of Chemical Safety and Pollution Prevention (OPPTS) guidelines, with the exception of the immunotoxicity and genotoxicity study. These two studies were performed in accordance to OPPTS guidelines only.

For saflufenacil, tables with relevant data are available at the end of this document. Physical chemical properties are presented in Table A A.3. A summary of toxicokinetic data is presented in Table A A.4. Acute toxicity data are presented in Table A A.5, subacute toxicity and subchronic toxicity data are presented in Table A A.6, and genetic toxicity data are presented in Table A A.7. Studies providing data relevant to hormone perturbation are summarized in Table A A.8 and studies providing data relevant to immune suppression for saflufenacil are presented in Table A A.9. MoA and mechanistic studies are summarised in Table A A.10. As stated above, the toxicological data were generated between 2005-2010, and data interpretations were used from the US EPA through DERs and risk assessment(s) conducted between 2014-2016. The data were considered reliable and acceptable as per the US EPA use of the studies to support the registration of saflufenacil. No additional assessment for reliability or acceptability was done for the purposes of this assessment.

Toxicokinetic data indicate saflufenacil is well absorbed and rapidly excreted (Table A A.4). Following a single dose, and regardless of the dose level, maximum blood concentrations (C_{max}) were achieved within 1 hour of dosing and declined rapidly thereafter. Excretion of orally dosed saflufenacil was essentially complete within 96 h, and the majority was eliminated within the first 24 to 48 h. There was a sex-dependent difference in the excretion of orally administered saflufenacil. Following single low and high dose administration or repeated-dose administration, the main route of elimination in male rats was via the faeces, while urinary excretion was the major route of elimination in females. The sex difference in route of excretion was due to the significantly higher biliary excretion of saflufenacil residues in males than in females. Radiolabelled analysis of blood showed that the majority of saflufenacil residues were in the plasma and were not bound to cellular elements such as red blood cells. At 168 h after dosing, saflufenacil residues remaining in tissues were very low and occurred mainly in carcass, liver, skin, and gut contents. Saflufenacil was metabolized by three major transformation steps: demethylation of the uracil ring system, degradation of the N-methyl-N-isopropyl group to NH2, and cleavage of the uracil ring, forming a sulfonylamide group. The predominant metabolites were M800H01, M800H03, and M800H07. There were no significant sex-related differences in metabolic profiles (Table A A.4). The toxicokinetic data support the sex-dependent difference in excretion. This difference was more pronounced at the low-dose level (4 mg/kg) and resulted in males having up to 3X higher internal exposures than females as measured by the plasma area under the concentration-time curve (AUC) (740 µg Eq × h/g in males compared to 247 µg Eq × h/g in females). Increasing the administered dose by a factor of 25 (4 mg/kg vs. 100 mg/kg) resulted in

less than proportional increases in plasma AUC values at 6.1 in males (741 µg Eq × h/g at 4 mg/kg and 4502 µg Eq × h/g at 100 mg/kg) and 12.4 in females (247 µg Eq × h/g at 4 mg/kg compared to 3057 µg Eq × h/g at 100 mg/kg).

Saflufenacil exhibited low acute toxicity via the oral, dermal, and inhalation routes of exposure (Toxicity Category III or IV) (US EPA, 2014a; US EPA, 2015). It was slightly irritating to the eyes (Toxicity Category III) but was not a dermal irritant or a dermal sensitizer. (Table A A.5).

The key toxicological effect of saflufenacil in sub-chronic oral studies in the rat, mouse, and dog were haematological changes indicative of anaemia and porphyria, which produced some systemic effects such as decreased body weights, adverse clinical signs and secondary effects in the spleen and liver (Table A A.6). The NOAEL values from 90-day studies were essentially the same in rat, mouse and dog studies (10.5, 12.4 and 10 mg/kg/day, respectively), so any of these three species might provide a relevant POD for risk assessments if dosed over a longer time interval.

Hormone perturbation was indirectly evaluated using the 2-generation reproductive toxicity study conducted in accordance with OECD 416 (2001), subchronic toxicity studies in mice, rats, and dogs, and developmental toxicity studies in rats and rabbits. However, it should be noted that hormonal measurements were not described, and several study parameters relevant for the assessment of endocrine disruption / hormonal perturbation are not determined in the available data. Within the rat and rabbit developmental toxicity studies, exposure to saflufenacil and resulting haematology changes did not produce an increase in malformations. Increased foetal susceptibility was noted based on foetal effects in the rat (decreased fatal body weights and increased skeletal variations), and in rabbit there were increased liver porphyrins at dose levels that were not maternally toxic. In the two-generation reproduction study in rats, the NOAEL values for parental effects and offspring effects were the same (15 mg/kg/day), but EPA reviewers noted that the offspring effects (e.g. increased stillborn pups, decreased viability and lactation indices, decreased pre-weaning body weight, change in haematological parameters) may be more severe than maternal effects at the same dose level (e.g. decreased food intake, body weight and changes in haematological parameters; US EPA, 2014c; US EPA, 2015).

The remainder of the database of toxicology studies with saflufenacil also did not show any evidence of hormone perturbation. This included a lack of effect on the thyroid gland, testis (and associated male organs), and ovaries (and associated female organs) in all of the subchronic studies. The haematological database supports the lack of oestrogen, androgen, steroidogenesis, and thyroid receptor-mediated effects (Table A A.8). In addition, ToxCast Model Predictions for oestrogen receptor agonist, antagonist and binding (CERAPP) are inactive and for androgen receptor agonist, antagonist, and binding activity (COMPARA) are also inactive. The US EPA ultimately concluded that the observed susceptibility was of low concern because clear NOAEL values were obtained, the dose-response relationship for the effects of concern was well characterized and the point of departure for risk assessments would be protective of the developmental and offspring effects (US EPA, 2015).

In a guideline immunotoxicity study, saflufenacil was administered in the diet to groups of male C57BL/6J Rj mice at dietary concentrations up to 250 ppm. Saflufenacil did not cause immunosuppression based on the lack of any changes in T-cell dependent antibody response (TDAR) in mice. In addition, there was no evidence of an effect of saflufenacil on the immune system in the toxicology database, including effects on histopathology changes in lymph nodes or thymus. As a PPO inhibitor, saflufenacil produced porphyria and regenerative anaemia, but it did not produce a clear deficit in white blood cell number and function, and there was no evidence of increased infections in repeat-dose studies (Table A A.9). The porphyria and regenerative anaemia produced characteristic changes in the spleen and bone marrow in certain studies, that reflected increased production of erythrocytes to compensate for the lower counts, but it did not reflect a deficiency in the immune system. Thus, based on the weight of the evidence, the EPA concluded that saflufenacil failed to induce toxicity specific to the immune system (US EPA, 2014a; US EPA, 2015).

4. Mode of action

Protoporphyrinogen oxidase (PPO) is responsible for the seventh step in biosynthesis of protoporphyrin IX in mammals (Figure A A.1). This porphyrin is the precursor to haemoglobin, the oxygen carrier in animals, and chlorophyll, the pigment at the heart of photosynthesis in plants. In mammals, this enzymatic step in haem synthesis occurs in the inner mitochondrial membrane. Inhibition of PPO in mammals can lead to an accumulation of protoporphyrinogen and possibly other precursors in the pathway, which is generically described as porphyria, meaning an increased concentration of porphyrins in the blood. An adverse outcome pathway for PPO inhibition is available and the toxicological mechanism of action and key events are well understood (AOP Wiki 131, 2023; Smith and Foster, 2018; Jakubek et al., 2021).

From some class analogues, the following AOP for hepatocellular tumour formation can be established (refer to oxadizon data): PPO inhibition, accumulation of protoporphyrinogen in the liver, hepatocyte injury, regenerative cell proliferation, proliferative lesions, liver tumour formation.

Special studies to support mode of action were conducted to examine the dose-response of porphyria-related effects in male and female rats (Table A A.10).

Figure A A.1. Pathway for heme synthesis in mammals

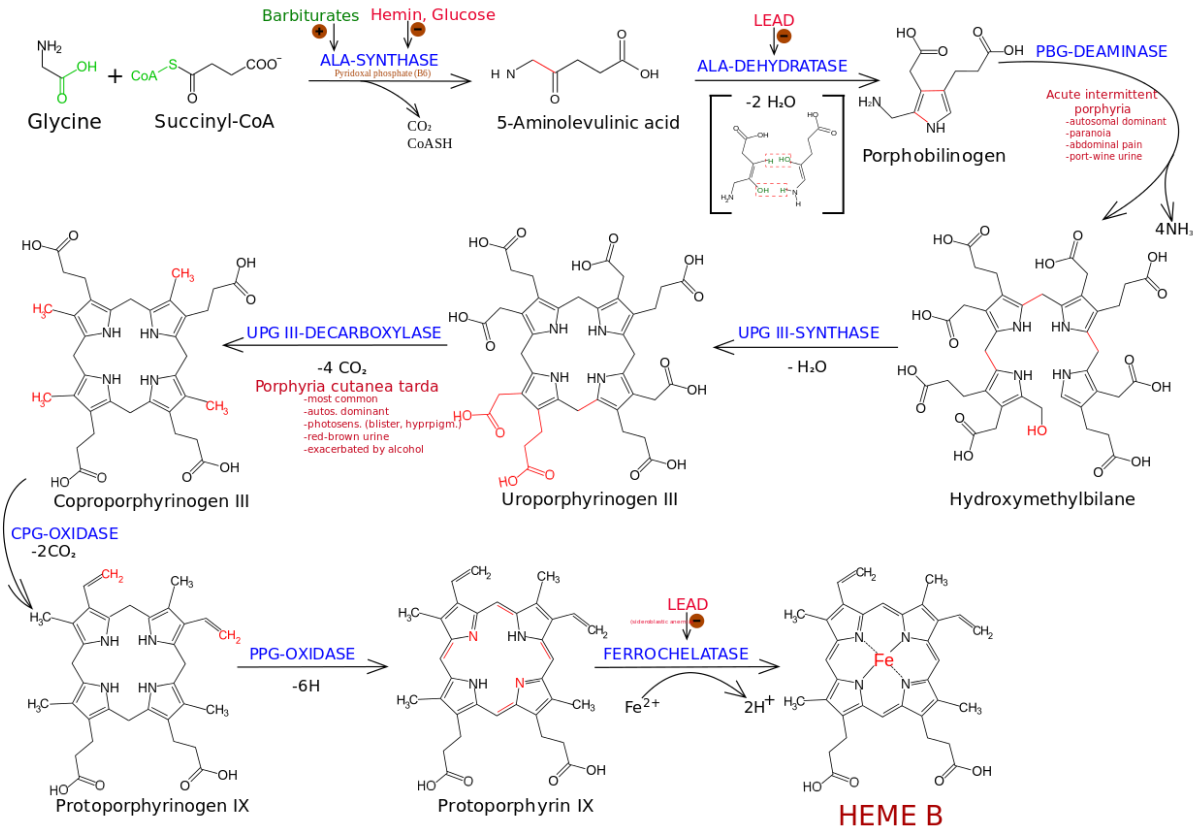


Image from Wikipedia (2016).

Note: PPG-Oxidase = PPO = protoporphyrinogen oxidase, the enzyme inhibited by saflufenacil in mammals

5. Read-across

Section note: The selection of analogues, and the associated chemical read-across described in this section were conducted in 2019 using data and read-across assessment tools which were publicly available at the time. It is noteworthy that following several rounds of feedback from the OECD IATA reviewers, a description of how the chemical read-across might be conducted in present day (e.g., 2024) will be further addressed in the uncertainties section of this IATA.

Saflufenacil belongs to the N-phenylimide class of chemicals (HRAC, 2019), which according to the 2019 HRAC, there are 29 herbicide active ingredients listed as PPO inhibitors (HRAC/WSSA Group 14 (Legacy HRAC Group E)), and nine subclassifications by structural classes (HRAC, 2019). For the purpose of this case study, analogues were included for the carcinogenicity read-across assessment by first searching for the closest structural similarity (based on Morgan fingerprints), followed by assessing the structurally similar analogues for common off-target mode of action in subchronic studies, and finally filtered by those that were also registered by the US EPA. This criterion reduced the initial list of 29 chemicals in HRAC down to six analogue chemicals for the original evaluation (conducted in 2019). The reasons for restricting to chemicals registered by the US EPA were 1) availability of data, and 2) assessment by a single agency for consistency of practice.

A quantitative metric of Morgan fingerprints was used to evaluate the structural similarity of analogue chemicals to saflufenacil, using the generalized read-across (GenRA) tool that is available on the US EPA CompTox website (US EPA GenRA, 2021). Morgan fingerprints make use of the length between atom groups i.e., the length of the vector and the radius uses the size of the atom groups. Similarity scores, which take chemical features and biological activity into account when calculating similarity, are provided in Table A A.11. The source analogues were analysed using Morgan fingerprints, which identified butafenacil (0.29) with the closest structural similarity to saflufenacil. The additional analogues belonging to different structural classes had slightly lower values (0.15 – 0.20). Physical chemical properties of saflufenacil and the six analogues selected for read-across assessment are summarised in Table A A.11. All molecules have molecular weights in the range of 345 – 501 g/mole. All of them are solids at room temperature, with the exception of carfentrazone-ethyl (a liquid). Saflufenacil has a lower vapor pressure than all other analogues, reflecting its lack of volatility. Saflufenacil and sufentrazone contain a weakly dissociable hydrogen because the NH groups are adjacent to polar sulfamoyl moieties and are weak acids (pKa 4.41 and 6.56). Log Kow values are in the range of 0.99 – 4.8 for all molecules, which is below the range (Log Kow value 5 – 8) where bioaccumulation is a concern. Bioaccumulation, if present, would lead to consideration as a persistent organic pollutant is warranted (ECETOC, 2000). Log Kow values are also used to consider the potential for systemic bioavailability, with more lipophilic compounds expected to be absorbed across cellular barriers than hydrophilic compounds.

The six selected analogue chemicals are PPO inhibitors from the N-phenylimide class (butafenacil), thiadizole class (fluthiacet-methyl), oxadiazole class (oxadiazon), triazolinone class (carfentrazone, sulfentrazone) and the ‘other’ class (flufenpyr-ethyl) (HRAC, 2020). The analogues each exhibit haematological changes that were evaluated in subchronic studies, which could relate to the off-target toxicological mode of action of saflufenacil. Structures of these read-across molecules and of saflufenacil are shown in Table A A.11. Each of these molecules has a halogenated phenyl ring bonded to a heterocyclic ring, with a bulky side chain (e.g. CF_3, CF_2, t-butyl) present on the heterocyclic ring. Thus, registered PPO inhibitors that are structurally similar to saflufenacil were selected for read-across to predict the carcinogenic potential of saflufenacil.

To further assess the similarity of the analogues, the ToxPrints tool was included to compare the biological properties of the chemicals and modes of action in the toxicity pathways, allowing refinement of the relevant analogues by structural and bioactivity similarities (Yang et al., 2015). The ToxPrints makes use of the library of chemotypes to identify biological properties of the chemicals to support the bioactivity similarity assessment (Figure A A.2). Figure A A.2 illustrates the proximity of the six selected analogues to saflufenacil. While additional chemicals belonging to the N-Phenyl-imides chemical class neighbour saflufenacil in this ToxPrints read-out, these were not included in the read-across analysis because they either did not exhibit similar MOA of toxicity, or they were not registered within the US (i.e., no publicly available information in 2019). It is also noteworthy, as the parameters used in the ToxPrints similarity assessments change, the categorisation and grouping of the chemicals may change. This furthers the necessity to justify and provide the rationale for which, and how, tools will be implemented in the read-across analysis.

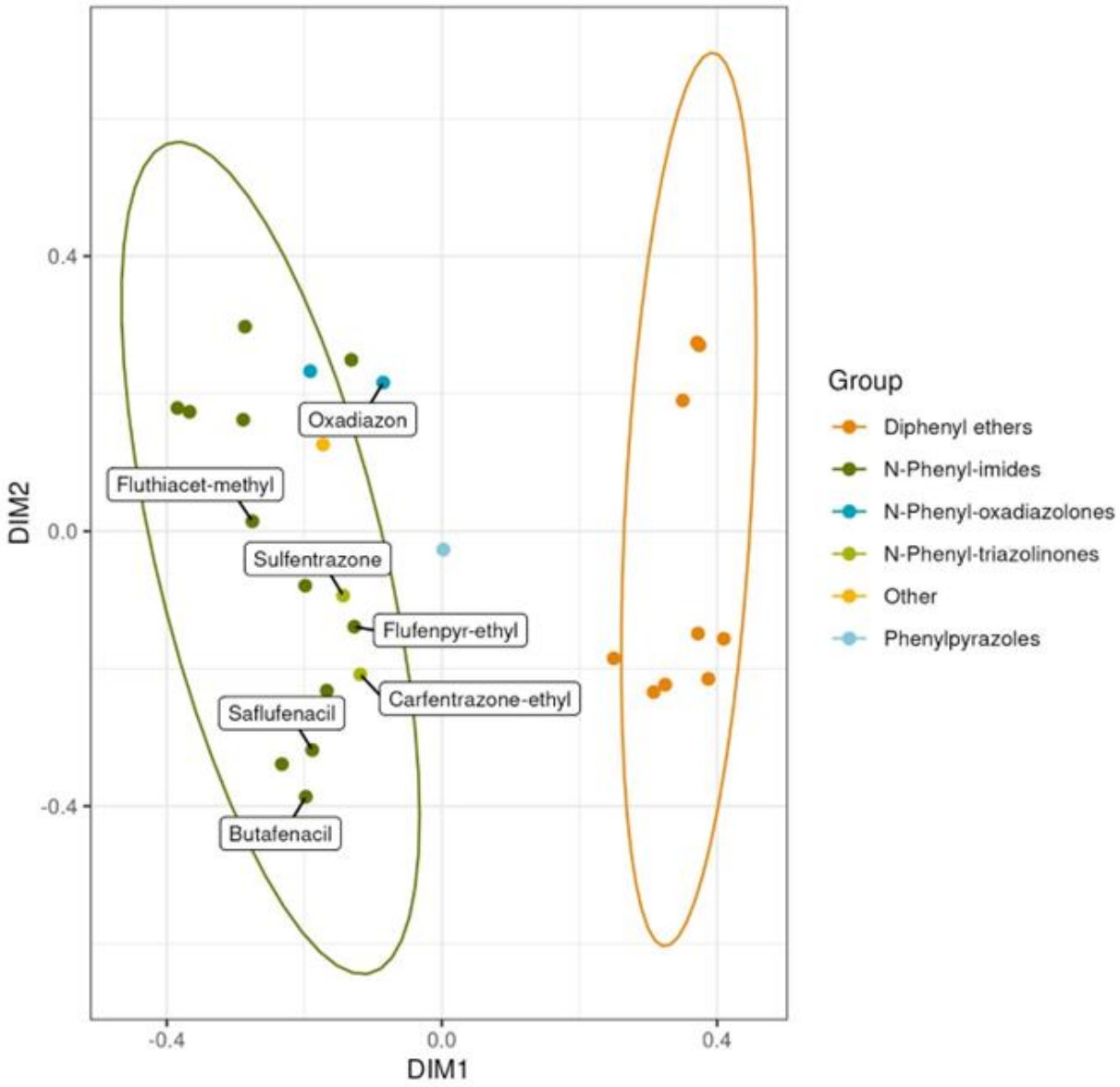
It is noteworthy that the use of read-across in this case study was not for the purpose of estimating the POD for chronic dietary risk assessment of the new active substance. This read-across analysis served to gain a better understanding of the common toxicity and bioactivity of this class of chemistry and support the hazard characterisation of the new active substance. The toxicity data for the read-across chemicals were obtained from publicly available human health risk assessments (HHRA) available at Regulations.gov. An overview of physical chemical properties and acute toxicity is presented in Table A A.11. A summary of the repeated dose studies and carcinogenicity for the analogue chemicals is presented in Table A A.12. Details of the subchronic effects of the read-across analogue chemicals are presented in Table A A.13. Details of the chronic toxicity and carcinogenicity studies for the analogue chemicals are presented in Table A A.14.

6. Evaluation of read-across analogues

The toxicological effects of the analogues used in the read-across assessment were similar to the target compound including acute toxicity, subchronic, genotoxic, and immunotoxic potential (Table A A.11-Table A A.13). Common key toxicological effects included markers of porphyria, anaemia and resulting changes in the liver. Butafenacil, the read-across compound in the same chemical class and the closest in structural similarity, reported the same key effects as saflufenacil including changes in spleen and bone marrow, general systemic toxicity, and mortality at the highest dose tested.

The read-across PPO inhibitors where toxicokinetic information was available, demonstrated extensive metabolism and rapid excretion, similar to the results reported in the above toxicokinetic sections for saflufenacil. Therefore, these herbicide molecules are not likely to bioaccumulate in chronic exposure. Unlike saflufenacil, kinetics appeared to be similar between male and female rats for most molecules, and no corresponding differences in NOAEL values between male and female test animals were observed. No common metabolite across the class of PPO inhibitors has been identified, and no particular metabolite / metabolic pathway has been indicated that leads to enhanced toxicity over that of the parent molecules.

Figure A A.2. Chemical clustering based on ToxPrints including the new active substance



This figure presents the results from the ToxPrints assessment to highlight proximity of the selected analogues to saflufenacil. In addition to structural similarity, ToxPrints makes use of the library of chemotypes to identify biological properties of the chemicals and modes of action in the toxicity pathways, allowing refinement of the relevant analogues by structural and bioactivity similarities (Yang et al., 2015).

Of the PPO inhibitor chemicals with publicly available cancer risk assessment information, four of six chemicals were classified as ‘Not likely to be carcinogenic to humans.’ Oxadiazon and fluthiacet-methyl were classified as ‘Likely to be carcinogenic to humans.’

Oxadiazon was classified based on liver adenomas and carcinomas in mice and in rats (both males and females). A mechanistic study (1991) indicated oxadiazon might be a peroxisome proliferator, a possible MoA for rodent liver tumours. The lack of evidence for peroxisome proliferation in the repeat-dose toxicity studies conducted with saflufenacil demonstrates a different biological response to these two PPO inhibitor pesticides. Based on a clear NOAEL for effects recorded in the liver and no evidence to suggest saflufenacil will induce peroxisome proliferation, it is considered to not be a tumorigenic chemical.

Fluthiacet-methyl was classified as 'Likely to be carcinogenic to humans' based on liver adenomas and carcinomas in male mice and pancreatic tumours in male rats. Similar effects on the erythropoietic system and liver were recorded in the subchronic toxicity study in mice with fluthiacet-methyl, demonstrating a temporal response to these effects. Similar effects were not recorded in repeat-dose toxicity studies conducted with saflufenacil. The pancreatic tumours were observed only in male rats treated with fluthiacet-methyl and were cited by the US EPA based on a trend towards an increase in pancreatic tumours in male rats (suggesting a low incidence). Therefore, they seem to be of questionable relevance to saflufenacil and saflufenacil was evaluated for any potential chronic toxicity or carcinogenic effects involving the pancreas.

Importantly, liver tumours were seen for the two carcinogenic analogue chemicals. Most of the analogues induced haematology changes (anaemia) and subsequent changes to the spleen, bone marrow, and liver were the predominant toxicity in rats, mice, and dogs. In the case of carfentrazone, fluorescence microscopy of the liver from treated rats revealed red fluorescent granules, which is characteristic of porphyrins. The liver is the primary site of excretion of excess porphyrins. Due to the molecular weight and non-polar characteristics of these molecules, deposition in the liver is known to occur when large increases in concentration of porphyrin in the blood occur (Gschnait et al., 1975; Smith and Foster, 2018). Deposition of porphyrins such as protoporphyrin IX or protoporphyrinogen in the liver has been shown to produce hepatotoxicity via oxidative stress and via precipitation of porphyrin granules in hepatocytes and in bile canaliculi, which results in inflammatory infiltration and blockage of bile canaliculi. Fatty change and necrosis of the hepatocytes eventually occur, and the diminished capacity to process and excrete the porphyrins further exacerbates liver toxicity (Gschnait et al., 1975; Sachar et al., 2016; Smith and Foster, 2018). Thus, the toxicity to the liver caused by saflufenacil as well as the analogues is likely to be a secondary effect related to PPO inhibition and porphyria.

For most of the read-across molecules, the extent of liver damage at the tested dose levels was limited to fatty change, vacuolation, and/or inflammation and did not include findings of liver necrosis and/or regenerative hyperplasia that would indicate the potential for hepatocellular adenomas. For oxadiazon and fluthiacet-methyl, the level of liver damage in 90-day studies (when available in US EPA reviews) was greater (e.g. necrosis, degenerative change, ↑ ALAT and ASAT in the blood), and consequently is consistent with the eventual generation of increased tumour response due to regenerative cell proliferation of the hepatocytes. Some read-across molecules (e.g. butafenacil) showed liver changes including necrosis and ↑ ALAT in the 90-day rat study but did not develop liver tumours in the carcinogenicity assays. The liver necrosis in the 90-day rat study with butafenacil occurred at ≥1000 ppm dietary feeding levels, whereas the highest dose used in the 2-year rat study was 300 ppm, and thus the selected dose levels are also a consideration in whether a PPO inhibitor will show liver necrosis and regenerative liver tumours in the long-term studies.

Overall, the evidence indicates that saflufenacil is unlikely to generate treatment-related tumours in rats or mice if a long-term set of studies were conducted. The 28-day mouse study showed some increases in ALAT or ASAT at higher dose levels, but the liver histopathology findings in this study and the 90-day studies in mice and rats were generally not indicative of necrosis or degenerative changes that would potentially lead to regenerative hyperplasia and liver tumours if continued for 18-24 months. In addition, the mechanism for herbicidal activity and mammalian toxicity (PPO inhibition) has been shown to lead to

dose-limiting porphyria and anaemia in the read-across molecules, but not a tumour response if the dose levels are not sufficiently high to trigger significant liver necrosis.

Minor liver histopathology changes were reported in subchronic studies with saflufenacil (unlike fluthiacet-methyl and oxadiazon), which gave a strong indication that liver tumours secondary to necrosis and regenerative responses are unlikely for saflufenacil if treated at similar dose level in chronic studies. Even if liver tumours did occur with saflufenacil, the MoA for hepatocellular adenomas and carcinomas with PPO inhibitors is a well-known response to liver necrosis that results from toxicity to liver deposition of excess porphyrins (Gschnait et al., 1975; Sachar et al., 2016; Smith and Foster, 2018). This type of response would be possible in humans if dose levels were high enough to induce sufficient porphyrin deposition and liver necrosis (Lefkowitch and Grossman, 1983; Smith and Foster, 2018), but they would not be of concern at lower saflufenacil exposure levels that do not produce porphyria and anaemia. In summation, the wealth of data available indicates that a threshold-based approach to carcinogenicity risk assessment is appropriate for saflufenacil and a margin of exposure approach in the chronic risk assessment that protects against all types of toxicity including porphyria, anaemia and resulting histopathology to spleen, liver and bone marrow would be protective against any potential carcinogenicity effects as well.

Overall, there are no clear signs of hormone disruption based on developmental studies with saflufenacil and selected analogues. While some effects on pup viability in a two-generation reproduction study occurred with saflufenacil and one analogue (sulfentrazone), the fact that these effects co-occur with haematology changes makes it likely that porphyria and anaemia are responsible for the observations in the pups, rather than a hormone-disruption mechanism with saflufenacil.

The chemicals used for PPO inhibitor read-across did not show signs of immunotoxicity using a TDAR study. The database includes 28-day immunotoxicity studies for fluthiacet-methyl and sulfentrazone, which did not show any evidence of immune suppression. Limited information was available for the remining four analogues; however, based on the data across multiple studies, there was no evidence of immunosuppression identified in the available analogue registration documents.

7. Toxicity Mode of action

There are no special studies or definitive MoA work conducted with any of the analogues used in the read-across assessment. However, the compounds share the same pMoA and biological effects. The key effects and target organs are consistent between the read-across and target compound and support the MoA proposed for saflufenacil.

8. WoE evaluation

In the absence of the results from the rat chronic toxicity/carcinogenicity study and mouse carcinogenicity studies with saflufenacil, a WoE approach was adopted to evaluate the potential for carcinogenicity and define a POD for chronic exposure. All of the toxicological information for saflufenacil was used in this approach. Information from the chemical analogues having similar pMOA or MOA as saflufenacil was also used to determine the potential chronic toxicity and carcinogenicity of this chemical class.

Separate endpoint(s) for cancer effects are not needed for saflufenacil because the chronic dietary POD will be protective for all potential carcinogenic and non-carcinogenic effects. Saflufenacil and analogues are all non-genotoxic, and they do not show any evidence of hormone perturbation or immune suppression.

The sub-chronic rat and mouse studies with saflufenacil produced changes in haematology parameters and associated tissues that were predictable based on the findings seen with other PPO-inhibiting molecules. These characteristic toxic effects, which are directly related to PPO inhibition, limit dose levels

in long-term studies where LOAEL and NOAEL levels are defined based on changes in haematological parameters, as well as secondary spleen, bone marrow, and liver histopathology.

In addition, the minor liver histopathology changes in 90-day studies with saflufenacil (unlike fluthiacet-methyl and oxadiazon) gave a strong indication that liver tumours secondary to necrosis and regenerative responses are unlikely for saflufenacil if treated at similar dose level in chronic studies. Even if liver tumours did occur with saflufenacil, the mode of action for hepatocellular adenomas and carcinomas with PPO inhibitors is a well-known response to liver necrosis that results from toxicity to liver deposition of excess porphyrins (Gschnait et al., 1975; Sachar et al., 2016; Smith and Foster, 2018). This type of response is possible in humans only if dose levels are high enough to induce sufficient porphyrin deposition and liver necrosis (Lefkowitch and Grossman, 1983; Smith and Foster, 2018), but they should not be of concern at lower saflufenacil exposure levels that do not produce porphyria and anaemia. This WoE assessment was based on the US EPA's regulatory hazard characterization and risk assessment. WoE assessments prepared for the EU must include ECHA hazard assessment and EFSA risk assessments.

When comparing the results of subchronic and chronic toxicity studies for the analogues used in the read-across assessment, there was a limited increase in toxicity over time (≤10-fold). With butafenacil, the most structurally similar of the read-across compounds to saflufenacil, the increase in toxicity over time (comparing both NOAELs and LOAELs) was ~3-fold. Only carfentrazone-ethyl demonstrated an increase in toxicity over time > 10-fold. The rapid and complete excretion of saflufenacil supports that toxicokinetics will not impact toxicity over time.

In summation, the wealth of data available indicates that a low-dose linear approach to carcinogenicity risk assessment is not appropriate for saflufenacil, and the margin of exposure approach in the chronic risk assessment that protects against all types of toxicity including porphyria, anaemia, and resulting histopathology to spleen, liver and bone marrow will be protective against any potential carcinogenicity effects as well.

9. Proposed PODs for chronic risk assessment

The proposed PODs for chronic risk assessments with saflufenacil, if the chronic/carcinogenicity studies in rats and mice are not conducted, are shown in Table A A.15.The NOAELs from the currently available 90-day subchronic studies for saflufenacil are 10.5, 12.4, and 10 mg/kg/day for rat, mouse, and dog studies (respectively), comparing only the achieved dose levels for the most sensitive effect in the most sensitive sex. These NOAEL values across three species are essentially the same, so the value for rats (10.5 mg/kg/day) is proposed to derive a chronic population-adjusted dose (cPAD). The selection of the NOAEL from the rat 90-day study will be conservative; it is based upon the defining toxicity of saflufenacil and other PPO inhibitors, and the induction of porphyria and anaemia (Figure A A.3). The RISK21® graph shown in Figure A A.4 demonstrates that the % cPAD values calculated from the 90-day rat NOAEL is below the US EPA’s Health Effects Division (HED) level of concern, after using an additional 10X uncertainty factor to derive the chronic point of departure (total of 1000-fold UF). For all subpopulations, the total exposure represented 32% - 87% of the allowable risk, which is below the HED level of concern of 100%.

Figure A A.3. Points of Departure (NOAELs) and LOAELs for toxicity studies conducted with saflufenacil

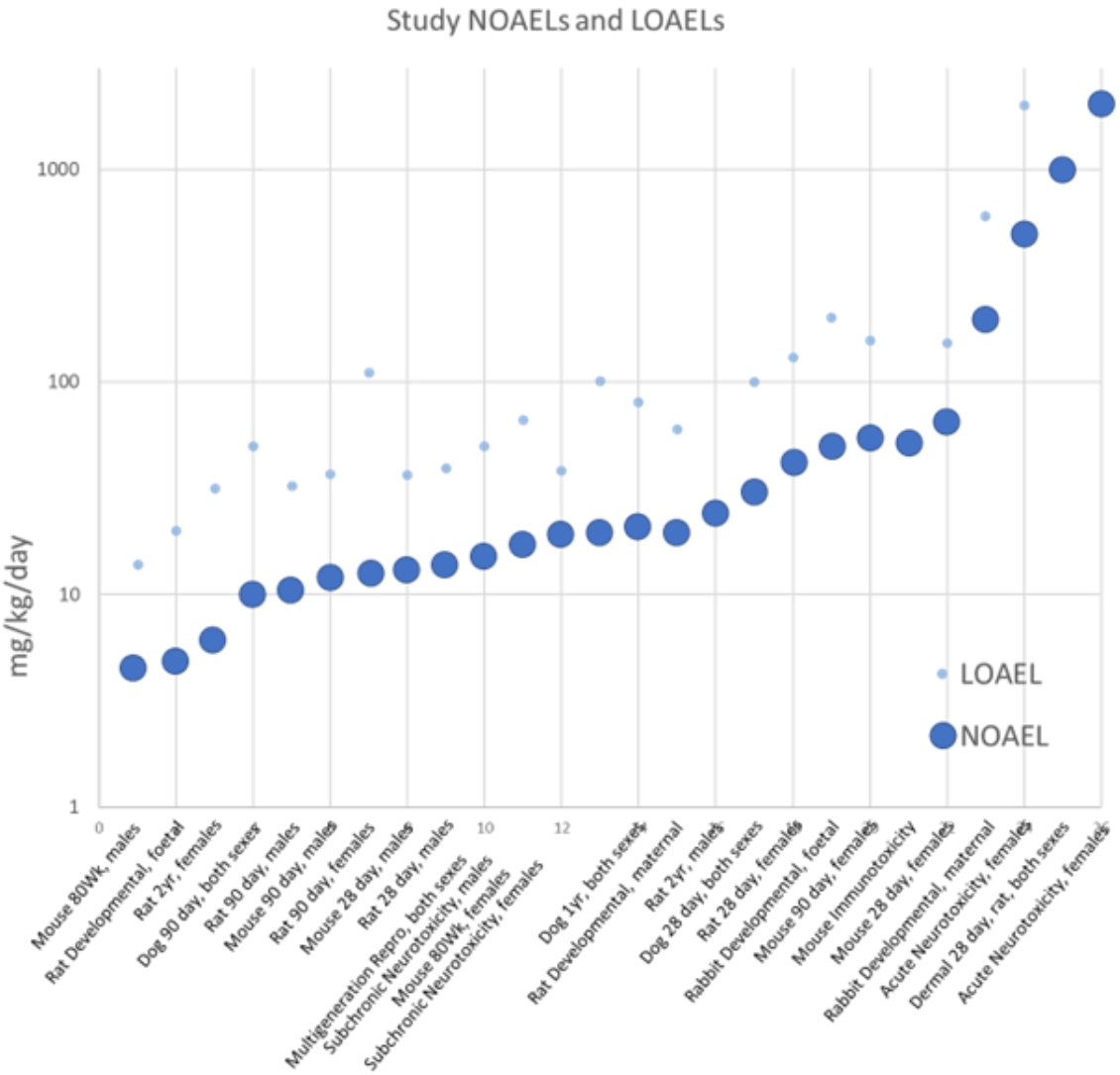
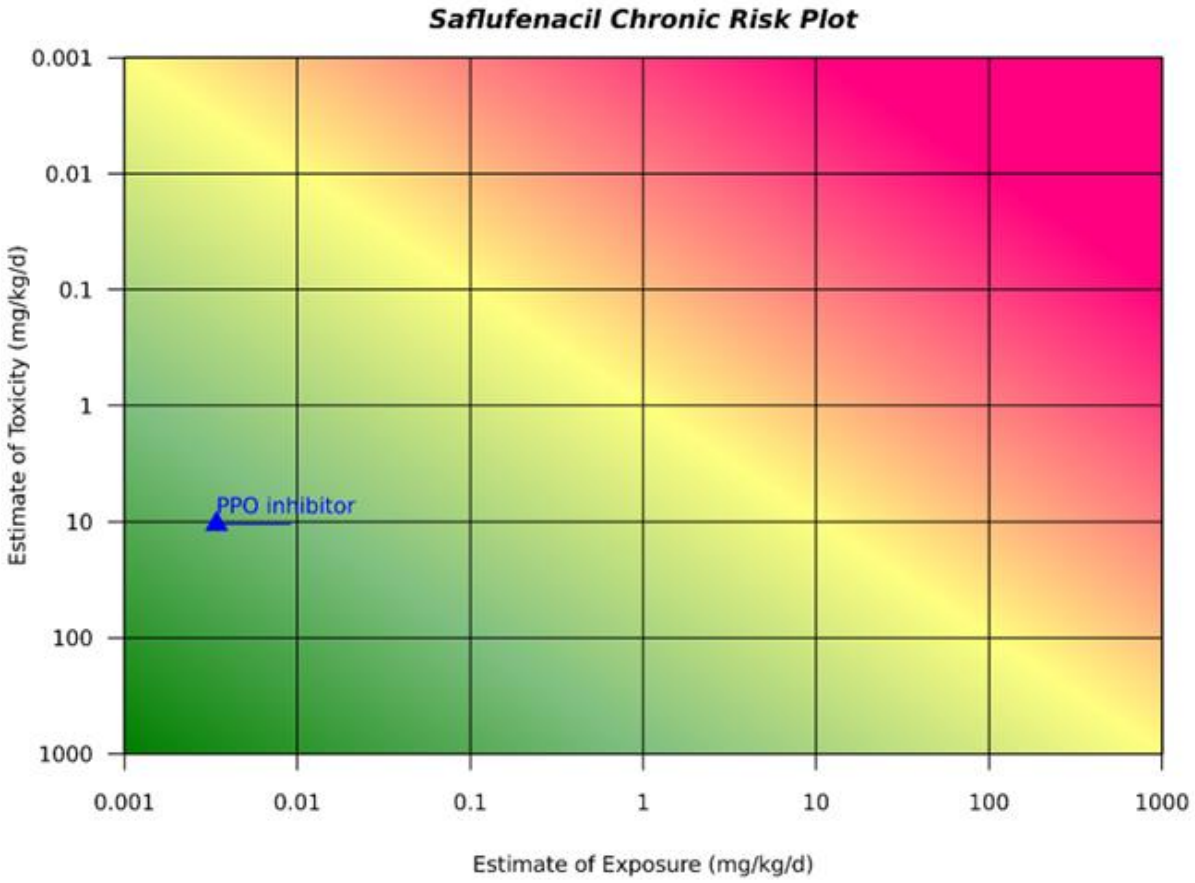


Figure A A.4. RISK21® plot evaluation of available exposure and hazard data for the safety assessment of saflufenacil



The yellow line in the RISK21® tool represents the margin of exposure between the 90-day toxicity study NOAEL (as an estimate of toxicity) and the registrant's modelled exposure values (as estimates of exposure) generated in US EPA's DEEM dietary risk software. The Health and Environmental Science Institute (HESI) provide RISK21® tools, which are available online through the following link: https://risk21.org/webtool/

10. Uncertainty

An uncertainty analysis is provided based on categories in an ordinal scale and is presented in Table A A.2. The scale is defined referring to the quality of the evidence generated/that was available supporting the assumptions of the case study and the overall weight of evidence, which is again related to the impact of the uncertainty on the hypothesis. For example, limited and poor-quality evidence is likely to lead to larger uncertainty and vice versa.

When considering the uncertainty of this approach it is important to evaluate that uncertainty against the methodology of traditional human health risk assessment. The current accepted methodology for conducting human health risk assessment includes an inherent level of uncertainty to which stakeholders have become accustomed. The uncertainty of this traditional approach is generally accepted and mitigated

by using the most sensitive adverse effect, in the most sensitive sex, in the assumed most sensitive species to define the hazard potential. Default uncertainty factors for interspecies or intraspecies toxicodynamic and toxicokinetic differences are additionally applied in accordance with regulatory policy.

Through a retrospective assessment of saflufenacil, the most sensitive effect, in the most sensitive species, in the most sensitive sex has been identified. The POD for risk assessment is supported by analogue chemicals used for read-across that function through the same toxicological mode of action. While Table A A.2 provides a level of certainty in using the IATA, this table is not prescriptive, and the factors assessed for uncertainty will be dependent upon the case.

Table A A.2. Level of certainty of this approach

Factor	Uncertainty (low, medium, high)	Impact of uncertainty on hypothesis
Uncertainty due to the lack of rodent chronic toxicity / carcinogenicity studies with saflufenacil	Low	This case study demonstrates that rodent chronic toxicity/ carcinogenicity studies are not always necessary to fulfil regulatory data needs for agrichemical safety assessment. In the case of saflufenacil, the data available combined with the toxicity data from chemical analogues demonstrate what is known and understood regarding the PPO inhibitor MOA/AOP, which are sufficient to estimate a chronic POD for saflufenacil without the chronic toxicity/carcinogenicity studies.
Weight of the evidence-based approach	Low	The approach used in the case study was a weight of the evidence (WoE) approach. While there are various guidance structures for WoE in the context of specific decisions, there is no specific guidance for the current use. The authors included lines of evidence, information that was pertinent and relevant, to assess the potential for carcinogenicity and knowledge gaps; supporting a conclusion that additional rodent chronic toxicity/carcinogenicity studies were not needed to estimate a POD protective against chronic risk.
Lack of measurement of hormones	Medium	An endocrine assessment has been completed for saflufenacil based on DERs generated by the EPA in 2006-2007 for developmental and reproductive effects. Under the United States FIFRA, the EPA concluded, based on the available data, that saflufenacil and the surrogate compounds do not result in toxicity via an endocrine MOA and thus not relevant for the selection of endpoints for risk assessment. The lack of hormone measurement does not affect the WoE assessment or outcome because a hormonal MoA relevant to carcinogenicity was not identified for the target chemical or analogues used in the read-across assessment. Effects due to perturbations of reproductive hormones were considered adequately evaluated by the US EPA in the toxicological database, including the repeated dose, reproductive, developmental, and ToxCast data. Medium uncertainty is reported because hormone perturbation was not assessed in accordance with other regulatory legal frameworks outside of FIFRA (e.g., Delegated Regulation on Classification, Labelling and Packaging (CLP) of chemicals).
No Inclusion of modelling or ToxCast data to support endocrine data gaps	Medium	A conclusion can be made that saflufenacil and the surrogate compounds do not result in toxicity via an endocrine MOA and thus there were no data gaps related to endocrine effects relevant to the selection of endpoints for carcinogenicity risk assessment. Effects due to endocrine disruption were considered adequately evaluated in the toxicological database, including the repeated dose, reproductive, and developmental toxicity data. Medium uncertainty is reported because hormone perturbation was not assessed in accordance with other regulatory legal frameworks outside of FIFRA, as authorized in the United States (E.g., Delegated Regulation on Classification, Labelling and Packaging (CLP) of chemicals).
Lack of details immunotoxicity endpoints	Medium	The human-relevant mode of action for carcinogenicity concerns is immunosuppression. An immunotoxicity assessment has been completed for saflufenacil by the EPA (2011a). The available data for saflufenacil and chemical analogues do not support immune suppression as a key event that would lead to carcinogenicity following long-term exposure to saflufenacil or its analogues used for the read-across assessment. Under the United States FIFRA, the US EPA

Factor	Uncertainty (low, medium, high)	Impact of uncertainty on hypothesis
		concluded that saflufenacil failed to induce toxicity specific to the immune system following guidance from EPA OPPTS 870.7800 (1998). Further evaluation of immunotoxicity would not impact the determination that immunosuppression is not a relevant carcinogenic MoA. Medium uncertainty is reported because immunotoxicity was not assessed in accordance to other regulatory legal frameworks outside of FIFRA, as authorized in the United States (e.g., Delegated Regulation on Classification, Labelling and Packaging (CLP) of chemicals). It was also noted during the IATA review process that cytokine levels were not measured in the *in vivo* studies and subtyping was not reported for WBC. Uncertainty could have been reduced by evaluating if proliferative stimulation of the bone marrow related to regenerative anaemia.
QSAR modelling – Domain of applicability	Not Applicable	Morgan fingerprints (GenRA) and ToxPrints were used to assess structural similarity and identify biological properties of the chemicals and modes of action in the toxicity pathways, to allow refinement of the relevant analogues by structural and bioactivity similarities (Yang et al., 2015). Most of the data were derived from toxicological studies – therefore QSAR modelling was not used for the prediction of general toxicity. More information would need to be collected through QSAR modelling to determine the level of certainty.
QSAR modelling – to address data gaps for endocrine endpoints	Medium	An endocrine assessment has been completed for saflufenacil in light of the requirement for the United States FIFRA in 2006-2007. A conclusion was made, based on the available data, that saflufenacil and the analogue compounds do not result in toxicity via an endocrine MOA and thus not relevant for selection of endpoints for risk assessment. Effects due to endocrine disruption were considered adequately evaluated in the toxicological database, including the repeated dose, reproductive, and developmental toxicity data. Given that recent OECD Test Guidelines to address endocrine disruption, and QSAR model models were not used to investigate endocrine effects, QSAR models would need to be run to reduce uncertainty.
Literature review (grey and white) compared to a systemic literature search	Medium	Grey literature was limited to reviews conducted by the US EPA. Literature search through PubMed was conducted but no useful publications were identified. A systematic review was not conducted.
Read-across – analogue selection	Medium	For this IATA, the closest structurally similar analogues by Morgan fingerprints were retained which also had similarities in toxicological MOA for off-target effects. The pMoA and MoA are similar in the group of PPO inhibitors. There are different chemical classes within the PPO inhibitors, of which, six analogues with EPA data belonging to five different chemical classes were selected. However, data was only used for one analogue, butafenacil, representing the chemical class of N-phenylimides, to which saflufenacil belongs. In cases where the MoA is understood, structural similarity and physical chemical properties drive the pharmacokinetics, not the pharmacodynamics of the compounds. While the % calculated similarity using Morgan fingerprint is = 0.29, the target organ toxicity was similar. The similarity of the analogue can be taken into consideration during the selection of key endpoints, if necessary. The approach used in this case study in estimating a POD was to select the most sensitive species effect (NOAEL) and add an additional 10x uncertainty factor for the extrapolation from subchronic to chronic effect. Read-across was used to evaluate common MOA, but was not used to support the estimation of the POD for this case example. Additional investigation into the analogue selection by chemical class (pMOA), as well as toxicity (MOA) would be needed to reduce uncertainty in the analogue selection for read-across assessment.
Read-across – exclusion of compound based on no EPA review	Medium	For the purpose of this IATA, the closest analogues by Morgan fingerprints were retained which also had similarities in toxicological MOA for off-target effects. Analogues were further filtered to include only the chemicals that had registration within the US EPA. The primary reasons for restricting inclusion to chemicals registered by the US EPA were 1) availability of data, and 2) assessment by a

Factor	Uncertainty (low, medium, high)	Impact of uncertainty on hypothesis
		singular agency to reduce variability in conclusions. The analogues each exhibit haematological changes that were evaluated in subchronic studies, which could relate to the off-target toxicological mode of action of saflufenacil. For this case study, one compound was excluded because it was not registered in the US and therefore no EPA review was available. This was an *a priori* decision made by the team to efficiently work through a case example, but a different approach to the read-across assessment could be made by others.
Mechanism of action AOP	Low	The AOP for this chemical class (PPO inhibitors) is well characterized. Peer-reviewed publications are available supporting this as well as an OECD AOP 131 Wiki entry. We saw no need to reiterate.
US based references	Low	This example case study was developed in the context of using information submitted to fulfil the United States FIFRA. The acceptability of the studies and the interpretation of the studies were those of the EPA as generated in their DERs between 2005-2010.
Reliability and acceptability of the data	Low	This example case study was developed in the context of using information submitted to fulfil the United States FIFRA. The acceptability of the studies and the interpretation of the studies were those of the EPA as generated in their DERs between 2005-2010. JMPR and PMRA also reviewed and interpreted the available studies.
Analysis of studies per current OECD guidelines	Low	This example case study was developed in the context of using information submitted to fulfil the United States FIFRA. The acceptability of the studies and the interpretation of the studies were those of the EPA as generated in their DERs between 2005-2010. JMPR and PMRA also reviewed and interpreted the available studies. It should be noted that OECD Test Guidelines are continually updated; thus some of the data used in the retrospective WOE do not reflect the most recent guideline improvements (e.g., genotoxicity and immuno). All data generated for saflufenacil was conducted in accordance to Good Laboratory Practice following OECD guidelines as well as US EPA Office of Chemical Safety and Pollution Prevention (OPPTS) guidelines, with the exception of the immunotoxicity study. This study was performed in accordance to OPPTS guidelines only.
Concordance and weight of evidence of all data used for justifying the hypothesis	Low	The targeted toxicity data for the compound of interest combined with data from the chemical analogues used in the read-across were assessed in a consistent manner to provide sufficient information to address the potential for chronic toxicity and carcinogenicity. Saflufenacil and the analogues used in the read-across assessment share a similar MoA and target organ toxicities. The data from the analogues identified liver cancer as a potential effect with corresponding precursory histopathological findings. These effects were not demonstrated in the toxicological database with saflufenacil. The lowest NOAEL from the 90-day toxicity studies was selected to estimate the chronic POD.
Overall uncertainty of the IATA	Medium	The saflufenacil case study provides an example of a WoE approach for waiving both the rat chronic toxicity/carcinogenicity and the mouse carcinogenicity studies. Given the retrospective nature of this exercise, uncertainties could be addressed in prospective cases.

Uncertainty ranking key: low = uncertainty is not a concern and will not affect the confidence of the IATA conclusion; medium = uncertainty is a moderate concern and may affect the confidence of the IATA conclusions; high = uncertainty is a concern and needs to be addressed.

11. WoE Based Assessment

The goal of this assessment was to use the available toxicological data for saflufenacil as well as read-across to determine if rodent chronic toxicity and carcinogenicity studies are needed to estimate a POD that is health protective for chronic toxicity and carcinogenicity. This approach considered publicly available

hazard and exposure information from the United States EPA, as regulated under FIFRA, for saflufenacil and the analogues chemicals selected for the read-across assessment.

Saflufenacil acts as an herbicide via the inhibition of the plant PPO, which is responsible for the seventh step in biosynthesis of protoporphyrin IX. This porphyrin is the precursor to haemoglobin, the oxygen carrier in animals, and chlorophyll, the pigment at the heart of photosynthesis in plants. In mammals, disruption of this enzymatic step in haem synthesis can result in porphyria at high dose levels with uninterrupted exposure. The toxicology properties of six PPO-inhibiting herbicides with publicly available EPA reviews were evaluated in this document to inform the need for chronic toxicity and carcinogenicity studies in rats and mice to assess risks following chronic exposure to saflufenacil.

Based on a WoE approach, the case study developer proposes that chronic toxicity/carcinogenicity studies not be required at this time for saflufenacil. Note, that while the author of the WoE case study proposes that chronic toxicity/carcinogenicity studies are not needed to support a health-protective POD, this is not a reflection of a regulatory decision. The regulatory interpretation of this case study would be dependent on a formal data submission and review, which was out of scope of this retrospective exercise.

To, summarize, here are observations from conducting the WoE-based assessment, including data gaps and uncertainties:

- Saflufenacil and the analogues selected for the read-across assessment were classified as non-genotoxic.
- The key toxicological effect of saflufenacil and the analogues selected for the read-across assessment is PPO inhibition. Common key toxicological effects included markers of porphyria, anaemia, and resulting changes in the liver. PPO inhibitors, including saflufenacil and the analogues, produced porphyria and regenerative anaemia, with characteristic changes in the spleen and bone marrow in certain studies, that reflected increased production of erythrocytes to compensate for the lower red blood cell counts. In the liver, deposition of porphyrins has been shown to produce hepatotoxicity via oxidative stress and via precipitation of porphyrin granules in hepatocytes and in bile canaliculi, which results in inflammatory infiltration and blockage of bile canaliculi. Fatty change and necrosis of the hepatocytes eventually occur, and the diminished capacity to process and excrete the porphyrins further exacerbates liver toxicity (Gschnait et al., 1975; Sachar et al., 2016; Smith and Foster, 2018). Thus, the toxicity to the liver caused by saflufenacil as well as the analogue chemicals used in the read-across assessment is likely to be a secondary effect related to PPO inhibition and porphyria.
- Saflufenacil and the analogue chemicals used in the read-across assessment demonstrated extensive metabolism and rapid excretion. Therefore, these herbicide molecules are not likely to bioaccumulate following chronic exposure. Unlike saflufenacil, kinetics appeared to be similar between male and female rats for most molecules, and no corresponding differences in NOAEL values between male and female test animals were observed. No common metabolite across the class of PPO inhibitors has been identified, and no particular metabolite / metabolic pathway has been indicated that leads to enhanced toxicity over that of the parent molecules. Based on the available toxicokinetic information, an increase in toxicity over time is not expected from subchronic to chronic exposure.
- There is no evidence in the toxicological data for saflufenacil or the analogues selected for chemical read-across assessment to support that hormone perturbation would be a relevant MoA for carcinogenicity for saflufenacil. No endocrine-related adverse effects relevant to this MoA were reported in the toxicological database for saflufenacil or the selected analogues used in the read-across assessment including subchronic, chronic, reproductive, and developmental toxicity studies. It should be noted that hormonal measurements were not described in accordance to data

requirements for certain regulatory assessments (E.g., Delegated Regulation on Classification, Labelling and Packaging (CLP) of chemicals), and several study parameters relevant for the assessment of endocrine disruption / hormonal perturbation are not determined in the available data. It should also be noted that while the US EPA risk assessments used to evaluate saflufenacil and selected analogues for developmental toxicity did not indicate developmental toxicity, other PPO inhibitor chemicals have been classified in Europe for developmental toxicity. Further investigation would be needed to better understand the potential implications of developmental toxicity in a WoE-based assessment for estimating a chronic POD.

- There is no evidence in the toxicological data for saflufenacil or the analogue chemicals used in the read-across assessment to support that immune suppression is an operative carcinogenic MoA for saflufenacil. As a PPO inhibitor, saflufenacil produced porphyria and regenerative anaemia, but it did not produce a clear deficit in white blood cell number and function, and there was no evidence of increased infections in repeat-dose studies (Table A A.9). The porphyria and regenerative anaemia produced characteristic changes in the spleen and bone marrow in certain studies, that reflected increased production of erythrocytes to compensate for the lower counts, but it did not reflect a deficiency in the immune system. Thus, based on the weight of the evidence, the data do not support immune suppression as a key event that would lead to carcinogenicity following long-term exposure to saflufenacil or its analogues used for the read-across assessment. It was noted during the IATA review process that cytokine levels were not measured in the *in vivo* studies and subtyping was not reported for WBC. Uncertainty could have been reduced by evaluating if proliferative stimulation of the bone marrow related to regenerative anaemia. Immunotoxicity was assessed following EPA guidance OPPTS 870.7800 (1998).
- The saflufenacil 90-day study NOAEL values were very similar for the rat, mouse, and dog sub-chronic studies (10 – 12 mg/kg/day). NOAEL values were lower in the 90-day studies for male rats and male mice than for females, which is consistent with the known differences in toxicokinetics between the sexes.
- When comparing the results of subchronic and chronic toxicity studies for the analogues used in read-across, there was a limited increase in toxicity over time (≤10X). The rapid and complete excretion of saflufenacil and analogue compounds supports this finding.
- The EPA classified four of six analogues used for the read-across assessment as PPO inhibitors as “Not Likely to be Carcinogenic to Humans”. Two PPO inhibitors (fluthiacet-methyl and oxadiazon) had liver tumours in rats and/or mice, and the degree of liver histopathology in 90-day studies (e.g. necrosis, hyperplasia), which is a precursor to carcinogenicity and a predictor of the long-term outcomes. Deposition of excess porphyrin in the liver due to porphyria has been shown to cause liver damage, and therefore dose levels of PPO inhibitors that are not sufficient to trigger these effects would have minimal relevance to human carcinogenic risk. Saflufenacil induced some histopathology changes in the liver after 28 or 90 days (fatty change, lymphoid infiltrate), but the lack of peroxisome proliferation, necrosis and degenerative changes suggests saflufenacil is unlikely to induce liver tumours in long-term studies.
- Several uncertainties were flagged as needing further investigation to reduce uncertainty related to the analogue selection for the read-across assessment (E.g., a clear explanation for the inclusion/exclusion criteria). At the time of the initial analogue selection in 2019, the general consensus of the workgroup was to use chemicals that had publicly available information for chemicals that had a risk assessment from the US EPA in order to maintain consistency of data interpretation from one regulatory authority. The analogues were first assessed based on structural similarity, followed by an assessment of common off-target effects. Uncertainty could potentially be reduced by evaluating all 9 analogues in the N-Phenyl-Imide chemical class as a first

assessment – regardless of their data availability by registration in the United States. The read-across assessment could start with the 9 substances in the N-Phenyl-Imide chemical class, and then evaluate both common toxicological effects and structural similarity. It is noteworthy that the number of chemicals in the N-Phenyl-Imide chemical class has expanded in the HRAC since the initial read-across assessment in 2019. In this case study example, the read-across assessment was not further modified to account for newly available information since 2019 because the read-across was ultimately *not used to estimate the POD*, but rather, to confirm the PPO-related MOA. In subsequent case studies, it will be important to execute a thorough read-across assessment beyond the information available under a specific regulatory authority.

- These PPO-related toxic effects are expected to limit dosing in long-term studies. The PPO-related toxicity (e.g., haematological effects) is not associated with any clear pattern of treatment-related tumours, which is supported based on results with the analogues used in read-across. Thus, saflufenacil is predicted to be non-carcinogenic in long-term rat and mouse studies.
- A POD for chronic risk assessment is proposed based on a 90-day rat study NOAEL, which is protective of other effects in the toxicological database reported at higher dose levels.

12. Retrospective assessment

In its human health risk assessments, which included the rat and mouse chronic toxicity and carcinogenicity studies, EPA used a POD of 4.6 mg/kg/day for the chronic risk assessments with saflufenacil, based on the 18-month mouse study and a total 100-fold uncertainty factor (UF) (EPA, 2014c; EPA, 2015). In this case study example, a POD of 10.5 mg/kg/day value was estimated based on the lowest 90-day study NOAEL between rats, mice, and dogs. In previous regulatory decisions to waive chronic testing, a 10x uncertainty factor (UF) is typically applied to the subchronic test by default when extrapolating to chronic risk, unless a scientifically defensible justification can be provided to illustrate an alternative UF. In this case, a 3-fold uncertainty factor could be used to estimate the chronic reference dose (cRfD) for saflufenacil from the 90-day study NOAEL, which would be conservative since the actual NOAEL in long-term studies (4.6 mg/kg/day) was only about 2-fold lower than the lowest 90-day NOAEL (10.5 mg/kg/day). If the chemical analogues included in the read-across assessment would have been used to estimate the POD, the average of the most sensitive species tested for carcinogenicity would have been the mouse with an average NOAEL of the analogues was 24.3 mg/kg/day. Thus, the 10.5 mg/kg/day value from the 90-day study was used to estimate the POD since it was the more conservative value.

The chronic POD from the JMPR (2011) and PMRA (2009; 2015) was also 4.6 mg/kg bw/day from the mouse oncogenicity study and the regulatory authorities agreed that saflufenacil was not carcinogenic in rats and mice. Ultimately, the EPA determined that saflufenacil was not likely carcinogenic to humans based on the lack of tumours in the mouse and rat carcinogenicity studies and lack of mutagenicity.

13. Strategy and Integrated Conclusion

The strategy developed within the framework addresses the need for the reduction in animal testing through a WoE assessment of the available data to determine if a POD can be estimated that is protective for chronic toxicity and carcinogenicity. The purpose of rodent chronic toxicity and carcinogenicity studies is to identify tumorigenic potential in rodents and assess their relevance to humans (hazard identification) (ICH, 2022). In addition, the results of rodent chronic toxicity and carcinogenicity studies are assessed in terms of the entire toxicological database to identify endpoints for human health risk evaluation. The strategy supporting this framework is based on the ability to use information in a read-across assessment,

as well as other assays used in toxicity assessment, to address the potential for chronic/carcinogenicity effects by estimating a health-protective POD.

The key to this strategy is the ability to identify the genotoxic potential of chemicals, including QSAR modelling, early in the process of screening agrichemical candidates. It should be noted that genotoxicity testing was limited when the requirements for rodent carcinogenicity were first issued to fulfil regulatory safety assessments. In 2024, our understanding of pMoA and non-carcinogenic and carcinogenicity Moas including their key events (precursor effects) allows us to have confidence in this strategy. Toxicological data generated on the agrichemical, excluding the rodent chronic toxicity/carcinogenicity studies, allow for the identification of endocrine Moas, immunotoxicity MoAs, target organs, and key toxicological effects. For saflufenacil, the MoA (PPO inhibition) is known and well-documented, with available AOPs and peer-reviewed literature. Therefore, the key target organs and critical effects for saflufenacil follow what would be expected (haematological effects with secondary liver effects at higher dose levels). In addition, chronic toxicity and carcinogenicity studies exist on other PPO inhibitors and were incorporated to support the WoE. This strategy is supported by the FDA's guidance for the need of long-term rodent carcinogenicity studies (Bourcier et al., 2024; ICH, 2022) and relevant peer-reviewed literature. (Cohen et al. 2019; Boobis et al. 2016; Corvi et al. 2017; Doe et al. 2019; Hilton et al., 2022; Reddy et al., 2010; Sistare et al., 2011).

Uncertainties can be further addressed in subsequent case studies that incorporate more data, especially from more closely related analogues of the N-phenylimide class, including mechanistic data to inform adverse outcome pathways, and read-across, while chronic toxicity/carcinogenicity studies are available. Additionally, uncertainties will be better addressed through discussions related to the development of guidance (e.g., sufficiency of data to support WoE).

The process described in this IATA, and the associated case study examples, provide a starting point that demonstrates the use of a WoE approach for waiving both the rat chronic toxicity/carcinogenicity and the mouse carcinogenicity studies. The saflufenacil case study is based on an available toxicological MoA (PPO inhibition), existing toxicology, and read-across assessment of saflufenacil. The saflufenacil case study indicates a low likelihood of a tumour response and the availability of sub-chronic values for use in risk assessments that are protective of both chronic effects and any potential carcinogenic effects. The same conclusions on the lack of potential for carcinogenicity reached in this document were also determined by EPA (2014c; EPA, 2015), JMPR (2011) and PMRA (2009; 2015), and the lowest POD from the subchronic toxicity testing with an additional 10X UF was protective of the chronic POD selected by the regulatory authorities.

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15. Tables

Table A A.3. Physical-chemical properties of saflufenacil

Parameter	Value
Molecular weight (g/mole)	500.9
Physical state at room temperature	Solid
Melting point	189.9°C.
Density at 20 °C.	0.661 kg/L
Water solubility (20 °C.)	In g/100 mL:
pH 4	0.0014
pH 5	0.0025
pH 7	0.21
pH 9	n.d. (degraded)
Solubility, organic solvents (20 °C.):	In g/100 mL:
Acetonitrile	19.4
Acetone	27.5
Ethyl acetate	6.55
Methanol	2.98
Isopropanol	0.25
Toluene	0.23
n-Heptane	<0.005
Vapor pressure (20 °C.)	4.5×10^{-15} Pa
Henry's law constant (atm-m^3/mol)	1.07×10^{-20}
Octanol/water partition coefficient Log (P_{ow}) at 25 °C	2.6
Dissociation constant in water, pKa	4.41 ± 0.025
pH (1% solution at 25°C.)	4.43

References:

- EPA (2014a)
- IUPAC (2021a)
- Pubchem (2021a)

Table A A.4. Summary table of toxicokinetic parameters and metabolism in Wistar rats for saflufenacil (OPPTS 870.7485 MRIDs 47128129 and 47128130)

Parameter	Group 1	Group 2	Group 3
Dose level (oral, mg/kg)	**4 mg/kg**	**20 mg/kg**	**100 mg/kg**
Purity	Radiolabel = 96% Non-radiolabel = 93.9%		
% in Urine	Major route of excretion in females		
% in Faeces	Major route of excretion in males		
Tissue distribution	GI tract, liver, kidneys, lungs, and thyroid		
T_{max}	1 hour		
Cmax (µg Eq/g)	M: 23.9 F: 23.0	M: 98.3 F: 84.8	M: 286.0 F: 258.3
AUC (µg Eq × h/g)	M: 741 F: 247	M: 2131 F:754	M: 4502 F: 3057

Initial T ½ (h)	M: 20.9 F:49.5	M: 22.6 F: 58.1	M: 33.5 F: 59.2
Major metabolites	M800H01, M800H03, and M800H07 (urine and faeces)		
Structure of M800H01			
Structure of M800H03			
Structure of M800H07			

References: EPA, 2014a; EPA, 2015. **Note:** only limited details of toxicokinetic studies were available in these EPA reviews. Structures obtained from EPA, 2011b and APVMA, 2012.

Table A A.5. Acute toxicity of saflufenacil

Study (Guideline(s), Reference(s))	**Result**	**Toxicity Category (US EPA)**
Acute oral – rat (Wistar) (OPPTS 870.1100) (MRID 47128101)	LD50 > 2000 mg/kg	III
Acute dermal – rat (Wistar) (OPPTS 870.1200) (MRID 47128102)	LD50 > 2000 mg/kg	III
Acute inhalation – rat (Wistar) (OPPTS 870.1300) (MRID 47128103)	LC50: > 5.3 mg/L;	IV
Acute eye irritation – rabbit (New Zealand White) (OPPTS 870.2400) (MRID 47128105 & 47128104)	Minimal irritation	III
Acute dermal irritation – rabbit (New Zealand White) (OPPTS 870.2500) (MRID 47128106)	Slightly irritating	IV
Skin sensitization – guinea pig (Dunkin Hartley) (OPPTS 870.2600) (MRID 47128107)	Not a dermal sensitizer in guinea pigs	Negative

References: EPA, 2014a; EPA, 2015

Table A A.6. Sub-chronic toxicity studies of saflufenacil

Study (Guideline(s), Reference(s))	**Study Details**	**Doses**	**Results**
28-Day Oral – Rat (Wistar) (OPPTS 870.3100) (MRID 47128108)	5/sex/dose 94.2% purity	Dietary Concentration: 0, 50, 150, 450, 1350, 4050 Intake Dose: M: 0, 4.5, 13.4, 39.2, 117, 357 F: 0, 5.0, 15.9, 43.6, 130.4, 376	NOAEL = 13.4/43.6 (M/F) LOAEL = 39.2/130.4 (M/F) In males and females: ↓ Hb, MCV and MCH.
28-Day Oral – Mouse (C57BL/6NCrl)	5/sex/dose 94.2% purity	Dietary Concentration: 0, 50, 150, 450, 1350, 4050	NOAEL = 12.8/63.4 (M/F) LOAEL = 36.6/153.1 (M/F)

(OPPTS 870.3100) (MRID 47128110)		Intake Dose: M: 0, 12.8, 36.6, 112, 335, 882 F: 0, 17.9, 63.4, 153.1, 446, 1630	In males: ↑ ALAT, ASAT, urea, total bilirubin; ↓ Hb and Hct. In liver, ↑ liver wt and centrilobular fatty change. In females: ↑ centrilobular fatty change in the liver
28 Day Oral – Dog (Beagle) (OPPTS 870.3150) (MRID 47128112)	4/sex/dose 93.8% purity Capsule	0, 30, 100, 300 mg/kg/day. Acceptable / Guideline	NOAEL = 30/30 (M/F) LOAEL = 100/100 (M/F) In males and females: ↓ MCV, MCHC and MCH, bone marrow hyperplasia, ↑ iron storage in liver, extramedullary haematopoiesis in spleen
90 Day Oral – Rat (Wistar) (OPPTS 870.3100) (MRID 47128109)	10/sex/dose 93.9% purity	Dietary Concentration: 0, 50, 150, 450 (males), 1350, or 4050 (females) Intake Dose: M: 0, 3.5, 10.5, 32.3, 94.7 F: 0, 4.3, 12.6, 110.5, 344.7 Acceptable / Guideline	NOAEL = 10.5/12.6 (M/F) LOAEL = 32.3/110.5 (M/F) In males and females: multiple haematological effects, ↑ spleen wt, extramedullary haematopoiesis
90 Day Oral – Mouse (C57BL/6NCrl) (OPPTS 870.3100) (MRID 47128111)	10/sex/dose 93.9% purity	Dietary Concentration: 0, 15 (males only), 50, 150, 450, and 1350 (females only) Intake Dose: M: 0, 3.6, 12.4, 36.7, 109.1 F: 0, 17.6, 51.8, 156.6, 471.2 Acceptable / Guideline	NOAEL = 12.4/51.8 (M/F) LOAEL = 36.7/156.6 (M/F) ↓ Body wt, body wt gains and food utilization. In males and females: multiple haematological changes, ↑ liver wt, liver centrilobular fatty change and lymphoid infiltrate
90 Day Oral – Dog (OPPTS 870.3150) (MRID 47128113)	5/sex/dose 93.8% purity Capsule	0, 10, 50, 150 mg/kg/day. Acceptable / Guideline	NOAEL = 10/10 (M/F) LOAEL = 50/50 (M/F) In males and females: ↓ MCV and MCH

References: EPA, 2014a; EPA, 2015;
Abbreviations: ALAT = alanine aminotransferase. ASAT = aspartate aminotransferase. Hb = haemoglobin. Hct = haematocrit. MCH = mean cell haemoglobin. MCHC = mean cell haemoglobin concentration. MCV = mean cell volume. RBC = red blood cell concentration. SDH = sorbitol dehydrogenase.

Table A A.7. Genetic toxicity studies for saflufenacil

Study Type (Guideline(s), Reference(s))	**Doses / Classification**	**Results**
Bacterial reverse mutation test (Salmonella typhimurium and Escherichia coli) OPPTS 870.5100 MRID 47128121 (2005) MRID 47128122 (2005)	0, 20, 100, 500, 2500, 5000 µg/plate Acceptable / Guideline	Negative (with or without metabolic activation)
In vitro Mammalian Cell Gene Mutation Test (Chinese Hamster Ovary cells) OPPTS 870.5300 MRID 47128123 (2005)	0, 312.5, 625, 1250, 2500, 5000 µg/plate Acceptable / Guideline	Negative
In vitro Mammalian Chromosome Aberration Test (V79 cells) OPPTS 870.5375 MRID 47128124 (2005)	-S9: 0, 5, 10, 20 µg/mL +S9: 0, 10, 20, 40 µg/mL Acceptable / Guideline	Considered clastogenic *in vitro* in V79 cells with metabolic activation, but not clastogenic without metabolic activation.
In vivo Mammalian Cytogenetics – Erythrocyte Micronucleus assay in mice	0, 500, 1000, 2000 mg/kg/day	Negative

OPPTS 870.5395 MRID 47128125 (2005)	Acceptable / Guideline	
In vivo Genotoxicity Testing – Unscheduled DNA Synthesis (Rat) OPPTS 870.5550 MRID 47128126 (2005)	0, 1000, 2000 mg/kg, single oral dose. Acceptable / Non-Guideline	Negative

References: EPA, 2014a; EPA, 2015

Table A A.8. Studies providing data relevant to hormone perturbation for saflufenacil

Study (Guideline(s), Reference(s))	Study Details	Doses	Results
Prenatal Developmental – Rat (Wistar) (OPPTS 870.3700a) (MRID 47128115)	25 females/dose Gestation day 6-19 93.8% purity Oral gavage	0, 5, 20, 60 mg/kg/day Acceptable / Guideline	Maternal NOAEL (mg/kg/day) = 20 LOAEL (mg/kg/day) = 60 Based on decreased Hb, Hct, MCV and MCH Developmental NOAEL (mg/kg/day) = 5 LOAEL (mg/kg/day) = 20 Based on decreased foetal body wt and increased skeletal variations
Prenatal Developmental – Rabbit (Crl:CHBB(HM)) OPPTS 870.3700b) (MRID 47128116)	25/females/dose Gestation day 6-28 93.8% purity Oral gavage	0, 5, 200, 600 mg/kg/day Acceptable / Guideline	Maternal NOAEL (mg/kg/day) = 200 LOAEL (mg/kg/day) = 600 Based on mortality and increased necropsy findings Developmental NOAEL (mg/kg/day) = 50 LOAEL (mg/kg/day) = 200 Based on increased liver porphyrins
Reproduction and Fertility – Rat (Wistar) (OPPTS 870.3800) (MRID 47128117)	25/sex/dose 0, 1, 3 and 10 PPM Dietary	0, 5, 15, 50 mg/kg/day Acceptable / Guideline	Parental NOAEL (mg/kg/day) = 15 LOAEL (mg/kg/day) = 50 Based on decreased food intake, body weight and changes in haematological parameters and organ weights indicative of anaemia Offspring NOAEL (mg/kg/day) = 15 LOAEL (mg/kg/day) = 50 Based on decreased number of live born pups, increased number of stillborn pups, decreased viability and lactation indices, decreased pre-weaning body weight and changes in haematological parameters Reproductive NOAEL (mg/kg/day) = 50 LOAEL (mg/kg/day) = Not Established

References: EPA, 2014a; EPA, 2015

Table A A.9. Studies providing data relevant to immune suppression for saflufenacil

Study (Guideline(s), Reference(s))	Study Details	Doses	Results, related to immune suppression
Immunotoxicity Study (male C57BL/6J Rj mice) (OPPTS 870.7800) (MRID 48233701)	8/males/dose Dosing: 4 weeks 93.8% purity Dietary	0, 50, 125 and 250 ppm (0, 10, 27 and 52 mg/kg/day)	NOAEL (systemic toxicity) = 10 mg/kg/day). LOAEL (systemic toxicity) = 27 mg/kg/day) based on significant changes in pathological and clinical pathology parameters. NOAEL (immunotoxicity) = 52 mg/kg/day. LOAEL for immunotoxicity was not identified. No effects on T-cell dependent antibody response (TDAR) at highest dose tested.

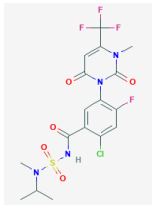
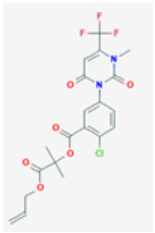
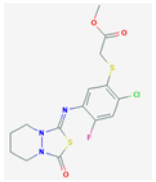
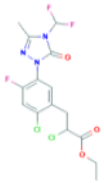
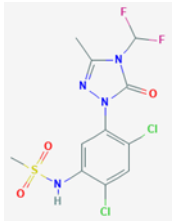
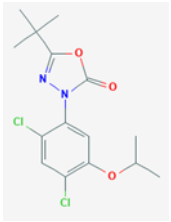
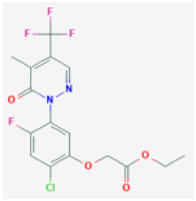
References: EPA, 2014a; EPA, 2015

Table A A.10. Mode of action and mechanistic studies for saflufenacil

Study (Guideline(s), Reference(s))	Study Details	Doses	Results
Mechanistic study – total porphyrin analysis in rat (Wistar) (Non-guideline) (MRID 47128132 (2006))	10/sex/dose Dosing: 8 weeks 94.2% purity Dietary	0, 10, 50, or 1000 ppm M: 0, 0.8, 4.1, 80.6 mg/kg/day F: 0, 0.9, 4.6, 89.5 mg/kg/day	Total porphyrins in faeces and liver provided the most reliable and sensitive data. Statistically significant effects on porphyrin metabolism could be detected at exposure concentrations well below those associated with adverse haematological effects NOAEL= 4.1 mg/kg/day. LOAEL = 80.6 mg/kg/day based on decreased Hb, Ht, MCV, MCH, and MCHC
Mechanistic study-porphyrin analysis supplementary in rat (Wistar) (Non-guideline) (MRID 47128131 (2005))	10/sex/dose Dosing: 8 weeks 94.2% purity Dietary	0, 1, 5, or 25 ppm M: 0, 0.1, 0.4, 2.0 mg/kg/day F: 0, 0.1, 0.5, 2.3 mg/kg/day	Dietary administration of saflufenacil at 25 ppm caused an increase in porphyrin in faeces of male (237%) and female (61%) rats, while saflufenacil at 5 ppm caused an increase in faecal porphyrin only in males. There were no effects on haematology parameters

References: EPA, 2014a; EPA, 2015

Table A A.11. Summary table: Physical-chemical properties and acute toxicity for read-across chemical analogues

	Target	Source Analogue 1	Source Analogue 2	Source Analogue 3	Source Analogue 4	Source Analogue 5	Source Analogue 6
Chemical Name	Saflufenacil	Butafenacil	Fluthiacet-methyl	Carfentrazone-ethyl	Sulfentrazone	Oxadiazon	Flufenpyr-ethyl
CASRN	372137-35-4	134605-64-4	117337-19-6	128639-02-1	122836-35-5	19666-30-9	188489-07-8
2D Structure							
Chemical Class	N-Phenyl-imides	N-Phenyl-imides	N-Phenyl-imides	N-Phenyl-triazolinones	N-Phenyl-triazolinone	N-Phenyl-oxadiazolones	Other
Structural similarity index (EPA GenRA)	1.0	0.29	n.d.	0.19	0.17	0.15	0.20
Physical form at 25 deg C	Solid	Solid	Solid	Liquid	Solid	Solid	Solid
MW (g/mole)	500.9	474.8	403.9	412.19	387.18	345.22	408.73
Physical Properties:							
Melting Point	189.9 °C	113 °C	106.0 °C.	-22.1 °C.	122.0 °C	88.5 °C	100 °C
Boiling Point	Not determined	270 °C	Decomposes at 249 °C	352.5 °C.	Not determined	282.1 °C	Not determined
Vapor Pressure	4.5×10^{-12} mPa	7.4×10^{-06} mPa	4.41×10^{-4} mPa	7.20×10^{-03} mPa	1.30×10^{-04} mPa	0.67 mPa	3.76×10^{-04} mPa
pKa	4.41	No dissociation	No dissociation	No dissociation	6.56	No dissociation	n.d.
Log70epaw	2.6 (log Pow)	3.19	3.77	3.36	0.99	4.8	2.99 (log P)
Acute Toxicity:							
Acute toxicity	LD50 > 2000	LD50 > 5000	LD50 > 5000	LD50 > 5000	LD50 = 2855 mg/kg	LD50 > 5000 mg/kg	LD50 > 5000 mg/kg

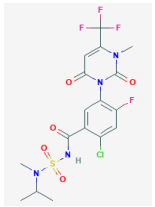
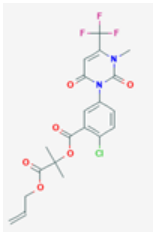
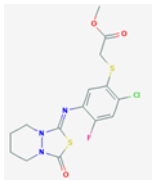
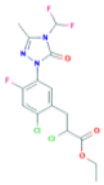
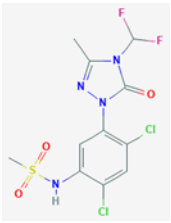
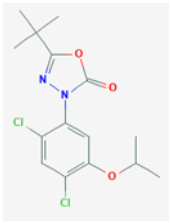
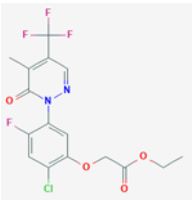
	Target	Source Analogue 1	Source Analogue 2	Source Analogue 3	Source Analogue 4	Source Analogue 5	Source Analogue 6
Chemical Name	Saflufenacil	Butafenacil	Fluthiacet-methyl	Carfentrazone-ethyl	Sulfentrazone	Oxadiazon	Flufenpyr-ethyl
CASRN	372137-35-4	134605-64-4	117337-19-6	128639-02-1	122836-35-5	19666-30-9	188489-07-8
2D Structure							
Chemical Class	N-Phenyl-imides	N-Phenyl-imides	N-Phenyl-imides	N-Phenyl-triazolinones	N-Phenyl-triazolinone	N-Phenyl-oxadiazolones	Other
(oral, rat unless indicated)	mg/kg	mg/kg	mg/kg	mg/kg			
Eye irritation (EPA class)	Minimal irritation (III)	Irritation resolved in 96 h (III)	Minimal irritation (III)	Minimal irritation (IV)	Irritation resolved in 96 h (III)	Mild irritation (III)	Minimal irritation (IV)
Skin sensitization	Not a sensitizer	Not a sensitizer	Not a sensitizer	Not a sensitizer	Not a sensitizer	Not a sensitizer	Not a sensitizer
References:	EPA, 2014a; EPA, 2015	EPA, 2003	EPA, 2016	EPA, 2006	EPA, 2014d	EPA, 2014c	EPA, 2014b

Table A A.12. Summary table: Repeated-dose toxicity and carcinogenicity for read-across chemical analogues

	Target	Source Analogue 1	Source Analogue 2	Source Analogue 3	Source Analogue 4	Source Analogue 5	Source Analogue 6
Chemical Name	Saflufenacil	Butafenacil	Fluthiacet-methyl	Carfentrazone-ethyl	Sulfentrazone	Oxadiazon	Flufenpyr-ethyl
Genotoxicity (*in vitro*)	Negative, except clastogenic in chromosome ab. test with S9	Negative	Positive clastogenicity in two mammalian cell lines (±S9). Negative for mutagenicity in	Negative	Negative	Negative	Negative

	Target	Source Analogue 1	Source Analogue 2	Source Analogue 3	Source Analogue 4	Source Analogue 5	Source Analogue 6
Chemical Name	Saflufenacil	Butafenacil	Fluthiacet-methyl	Carfentrazone-ethyl	Sulfentrazone	Oxadiazon	Flufenpyr-ethyl
			CHO, Ames, and rat hepatocyte systems. Negative in a mammalian UDS assay				
Genotoxicity (*in vivo*)	Negative (micronucleus and UDS tests)	Negative	Negative in mouse bone marrow assay; positive in rat liver micronucleus assay. EPA considered not a concern for mutagenicity.	Negative	Negative	Negative	Negative
Repeated Dose Toxicity:							
28 day study	**Rat, Mouse, Dog:** markers of porphyria, anaemia & resulting changes in liver, spleen & bone marrow	Not described in detail. See 90-day summary	Not described in detail. See 90-day summary	Not described in detail. See 90-day summary	Not described in detail. See 90-day summary	Not described in detail. See 90-day summary	Not described in detail. See 90-day summary
90 day study	**Rat, Mouse, Dog:** markers of porphyria, anaemia & resulting changes in liver, spleen & bone marrow. **Also (rat):** General systemic toxicity + some deaths at HDT	**Rat, Mouse, Dog:** markers of porphyria, anaemia & resulting changes in liver, spleen & bone marrow. **Also (rat):** General systemic toxicity + some deaths at HDT	**Rat, Mouse:** markers of porphyria, anaemia & changes to liver, urinalysis. **Also (Mouse):** changes to bone marrow & liver (fatty change, chronic inflam., necrosis, pigmentation). ↑ ALAT, ASAT, SDH, bile acids. **Dog:** ↓ body wt. gain	**Rat:** markers of porphyria, anaemia & changes to liver. General systemic toxicity (↓ Body wt, food consumption) **Mouse:** liver histopathology changes. **Dog:** ↓ body wt. gain; ↑ porphyrin levels	**Rat, Mouse, Dog:** markers of porphyria, anaemia & resulting changes in liver, spleen & bone marrow.	**Rat:** markers of porphyria, anaemia & changes to liver (↑ liver wt., fatty change, necrosis). ↓ Body wt **Mouse:** study not summarized in available reviews. **Dog:** ↑ Thyroid wt. in males	**Rat:** No adverse effects up to 1434 / 1591 mkd (M/F) **Mouse:** ↑ Liver wt., liver hepatocellular vacuolation. **Dog:** ↓ Body wt, food consumption, food efficiency, ↑ vomiting

	Target	Source Analogue 1	Source Analogue 2	Source Analogue 3	Source Analogue 4	Source Analogue 5	Source Analogue 6
Chemical Name	Saflufenacil	Butafenacil	Fluthiacet-methyl	Carfentrazone-ethyl	Sulfentrazone	Oxadiazon	Flufenpyr-ethyl
Repeated Dose Toxicity:							
Reproductive Toxicity	No evidence a selective hormonal or reproductive effects (rat). **Note:** added measure of haematology of adults and pups showed changes reflecting anaemia	No evidence a selective hormonal or reproductive effects (rat)	No evidence a selective hormonal or reproductive effects (rat)	No evidence a selective hormonal or reproductive effects (rat)	↑ duration of gestation (F), degeneration and/or atrophy of the germinal epithelium of the testes + oligospermia and intratubular degenerated seminal material in the epididymis of F1 males. In offspring, ↓ litter size, ↑ stillborn pups, ↓ pup and litter postnatal survival, and ↓ pup body weights	No evidence a selective hormonal or reproductive effects (rat)	No evidence a selective hormonal or reproductive effects (rat)
Developmental	No evidence a selective hormonal or developmental effects (rat, rabbit)	No evidence a selective hormonal or developmental effects (rat, rabbit)	No evidence a selective hormonal or developmental effects (rat, rabbit)	No evidence a selective hormonal or developmental effects (rat, rabbit)	No evidence a selective hormonal or developmental effects (rat, rabbit)	No evidence a selective hormonal or developmental effects (rat, rabbit)	No evidence a selective hormonal or developmental effects (rat, rabbit)
Repeated Dose Toxicity:							
Hormone perturbation	No specific studies.	No specific studies.	No specific studies.	No specific studies.	No specific studies.	No specific studies.	No specific studies.
Immune suppression	No evidence of immune suppression (mouse TDAR study)	No specific studies	No evidence of immune suppression (TDAR study)	No specific studies	No evidence of immune suppression (TDAR study)	No specific studies	No specific studies
Carcinogenicity	WoE-based assessment requested for rat	Rat: None Mouse: None	Rat: ↑pancreatic adenomas (males). Mouse: ↑	Rat: None Mouse: None	Rat: None Mouse: None	Rat: ↑ hepatocellular adenomas &	Rat: None Mouse: None

	Target	Source Analogue 1	Source Analogue 2	Source Analogue 3	Source Analogue 4	Source Analogue 5	Source Analogue 6
Chemical Name	Saflufenacil	Butafenacil	Fluthiacet-methyl	Carfentrazone-ethyl	Sulfentrazone	Oxadiazon	Flufenpyr-ethyl
	and mouse studies, based on this document.	EPA class: Not likely	hepatocellular adenomas and carcinomas (males). EPA class: Likely (Q1*)	EPA class: Not likely	EPA class: Not likely	carcinomas (males). Mouse: ↑ hepatocellular adenomas & carcinomas (males & females). EPA class: Likely (Q1*)	EPA class: Not likely
Repeated Dose Toxicity:							
Special studies	Dose-response of ↑ porphyrin levels determined in 2 special studies in rats. ↑ porphyrin levels seen (urine, faeces, plasma, liver) at dose levels with no other changes.	No specific studies	No specific studies	No specific studies	No specific studies	Mechanistic study that suggested oxadiazon might be a peroxisome proliferator, a possible MoA for liver tumours. Earlier study (1991); not rigorous by today's MoA frameworks (e.g. Meek, 2014).	No specific studies
References:	EPA, 2014a; EPA, 2015	EPA, 2003	EPA, 2016	EPA, 2006	EPA, 2014d	EPA, 2014c	EPA, 2014b

Abbreviations: CHO = Chinese hamster ovary. HDT = highest dose tested. ALAT = alanine aminotransferase. ASAT = aspartate aminotransferase. SDH = sorbitol dehydrogenase. TDAR = T-cell dependent antibody response. UDS = unscheduled DNA synthesis.

Table A A.13. Detailed sub-chronic effects of read-across chemical analogues

	90-Day Rat	90-Day Mouse	90-Day Dog
Butafenacil	NOAEL= 19/21 mg/kg/day	NOAEL= 4/6 mg/kg/day	NOAEL = 200 mg/kg/day

	90-Day Rat	90-Day Mouse	90-Day Dog
(EPA, 2003).	Effects (62/69 mg/kg/day in M/F): ↓ wt gain, Haematology changes of ↓ Hb, Hct, MCV and MCH; bone marrow hypercellularity; ↑ bilirubin and urobilinogen; ↑ ALAT. Liver: hepatocyte necrosis, inflammatory cell infiltration	Effects (14/20 mg/kg/day in M/F): Liver histopathology: fatty change, glycogen deposition, hypertrophy	Effects (1000 mg/kg/day): Haematology changes of ↓ MCV and MCH, ↑ RDW, HDW, platelets and triglycerides. Hemosiderosis in spleen and liver; extramedullary haematopoiesis in spleen
Flutiest-methyl (EPA, 2016).	NOAEL = 6/7 mg/kg/day Effects (216/249 mg/kg/day in M/F): ↓ wt gain, changes in haematology, clinical chemistry, urinalysis, ↑ liver weight and microscopic pathology	NOAEL= 1 mg/kg/day Effects (66 mg/kg/day): Effects on erythropoietic system (↓ Hb, ↓ Hct, ↓ MCH and ↓ MCHC); ↑ bone marrow granulopoiesis and extramedullary haematopoiesis. Effects on liver: ↑ liver wt., ↑ ALAT, ASAT, SDH, bile acids; histopathology of ↑ fatty change, chronic inflammation, karyomegaly, single cell necrosis, ceroid/lipofuscin pigmentation	6-Week dog study: NOAEL = 78 mg/kg/day Effects (232 mg/kg/day): ↓ body wt. gain
Carfentrazone (EPA, 2006).	NOAEL = 226/284 mg/kg/day Effects (470/578 mg/kg/day in M/F): ↓ Body wt, ↓ food consumption, Histopathology lesions indicating liver toxicity	NOAEL = 571 mg/kg/day Effects (1143 mg/kg/day): Liver pathology indicating liver toxicity	NOAEL = 50 mg/kg/day Effects (150 mg/kg/day): ↓ body wt. gain; ↑ porphyrin levels
Sulfentrazone (EPA, 2014d)	NOAEL = 11/13 mg/kg/day Effects (32/111 mg/kg/day in M/F): Multiple haematological effects (↓ Hb, Hct, MCV, MCH) and increased spleen weight and extramedullary haematopoiesis	NOAEL = 12/52 mg/kg/day Effects (37/157 mg/kg/day in M/F): Haematological effects (↓ Hb, Hct, MCV, MCH) and ↑ liver wt., fatty change, lymphoid infiltrate	NOAEL = 10 mg/kg/day Effects (50 mg/kg/day): Haematological effects (↓ MCV, MCH)
Oxadiazon (EPA, 2014c).	NOAEL = 25 mg/kg/day Effects (100 mg/kg/day): ↓ Body wt, Haematological effects (↓ Hb, Hct, RBC) and ↑ liver wt. and pathology effects	90-day mouse study not summarized.	NOAEL <25 mg/kg/day Effects (25, 100, 1000 mg/kg/day): ↑ Thyroid wt. in males

	90-Day Rat	90-Day Mouse	90-Day Dog
	associated with liver damage (fatty change, hepatocellular necrosis)		
Flufenpyr-ethyl (EPA, 2014b). .	NOAEL > 1434/1591 mg/kg/day in M/F Effects No adverse effects	NOAEL = 395 mg/kg/day Effects (908 mg/kg/day): ↑ Liver wt., liver hepatocellular vacuolation	NOAEL = 300 mg/kg/day Effects (1000 mg/kg/day): ↓ Body wt, food consumption, food efficiency, ↑ vomiting

NOAEL and LOAEL values (mg/kg/day) are shown for Male/Female animals, where applicable.
Abbreviations: ALAT = alanine aminotransferase. ASAT = aspartate aminotransferase. Hb = haemoglobin. Hct = haematocrit. MCH = mean cell haemoglobin. MCHC = mean cell haemoglobin concentration. MCV = mean cell volume. RBC = red blood cell concentration. SDH = sorbitol dehydrogenase.

Table A A.14. Detailed carcinogenicity and/or chronic toxicity results, classification and POD for read-across chemical analogues

Read-across Chemical	Carcinogenicity Classification (U.S. EPA)	2-Year Rat Study Results	18-Month Mouse Study Results	Chronic POD (Study, UF)
Butafenacil (EPA, 2003).	Not likely to be carcinogenic to humans	No carcinogenicity Liver: fatty change, increased mitotic activity. Haematology effects (see 90-day rat study) NOAEL = 3.76/4.43 mg/kg/day (M/F) LOAEL = 11.4/13.0 mg/kg/day (M/F)	No carcinogenicity Liver: ↑ liver wt., Kupffer cell hyperplasia, inflammatory cell infiltrate, single cell necrosis, lipofuscin deposits. NOAEL = 1.17/1.20 mg/kg/day (M/F) LOAEL = 6.96/6.59 mg/kg/day (M/F)	cPAD = 0.012 mg/kg/day (18-Mo. Mouse study, 100X)
Fluthiacet-methyl (EPA, 2016).	Likely to be carcinogenic to humans (Q1*)	↑pancreatic adenomas (males). Liver toxicity, pancreatic toxicity, uterine toxicity (haemorrhage, inflammation). Haematology changes assumed (not in EPA summary) NOAEL = 2.1 mg/kg/day LOAEL = 130 mg/kg/day	↑ hepatocellular adenomas and carcinomas (males). Liver: cell degeneration, necrosis, histiocytic pigmentation, karyomegaly, chronic active inflammation. Haematology changes (see 90-day study). NOAEL = 0.1 mg/kg/day LOAEL = 1.0 mg/kg/day	cPAD = 0.001 mg/kg/day (18-Mo. Mouse study, 100X) Q1* = 0.207 $(mg/kg/day)^{-1}$ based on male mouse liver tumour response at ≥10 mg/kg/day

Read-across Chemical	Carcinogenicity Classification (U.S. EPA)	2-Year Rat Study Results	18-Month Mouse Study Results	Chronic POD (Study, UF)
Carfentrazone (EPA, 2006)	Not likely to be carcinogenic to humans	No carcinogenicity. Liver: hepatotoxicity. Haematology changes (see 90-day study) NOAEL = 3.0 mg/kg/day LOAEL = 12.0 mg/kg/day	No carcinogenicity. Liver: histopathology. Haematology changes (see 90-day study) NOAEL = 10/12 mg/kg/day (M/F) LOAEL = 110/119 mg/kg/day (M/F)	cPAD = 0.03 mg/kg/day (2-yr. rat study, 100X)
Sulfentrazone (EPA, 2014d).	Not likely to be carcinogenic to humans	No carcinogenicity. ↓ Body wt., food consumption. Haematology changes, ↑ reticulocytes in bone marrow. NOAEL = 40/36.4 mg/kg/day (M/F) LOAEL = 82.8/67 mg/kg/day (M/F)	No carcinogenicity. Haematology changes (↓ Hb, Hct). NOAEL = 93.9/116.9 mg/kg/day (M/F) LOAEL = 160.5/198.0 mg/kg/day (M/F)	cPAD = 0.14 mg/kg/day (2-gen. reproduction study, 100X)
Oxadiazon (EPA, 2014c).	Likely to be carcinogenic to humans (Q1*)	In 2 studies: Evidence of carcinogenicity: ↑ incidence hepatocellular adenomas & carcinomas (males). Liver: ↑ liver wt., serum protein (females). Haematology & liver-related clinical chemistry changes (see 90-day study) NOAEL = 0.36/0.5 mg/kg/day, M/F LOAEL = 3.5/4.8 mg/kg/day (Wistar/F344)	In 2 studies: Evidence of carcinogenicity: ↑ incidence hepatocellular adenomas & carcinomas (males & females). Haematology changes (anaemia). Liver: ↑ liver wt., histopathology changes. Kidneys (males): histopathology changes NOAEL = 1.09 mg/kg/day LOAEL = 10.6 mg/kg/day (2nd study)	cPAD not established since no dietary uses. Q1* = 7.11 $(mg/kg/day)^{-1}$ based on liver tumours in male mice at ≥9.3 mg/kg/day
Flufenpyr-ethyl (EPA, 2014b).	Not likely to be carcinogenic to humans	No carcinogenicity. No evidence of toxicity at doses ≥778 / 1025 mg/kg/day (M/F). NOAEL = 777.8/1024.7 mg/kg/day (M/F). LOAEL not established	No carcinogenicity. Haematology changes (mild anaemia). Liver toxicity (males & females) NOAEL = 39.9/43.7 mg/kg/day (M/F). LOAEL = 401.8/447.9 mg/kg/day (M/F)	cPAD = 0.4 mg/kg/day (18-mo. Mouse study, 100X)

Classification, POD and NOAEL/LOAEL values are based upon U.S. EPA reviews.
Abbreviations: POD = point of departure. cPAD = chronic population-adjusted dose. Q1* = cancer potency factor.

Table A A.15. Proposed POD for chronic risk assessment with saflufenacil

Chronic Dietary (all subpopulations)	NOAEL = 10.5 mg/kg/day	UF_A = 10X UF_H = 10X UF_E = 10X	cPAD = 0.0105 mg/kg/day ADI = 0.0105 mg/kg/day	Subchronic (90-day) Study in Rats NOAEL = 10.5 mg/kg/day LOAEL = 32.3 mg/kg/day (M) Based on multiple haematological effects (↓ Hb, ↓ Hct, ↓ MCH, ↓ MCV, ↓ MCHC and ↑ reticulocytes) related to porphyria and anaemia, with related increase in spleen weight and extramedullary haematopoiesis. Effects in female rats were similar (NOAEL = 12.6 mg/kg/day)
Cancer (Oral, dermal, inhalation)	Separate endpoint(s) for cancer effects should not be needed for saflufenacil, as the chronic dietary risk assessment (cPAD) should be protective for all effects.			

Abbreviations: POD = point of departure. NOAEL = no-observed adverse-effect level. LOAEL = lowest-observed adverse-effect level. UF = uncertainty factor. UF_A = extrapolation from animal to human (interspecies). UF_H = potential variation in sensitivity among members of the human population (intraspecies). UF_E = extrapolation from 90-day to chronic (2-year) endpoint. PAD = population-adjusted dose (a = acute, c = chronic). ADI = acceptable daily intake.

Annex B. Example 2: Spiropidion

The purpose of this example case study is to evaluate the toxicological data that was available for spiropidion and publicly available risk assessments and data evaluation records (DER) generated by the US EPA for the identified read-across compounds to determine if a health-protective POD can be established without the need for rodent chronic toxicity and carcinogenicity studies (OECD TG 451, 452, 453). Based on the toxicity profile for spiropidion and assessment for the potential of carcinogenicity following the mode of action research, assessment for any evidence of preneoplastic lesions in the subchronic studies, and read-across analysis of similar chemicals, the classification for carcinogenicity can be determined for spiropidion. Specifically, the case study presented herein provides an example of a WoE-based assessment to fulfil regulatory carcinogenicity data needs stipulated by the United States United States Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Title 40 Part 158, as regulated by the US EPA (CFR, 2012). Other regulatory legal statutes may require different types of information, level of detail, or toxicity assessment that is not similar in regulation to FIFRA, and thus may result in data requirements that are unique by regulatory region (e.g., Commission Regulation (EU) No 283/2013; Canada's Pest Control Products Act).

The case study was developed within the ReCAAP initiative, which was supported by the United States EPA's Guiding Principles for Data Requirements that outlines their availability to accept a WoE-based safety assessment for agrichemicals (US EPA 2013a; Craig et al., 2019). Given the available landscape to develop a WoE-based safety assessment, in this case, for estimating a POD that is protective against chronic outcomes, the data requirements under the US EPA were the starting point to explore the sufficiency of data needed to achieve a health-protective outcome. While the focus of this case study was fulfilling agrichemical data requirements for the United States, additional case studies are encouraged to help identify the sufficiency of data in other regions that fulfil different regulatory statutes in addressing chronic toxicity and carcinogenicity.

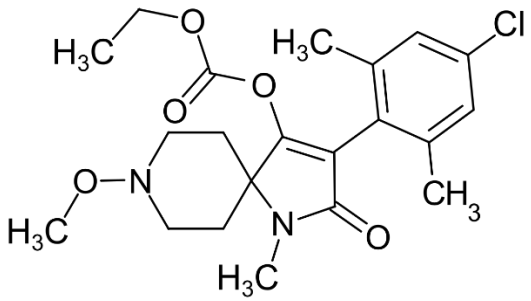
It should be noted that this case study on spiropidion was developed in 2019-2020, the contents of which are reported in this IATA. The 'uncertainties section' of this IATA case study explores data gaps, as well as the learnings acquired from the OECD case study project review that are important to consider in the present day (2024) and beyond. The idea is to clearly identify gaps and uncertainties in this case to inform the development of subsequent case studies on carcinogenicity.

1. Data

For this example case study, relevant data was used to obtain the necessary information on the target compound spiropidion and read-across analogues. The data for spiropidion was obtained from OECD guideline studies and regulatory assessments, and the data for the read-across compounds was obtained from regulatory assessments conducted by the United States Environmental Protection Agency (EPA, 2011, 2016, 2017, 2018, 2020, 2021, 2022, 2023) and publicly available domains (JMPR, 2021; PPDB, 2023; Kim, 2023; CompTox, 2023). The data were considered reliable and acceptable as per the US EPA use of the studies to support the establishment of permanent tolerances of the first food uses for spiropidion in fulfilment of the United States FIFRA. OECD Test Guidelines were used for the following studies

evaluated in the WoE: 408, 409, 414, 416, 417, 424, 452, 453, 471, 474, 476, 487. The immunotoxicity studies conducted on the read-across analogues followed the US EPA guidelines available at the time the studies were conducted (OPPTS 870,7800, 1998). The genotoxicity studies were conducted according to the current OECD guidelines at the time the studies were conducted. The currently available revisions of the OECD guidelines were not taken into account to determine the reliability of the available studies beyond what the US EPA classifies as acceptable.[3] An additional assessment of reliability or acceptability was not conducted for the purposes of this assessment. The nomenclature and chemical structure of spiropidion are detailed in Table A B.1.

Table A B.1. Nomenclature for spiropidion

Parameter	Result
Common Name	Spiropidion
IUPAC Chemical Name	[3-(4-chloro-2,6-dimethylphenyl)-8-methoxy-1-methyl-2-oxo-1,8-diazaspiro[4.5]dec-3-en-4-yl] ethyl carbonate
CAS Registry Number	1229023-00-0
Synonyms	SYN546330
Chemical Class	Tetramic acid derivative
Molecular Formula:	C21H27ClN2O5
2DStructure	

The intended pMoA of spiropidion is inhibition of the enzyme Acetyl CoA carboxylase (ACCase) which places it in the Insecticide Resistance Action Committee (IRAC) group 23 for resistance management considerations (IRAC, 2023).

Spiropidion (SYN546330) is a new compound in the tetramic and tetronic acid family (TAs/TADs) (IRAC Group 23). This novel active substance incorporates a spiro methoxy-piperidine ring as a main chemical innovation. Spiropidion is a proinsecticide which upon hydrolysis releases its potent active principle, a 2-aryl- cyclic-1,3-dione derivative (SYN547305), responsible for the target site binding in insects. Spiropidion and the TAs/TADs family disrupt fatty acid biosynthesis in insects through the inhibition of the enzyme Acetyl CoA carboxylase (ACCase). Spiropidion has been specifically designed to limit the toxicities demonstrated by the legacy TAs/TADs.

[3] All studies were conducted in accordance with the latest version of the OECD Test Guidelines at that time. The studies for saflufenacil were conducted before 2010 and subsequent revisions to OECD guidelines may affect the results. While new studies should not be initiated using old versions of the OECD TG, results of studies conducted of an older test guideline (that was current at the time the study was initiated) are still accepted under the agreement on Mutual Acceptance of Data (OECD; Section 4 – Health effects: replaced and cancelled Test Guidelines).

The ester group in the main metabolite of spiropidion, SYN547305, is rapidly hydrolysed in aqueous conditions. The genotoxicity and repeat dose toxicity studies for spiropidion are conducted in aqueous conditions, thus significant exposure to SYN547305 occurs in the studies conducted with spiropidion. As such, SYN547305 is considered to be toxicologically equivalent to spiropidion and the toxicology studies conducted in the parent compound, spiropidion, apply to SYN547305. The repeat dose package and applicable points of departure for spiropidion can be applied to SYN547305.

This insecticide provides excellent control of aphids, whiteflies, psyllids, scales and mites in vegetables and specialty crops. With no cross-resistance to neonicotinoids, this non-neuronal acting chemistry is anticipated to be a good fit for Integrated Pest Management (IPM). Spiropidion is proposed to be used as foliar application on fruiting vegetables (Crop Group – CG-8), Cucurbit Vegetables (CG-9), Citrus Fruit (CG-10), Potato, Soybean, and Cotton. No non-food or residential uses are proposed at this time.

2. Evaluation of genotoxic potential

Spiropidion is a proinsecticide which upon hydrolysis releases its potent active principle, SYN547305. This metabolite SYN547305 is readily formed under aqueous conditions and is therefore formed and adequately tested in both the *in vitro* and *in vivo* genotoxicity studies. A full set of OECD guideline acceptable genotoxicity studies are available for spiropidion, as shown in Table A B.9. Two bacterial reverse gene mutation studies and an *in vitro* forward gene mutation study were each negative, both with and without S9 metabolic activation. An *in vitro* micronucleus assay was also negative, both with and without S9 metabolic activation. In the *in vitro* chromosome aberration assay, there was a statistically significant increase in the incidence of chromosomal aberrations (excluding gaps) in both the presence and the absence of S9 metabolic activation. There was no increase in the incidence of polyploid metaphases in any experimental conditions in that study. Follow-up *in vivo* micronucleus studies in the rat were carried out with treatment by oral gavage and confirmation, proof of exposure, demonstrating the bone marrow was exposure via LC-MS/MS analysis. The presence of spiropidion and its major metabolite SYN547305 were both confirmed using a validated analytical method. A follow-up *in vivo* rat bone marrow chromosome aberration assay was investigated; treatment was administered by oral gavage and bone marrow samples were collected and assessed for toxicity by using the Mitotic Index. The two *in vivo* micronucleus studies and an *in vivo* chromosome aberration assay were each negative. Overall, it is concluded that spiropidion is not genotoxic.

3. Data review of target chemical

All data generated for spiropidion reported herein was conducted in accordance with Good Laboratory Practice following OECD guidelines as well as US EPA Office of Chemical Safety and Pollution Prevention (OPPTS) guidelines.

Toxicokinetic data in rats indicate spiropidion is well absorbed and rapidly excreted (Table A B.6).

For spiropidion, tables with relevant data are available at the end of this example case study. Physical chemical properties are presented in Table A B.5. A summary of toxicokinetic data is presented in Table A B.6. Acute toxicity data are presented in Table A B.7, short-term toxicity and subchronic toxicity data are presented in Table A B.8 and genetic toxicity data are presented in Table A B.9. Studies providing data relevant to hormone perturbation related to reproductive and developmental toxicity studies are summarised in Table A B.10 and studies providing data relevant to immune suppression for spiropidion are presented in Table A B.11. MoA and mechanistic studies are summarised in Table A B.12.

Toxicokinetic data in rats indicate spiropidion is well absorbed and rapidly excreted (Table A B.6). Following a single oral dose, at 5 or 250 mg/kg bodyweight, maximum blood concentrations (Cmax) were achieved within 1-4 hrs of dosing and declined rapidly thereafter. Excretion of orally dosed spiropidion was essentially complete within 168 hrs, and the majority (>94 %) was eliminated within the first 48 hr. There was no sex-dependent difference in the excretion of orally administered spiropidion. Following single low and high dose oral administration or repeated dose administration, the main routes of elimination were the urine and faeces. Radiolabelled analysis of blood showed that the majority of spiropidion residues were in the plasma and were not bound to cellular elements such as red blood cells. At 168 hrs after dosing, spiropidion residues remaining in tissues were very low (<0.7%), and occurred mainly in carcass, liver, skin, and gut contents. The predominant biotransformation pathway observed for spiropidion was initial ester hydrolysis of the ethoxy carbonyl moiety to form the enol metabolite SYN547305. SYN547305 underwent subsequent loss of the methoxy moiety from the piperidine ring to form the metabolite SYN548430. No cleavage of the ring cycles were observed. The biotransformation pathway for spiropidion is further summarized in Figure A B.1. There were no significant sex-related differences in metabolic profiles (Table A B.6 and Table A 0.4).

The pharmacokinetics of spiropidion (SYN546330) were investigated in the rat following repeated oral or single intravenous administration. Spiropidion was rapidly converted to the keto-enol (SYN547305) after both oral and intravenous administration of spiropidion. After intravenous administration of spiropidion, blood concentrations of spiropidion were generally very low (<10 ng/mL) and were below the limit of quantification at numerous time points throughout the concentration/time profile. Likewise, after oral administration of spiropidion, concentrations of unchanged spiropidion in blood were generally <10 ng/mL. The low and variable blood concentrations of unchanged spiropidion is due to the rapid hydrolysis of spiropidion to metabolite SYN547305. This is supported by the concentrations of metabolite SYN547305 observed after intravenous and oral administration of spiropidion.

The most abundant metabolite, desmethoxy SYN547305, was detected in all urine and faeces samples and accounted for up to 78% of the administered dose following oral administration and up to 84% following intravenous administration of spiropidion. Only two other metabolites, (hydroxy oxidized desmethoxy SYN547305 and hydroxy desmethoxy SYN547305a) accounted for greater than 5% of the administered dose in excreta. Hydroxy oxidized desmethoxy SYN547305 accounted for up to 19% of the administered dose and hydroxy desmethoxy SYN547305a accounted for up to 7.8% of the administered dose.

SYN548430, SYN547305, and hydroxy oxidized desmethoxy SYN547305 were the main circulating metabolites. SYN547305 was the largest, accounting for up to ca 48% TRAUC (systemic exposure of total radioactivity) in the 5 and 250 mg/kg samples. At least 5 other unidentified radiolabelled circulatory components were detected in the plasma, with no single component accounting for >3.5% TRAUC. Thus, the enol metabolite SYN547305 is the major circulating metabolite in the mammalian system when orally administered spiropidion and adequately assessed for its safety in the toxicology data package for spiropidion.

Spiropidion exhibited low acute toxicity via the oral, dermal, and inhalation routes of exposure (Toxicity Category III or IV) (EPA 2022, 2023). Spiropidion was not a skin irritant, was minimally irritating to the eye, was a skin sensitizer, and was not phototoxic. (Table A B.7).

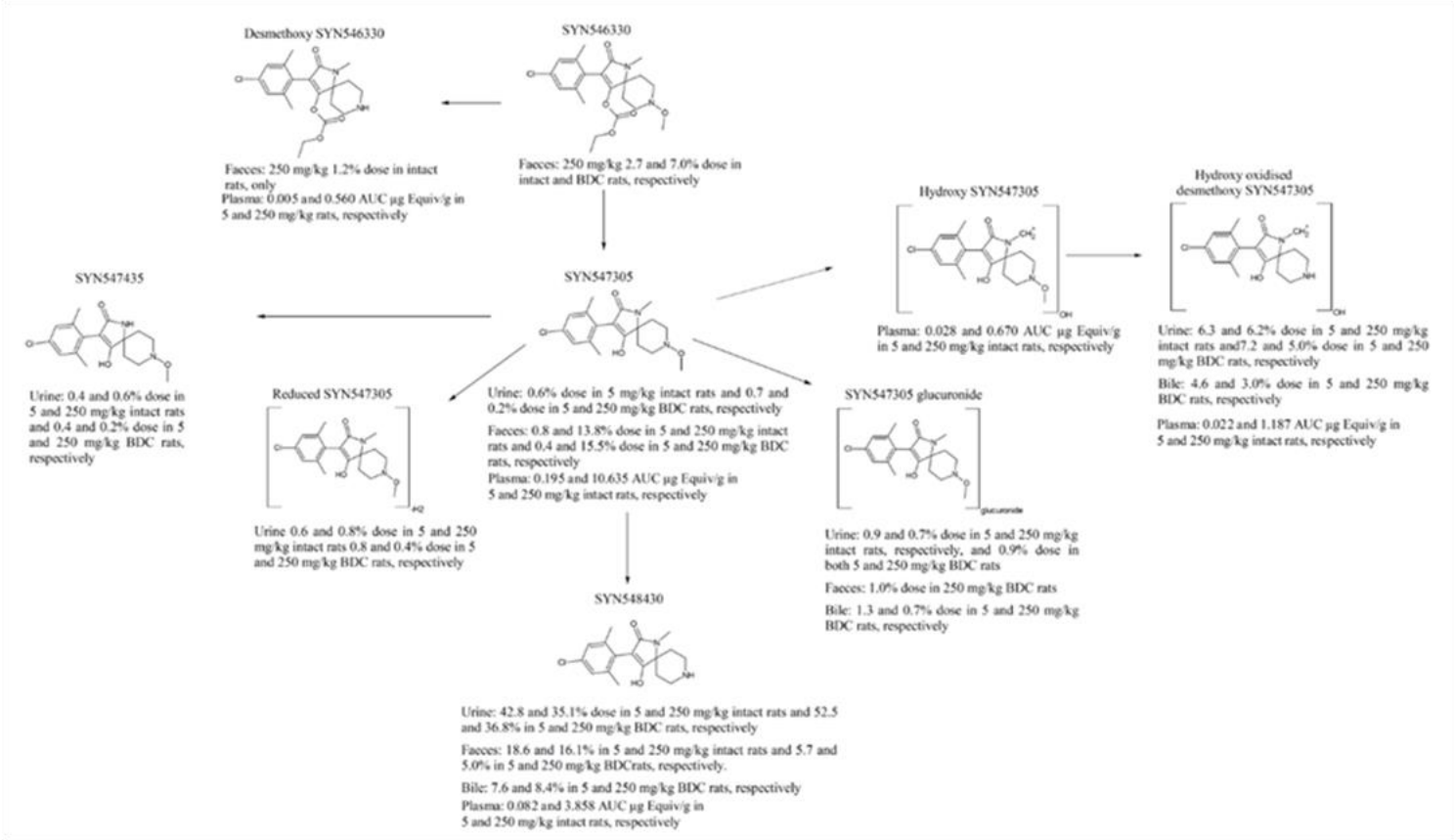
The key toxicological effects of spiropidion in sub-chronic oral studies in the rat, mouse, and dog included liver effects in mice, liver and thyroid effects in rats, and clinical effects in dogs (Table A B.8). In dog studies, adverse clinical signs included salivation, unsteady on feet, ataxia, subdued, twitching, abnormal breathing, uncoordinated, and unaware of surroundings. The NOAEL values from 90-day studies identified the dog NOAEL as the most protective endpoint (NOAEL for dog, rat, and mouse were 15, 31.5, and 105 mg/kg/day, respectively). In addition to the available short-term and subchronic studies, three available

(rat and rabbit) toxicology studies on reproduction and developmental toxicity with spiropidion did not show any evidence of reproductive hormone perturbation (Table A B.10).

Hormone measurements were not measured in the data package for spiropidion. Hormone perturbation was evaluated using the two-generation reproductive toxicity study, subchronic toxicity studies in mice, rats, and dogs, and developmental toxicity studies in rats and rabbits. Within the developmental toxicity studies in the rat and rabbit, there was no increase in malformations. There was no evidence of increased pre- or post-natal sensitivity or susceptibility observed in the database. No adverse parental, offspring, or reproductive effects were observed in the two-generation reproductive toxicity study up to the highest dose tested. No adverse parental or developmental effects were observed in the rat and rabbit developmental toxicity studies up to the highest dose tested.

Thyroid follicular cell hypertrophy and/or colloid contraction were observed in the short-term, subchronic, and F1 females of the developmental toxicity studies in the rat with spiropidion. In rats, minimal to mild thyroid follicular cell hypertrophy was consistently observed across subchronic durations. Mode of action research identified the increased uridine diphosphate-glucuronosyltransferases (UDP-GT) induction in the rodent liver, and lack of treatment-related effects in the thyroid peroxidase (TPO) assay, suggesting that the thyroid findings may be secondary. However, other modes of action are also possible and have not been investigated further.

Figure A B.1. Biotransformation Pathway for Spiropidion Following Oral Administration to Rats



The remainder of the toxicology data package with spiropidion also did not show any evidence of hormone perturbation. This included a lack of effect on the E-modality, A-modality, and/or steroidogenesis; however, it should be noted that the OECD TG studies were conducted before specific endocrine parameters were introduced. The findings in the thyroid were only observed in the rat. The human non-relevance case for the minimal thyroid follicular hypertrophy, based on prior established and well-recognised mechanisms known to be non-human relevant (e.g., thyroid follicular cell hypertrophy and hyperplasia) was supported by the increased induction of hepatocellular UDP-GT, lack of TPO activity, and lack of developmental effects in the developmental and reproductive toxicity studies conducted with spiropidion (Bourcier et al., 2024, Huisinga et al., 2020, Vansell, 2022). However, other modes of action (e.g., via deiodinase inhibition, sodium iodide symporter) are also possible and have not been investigated further for exclusion of human relevance. In addition, there is no comparison of enzyme induction in different species, including humans (*in vitro* assay), as would be required by current practice.

The database of repeat dose toxicology studies with spiropidion did not show any evidence of immune-related effects. There was no evidence of treatment-related changes in any immune-related parameters, including mortality due to infections, haematology, globulin levels, organ weights (adrenals, spleen, thymus), macroscopic findings, or histopathology (adrenals, bone marrow, lymph nodes, spleen, thymus). This class of insecticides was represented in the EPA's 2012 retrospective analysis of compounds for which immunotoxicity studies did not impact the human health risk assessment. EPA evaluations of other insecticidal compounds in the same class of chemistry as spiropidion found no evidence of functional immunotoxicity. Overall, there is no evidence that spiropidion or other compounds in this class of chemistry directly target the immune system (US EPA, 2013b); therefore, spiropidion is considered unlikely to be immunotoxic.

3. Mode of action

Spiropidion and the TAs/TADs family disrupt fatty acid biosynthesis in insects through the inhibition of the enzyme Acetyl CoA carboxylase (ACCase). ACCase is a biotin-containing enzyme that catalyses the ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA. This reaction is the first step in the synthesis of fatty acids: malonyl-CoA is the first building element of fatty acids. Two forms of ACCase occur in plants, prokaryotic and eukaryotic forms. The prokaryotic form is insensitive to these ACCase-inhibiting herbicides and is found only in the plastids of dicotyledonous plants. The eukaryotic form is found in the cytoplasm of all plants and in the plastids of grasses, in which the plastid-encoded prokaryotic form was lost and replaced by the eukaryotic, nuclear-encoded form with a transit peptide to target it for the plastid. The eukaryotic form is sensitive to ACCase-inhibiting herbicides. Similarly, the insecticidal pMoA is through the inhibition of fatty acid synthesis. Although ACCase is found across species, ACCase-inhibiting herbicides/insecticides do not inhibit mammalian, fungal, or broadleaf plant ACCase.

The design and development of this new ACCase inhibitor pesticide is derived from a sustained investigation of this class of chemistry and its chemical subclasses. Through time, and further understanding of this chemistry, the selection of ACCase sub-classes has decreased the probability that potential new active ingredients would exhibit developmental toxicity in rat and rabbit OECD guideline studies. This was achieved by lowering the predicted systemic exposure whilst maintaining the ability of the subclass to have a range of inhibitory potencies with high selectivity between plant and human ACC1 to maintain efficacy and resistance-breaking properties. This was achieved mainly by designing and selecting molecules with lower logP resulting in higher fraction unbound. Ultimately, this resulted in lower systemic exposure. Additional contributions to this development were made by lower absorption through reduced intestinal permeability and solubility. Such enhancement in developing new ACCase active ingredients has influenced some changes in the toxicity profiles noted across the chemical analogues. Despite these improvements, the read-across assessment includes all available data on the relevant analogues to provide a thorough assessment.

4. Read-across

Section note: The selection of analogues, and the associated chemical read-across described in this section were conducted in 2019 using data and read-across assessment tools which were publicly available at the time. It is noteworthy that following several rounds of feedback from the OECD IATA reviewers, a description of how the chemical read-across might be conducted in the present day (e.g., 2024) will be further addressed in the uncertainties section of this IATA.

For this assessment, the first step in identifying relevant compounds for read-across was to identify compounds with the herbicide or insecticide ACCase inhibition pMoA. In general, the ACCase inhibitor pesticides work through the inhibition of lipid biosynthesis and work by interfering with growth regulation. Potential compounds for use in read-across were selected based on the ACCase inhibition pMoA, defined by the Herbicide Resistance Action Committee (HRAC) and Insecticide Resistance Action Committee (IRAC). The ACCase inhibitor herbicides (HRAC Class 1) included several different chemical groups, namely phenylpyrazolin (DENs), cyclohexanedione (DIMs), and aryloxyphenoxypropionate (FOPs) chemistries. The ACCase inhibitor insecticides (IRAC Class 23) included the tetronic and tetramic acid derivatives (TAs/TADs). There were 23 herbicides and insecticides identified as ACCase inhibitor chemicals representing the identified chemical groups.

Structural similarity was defined based on ToxPrints, using a selection of 729 binary chemical fingerprints broadly associated with toxicity (Yang et al., 2015). ToxPrints are generally available for many molecules

in the CompTox Chemicals Dashboard, and they can also be generated using the Chemotype application. ToxPrints were gathered for all 23 ACCase inhibitors, and among the 729 defined fingerprints, 96 were present in one or more ACCase inhibitor. The other ToxPrints were excluded, as they did not provide additional information to compare structural similarity. A principal coordinate analysis was conducted based on the binary distance matrix between each ACCase inhibitor (Figure A B.2). Additionally, a clustering analysis was conducted (Figure A B.3). Both approaches clearly indicate grouping of the ACCase inhibitor classes, with spiropidion most similar to other TAs/TADs and DENs and distinct from other ACCase inhibitor classes. The phenylpyrazolin, pinoxaden, did categorise with the TAs/TADs based on ToxPrints fingerprints associated with toxicity. However, this active substance was not used in the read-across based on the difference in target organs of toxicity (kidney) and endpoints used in risk assessment (maternal toxicity in the developmental toxicity studies). Pinoxaden did not present with evidence of carcinogenicity thus exclusion of this chemical as an analogue was considered of low concern.

Following the structural similarity assessment, a review of the distinguishing factors of the different ACCase chemistries, the ACCase enzyme and its physiological function, was included to ascertain whether there were significant changes in the subcategories of this class and assess the reliability of the potential read-across analogues (Délye 2005; Rendina 1990, Takano 2021, Xia 2016, Yu 2010). The strategy, to start the read-across analysis with all ACCase chemistries and data that contributed to the overall understanding of the chemical and overall safety assessment in the read-across analysis, is considered to be a conservative approach based on the improvements made to this class of chemistry over time.

Based on the review of the available data, read-across with the TAs/TADs chemical group compounds was determined to be the most relevant for the assessment of spiropidion. This conclusion was based on pMoA, bioactivity, and structural similarity. The structures of the TAs/TADs are presented in Figure A B.4 and Table A B.2, Table A B.13 and Table A 0.3.

Although ACCase is found across species, ACCase-inhibiting herbicides/insecticides do not potently inhibit mammalian, fungal, or broadleaf plant ACCase. Therefore, although the TAs/TADs have the same insecticidal pMoA, there is not a known, shared MoA available for the TAs/TADs.

The liver-induced UDP-GT thyroid MoA has been identified as a relevant MoA for spiropidion. This MoA is a general adaptive response and secondary to increased Phase II enzyme induction in the liver. However, to exclude human relevance, there is no comparison of enzyme induction in different species, including humans (in vitro assay, as would be required by current practice).

The use of read-across in this case study was not for the purpose of estimating the POD for chronic dietary risk assessment of the new active substance. This read-across analysis served to gain a better understanding of the common toxicity and bioactivity of this class of chemistry and support the hazard characterisation of the new active substance.

A detailed read-across analysis with a comparison of the toxicological effects is included in the supplemental data in Appendix A: SUPPLEMENTAL DATA: ACCase Inhibitors Toxicological Read-across for Tetronic and Tetramic Acid Derivatives**.**

Figure A B.2. Chemical clustering based on ToxPrints including the new active substance

This figure presents the results from the ToxPrints assessment. In addition to structural similarity, ToxPrints makes use of the library of chemotypes to identify biological properties of the chemicals and modes of action in the toxicity pathways, allowing refinement of the relevant analogues by structural and bioactivity similarities (Yang et al., 2015).

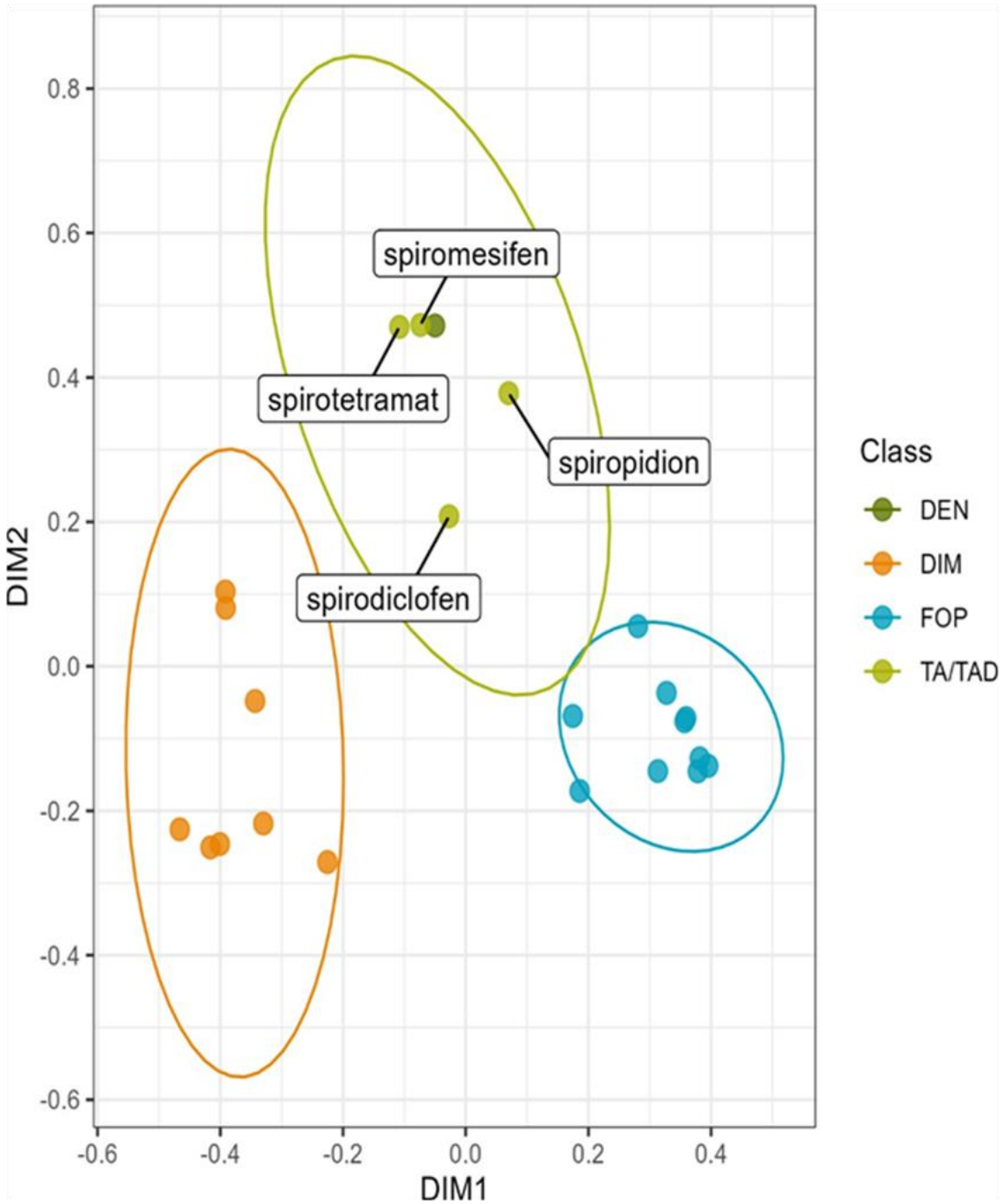


Figure A B.3. Chemical clustering based on ToxPrints

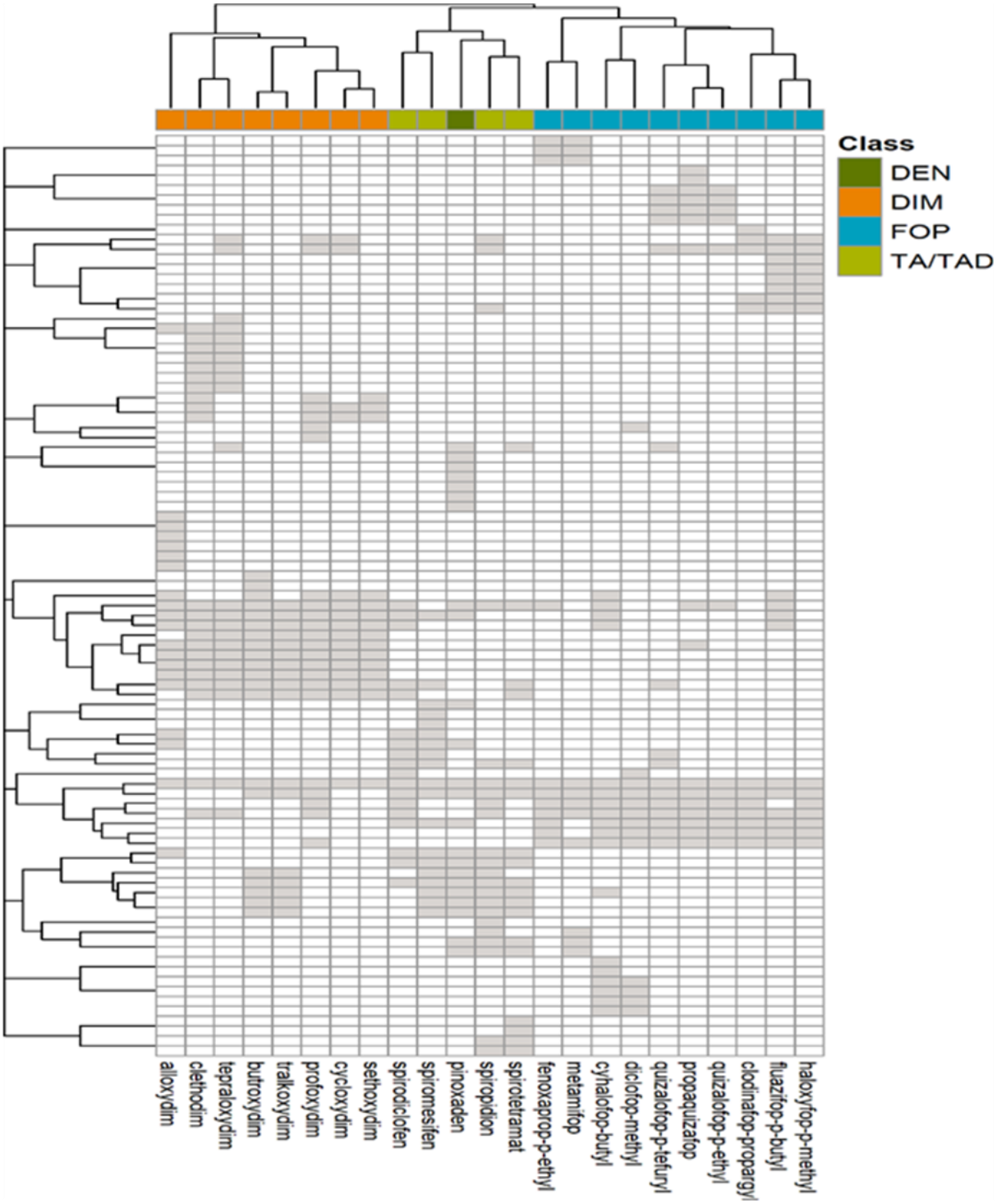


Figure A B.4. Chemical structures of the tetronic and tetramic acid derivatives

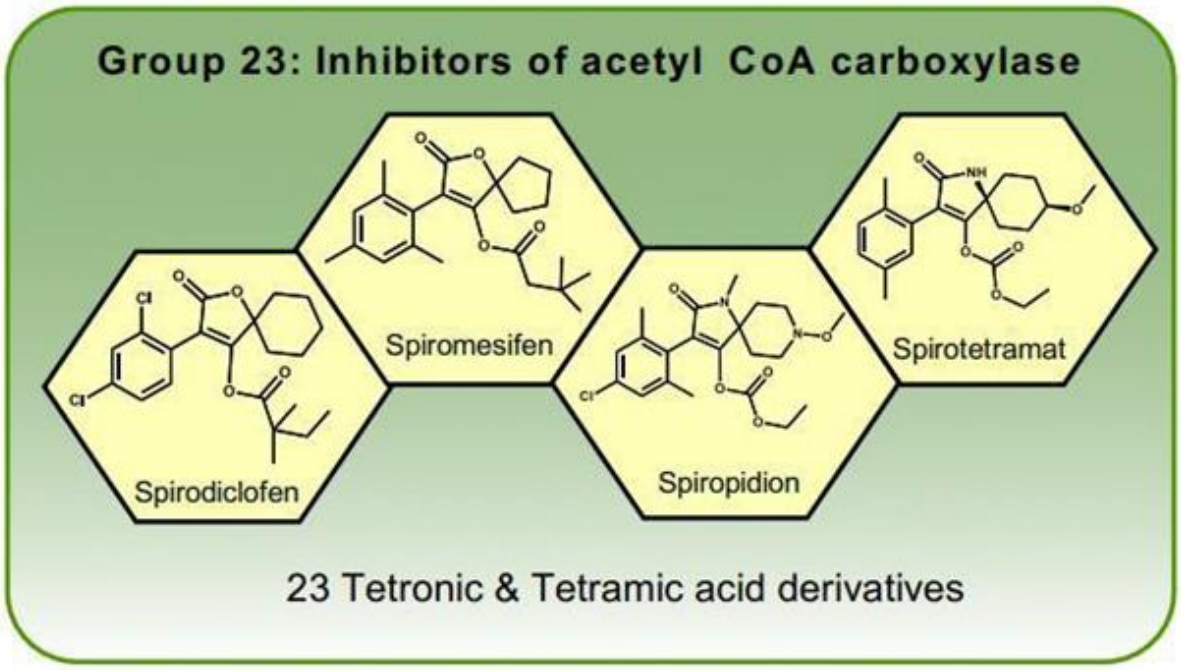
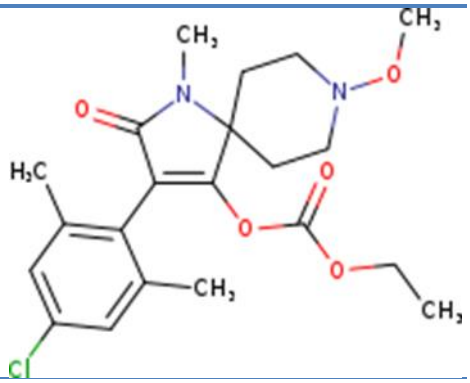
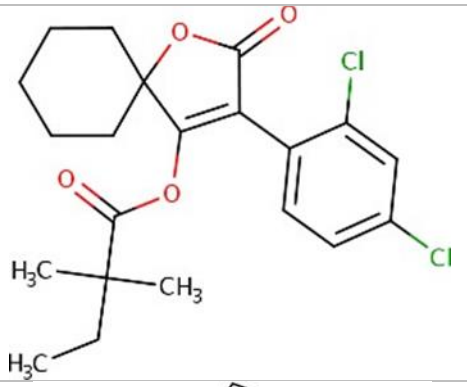
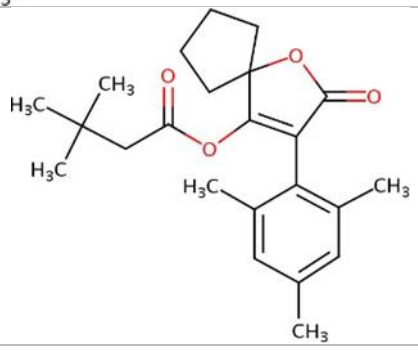
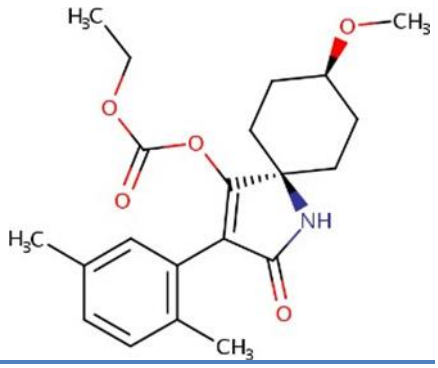


Table A B.2. Read-across compounds selected for spiropidion

Spiropidion	1229023-00-0	
Read-Across Analogues		
Spirodiclofen	148477-71-8	
Spiromesifen	283594-90-1	
Spirotetramat	203313-25-1	

6. Evaluation of read-across analogues

The toxicology of the structurally similar chemicals spirodiclofen, spiromesifen, and spirotetramat were evaluated and compared to those reported with spiropidion. Overall, there is no genotoxic concern for the TAs/TADs, including spiropidion. The review of the structurally similar read-across chemicals identified spiromesifen as the most biologically similar compound to spiropidion based on the overall bioactivity similarity. The effects described with the TAs/TADs were used to identify any precursor effects that would be indicative of similar toxicity.

In terms of toxicokinetics, spiropidion and the read-across compounds were extensively metabolized, the highest tissue distribution was to the liver and kidneys, and the compounds were rapidly excreted mainly via the urine. Except for spiropidion, the structurally related chemicals demonstrated a sex-related difference in absorption, distribution, and metabolism with males receiving a greater systemic exposure than female rats. The sex difference in systemic exposure was factored into interpreting the bioactivity of the analogues and selecting the health-protective, conservative, points of departure.

The acute toxicity profiles of the TAs/TADs, including spiropidion, were similar, including positive skin sensitization results.

For the TAs/TADs, the target organs were the adrenal glands, liver, thyroid, and testes, with effects in the target organs detected following 90 days of oral exposure. Reproductive effects including effects on male reproductive organs, sperm production and Leydig cells were reported in the reproductive toxicity studies with spirodiclofen and/or spirotetramat. Following chronic exposure, studies with spiromesifen reported testicular effects in the chronic oral toxicity in dogs and spirotetramat reported thymus effects in dogs. The spiropidion database was reviewed for similar studies or precursor effects, and no effects were noted on the reproductive system. Spiropidion was evaluated for the potential to result in rat Leydig cell and uterine tumours and mouse liver tumours; no precursor effects were reported to indicate a similar response with spiropidion following chronic exposure.

Following the regulatory reviews conducted by the US EPA (2011, 2021), spirodiclofen was the only compound classified as likely to be carcinogenic to humans with a Q1* assigned based on increased liver tumours in mice and increased testes Leydig cell adenoma in male rats and increased adenoma/adenocarcinoma in female rats. All other TAs/Tads were classified as not likely to be carcinogenic to humans.

Refer to the supplemental data for more details on the toxicology profiles of spiropidion and the read-across compounds.

Toxicokinetics

The TAs/TADs compounds spirodiclofen, spiromesifen, and spirotetramat, were evaluated for absorption, distribution, metabolism, and elimination in male and female rats following single intravenous, and oral dosing and after repeated oral dosing. A summary table of toxicokinetic parameters and metabolism in rats for spiropidion can be found in Table A B.6. The toxicokinetic data for the TAs/TADs can be found in Table A 0.4. Spiropidion, spirotetramat and spirodiclofen had similar oral absorption; the oral absorption for spiromesifen was lower. Time to peak concentration varied slightly; peak blood and tissue concentration was reached after 1-4 hrs with spiropidion, 3 or 4-8 hrs for spirodiclofen (dependent on methodology for determining concentrations), 1 hr for spiromesifen, and 0.2-1 hr for spirotetramat. Saturation of absorption was reported for spirodiclofen and spiromesifen, but not for spiromesifen or spiropidion.

The TAs/TADs, including spiropidion, demonstrate rapid and complete excretion with >90% of the administered dose excreted within 48 hrs of exposure. All compounds including spiropidion were pro-

insecticides, with the enol as the active metabolite. Extensive metabolism was reported with all compounds and excretion was primarily via the urine. There was no evidence of bioaccumulation in any specific organs or tissues. Incomplete absorption was supported for spirodiclofen and spiromesifen based on increased recovery by the faecal route.

Spirodiclofen was highest in the liver, kidney, plasma, gastrointestinal tract (GIT) and skin; organ and tissue levels were 5-15 times lower in females compared to male rats. Spiromesifen was measured highest in the liver, GIT, bladder and blood within the heart, with a slight sex-related difference supporting the lower systemic exposure in female rats. Spirotetramat was measured highest in the liver and kidney; the higher Cmax and AUC in males indicated greater systemic exposure than female rats. In comparison, spiropidion did not demonstrate a sex-related difference in toxicokinetics, and the highest concentrations were reported in the carcass, liver, skin, and GIT.

Acute Toxicity

The TAs/TADs, including spiropidion, are generally of low acute toxicity via oral, dermal, and inhalation routes of exposure and have a low irritation potential (Categories IV), except for spirotetramat which is a Category III for eye irritation. Acute toxicity results for spiropidion can be found in Table A B.7. Summary tables of physical-chemical properties and acute toxicity for read-across chemical analogues can be found in Table A 0.4 and Table A 0.5 Spiropidion and the read-across TAs/TADs were skin sensitisers.

Repeated Dose Toxicity

With spiropidion, the target organs were the liver in mice, the liver and thyroid in rats, and clinical signs of toxicity in dogs. In short-term oral toxicity studies with spirodiclofen in mice, rats, and dogs, the primary target organs of toxicity were the adrenal glands and testes. The predominant finding was vacuolation of the adrenal cortex, which was noted in the mouse, rat, and dog studies and was often accompanied by increased adrenal weight. In repeated-dose short-term oral toxicity studies with spiromesifen in mice, rats, and dogs, the most sensitive effect was the reduction of plasma cholesterol. Common findings were effects on body weight, liver (including liver enzyme induction), thyroids (rats and dogs), and adrenals. In short-term toxicity studies with spirotetramat in mice and rats, no adverse effects were observed at the highest doses tested. In dogs, the LOAEL was the highest dose tested based on decreased thymus size and weight and decreased body weight and food consumption.

In repeated dose subchronic oral toxicity studies effects were similar across the three TAs/TADs, with liver and kidney toxicity in rats and dogs. Sub-chronic toxicity studies of spiropidion can be found in Table A B.8. A summary table of repeated-dose for read-across chemical analogues can be found in Table A 0.6. Detailed sub-chronic effects of read-across chemical analogues can be found in Table A 0.7. Other targets of toxicity were thyroid, clinical biochemistry, and general systemic toxicity effects (body weight/food consumption) in rats, mice, and dogs. The lowest NOAELs/LOAELs were in the dog for both spiropidion and spirodiclofen.

Although statistically significant decreases in plasma cholesterol and triglycerides were observed in rats with spiropidion administration, the difference from concurrent controls was relatively small. All cholesterol values, even at the highest dose levels of spiropidion, were either within the normal range of the historical control data (HCD) or only slightly lower. In the absence of other correlated findings, this slight reduction in plasma lipid levels would not impair the normal function of the organism and therefore would not be considered an adverse effect. There were no associated adverse effects, including the functional neurobehavioral battery. It is also important to note that the reduction in plasma lipids was not consistently replicated in female rats and that there was no evidence of progression over time in male rats, suggesting that this effect was transient (reversible) and non-adverse.

Cholesterol is an important biological molecule as it is a precursor for the synthesis of steroid hormones, bile acids and vitamin D (Lin et al., 2015). Essential levels of cholesterol are synthesized by the body, and various processes regulate the levels of plasma cholesterol. The health risks of excessive cholesterol are well-known, and it has been established that lipid-lowering medications in humans improve cardiovascular and mortality outcomes. Although lower cholesterol levels have been correlated with certain neuropsychiatric conditions, a causal relationship is unlikely because there are no known neuropsychiatric risks associated with cholesterol-lowering medications. For example, new medications that inhibit PCSK9 have been found to lower cholesterol by up to 60% in addition to maximal statin therapy, without any associated adverse events (Katzmann et al., 2020). In animal toxicity studies, alterations in plasma lipid levels may be corroborating evidence of toxicity in target organs such as the liver, thyroid, or adrenal gland, but are not an adverse effect per se in the absence of correlated findings.

The decrease in cholesterol in the short-term 28-day toxicity study was statistically significant in males at all dose levels and females >2000 ppm. However, the variability in each treatment group demonstrated there was an overlap in the levels measured between concurrent control animals and the lowest dose level (500 ppm). The range of cholesterol levels in the concurrent control male animals (1.4-1.9 mmol/L) overlapped with treatment group 1 males (0.7-1.6 mmol/L). Historical control data for this strain of rat at this Lab was 1.95 +0.65 mmol/L (1.3-2.6 mmol/L). In the 90-day oral (dietary) toxicity in the Wistar Han rat, similar to the 28-day toxicity study, based on the inter-animal variability, comparison with background HCD for this strain at the Lab, and lack of any other correlated findings at the low and intermediate dose levels, this decrease in plasma cholesterol and triglyceride levels was considered non-adverse.

Reproductive and Developmental Toxicity (Reproductive Hormone Perturbation)

For spiropidion, no reproductive or offspring effects were reported in the rat reproduction study and parental toxicity included effects on thyroid. Studies providing data relevant to hormone perturbation (reproductive and developmental toxicity studies) for spiropidion can be found in Table A B.10. A summary of reproductive/developmental toxicity studies with the read-across TAs/TADs can be found in Table A 0.12. With spirodiclofen, parental toxicity included effects on liver and adrenals and there were additional effects observed at higher doses (i.e., delayed sexual maturation, increased severity of ovarian luteal cell vacuolation/degeneration, decreased testicular spermatid and epididymal sperm counts, and atrophy of the testes, epididymides, prostate, and seminal vesicles); offspring effects (body weight decrements). With spiromesifen, reproductive toxicity in females (increased primordial follicles, decreased growing follicles, and decreased corpora lutea) was observed at the same dose causing parental effects and decreased pup body weight was observed at a lower dose than parental effects. Effects of TAs/TAD were observed in the male reproductive tract; including hypertrophic Leydig cells and histopathological findings in the testes, epididymis, and prostate following treatment with spirodiclofen, and decreased testis weight, testicular degeneration and vacuolation, hypospermia in the epididymis, and abnormal spermatozoa following treatment with spirotetramat; however, these effects were not reported with spiropidion.

For spirotetramat, at high dose levels (419 mg/kg/day in males) and in the presence of systemic toxicity, reproductive toxicity effects in the sperm were reported with reproductive performance affected in F1 male rats. Overall, the reproductive toxicity was observed secondarily to general systemic toxicity; that is reproductive toxicity was observed at dose levels at which systemic toxicity was present or at dose levels higher than those resulting in systemic toxicity.

The potential for spiropidion to result in male reproductive effects similar to spirotetramat and spirodiclofen was evaluated, including effects on the Leydig cells. No similar effects and no precursor effects were found in the toxicological database for spiropidion.

Developmental toxicity studies in rats and rabbits with spiropidion or the read-across chemicals generally did not indicate a concern for selective toxicity to the developing foetus. With spiropidion, decreased mean body weight, body weight gain, and food consumption were reported in maternal rats and rabbits with no evidence of developmental toxicity in rats and decreased foetal body weights in the presence of maternal effects in rabbits. For spirodiclofen, an increased incidence of slight dilatation of the renal pelvis was observed in foetuses at the limit dose (1000 mg/kg/day), in the absence of maternal toxicity in the rat developmental toxicity study for spirodiclofen. EPA (2022) noted that concern for pre- or post-natal sensitivity or susceptibility is low since the effect was slight, only seen at the limit dose, occurred without statistical significance, and did not display a clear dose-response. In the rabbit developmental study, there were no developmental effects and maternal toxicity was evidenced by body weight decrements. With spiromesifen, maternal effects were reported in the rat (clinical signs reported as convulsions in rats and increased incidence of abortion and total resorptions in rabbits. No developmental effects were reported in rats or rabbits. For spirotetramat, there was evidence of increased qualitative susceptibility in the rat developmental study with reduced foetal weight and increased incidences of malformations and skeletal deviations observed at the limit dose, while maternal effects at this dose consisted of only body-weight decrements. EPA (2022) concluded that there was no evidence of increased quantitative or qualitative susceptibility to offspring following pre- or post-natal exposure to spirotetramat in the rabbit developmental.

ToxCast pathway model data were generated and the data available on the TAs/TADs predicted no interaction with the oestrogen or androgen pathways (CompTox, 2023). Studies providing data relevant to hormone perturbation for spiropidion can be found in Table A B.10. A summary of reproductive toxicity for read-across chemical analogues can be found in Table A 0.12.

As stated previously, the liver-induced UDP-GT thyroid effects are a relevant MoA for spiropidion and spiromesifen. This is addressed further in the MoA section. However, to exclude human relevance, there is no comparison of enzyme induction in different species, including humans (*in vitro* assay, as would be required by current practice).

Immunotoxicity

The chemicals used for ACCase read-across did not show signs of immunotoxicity in the T-cell dependent antibody response (TDAR) assays used. In all available studies, there was no evidence of an immunosuppressive effect up to the highest dose level tested. The database includes 28-day immunotoxicity studies for spirodiclofen, spiromesifen, and spirotetramat, which did not show any evidence of immune suppression. Studies providing data relevant to immune suppression for spiropidion can be found in Table A B.11.

Neurotoxicity

Acute neurotoxicity studies in rats reported clinical signs of toxicity with spiropidion and spirotetramat. While not reported in the acute neurotoxicity study, clinical signs of toxicity (convulsions) were reported in maternal rats in the developmental toxicity study with spiromesifen. No adverse effects were reported in the acute neurotoxicity study in rats with spirodiclofen or spiromesifen. No notable findings were reported in subchronic neurotoxicity studies with spirodiclofen, spiromesifen, or spirotetramat. Clinical signs of toxicity were reported with spiropidion in the dog studies; the other TAs/TADs did not report similar findings in the dog.

Chronic Toxicity and Carcinogenicity

In chronic toxicity and carcinogenicity studies with the read-across compounds, effects similar to those reported in the respective subchronic toxicity studies were reported with spiropidion and the TAs/TADs.

Spiromesifen and spirotetramat were classified by EPA or JMPR as “not likely” or “unlikely” to be carcinogenic to humans.

Spirodiclofen was classified as ‘Likely to be carcinogenic to humans’ by the EPA (2015). Spirodiclofen was classified based on increased liver tumours in mice and increased testes Leydig cell adenoma in male rats and increased uterine adenoma/adenocarcinoma in female rats. Evidence for this carcinogenic potential was observed in the subchronic toxicity studies. Based on precursor events evident in the subchronic studies, this afforded the ability to predict the long-term effects for spirodiclofen. Subchronic studies with spiropidion did not present with such precursor events, indicating the likelihood for these long-term effects was of low concern for this active substance. In toxicity studies with spirodiclofen, precursor effects in the liver of mice were reported in subchronic toxicity studies and Leydig cell effects were reported in subchronic duration studies in both rats (Leydig cell vacuolation) and mice (hypertrophic Leydig cells in the testes). The only exception to this is the uterine tumours in rats with spirodiclofen, where no precursor findings in the uterus were reported in the repeated dose or reproductive toxicity studies. The uterine tumours were in the presence of other tumours and systemic toxicity and were only present at the highest dose level tested (152.9 mg/kg/day). The lack of identification of precursor findings to predict this effect was not crucial to the assessment of spiropidion because the effect was present with other effects, which could be evaluated in the subchronic studies.

Detailed carcinogenicity and/or chronic toxicity results, classification, and POD for read-across chemical analogues can be found in Table A 0.10 and Table A 0.23

Thyroid MoA

Spiropidion, spiromesifen, and spirotetramat were associated with thyroid effects. As described above, the thyroid is a target organ for spiropidion with effects reported in rat studies and the liver induced UDP-GT as the MoA for thyroid effects. However, to exclude human relevance, there is no comparison of enzyme induction in different species, including humans (*in vitro* assay, as would be required by current practice). With spiromesifen, the thyroid gland was a target organ of toxicity in the subchronic rat studies with increases in the incidence and severity of thyroid follicular cell hypertrophy and colloidal alternation, increases in TSH and thyroxine-binding capacity (TBC), and decreased T3 and T4; decreased T4 was reported in dogs. For spirotetramat, in subchronic dog studies, lower circulating levels of the thyroid hormones T3 and T4 were recorded in both sexes. Decreases in T4 were also observed in a one-year study in dogs. The histopathological change in the thyroid was a reduction in follicle size in the high-dose group, a possible indication of a reduced amount of colloid. There were no correlative changes in thyroid weight, thyroid histopathology, or TSH in either sex. Based on this information spiromesifen shares the liver induced UDP-GT rodent thyroid MoA with spiropidion. Although there were no histopathological changes observed in the dog toxicity studies with spiropidion, hormone measurements were not measured in the studies. MoA and mechanistic studies for spiropidion can be found in Table A B.12 and Table A 0.15 through Table A 0.19.

Increase in Toxicity over Time

When comparing the results of subchronic and chronic toxicity studies, there was a limited increase in toxicity over time (<10X) for the TAs/TADs read-across compounds. The rapid and complete excretion of the TAs/TADs supports this finding.

7. Toxicity Mode of action

Thyroid follicular cell hypertrophy and/or colloid contraction were observed in the short-term and subchronic toxicity studies in the rat with spiropidion. In the 28-day oral (dietary) toxicity study in rats, minimal follicular cell hypertrophy was observed in the thyroid glands of animals that received spiropidion at dose levels of 2000 or 3000 ppm (177 or 259 mg/kg/day, respectively). In the 90-day toxicity study in rats, microscopic findings of diffuse follicular cell hypertrophy and colloid contraction were observed in the thyroid glands in the high-dose groups only (2500 and 1500 ppm: equivalent to 159 and 110 mg/kg/day in males and females, respectively). There was no increase in thyroid weight in any treatment group of these studies in the rat. There was no change in thyroid weight or histopathological changes in the mouse or dog.

Additional mechanistic studies were conducted to further understand the thyroid MoA in the rat (Table A B.12). A study to assess the potential induction of UDP-GT enzymes in liver samples from Han Wistar rats was conducted following oral administration of spiropidion by diet at inclusion levels of 2500 and 1500 ppm (males and females, respectively) for 13 weeks. Statistically significant increases in the rate of thyroxine glucuronidation were observed. It was concluded that spiropidion is an inducer of hepatic UDP-GT activity in both male and female Han Wistar rats. UDP-GT activity was increased at the dose levels that caused effects on the thyroid, thus effects on the thyroid following exposure to spiropidion are secondary to liver enzyme induction (Table A 0.15 through Table A 0.19). As stated previously, the liver-induced UDP-GT thyroid effects is a relevant MoA for spiropidion; however, to exclude human relevance, there is no comparison of enzyme induction in different species, including humans (*in vitro* assay, as would be required by current practice).

Thyroid peroxidase (TPO) inhibition as an alternative mode of action for thyroid effects was found to be inactive for spiropidion. An *in vitro* study was conducted to evaluate the effect of spiropidion and its metabolite SYN547305 on rat (Han Wistar) TPO activity. Treatment had no significant effect on rat TPO activity at any concentration tested. It was concluded that spiropidion and its metabolite SYN547305 were not inhibitors of rat thyroid peroxidase activity *in vitro*. However, other modes of action (e.g. via deiodinase inhibition, sodium iodide symporter) are also possible and have not been investigated further for exclusion of human relevance. In addition, there is no comparison of enzyme induction in different species, including humans (*in vitro* assay).

The available data for spiropidion support a proposed MoA in rats involving liver-induced UDP-GT, resulting in increased conjugation and excretion of triiodothyronine (T3) and thyroxine (T4). Under the chronic proliferative stimulus of thyroid stimulating hormone (TSH), thyroid follicular cells undergo hypertrophy, and eventually hyperplasia and progress to form follicular cell adenomas and/or carcinomas. This MoA is well understood and documented in the peer reviewed literature (Dellarco et al., 2006; Hard, 1998; Bartsch et al, 2018, AOP Wiki, 162, 2023). An additional factor that may play a role in interspecies quantitative sensitivity to thyroid stimulation deals with the influence of protein carriers of thyroid hormones in the blood (Table A A.2).

Table A B.3. Inter- and Intra-species Differences

Parameter	Human	Rat
Thyroxine-binding globulin	present	essentially absent
T4 Half-life	5-9 days	0.5-1 day
T3 Half-life	1 day	0.25 day
T4 Production rate/kg bw	1 x	10 x that in humans
TSH	1 x	6-60 x that in humans
Follicular cell morphology	Low cuboidal	Cuboidal
Sex differences		

Serum TSH	Sexes equal	M ≤ 2 x F
Cancer sensitivity	F = 2.5 x M	M > F

T3: triiodothyronine; T4 thyroxine; TSH: thyroid stimulating hormone; F: Female; M: Male

Both humans and rodents have nonspecific low-affinity protein carriers of thyroid hormones (e.g., albumin). However, in humans, other primates, and dogs there is a high-affinity binding protein, thyroxine-binding globulin, which binds T4 (and T3 to a lesser degree); this protein is essentially missing in adult rodents and lower vertebrates (Savu et al., 1989; Rouaze-Romet et al, 1992). As a result, more T4 remains bound to proteins with lower affinity in the rodent and is more susceptible to removal from the blood, metabolism, and excretion from the body. In keeping with this finding, the serum half-life of T4 is much shorter in rats (less than 1 day) than in humans (5 to 9 days); this difference in T4 half-life results in a 10-fold greater requirement for exogenous T4 in the rat with a non-functioning thyroid than in the adult human (Döhler et al., 1979). Serum T3 levels also show a species difference; the half-life in rats is about 6 hr while that in humans is about 24 hr (Oppenheimer, 1979; Larsen, 1982).

There is a morphological consequence to these hormone differences. High thyroid hormone synthetic activity is demonstrated in follicles in rodents: they are relatively small, surrounded often by cuboidal epithelium. Follicles in primates demonstrate less activity and are large with abundant colloid, and follicular cells are relatively flattened (low cuboidal) (McClain, 1992).

The accelerated production of thyroid hormones in the rat is driven by serum TSH levels that are about 6- to 60-fold higher than in humans. This assumes a basal TSH level in rats and humans of 200 ng/ml and 5 U/ml, respectively, and a potency of human TSH of 1.5 to 15 U/mg of hormone (NIDDK, 1994). Thus, it appears that the rodent thyroid gland is chronically stimulated by TSH levels to compensate for the increased turnover of thyroid hormones. It follows that increases in TSH levels above basal levels in rats could more readily move the gland towards increased growth and potential neoplastic change than in humans. Interestingly, adult male rats have higher serum TSH levels than females (Chen, 1984), and they are often more sensitive to goitrogenic stimulation and thyroid carcinogenesis. In humans, there is no sex difference in hormone levels, but females more frequently develop thyroid cancer (Boring et al., 1994). With a disruption in thyroid-pituitary functioning, there is typically a reduction in both circulating serum T4 and T3 concentrations and an increase in TSH levels within days or a few weeks of chemical administration. In some cases, T4 levels may be lowered while T3 levels are maintained within normal limits.

The available data demonstrate that a threshold exists for the induction of the thyroid key events and that the liver-induced UDP-GT thyroid MoA is not quantitatively relevant for human cancer hazard/risk assessment purposes due to quantitative differences in response to UGT induction and increased T3/T4 clearance between rats and humans (Dellarco et al., 2006; Hard, 1998; Bartsch et al, 2018, AOP Wiki, 162, 2023). In summary, the data support the conclusion that spiropidion does not pose a carcinogenic hazard to humans based on quantitative differences between rats and humans, and a margin of exposure-based approach for human health risk assessment is appropriate for spiropidion.

8. WoE evaluation

In the absence of the results from the rat chronic toxicity/carcinogenicity study and mouse carcinogenicity studies with spiropidion, a WoE approach was adopted to evaluate the potential for carcinogenicity and select a POD for chronic exposure. All the available hazard and exposure information for spiropidion, including short-term and subchronic toxicity studies, MoA, read-across to TAs/TADs compounds in the same pMoA class as spiropidion, biological effects, along with the proposed use patterns for spiropidion

were used in this approach. Based on the pesticidal MOA, structural, and bioactivity similarity of the TAs/TADs the safety profiles of spirodiclofen, spiromesifen, and spirotetramat were included in the read-across assessment. Pinoxaden is structurally similar to spiropidion; however, it was not used in the read-across based on the difference in bioactivity and target organ toxicity.

Spiropidion and the TAs/TADs are non-genotoxic, and they do not show any evidence of estrogenic or androgenic hormone perturbation or immune suppression.

Short-term and subchronic exposures to spiropidion in mice, rats, and dogs indicated the target organs of toxicity were different for separate species. The critical effects following oral administration of spiropidion were loss of body weight at the highest dose tested in all species and changes to the thyroid consistently observed across subchronic durations in rats, increased liver weight in the mouse, and adverse clinical signs recorded in dogs. The thyroid effects in the rat consisted of minimal to diffuse follicular cell hypertrophy and colloid contraction in the thyroid gland. In dog studies, adverse clinical signs included salivation, unsteady on feet, ataxia, subdued, twitching, abnormal breathing, uncoordinated, and unaware of surroundings.

The available data for spiropidion support the liver-induced UDP-GT thyroid MoA in rats involving the induction of hepatic UDP-GT resulting in increased conjugation and excretion of T3 and T4. Chronic exposure to significant levels results in chronic proliferative stimulus of TSH, resulting in thyroid follicular cells hypertrophy, and potentially hyperplasia, progressing to form follicular cell adenomas and/or carcinomas. The available data also demonstrate that a health-protective threshold exists for the induction of the key events and that this MoA is not quantitatively relevant for human hazard/risk assessment purposes due to differences in response to UDP-GT induction and increased T3/T4 clearance between rats and humans. This MoA and the quantitative lack of relevance to humans is well understood and documented in the peer reviewed literature (Dellarco et al., 2006; Hard, 1998; Bartsch et al, 2018; Oppenheimer, 1979; Larsen, 1982; Boring et al., 1994; Chen, 1984; McClain, 1992). This WoE assessment was based on the US EPA's regulatory hazard characterization and risk assessment. Weight of evidence assessments prepared for the EU must include ECHA hazard assessment and EFSA risk assessments."

The effects demonstrated by the TAs/TADs were used to ensure that effects leading to chronic toxicity or carcinogenicity were not present with spiropidion. Common effects to the read-across compounds included findings in the adrenal glands (cytoplasmic vacuolation in the cortex) in rats, mice and/or dogs with spirodiclofen and spiromesifen; these effects were not recorded with spiropidion. Effects were observed in the male reproductive tract; including hypertrophic/hyperplastic Leydig cells and histopathological findings in the testes, epididymis and prostate following treatment with spirodiclofen, and decreased testis weight, testicular degeneration and vacuolation, hypospermia in the epididymis and abnormal spermatozoa following treatment with spirotetramat; these effects were not recorded with spiropidion[4]. Thymus atrophy was observed following treatment with spirodiclofen and spirotetramat in the dog; no effects on the thymus were recorded with spiropidion. Thyroid effects in the dog were reported with spiromesifen and spirotetramat; these effects were not reported with spiropidion. Additionally, the potential for direct action thyroid MoA relevant to the dog including thyroid peroxidase inhibition (TPO) was evaluated and not active with spiropidion.

Although the TAs/TADs demonstrated structural similarity and similarity in rapid and complete excretion and acute toxicity effects including skin sensitization effects, the repeated dose biological effects are different between the TAs/TADs including spiropidion. Although the TAs/TADs were structurally related

[4] JMPR (2021) reported treatment-related Leydig cell tumours with spiropidion. However, the available data support that the Leydig cell tumours are not treatment-related as the incidence was within the historical controls for the conducting laboratory.

and had the same pMoA, the TAs/TADs demonstrated weak alignment across the toxicity profiles. This is in part due to the improvements in design and development with this class of chemistry i.e., lower logP resulting in higher fraction unbound resulting in lower systemic exposure, and lower absorption through reduced intestinal permeability and solubility. However, the liver-induced MoA for thyroid effects was demonstrated for spiropidion, and the spiropidion database was evaluated for effects or precursor effects reported with the other TAs/TADs.

For compounds that show liver induction of UDP-GT and secondary thyroid effects in 90-day studies, conducting MoA data on early liver key initiating events will assess the biological effect and potential for human relevance. A point of departure should be selected that is protective of the liver-induced UDP-GT thyroid MoA or subsequent changes in thyroid hormone levels, which pre-empt the adverse outcome. With spiropidion, a LOAEL of 110 mg/kg/day for UDP-GT induction was identified in a 90-day mechanistic study and LOAEL of 58 mg/kg/day for histopathological change in the multigeneration reproductive toxicity study. Thus, the subchronic 90-day dog oral toxicity study NOAEL of 15 mg/kg/day was considered protective of the thyroid effects in rats. A cancer classification of “Not Likely to be Carcinogenic to Humans” at doses that do not induce changes in thyroid hormone levels should be assigned to spiropidion. The data from a mouse or rat carcinogenicity study is highly unlikely to support a different cancer classification for spiropidion based on the lack of precursor findings, immunotoxicity, and reproductive hormonal perturbations in the toxicological database. In summary, the data support the conclusion that spiropidion does not pose a quantitative carcinogenic risk to humans, and a margin of exposure-based approach for human health risk assessment is appropriate for spiropidion. The selection of the NOAEL from the subchronic toxicity study in dogs is protective of any potential effects in rodents, including carcinogenicity. Separate endpoint(s) for cancer effects are not needed for spiropidion because the chronic dietary POD should be predictive for all potential carcinogenic and non-carcinogenic effects. It should be noted here that there are unique regional regulatory requirements including criteria and information needs for assessing risk to compounds. Depending on the intended region for submission, the authors should consult with regulatory authorities to examine how to best use the WoE to fulfil the requirements under the regulatory legislations (e.g., Classification, Labelling and Packaging).

9. Proposed PODs for chronic risk assessment

We propose to use the 90-day dog NOAEL as the POD (15 mg/kg/day) for chronic risk assessment and waive the chronic/carcinogenicity studies in both rat and mouse for spiropidion based on the WoE approach described herein. This approach is protective for all potential thyroid, liver, reproductive, immunotoxicity, hormonal perturbation, and potential carcinogenic effects at higher dose levels (Figure A B.5).

Figure A B.5. Points of Departure (NOAELs) and LOAELs for toxicity studies conducted with spiropidion

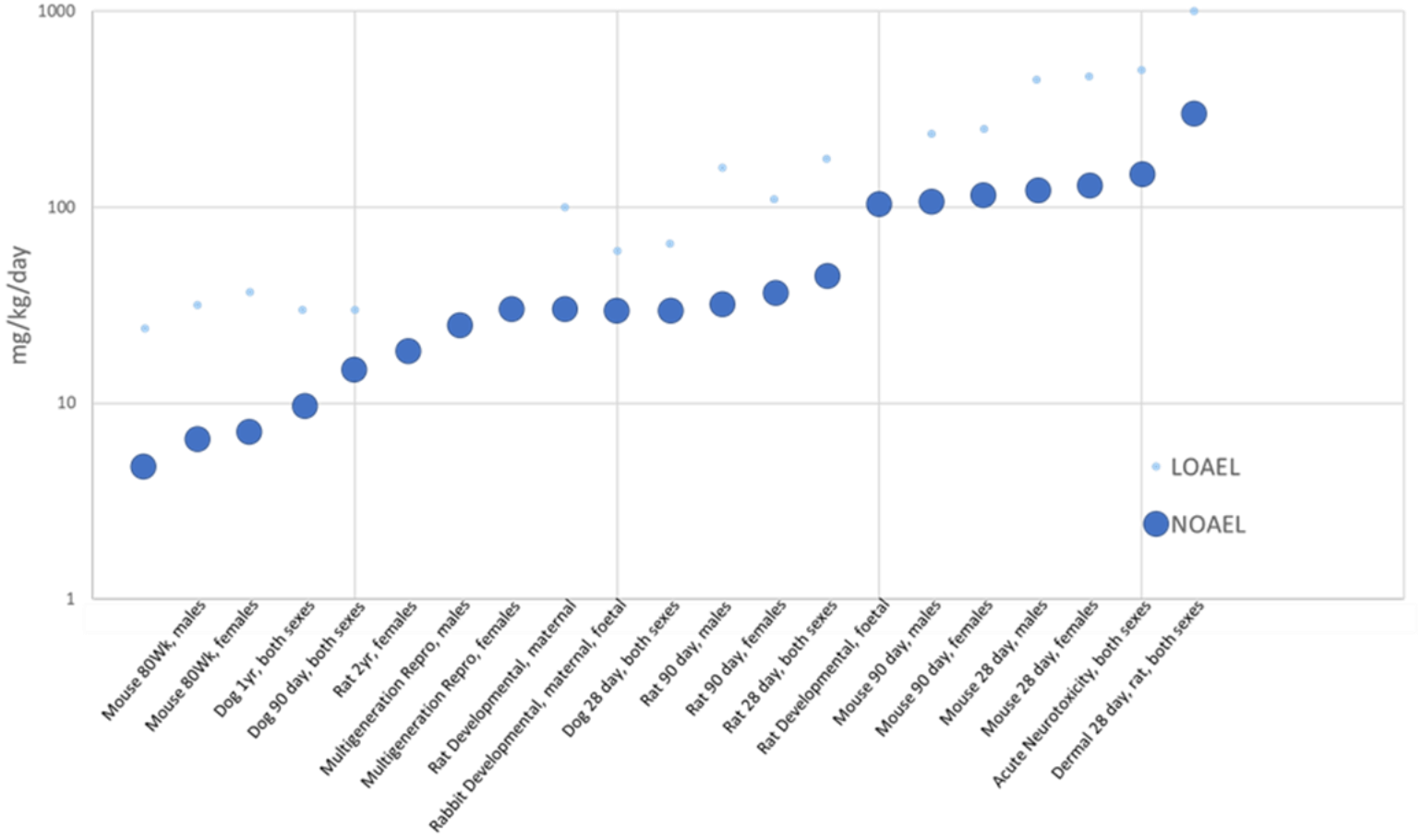
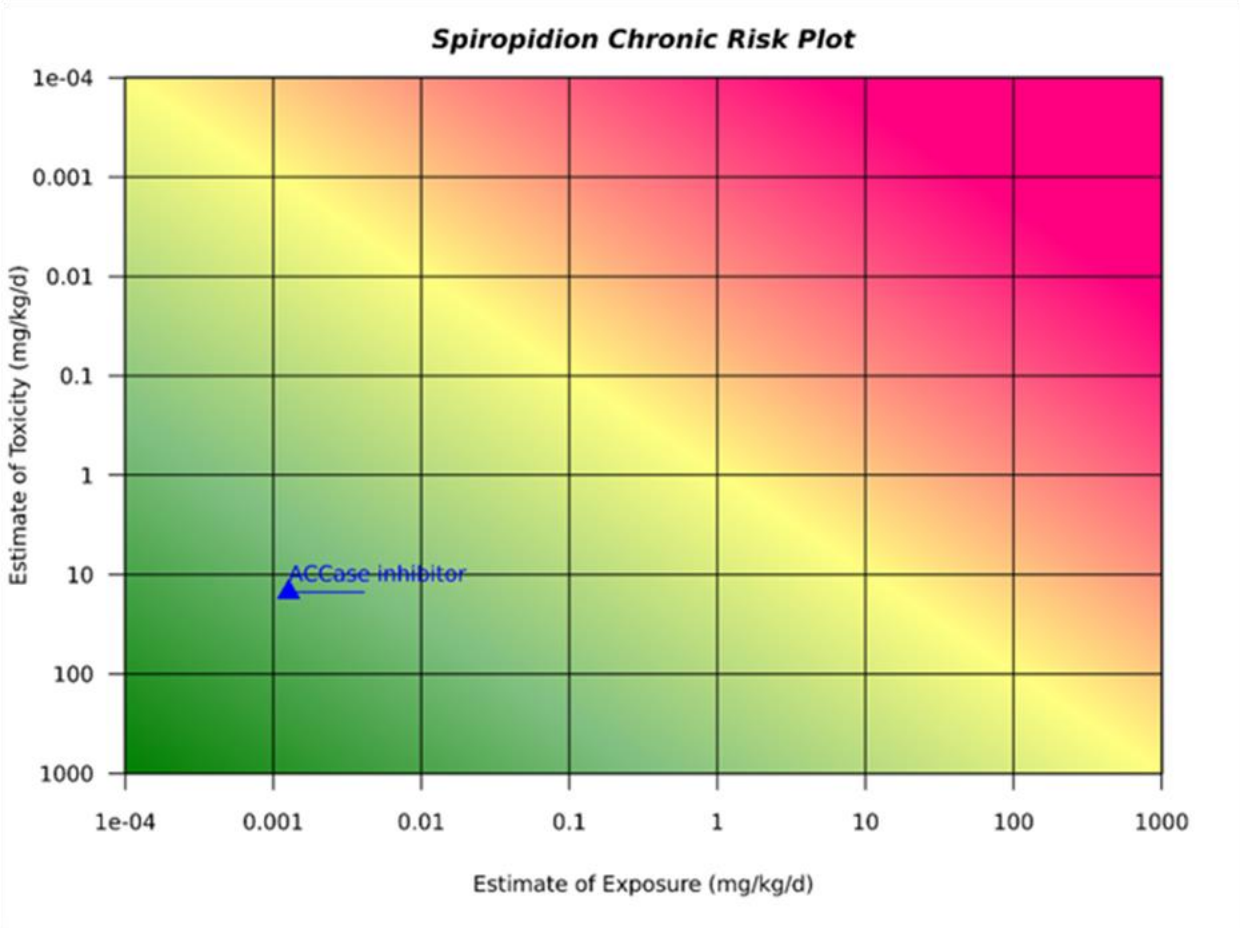


Figure A B.6. RISK21® plot evaluation of available exposure and hazard data for the safety assessment of spiropidion



The yellow line in the RISK21® tool represents the margin of exposure between the 90-day toxicity study NOAEL (as an estimate of toxicity) and the registrant's modelled exposure values (as estimates of exposure) generated in US EPA's DEEM dietary risk software. The Health and Environmental Science Institute (HESI) provides RISK21® tools, which are available online through the following link: https://risk21.org/webtool/

10. Uncertainty

An uncertainty analysis is provided based on categories on an ordinal scale and is presented in Table A B.4. The scale is defined as referring to the quality of the evidence generated/that was available supporting the assumptions of the case study and the overall weight of evidence which is again related to the impact of the uncertainty on the hypothesis. For example, limited and poor-quality evidence is likely to lead to larger uncertainty and vice versa.

When considering the uncertainty of this approach it is important to evaluate that uncertainty against the methodology of traditional human health risk assessment. The currently accepted methodology for conducting human health risk assessment includes an inherent level of uncertainty to which stakeholders have become accustomed. The uncertainty of this traditional approach is generally accepted and

mitigated by using the most sensitive adverse effect, in the most sensitive sex, in the assumed most sensitive species to define the hazard potential. Default uncertainty factors for interspecies or intraspecies toxicodynamic and toxicokinetic differences are additionally applied in accordance with regulatory policy. Through a prospective assessment of spiropidion, the most sensitive effect, in the most sensitive species, in the most sensitive sex has been identified. The POD for risk assessment is supported by read-across compounds that function through the same toxicological mode of action. While Table A B.4 provides a level of certainty in using the IATA, this table is not prescriptive, and the factors assessed for uncertainty will be dependent upon the case.

Table A B.4. Level of certainty of this approach

Factor	Uncertainty (low, medium, high)	Impact of uncertainty on hypothesis
Uncertainty due to the lack of rodent chronic toxicity/carcinogenicity studies with spiropidion	Low	This case study demonstrates that rodent chronic toxicity/carcinogenicity studies are not always necessary to fulfil regulatory data needs for agrichemical safety assessment. In the case of spiropidion, the data available combined with the testing results of read-across compounds and what is known and understood regarding the UDP-GT MoA/AOP is sufficient to derive a chronic oral POD for spiropidion without the chronic toxicity/carcinogenicity studies.
Weight of the evidence-based approach	Low	The approach used in the case study included a WoE approach. While there are various guidance structures for WoE in the context of specific decisions, there is no specific guidance for the current use. The authors included lines of evidence, information that was pertinent and relevant, to assess the potential for carcinogenicity and knowledge gaps; supporting a conclusion that additional rodent chronic toxicity/carcinogenicity studies were not needed to derive a POD to estimate chronic risk.
Lack of measurement of hormones	Medium	An endocrine assessment has been completed for spiropidion based on the Data Evaluation Records (DERs) generated by the US EPA (2022). Under the United States FIFRA, the EPA concluded, based on the available data, that spiropidion does not result in toxicity via an endocrine MoA and thus not relevant for the selection of endpoints for risk assessment (US EPA, 2022, 2023). The lack of hormone measurement does not affect the WoE assessment or outcome because a hormonal MoA relevant to carcinogenicity was limited to thyroid, for which mechanistic data are available to address human non-relevance and/or justification for a margin of exposure-based approach for chronic risk assessment. Effects due to perturbations of reproductive hormones were considered adequately evaluated by the US EPA in the toxicological database, including the repeated dose, reproductive, developmental and ToxCast data. Medium uncertainty is reported because hormone perturbation was not assessed in accordance with other regulatory legal frameworks (e.g., Delegated Regulation on Classification, Labelling and Packaging (CLP) of chemicals).

Lack of details immunotoxicity endpoints	Low	For immunotoxicity, the human-relevant mode of action for carcinogenicity concern is immunosuppression. The available data for spiropidion and surrogates support that there is no immunosuppression and that general immunotoxicity would not be used for endpoint selection in carcinogenicity assessment and risk assessment. The US EPA waived the conditional requirement for a short-term immunotoxicity study based on the absence of consistent effects on immune-related parameters in the available repeat-dose studies with spiropidion and the lack of immunotoxicity with other insecticidal compounds in the same class of chemistry as spiropidion (US EPA, 2022). Further evaluation of immunotoxicity would not impact the determination that immunosuppression is not a relevant carcinogenic MoA for spiropidion.
QSAR modelling - Domain of applicability	Not applicable	ToxPrints was used to identify biological properties of the chemicals and modes of action in the toxicity pathways, to allow refinement of the relevant analogues by structural and bioactivity similarities (Yang et al., 2015). The data for spiropidion and read-across analogues was derived from toxicological and mechanistic research studies – therefore QSAR modelling was not used for the prediction of general toxicity..
QSAR modelling – to address data gaps for endocrine endpoints	Low	An endocrine assessment has been completed for spiropidion in the light of the requirement for the United States FIFRA in 2006-2007. A conclusion was made, based on the available data, that spiropidion does not result in toxicity via an endocrine MoA and thus not relevant for selection of endpoints for risk assessment. The lack of QSAR modelling to address oestrogen, androgen, steroidogenesis or thyroid MoAs will not impact the WoE or outcome of this assessment because a hormonal MoA relevant to carcinogenicity was limited to thyroid, for which mechanistic data are available to address human non-relevance and/or justification for a margin of exposure-based approach for chronic risk assessment. However, hormone measurements were not measured in the data package for spiropidion and there was no comparison of enzyme induction in different species, including humans (*in vitro* assay, as would be required by current practice) to exclude human relevance.
Literature review (grey and white) compared to a systemic literature search	Medium	No literature research was conducted for the new active substance as the compound is new. No systematic literature search with reporting was performed. Grey literature was limited to reviews conducted by the US EPA and JMPR. A literature search through PubMed was conducted but no useful publications were identified.
Read-across – analogue selection	Medium	Although structurally similar read-across compounds were identified within the pMoA ACCase inhibitors, there was no common MoA except for the UDP-GT MoA for spiromesifen and spiropidion. This was based on an evaluation of the publicly available toxicological datasets for all ACCase compounds, which demonstrated slightly different target organs between the chemical classes. The similarity of the analogue can be taken into consideration during the selection of key endpoints, if necessary, but the approach used in this case study was to use the read-across compounds to evaluate the potential for toxicity with spiropidion as precursor effects in the toxicological database. The use of the read-across compounds was used in this case to decrease the

		uncertainty of chronic toxicity and carcinogenicity with spiropidion. The primary reasons for refining the list of read-across analogues registered by the US EPA was 1) differences in the bioactivity of the chemistries (e.g. the aryloxyphenoxypropionate (FOPs) are peroxisome proliferators thus excluded from the read-across) and 2) assessment by a singular agency (to reduce variability in conclusions). For this case study, the phenylpyrazolin (DENs) pinoxaden was also excluded from the read-across because of the difference in target organs of toxicity with the TAs/TADs thus a less relevant analogue. This was an *a priori* decision made by the team to efficiently work through the case example, but a different decision could be made by others. Additionally, a search for structurally similar analogues beyond the class of chemistry with a similar toxicity mode of action was not assessed. This too, should be a consideration to support the justification for the selection of source analogues.
Mechanism of action	Low	The thyroid UDP-GT MoA was shared between spiropidion and spiromesifen. No other common MoA was identified for the TAs/TADs. Other MoAs were not present as demonstrated by the lack of precursor effects with spiropidion. The endpoint for risk assessment was protective of the thyroid UDP-GT MoA.
US based references	Low	This example case study was developed in the context of using information submitted to fulfil the United States Federal Insecticide Fungicide and Rodenticide Act. The acceptability of the studies and the interpretation of the studies were those of the EPA (2022).
Reliability and acceptability of the data	Low	This example case study was developed in the context of using information submitted to fulfil the United States Federal Insecticides Fungicide and Rodenticide Act. JMPR and PMRA also reviewed and interpreted the available studies. The acceptability of the studies and the interpretation of the studies were those of the Regulatory Agencies.
Analysis of studies per current OECD guidelines	Low	This example case study was developed in the context of using information submitted to fulfil the United States Federal Insecticide Fungicide and Rodenticide Act. The acceptability of the studies generated for spiropidion, spirodiclofen, spiromesifen, and spirotetramat and the interpretation of the studies were those of the Regulatory Agencies ECHA, EFSA, JMPR and the US EPA, as generated between 2007-2023. All data generated for spiropidion and read-across analogues were conducted in accordance to Good Laboratory Practice following OECD guidelines.
Concordance and weight of evidence of all data used for justifying the hypothesis	Low	The targeted toxicity data for the compound of interest combined with data from read-across compounds was assessed in a consistent manner to provide sufficient information to address the potential for chronic toxicity and carcinogenicity. The endpoint selected was protective of key events in the identified thyroid UDP-GT MoA.
Overall uncertainty of the IATA	Medium	The process described and the spiropidion case study provides an example of a WoE approach for waiving both the rat chronic toxicity/ carcinogenicity and the mouse carcinogenicity studies.

Uncertainty ranking key: low = uncertainty is not a concern and will not affect use of IATA for regulatory decisions; medium = uncertainty is a moderate concern may affect use of IATA for regulatory decisions on a case-by-case basis; high = uncertainty is a concern, and needs to be addressed.

11. WoE Based Assessment

The goal of this assessment was to use the available toxicological data for spiropidion as well as read-across information to determine if rodent chronic toxicity and carcinogenicity studies are needed to estimate a POD that is health protective for chronic toxicity and carcinogenicity. Spiropidion is an ACCase inhibitor and acts as an insecticide via the inhibition of the enzyme acetyl-CoA carboxylase, which catalyses the ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA, the first step in the synthesis of fatty acids. Three TAs/TADs ACCase inhibiting insecticides were identified as the most appropriate relevant read-across compounds based on structural similarity, pMOA, and similarity of biological effects and toxicological endpoints (NOAEL/LOAELSs) from the other ACCase inhibitor pesticides. The toxicology properties of the TAs/TADs ACCase-inhibiting insecticides were evaluated in this document to provide evidence and inform the need for chronic toxicity and carcinogenicity studies in rats and mice to assess risks following chronic exposure to spiropidion.

Based on a WoE-based approach, the case study developer proposes that the rat and mouse chronic/carcinogenicity bioassays not be required at this time for spiropidion. Note, that while the author of the WoE case study proposes that chronic toxicity/carcinogenicity studies are not needed to support a health-protective POD, this is not a reflection of a regulatory decision. The regulatory interpretation of this case study would be dependent on a formal data submission and review, which was out of scope for this prospective exercise.

To summarize, here are the observations from conducting the WoE-based assessment including data gaps and uncertainties:

- Spiropidion and the analogues selected for the read-across assessment were classified as non-genotoxic.
- The key toxicological effect of spiropidion in sub-chronic oral studies in the rat, mouse, and dog included liver effects in mice, liver and thyroid effects in rats, and clinical effects in dogs. (Table A A.8). In dog studies, adverse clinical signs included salivation, unsteady on feet, ataxia, subdued, twitching, abnormal breathing, uncoordinated, and unaware of surroundings. The NOAEL values from 90-day studies identified the point of departure in the dog studies as the health-protective endpoint for risk assessment (NOAEL for dog, rat, and mouse were 15, 31.5, and 105 mg/kg/day, respectively). The NOAEL from the 90-day study in dogs was the lowest NOAEL between the tested species, supporting the selection of 15 mg/kg/day as the relevant POD for risk assessments.
- The relevant carcinogenic MoA for spiropidion is the liver-induced UDP-GT MoA for thyroid effects This MoA is shared with spiromesifen. Mechanistic studies addressed the key events in this MoA, including the induction of UDP-GT enzymes. Spiropidion did not inhibit rat thyroid peroxidase activity *in vitro*, thus supportive evidence that there is no direct effect on the thyroid. The available data support a non-genotoxic, threshold-based carcinogenic MoA, This MoA and the quantitative lack of relevance to humans is well understood and documented in the peer reviewed literature (Dellarco et al., 2006; Hard, 1998; Bartsch et al, 2018; Oppenheimer, 1979; Larsen, 1982; Boring et al., 1994; Chen, 1984; McClain, 1992) and supports the use of a threshold-based POD for chronic toxicity and carcinogenicity risk evaluation. The endpoint selected from the dog study of 15 mg/kg/day is protective of the induction of hepatic UPD-GT, which was demonstrated at 110 mg/kg/day in rats.

- Spiropidion does not have an estrogenic/androgenic hormonal mode of action and no adverse effects relevant to this MoA were reported in the toxicological database including the subchronic, reproductive, and developmental toxicity studies as well as available ToxCast data. The read-across compounds were evaluated for this potential and effects reported were not found in the toxicological database with spiropidion.
- Spiropidion is well absorbed and rapidly excreted. The predominant biotransformation pathway observed for spiropidion was via rapid and complete ester hydrolysis of the ethoxy carbonyl moiety to form the enol metabolite SYN547305. There were no significant sex- related differences in metabolic profiles and the toxicokinetic data support that there was no differences irrespective of dose or sex. As there is rapid absorption and complete excretion of spiropidion, with very small amount of radioactivity (<2% AD) found in the tissues and carcass, there is no accumulation of spiropidion in tissues and doses can be considered as individual exposure events with the amount of spiropidion biologically available transiently following exposure. This indicates no toxicokinetic increase in toxicity over time from subchronic to chronic exposure (Hilton et al., 2022). The read-across compounds all had similar rapid and complete excretion profiles, supporting this conclusion.
- Analysis of compounds for which immunotoxicity studies did not impact the human health risk assessment. Overall, there is no evidence that spiropidion or other compounds in this class of chemistry directly target the immune system (EPA, 2013b).
- For the TAs/TADs, spiromesifen and spirotetramat were classified by EPA or/and JMPR as “not likely” or “unlikely” to be carcinogenic to humans. Spirodiclofen was classified as ‘Likely to be carcinogenic to humans’ by the EPA based on increased liver tumours in mice and increased testes Leydig cell adenoma in male rats and increased uterine adenoma/adenocarcinoma in female rats. Spirodiclofen demonstrated effects on reproductive organs in the reproductive toxicity and 90-day toxicity studies, supporting the findings in the chronic toxicity and carcinogenicity study with spirodiclofen. Precursor effects with spirodiclofen were identified for the liver and Leydig cell effects. However, precursor effects in the uterus were not identified, the uterine tumours were present concurrently with other tumour types and systemic toxicity. Spirodiclofen is not genotoxic. Spiropidion did not demonstrate effects on reproductive organs in any of the available studies. Precursor effects observed in the subchronic studies with spiropidion included an increase in liver weights in the subchronic study in mice, and follicular cell hypertrophy and colloid contraction in the thyroid glands in the subchronic study in rats. Based on these observed effects, mode of action research was initiated to better understand the findings. A limited increase in toxicity over time was demonstrated when evaluating the progression of toxicity from the subchronic to chronic duration studies. The toxicokinetic data on the TAs/TADs support this lack of progression. Based on the MOA research and a health-protective threshold for the biological effects, the subchronic dog study point of departure is considered protective for the chronic dietary risk assessment of spiropidion.
- In summary, the data support the conclusion that spiropidion does not pose a carcinogenic risk to humans, and a margin of exposure-based approach for human health risk assessment is appropriate for spiropidion. This is the same conclusion reached by the US EPA in their evaluation of spiropidion, which included the rat combined carcinogenicity study and mouse carcinogenicity study (EPA 2022, 2023).

A POD for chronic risk assessment is proposed based on a 90-day dog study NOAEL, which is protective of other effects in the toxicological database reported at higher dose levels.

12. Retrospective assessment

In its human health risk assessments, which included the rat and mouse chronic toxicity and carcinogenicity studies, EPA used the same POD as this assessment of 15 mg/kg/day for the chronic risk assessments with spiropidion, based on the 90-day subchronic and 1-year chronic oral dog study and a total 100-fold uncertainty factor (EPA 2022, 2023). In our assessment, the POD of 15 mg/kg/day value was estimated based on the lowest 90-day study NOAEL between rats, mice and dogs with an additional 10-fold uncertainty factor for extrapolation from a subchronic study to chronic exposures (1000X). The use of the additional 10-fold UF to derive an estimated chronic reference dose (cRfD) for spiropidion from the 90-day study NOAEL was shown to be quite conservative, since the actual NOAEL in long-term studies (15 mg/kg/day) was the same as the lowest 90-day NOAEL (15 mg/kg/day). EPA also determined that spiropidion was not likely carcinogenic to humans based on the lack of tumours in the mouse and rat carcinogenicity studies and lack of mutagenicity.

The JMPR (2021) review of spiropidion concluded that the equivocal Increase in Leydig cell tumours was treatment-related in the rat chronic toxicity/carcinogenicity study. The potential for spiropidion to result in Leydig cell effects was evaluated, based on the effects of the tAs/TADs. The incidence of interstitial (Leydig) cell adenomas was not statistically significant, lacked a dose-response relationship, and was within the historical control data range for this age and strain of rats at the CRO Lab as well as the global RITA database. Based on the nature of this commonly observed finding in this strain and age of rats, the incidence of Leydig cell adenomas was considered not to be treatment-related in the combined chronic toxicity & carcinogenicity study in rats with spiropidion (SYN546330).

The chronic POD from the JMPR (2021) was 2.4 mg/kg bw/day from the from the rat chronic toxicity/carcinogenicity study. Based on the use of the additional 10-fold UF to derive a cRfD or acceptable daily intake (ADI) for spiropidion from the 90-day study, the NOAEL assigned in this evaluation from the dog study was still quite conservative, since the chronic POD assigned by JMPR of 2.4 mg/kg/day was less than 6.25-fold lower than the lowest 90-day NOAEL (15 mg/kg/day). JMPR (2021) also concluded that spiropidion “was unlikely to be genotoxic” and “In view of the lack of genotoxicity, the absence of carcinogenicity in mice and the fact that only an equivocal increase in testicular interstitial cell adenomas and ependymomas were seen in male rats, the Meeting concluded that spiropidion is unlikely to pose a carcinogenic risk to humans from the diet.”

13. Strategy and Integrated Conclusion

The strategy developed within the framework addresses the need for the reduction in animal testing through a WoE assessment of the available data to determine if a POD can be estimated that is protective for chronic toxicity and carcinogenicity. The purpose of rodent chronic toxicity and carcinogenicity studies is to identify tumorigenic potential in rodents and assess relevance to humans (hazard identification) (ICH, 2022). In addition, the results of rodent chronic toxicity and carcinogenicity studies are assessed in terms of the entire toxicological database to identify endpoints for human health risk evaluation. The strategy is based on the ability to use read-across. The key to this strategy is the ability to identify the genotoxic potential of chemicals, including QSAR modelling early in the process of screening agrichemical candidates during the research phase It should be noted that genotoxicity testing was limited when the requirements for rodent carcinogenicity were first issued to fulfil regulatory safety assessment. In 2023, our understanding of pesticidal and toxicological modes of action and key events, carcinogenicity MoAs and key events (including precursor effects) allows us to have confidence in this strategy. Toxicological data generated on the agrichemical, excluding the rodent chronic toxicity/carcinogenicity studies, allow for the identification of endocrine MoAs, and immunotoxicity MoAs. For spiropidion, the key target organ is the liver with

secondary thyroid. In addition, chronic toxicity and carcinogenicity studies exist on other TAs/TADs ACCase inhibitors and were incorporated to support the WoE. This strategy is supported by FDA's guidance for the need of long-term rodent carcinogenicity studies (ICH, 2022) and relevant peer-reviewed literature (Cohen et al. 2019; Boobis et al. 2016; Corvi et al. 2017; Doe et al. 2019; Hilton et al., 2022; Reddy et al., 2010; Sistare et al., 2011).

Uncertainties can be further addressed in subsequent case studies that incorporate more data availability, including mechanistic data to inform adverse outcome pathways, and read-across, while chronic toxicity/carcinogenicity studies are available. Additionally, uncertainties will be better addressed through discussions related to the development of guidance (e.g., sufficiency of data to support WoE).

The process described in this document, and the case study discussed in this IATA, provide a template and an example, that demonstrates the use of a WoE approach for waiving both the rat chronic toxicity/carcinogenicity and the mouse carcinogenicity studies. The spiropidion case study is based on available existing toxicology and read-across data on spiropidion and read-across compounds. The spiropidion case study indicates a low likelihood of a quantitively human-relevant tumour response and the availability of subchronic values for use in risk assessments that are protective of both chronic effects and any potential carcinogenic effects. The same conclusions reached in this document on the lack of potential for carcinogenicity were also determined by the EPA (2022, 2023) and the lowest POD from the subchronic toxicity testing with an additional 10X UF was protective of the chronic POD selected by the EPA (2022, 2023) and JMPR (2021).

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15. Tables

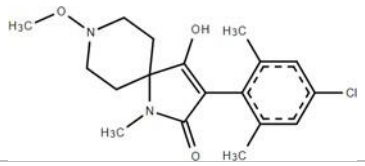
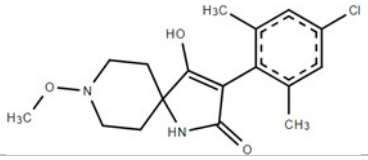
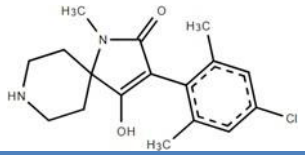
Table A B.5. Physical-chemical properties of spiropidion

Parameter	Value
Molecular weight (g/mole)	422.9
Physical state at room temperature	Off-white powder
Melting point	134.3°C
Boiling point	thermal decomposition before boiling
Density at 20 °C	1.28 g/cm³
Water solubility (25 °C)	46 mg/L
Vapor pressure (25 °C)	< 5 x 10^{-6} Pa (< 3.75 x 10^{-8} mmHg)
Henry's law constant at 25 °C (volatility)	< 46 x 10^{-6} Pa m³/mol
Octanol/water partition coefficient Log (P_{ow}) at 25 °C	3.2

References: US EPA, 2022; PPDB, 2023

Table A B.6. Summary table of toxicokinetic parameters and metabolism in rats for spiropidion

Parameter	Group1	Group2
Dose level (oral, mg/kg)	**5 mg/kg**	**250 mg/kg**
Absorption	69-86%	
% in Urine	57-61% for low dose, 49-53% for high dose	
% in Feces	29-36% for low dose, 45-47% for high dose	
% Biliary	15% for low dose; 13% for high dose	
Major metabolites	SYN547305, SYN547435, and SYN548430 (urine and faeces)	

T_{max}	2 hours [PH-^{14}C]-spiropidion: 1 hour	4 hours (males); 2 hours females [PH-^{14}C]-spiropidion: 1 hour
StructureofSYN547305		
StructureofSYN547435		
StructureofSYN548430		

References: US EPA (2022); JMPR (2021)

Table A B.7. Acute toxicity of spiropidion

Study	Result	Toxicity Category (US EPA)
Acute oral – rat	LD50 > 2000 mg/kg bw	III
Acute dermal – rat	LD50 > 5000 mg/kg bw	IV
Acute inhalation – rat	LC50: > 1.12 mg/L	III
Acute eye irritation – rabbit	Mildly irritating	IV
Acute dermal irritation – rabbit	Non-irritating	IV
Skin sensitization – mouse	Skin sensitizer EC3 value: 0.13%	Sensitizer
Phototoxicity – BALB/c 3T	Not phototoxic	Not phototoxic

References: US EPA, 2022; JMPR, 2021

Table A B.8. Sub-chronic toxicity studies of spiropidion

Study (Guideline(s), Reference(s))	Doses	Results
28-Day Oral, Mouse (OPPTS 870.3100)	Dietary Concentration: 0, 250, 700, 2500 Intake Dose: M: 0, 42.1, 117, 449; F: 0, 45.2, 126, 465	NOAEL 117/126 (M/F); LOAEL 449/465 (M/F) Premature deaths, ↓ body weights

13-week Oral, Mouse (OPPTS 870.3100) (MRID 51136210)	Dietary Concentration: 0, 250, 700, 1500 Intake Dose: M: 0, 35.2, 105, 236; F: 0, 44.1, 115, 252	NOAEL 105/115 (M/F); LOAEL 236/252 (M/F) In males: ↑ liver weight, urea, and BUN; ↓ food utilization. In females: ↑ alkaline phosphatase; ↓ albumin and albumin/ globulin ratio In males and females: ↓ BW gain
28-Day Oral, Rat (OPPTS 870.3050) (MRID 51136206)	Dietary Concentration: 0, 500, 2000, 3000 Intake Dose: M: 0, 44, 177, 259; F: 0, 44, 178, 264	NOAEL 177/178 (M/F); LOAEL 253/264 (M/F) Decreased bodyweight in both sexes.
13 Week Oral, Rat (OPPTS 870.3100) (MRID 51136207)	Dietary Concentration: 0, 100, 500, 2500 (M)/1500 (F) Intake Dose: M: 0, 6.2, 31.5, 159; F: 0, 7.0, 36.1, 110	NOAEL 31.5/36.1 (M/F); LOAEL 159/110 (M/F) In males and females: ↑incidence and severity of follicular cell hypertrophy and colloid contraction of the thyroid
28 Day Oral, Dog (OPPTS 870.3050) (MRID 51136212)	Intake Dose (Capsule): 0, 10, 30, and 2x does at 100 followed by 26-day washout then 4x doses at 65 mg/kg/day.	NOAEL 30 (both sexes); LOAEL 100/65 (M/F) One male and 2 female animals at 100 mg/kg/day or 100/65 mg/kg/day were euthanized due to adverse clinical observations (subdued behaviour, unsteady on feet, uncoordinated, and unaware of surroundings). Clinical signs were observed on Day 2 in all dogs at 100 mg/kg/day and in all dogs at 65 mg/kg/day.
13-week Oral, Dog (OPPTS 870.3150) (MRID 51136213)	0, 5, 15, 30 mg/kg/day (Capsule)	NOAEL 15 (both sexes); LOAEL 30 (both sexes) Clinical signs beginning on Day 13 (including unawareness of surroundings, body tremors, no coordination of the hind limbs, and subdued behaviour) leading to human euthanasia.
28-day dermal, Rat (OPPTS 870.3200) (MRID 51174625)	100, 300, 1000 mg/kg/day	NOAEL 300; LOAEL 1000 (both sexes) Decreased ↓BW in both sexes; in female thyroid gland, minimal follicular cell hypertrophy

References: US EPA, 2022; JMPR, 2021

Table A B.9. Genetic toxicity studies for spiropidion

Study Type (Guideline)	Results
In vitro gene mutation test Ames: Salmonella typhimurium (TA100, TA98, TA1535 and TA1537) and Escherichia coli (WP2 and WP2uvrA) (OPPTS 870.3200)	Negative
In vitro gene mutation test Ames: Salmonella typhimurium (TA100, TA98, TA1535 and TA1537) and Escherichia coli (WP2 and WP2uvrA) (OPPTS 870.3200)	Negative

In vitro Mammalian Chromosome Aberration Test (V79 Cells) (OPPTS 570.5375)	Positive
In vitro forward mutation assay Mouse lymphoma L5178Y cells, TK locus (OPPTS 870.5300)	Negative
In vitro micronucleus Human lymphocytes (OECD 478)	Negative
In vivo micronucleus Rat (Wistar) (gavage) (OPPTS 870.5395)	Negative
In vivo micronucleus Rat (Wistar) (gavage) (OPPTS 870.5395)	Negative
In vivo chromosome aberration Rat (Wistar) (gavage) (OPPTS 870.5395)	Negative

Reference: US EPA, 2022; JMPR, 2021

Table A B.10. Studies providing data relevant to hormone perturbation for spiropidion

Study (Guideline(s), Reference(s))	**Doses**	**Results (mg/kg/day)**
Reproduction and Fertility - Rat (OPPTS 870.3800) (MRID 51174674)	Dietary: 0, 50, 100, 500/300 (M/F) mg/kg/day P: 0, 3/4, 6/8, 31/24 (M/F) mg/kg bw/day F1: 0, 4/5, 8/10 39/28 (M/F) mg/kg bw/day	Parental NOAEL = 31/39 Males P/F1 NOAEL = 24/28 Females P/F1 LOAEL = Not observed Development NOAEL = 24/28 Females P/F1 LOAEL = Not observed Reproduction NOAEL = 31/39 Males P/F1 NOAEL = 24/28 Females P/F1 LOAEL = Not observed No adverse effect
Tolerability (7-days)– Rat, gavage	0, 200, 150, 75 mg/kg bw/day	Suitable high dose (repeat dose studies): 75 or 150 mg/kg bw/day. Based on ↓food intake, ↓body weight at 200, slight decrease at 150 and 75
Preliminary Developmental – Rat	0, 25, 75, 100, 125, 150 mg/kg bw/day	↓BW, ↓BW gain, ↓food intake leading to early termination at 125 and 150; no maternal effects at 25; no foetal effects at 25 or 75 (all mg/kg bw/day)
Developmental – Rat, gavage (OPPTS 870.3700) (MRID 51174638)	0, 10, 30, 100 mg/kg bw/day	Maternal NOAEL = 30; LOAEL = 100 Based on initial body weight loss, ↓body weight gain, ↓food intake. Foetal NOAEL = 100 LOAEL = >100. No adverse effect
Tolerability – Rabbit, gavage	0, 50, 85, 125, 250 mg/kg bw/day	Suitable high dose (repeat dose studies): 50 or 85 mg/kg bw/day. Based on marked BW loss at >125; ↓BW loss > 50; ↓food intake at 85 mg/kg bw/day.
Preliminary developmental – Rabbit, gavage	0, 15, 50, 75 mg/kg bw/day	↓body weight gain, ↓food intake associated with ↓group mean foetal weights at 75; no maternal effects at 15 and no foetal effects at 15 or 50; ↓food intake at 50 mg/kg bw/ day.

Developmental – Rabbit, gavage (OPPTS 870.3700) (MRID 51174640)	0, 10, 30, 60 mg/kg bw/day	Maternal NOAEL = 30; LOAEL = 60 Based on initial body weight loss, ↓body weight gain, ↓food intake. Foetal NOAEL = 30; LOAEL = 60 Based on ↓mean foetal weight and slight developmental delay.

References: US EPA, 2022; JMPR; 2021

Table A B.11. Studies providing data relevant to immune suppression for spiropidion

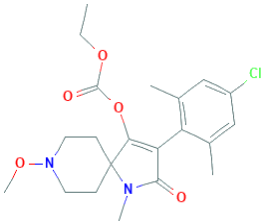
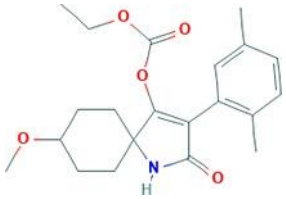
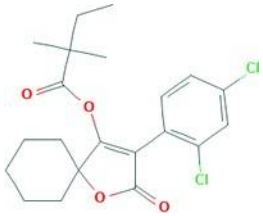
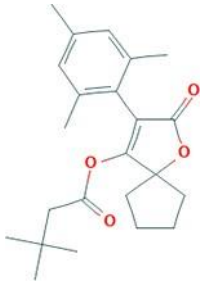
Study (Guideline(s), Reference(s))	Doses	Results, related to immune suppression
Spirotetramat Immunotoxicity Study (OPPTS 870.7800) (MRID 48525901) (US EPA, 2017)	Acceptable/guideline 500, 2500, or 12,000 ppm 33, 164, or 795 mg/kg/day	The functional immunotoxicity NOAEL is 12,000 ppm (equivalent to 795 mg/kg/day, the highest dose tested); the LOAEL was not established. The systemic toxicity LOAEL for spirotetramat in male rats is 12,000 ppm (equivalent to 795 mg/kg bw/day) based on decreased body weights, lowered food consumption, lower thymus weight, and observations of atrophic thymus. The NOAEL for systemic toxicity is 2500 ppm (equivalent to 164 mg/kg bw/day).
Spirodiclofen Immunotoxicity Study (OPPTS 870.7800) (MRID 49072601) (US EPA, 2021)	Acceptable/guideline 0, 500, 1500, 5500 ppm 0, 108, 334, 1216 mg/kg/day	NOAEL = 1216 mg/kg/day LOAEL = not established
Spiromesifen Immunotoxicity Study 4-Week Immunotoxicity (rat) (OPPTS 870.7800) (MRID 45819608) (US EPA, 2020)	Acceptable/guideline 0, 100, 500, or 3000 ppm M: 0, 9.6, 52.8, 291.6 mg/kg/day F: 0, 10.7, 45.7, 288.6 mg/kg/day	NOAEL = 52.8/45.7 mg/kg/day [M/F]. LOAEL = 291.6/288.6 mg/kg/day [M/F] Based on mortality (females), clinical signs (both sexes), and decreased body weights and body-weight gains (both sexes).

Spiromesifen Immunotoxicity Study Plaque-forming cell assay (mouse) (OPPTS 870.7800) (MRID 45819615) (US EPA, 2020)	Acceptable/guideline 0, 100, 500, or 3500 ppm M: 0, 30.5, 162.5, 1226.6 mg/kg/day F: 0, 47.9, 278.7, 1510.2 mg/kg/day	NOAEL = 162.5/278.7 mg/kg/day [M/F] LOAEL = 1226.6/1510.2 mg/kg/day [M/F] Based on decreased body weights and body-weight gains (decreased 38%) in males and decreased water consumption and spleen weights in both sexes. Under conditions of this study, no immunotoxicity (as detected by plaque forming cells) was observed.

Table A B.12. Mode of action and mechanistic studies for spiropidion

Study (Guideline(s), Reference(s))	**Doses**	**Results**
Mechanistic study – spiropidion and SYN547305 – Effect on Rat Thyroid Peroxidase Activity *In vitro* (Non-guideline)	0 (DMSO), 0.05, 0.5, 2, 5, or 10 µM	Thyroid peroxidase activity was assayed by determining the monoiodination of L-tyrosine. spiropidion and SYN547305 are not inhibitors of rat thyroid peroxidase activity *in vitro*.
Mechanistic study- Spiropidion 90-day UDP-GT (Non-guideline) (MRID 51136207)	Control and high dose groups from the 90-day oral (dietary) toxicity study 0, 2500/1500 ppm [M/F] 0, 159/110 mg/kg/day [M/F])	Assessment of UDP-GT induction following a 13- week dietary toxicity study in Han Wistar rats. Dietary exposure of male and female animals to spiropidion for 13 weeks at dietary inclusion levels of 2500 and 1500 ppm for males and females, respectively resulted in statistically significant increases in the rate of T4 glucuronidation. In males, a 2.5-fold increase was observed whilst in females a 2-fold increase was observed. spiropidion is an inducer of hepatic UDP-GT activity in both male and female Han Wistar rats.

Table A B.13. Summary table: Physical-chemical properties and acute toxicity for read-across chemical analogues.

	Target	SourceAnalogue1	SourceAnalogue2	SourceAnalogue3
Chemical Name	Spiropidion	Spirotetramat	Spirodiclofen	Spiromesifen
CASRN	1229023-00-0	203313-25-1	148477-71-8	283594-90-1
2DStructure				
Chemical Class	Tetramic acid derivative	Tetramic acid derivative	Tetramic acid derivative	Tetramic acid derivative
Physical form at 25°C				
MW (g/mole)	422.9			
Physical Properties:				
Melting Point	134.3 °C			
Boiling Point	thermal decomposition before boiling.			
Vapor Pressure	< 5 x 10^{-6} Pa			
pKa	4.41			
Log Kow	3.2 (log Pow)			
Acute toxicity (oral, rat unless indicated)	LD50 > 2000 mg/kg	LD50 > 2000 mg/kg	LD50 > 2000 mg/kg	LD50 > 2000 mg/kg

Skin irritation (EPA class)	Not an irritant (IV)	Not an irritant (IV)	Not an irritant (IV)	Not an irritant (IV)
Eye irritation (EPA class)	Minimal irritation (IV)	Minimal irritation (III)	Not an irritant (IV)	Not an irritant (IV)
Skin sensitization	Skin sensitizer EC3 value: 0.13%	Positive	Positive	Potential moderate contact sensitizer

Table A B.14. Summary table: Repeated-dose toxicity and carcinogenicity for read-across chemical analogues

	Target	**Source Analogue 1**	**Source Analogue 2**	**Source Analogue 3**
Chemical Name	Spiropidion	Spirotetramat	Spirodiclofen	Spiromesifen
Genotoxicity (*in vitro*)	Negative, except clastogenic in chromosome ab. test with S9	Negative	Positive clastogenicity in two mammalian cell lines (±S9). Negative for mutagenicity in CHO, Ames, and rat hepatocyte systems. Negative in a mammalian UDS assay	Negative
Genotoxicity (*in vivo*)	Negative (micronucleus and chromosome aberration studies)	Negative	Negative in mouse bone marrow assay; positive in rat liver micronucleus assay. EPA considered not a concern for mutagenicity.	Negative
28-day study	**Mouse:** well tolerated at doses below 2500 ppm **Rat:** minimal follicular cell hypertrophy in the thyroid gland at 2000 ppm and above in both sexes **Dog:** tolerated at dose levels at, below 30 mg/kg/day	Not described in detail. See 90-day summary	Not described in detail. See 90-day summary	Not described in detail. See 90- day summary
	Rat: NOAEL 31.5 (both sexes)	**Rat:**	**Rat, Mouse:** NOAEL 32.1/8.1	**Rat:** NOAEL 31.7/7.7 (M/F)

90-day study	LOAEL 110 (both sexes) In males and females: ↓foot splay, cholesterol, triglyceride, total protein and albumin levels; ↑white blood cell count, alanine aminotransferase; microscopic pathology findings of diffuse follicular cell hypertrophy and colloid contraction in the thyroid glands at >1500 ppm. In females: ↓BW gain and food utilization **Mouse:** NOAEL 105 (both sexes) LOAEL 236 (both sexes) In males: ↑ liver weight, urea, and BUN; ↓ food utilisation. In females: ↑ alkaline phosphatase; ↓ albumin and albumin/ globulin ratio in males and females: ↓ BW gain. **Dog:** NOAEL 15 (both sexes) LOAEL 30 (both sexes) Single female mortality at highest dose tested	NOAEL 148/188 (M/F) LOAEL 616/752 (M/F) Alveolar macrophages in both sexes; in males ↓ BW, abnormal spermatozoa, hypospermia in the epididymis, decreased testicular weight, testicular degeneration and vacuolation. **Mouse:** NOAEL 1305/1515 (M/F) LOAEL not observed **Dog:** NOAEL 81/32 (M/F) LOAEL 72 (both sexes) ↓ food consumption, depressed RBC parameters (red blood cell count, haemoglobin level and haematocrit), and thymus atrophy	(M/F), LOAEL 166.9/47.1 (M/F) Males: ↑ incidence and severity of small cytoplasmic vacuolation in the cortex of adrenal glands, ↓ cholesterol (week 5 and 13), and ↓ triglycerides (wee 5). Females: ↑ incidence of cytoplasmic vacuolation of the adrenal cortex. **Mouse:** NOAEL 15/30 (M/F) LOAEL 164/234 (M/F) Males: ↑ incidence of hypertrophic Leydig cells in the testes. Females: ↑ incidence of cytoplasmic vacuolation of the adrenal cortex. **Dog:** NOAEL 7.7/<8.4 (M/F) LOAEL 26.6/8.4 (M/F) Males: ↓ BW gain, ↑ liver and adrenal weights, ↓ prostate weights, and histopathology findings in the adrenal glands, testes, epididymis, thymus, and prostates. Females: ↑ adrenal gland weight and cytoplasmic vacuoles in the Zona fasciculata.	LOAEL 204.0/36.6 (M/F) Males: effects in the thyroid (colloidal alteration, thyroid follicular cell hypertrophy, decreased T3 and T4, and ↑ TBC and TSH), kidney (Hyaline droplets) and liver (↑ ALP, ALAT). Females: effects in the thyroid (↑ TSH, thyroxine binding capacity, thyroid follicular cell hypertrophy), kidney (mineralization), and liver (↑ ALP).) **Mouse:** NOAEL 11.5/20.3 (M/F) LOAEL 21.7/35.3 (M/F) Discoloured and microscopic changes (↓ in fine vesiculation, and the presence of cytoplasmic eosinophilia in zona fasciculata cells) in the adrenal glands, both sexes. Note: Some of the guideline parameters were not measured. NOAEL derived from second non-guideline study with reduced (only two) dose levels. **Dog:** NOAEL 9.2; LOAEL 71 Effects on clinical chemistry (↑ ALP) and liver histopathology (cytoplasmic changes).
Reproductive Toxicity	No evidence a selective hormonal or reproductive effects (rat). **Note:** added measure of haematology of adults and pups showed changes reflecting anaemia	No evidence a selective hormonal or reproductive effects (rat)	No evidence a selective hormonal or reproductive effects (rat)	No evidence a selective hormonal or reproductive effects (rat)

Developmental	No evidence a selective hormonal or developmental effects (rat, rabbit)	No evidence a selective hormonal or developmental effects (rat, rabbit)	No evidence a selective hormonal or developmental effects (rat, rabbit)	No evidence a selective hormonal or developmental effects (rat, rabbit)
Hormone perturbation	No specific studies.	No specific studies.	No specific studies.	No specific studies.
Immune suppression	No evidence of immune suppression (mouse TDAR study)	No specific studies	No evidence of immune suppression (TDAR study)	No specific studies
Carcinogenicity	WoE-based assessment requested for rat and mouse studies, based on this document.	**Rat**: None Mouse: None EPA class: Not likely	**Rat**: ↑pancreatic adenomas (males). **Mouse**: ↑ hepatocellular adenomas and carcinomas (males). EPA class: Likely (Q1*)	**Rat**: None Mouse: None EPA class: Not likely
Special studies	There was evidence of increased UGT activity in the liver following exposure to spiropidion. Thyroid Peroxidase (TPO) assay demonstrated no direct effects on the thyroid.	No specific studies	No specific studies	No specific studies
References:	EPA, 2022	EPA, 2003	EPA, 2016	EPA, 2006

Abbreviations: CHO = Chinese hamster ovary. HDT = highest dose tested. ALAT = alanine aminotransferase. ASAT = aspartate aminotransferase. SDH = sorbitol dehydrogenase. TDAR = T-cell dependent antibody response. UDS = unscheduled DNA synthesis.

Table A B.15. Detailed sub-chronic effects of read-across chemical analogues

	90-Day Rat	90-Day Mouse	90-Day Dog

	mg/kg/day(males/females)	mg/kg/day (Males/Females)	mg/kg/day(males/females)
Spiropidion	NOAEL 31.5; LOAEL 110	NOAEL 105; LOAEL 236	NOAEL 15; LOAEL 30
Spirotetramat (USEPA,2017).	NOAEL 148/188; LOAEL 616/752 Alveolar macrophages in both sexes; in males decreased BW, abnormal spermatozoa, hypospermia in the epididymis, decreased testicular weight, testicular degeneration and vacuolation.	NOAEL 1305/1515; LOAEL not observed	NOAEL 81/32; LOAEL 72 Decreased food consumption, depressed RBC parameters (red blood cell count, haemoglobin level and haematocrit), and thymus atrophy.
Spirodiclofen (USEPA,2011).	NOAEL 32.1/8.1; LOAEL 166.9/47.1 Males: increased incidence and severity of small cytoplasmic vacuolation in the cortex of adrenal glands, decreased cholesterol (week 5 and 13), and decreased triglycerides (week 5). Females: increased incidence of cytoplasmic vacuolation of the adrenal cortex.	NOAEL 15/30; LOAEL 164/234 Males: Increased incidence of hypertrophic Leydig cells in the testes. Females: increased incidence of cytoplasmic vacuolation of the adrenal cortex.	NOAEL 7.7/<8.4; LOAEL 26.6/8.4 Males: decreased BW gain, increased liver and adrenal weights, decreased prostate weights, and histopathology findings in the adrenal glands, testes, epididymis, thymus, and prostates. Females; increased adrenal gland weight and cytoplasmic vacuoles in the Zona fasciculata.
Spiromesifen (USEPA,2015a, 2018).	NOAEL 31.7/7.7; LOAEL 204.0/36.6 Males: effects in the thyroid (colloidal alteration, thyroid follicular cell hypertrophy, decreased T3 and T4, and increased TBC and TSH), kidney (Hyaline droplets) and liver (increased ALP, ALAT). Females: effects in the thyroid (increased TSH, thyroxine binding capacity, thyroid follicular cell hypertrophy), kidney (mineralization), and liver (increased ALP).	NOAEL 11.5/20.3; LOAEL 21.7/35.3 Discoloured and microscopic changes (decrease in fine vesiculation, and the presence of cytoplasmic eosinophilia in zona fasciculata cells) in the adrenal glands, both sexes. **Note**: Some of the guideline parameters were not measured. NOAEL derived from second non-guideline study with reduced (only two) dose levels.	NOAEL 9.2; LOAEL 71 Effects on clinical chemistry (increased ALP) and liver histopathology (cytoplasmic changes).

NOAEL and LOAEL values (mg/kg/day) are shown for Male/Female.
Abbreviations: ALAT = alanine aminotransferase. ALP = alkaline phosphatase. BW = body weight. RBC = red blood cell concentration. T3 = triiodothyronine. T4 = thyroxine. TBC = thyroxine-binding capacity. TSH = thyroid stimulating hormone

Table A B.16. Detailed carcinogenicity and/or chronic toxicity results, classification and POD for read-across chemical analogues

Read-across Chemical	Carcinogenicity Classification (U.S. EPA)	2-Year Rat Study Results (mg/kg/day)	18-Month Mouse Study Results (mg/kg/day)	Chronic POD (Study, UF)
Spirotetramat (EPA, 2008)	Not likely to be carcinogenic to humans based on lack of evidence in two oral rodent carcinogenicity studies.	No carcinogenicity. NOAEL = 12.5 (M/F) LOAEL = 169 (M/F) Based on ↓ kidney weight and renal tubular dilation.	No carcinogenicity. NOAEL = 1022 (M/F) LOAEL not observed	5 mg/kg/day Dog 1-year UF = 100X
Spirodiclofen (EPA, 2009)	Likely to be Carcinogenic to Humans; Q1* (mg/kg/day)-1 = 1.49 x 10-2	NOAEL = 14.7/19.9 (M/F) LOAEL = 110.1/152.9 (M/F) Increased testes Leydig cell adenoma in males, increased uterine adenoma and/or adenocarcinoma in females ↓ cholesterol and triglyceride levels, ↑ incidences of Leydig cell hyperplasia (males). ↑ TSH, uterus nodules (females) ↓ BW and BW gain, ↑ APh levels and vacuolated jejunum enterocytes	NOAEL = 4.1 (M/F) LOAEL = 610/722 (M/F) Increased hepatocellular adenoma and carcinoma. ↑ liver weight, enlarged adrenal gland, discoloured testis, and interstitial cell degeneration of the testes (males). ↑ incidences of adrenal gland pigmentation (females) ↑ adrenal weights and adrenal vacuolization	1.38 mg/kg/day Dog 1-year UF = 100X
Spiromesifen (EPA, 2008)	Not likely to be carcinogenic to humans	No evidence of carcinogenicity. NOAEL = 14.8 (M/F) LOAEL = 40.0 (M/F) Clinical signs: palpable masses, vaginal bleeding, and pallor. Gross necropsy: discoloured area in the lungs and nodules/dilation of uterus Histopathology: osseus metaplasia and granulomatous inflammation of the lungs in the males, liver necrosis, endometritis/ metritis, endometrial hyperplasia of the cervix uteri, and colloidal alteration of the thyroid gland in females. ↑ TSH in females.	No evidence of carcinogenicity. NOAEL = 3.3 (M/F) LOAEL = 22 (M/F) Gross changes: enlarged adrenal gland in males. Microscopic changes: cytoplasmic eosinophilia, ceroid deposits, and diffuse fatty changes of the adrenal cortex and pancreatic amyloidosis in both sexes.	3.8 mg/kg/day Multigeneration reproduction UF = 1000X

Classification, POD and NOAEL/LOAEL values are based upon U.S. EPA reviews.
POD = point of departure. cPAD = chronic population-adjusted dose. Q1* = cancer potency factor.

Table A B.17. Proposed POD for chronic risk assessment with spiropidion

Exposure Scenario	POD	UF	cPAD or ADI	Study and Toxicological Effects
Chronic Dietary (General Population Including infants & children)	NOAEL = 15 mg/kg/day	UF_A = 10X UF_H = 10X FQPA SF = 1X PCPA SF = 1X	cPAD = 0.01 mg/kg/day cRfD = 0.1 mg/kg/day	Dog 90-day dietary toxicity study NOAEL 15 mg/kg/day based on single female mortality at 30 mg/kg/day.
Cancer (Oral, dermal, inhalation)	Proposed Classification: Not likely to be carcinogenic to humans			

POD = point of departure. NOAEL = no-observed adverse-effect level. LOAEL = lowest-observed adverse-effect level. UF = uncertainty factor. UF_A = extrapolation from animal to human (interspecies). UF_H = potential variation in sensitivity among members of the human population (intraspecies). PAD = population-adjusted dose (a = acute, c= chronic).

The 90-day subchronic toxicity study in the dog was selected as sufficient to evaluate chronic dietary exposure. In the chronic dietary study (capsule), effects observed at the LOAEL (30 mg/kg/day) included clinical signs (decreased activity, hypersensitivity, lying on side, tremors, subdued behaviour, severe salivation and uncoordinated, trying to stand) leading to humane euthanasia (NOAEL = 10 mg/kg/day). In the subchronic study (capsule), effects were based on clinical signs (including unawareness of surroundings, body tremors, no coordination of the hind limbs, and subdued behaviour) leading to humane euthanasia observed at the LOAEL of 30 mg/kg/day (NOAEL = 15 mg/kg/day). Although it was a 90-day study, the endpoints from the subchronic dog study were still considered appropriate for a chronic assessment because they were similar to the findings in the chronic dog study and did not progress over time. Chronic dog studies generally do not provide a different POD than the subchronic dog studies and are no longer required for pesticide registration. This is supported by the similarity between the LOAEL (30 mg/kg/day) in the 90-day oral dog study and the LOAEL (30 mg/kg/day) from the 1-year oral dog study. Additionally, the NOAEL from the subchronic dog study (15 mg/kg/day), which is the selected POD, is protective of effects observed at the LOAEL in both studies (30 mg/kg/day). The cPAD of 0.15 mg/kg/day was derived from a NOAEL of 15 mg/kg/day and a 100X UF (10X interspecies extrapolation, 10X intraspecies variability, 1X FQPA SF).

Appendix A: SUPPLEMENTAL DATA: ACCase Inhibitors Toxicological Read-across for Tetronic and Tetramic Acid Derivatives

A.1 Summary

All of the acetyl CoA carboxylase (ACCase) inhibitor herbicides and insecticides share the same pesticidal mode of action (pMoA), namely inhibition of lipid biosynthesis. The ACCase inhibitor herbicides included several different chemical groups, namely phenylpyrazolin (DENs), cyclohexanedione (DIMs), and aryloxyphenoxypropionate (FOPs) chemistries. Tetronic and tetramic acid derivatives (TAs/TADs) are insecticides with ACCase inhibitor pMoA.

Toxicology data were compiled for 17 compounds across all the ACCase chemical groups and included toxicokinetics, acute, subacute, subchronic, chronic/carcinogenicity, genotoxicity, reproductive and development, hormone perturbation, neurotoxicity, immunotoxicity, and mechanistic studies.

Read-across with the TAs/TADs chemical group compounds was determined to be the most relevant for the assessment of spiropidion. This conclusion was based on both biological and structural similarity. First, biological similarity was defined based on the pesticidal mode of action. The HRAC and IRAC classification schemes were used to identify molecules known to inhibit ACCase. ToxPrints were gathered for all 23 ACCase inhibitors, and among the 729 defined fingerprints, 96 were present in one or more ACCase inhibitors. A principal coordinate analysis was conducted based on the binary distance matrix between each ACCase inhibitor. Additionally, a clustering analysis was conducted. Both approaches clearly indicate grouping of the ACCase inhibitor classes, with spiropidion most similar to other TAs/TADs and distinct from other ACCase inhibitor classes.

For the TAs/TADs, the target organs were the adrenal glands, liver, thyroid, and testes, with effects in the target organs detected following 90 days of oral exposure. The TAs/TADs were not associated with genotoxicity. Spirodiclofen was the only compound classified as likely to be carcinogenic to humans with a Q1* assigned. All other compounds were classified as not likely to be carcinogenic to humans. Two compounds are not associated with any acute adverse effects and two compounds have acute endpoints for general population assigned based on the acute neurotoxicity study or the 28-day oral toxicity study in dogs. For chronic dietary risk assessment, two compounds shared testicular effects in chronic oral toxicity in dogs or the chronic toxicity/carcinogenicity study in rats as the basis for the point of departure, the adverse effects in another study were based on decreased pup body weight in the 2-generation rat reproduction study, and one compound was associated with thymus involution in dogs as the critical effect.

A.2 Preliminary read-across analysis

Acetyl CoA carboxylase (ACCase) inhibitors include herbicides and insecticides belonging to several different chemistry groups, namely cyclohexanedione (DIMs), phenylpyrazolin (DENs), and aryloxyphenoxypropionate (FOPs) chemistries. Tetronic and tetramic acid derivatives (TAs/TADs) are insecticides with similar pMoAs of activity.

There were 23 herbicides and insecticides identified as ACCase inhibitor chemicals. Of these, 14 were registered by the EPA with publicly available Human Health Risk Assessment (HHRA) documents, two had peer-reviewed risk assessments conducted by the European Food Safety Authority (EFSA), and one was included in a Joint Food and Agriculture Organization of the United Nations and World Health Organization Meeting on Pesticides Residues (JMPR) report.

These 17 compounds cover the following chemical classes:

- Phenylpyrazolin (DENs)
- Cyclohexanediones (DIMs)
- Aryloxyphenoxy-propionates (FOPs)
- Tetronic and tetramic acid derivatives (TAs/TADs)

Pinoxaden, a DEN herbicide, is rapidly absorbed by weed foliage and translocated to the growing points of leaves and stems where it inhibits the acetyl CoA carboxylase enzyme.

The DIMs (clethodim, cycloxydim, sethoxydim, tralkoxydim, and tepraloxydim) are post- emergent herbicides whose primary pMoA involves inhibition of lipid biosynthesis and interference with growth regulation. Clethodim is a selective postemergence cyclohexenone herbicide registered for use on a variety of field and vegetable crops for the control of annual and perennial grasses; it does not control sedges or broadleaf weeds. Cycloxydim is used in outdoor foliar spraying against perennial grasses in rape, sugar beet, potato, green bean, field bean and tolerant maize. Sethoxydim is registered for use on turf (e.g. golf courses, recreational parks, home lawns, and sod farms) and ornamentals (residential landscape areas), as well as numerous agricultural crops. Tralkoxydim selectively controls annual grass weeds in cereal grain crops. Tepraloxydim is intended for postemergence control of certain annual grasses and quackgrass.

The FOPs include clodinafop-propargyl, cyhalofop-butyl, diclofop-methyl, fenoxaprop-P-ethyl, fluazifop-P-butyl, propaquizafop, and quizalofop-P-ethyl. Clodinafop-propargyl belongs to the exyphenoxy acid ester class of herbicides and is a liquid post-emergent herbicide used for the control of grass weeds in wheat. Cyhalofop-butyl is a highly selective aryloxyphenoxy-propionate post-emergence herbicide registered for use on rice and wild rice and is used to control many annual and seedling perennial grasses, including barnyard grass and sprangletop in drill-seeded and water-seeded rice. Diclofop-methyl may be applied pre-plant, pre-emergent, or post-emergent and is registered for use on golf course turf. Fenoxaprop-p-ethyl is a selective aryloxy phenoxy-propionate herbicide used to control grass weeds after emergence. Fluazifop-P-butyl is a selective herbicide registered for use to provide postemergence control of perennial and annual grass weeds. Propaquizafop is used as a systemic herbicide for annual and perennial grasses. Quizalofop-P- ethyl is a selective herbicide used for the control of annual and perennial grasses in crop- and non-croplands.

TAs/TADs are insecticides that also work through the inhibition of lipid biosynthesis and include spirodiclofen, spiromesifen, spirotetramat, and spiropidion. Spirodiclofen is a tetronic acid acaricide, which acts by interfering with mite development (active by contact with mite eggs, all nymphal stages, and adult

females (adult males are not affected). Spiromesifen is a tetronic acid insecticide/miticide used for control of mites and whiteflies in coffee, corn (field, pop, sweet), cotton, low-growing berry crop subgroup 13-07G, tea, mint, and several vegetable crops. Spirotetramat is a tetramic acid derivative (ketoenole), which is active against sucking insects in a variety of plants (systemic (xylem and phloem mobile) and can control hidden pests and protect new shoots). Spiropidion is a pro-insecticide incorporating a novel tetramic acid derivative that can be used to protect a wide array of crops from some of the most damaging, and difficult-to-control, sucking pests.

Based on the review of the available data, read-across with the TAs/TADs chemical group compounds was determined to be the most relevant for the assessment of spiropidion. This conclusion was based on both biological and structural similarity. First, biological similarity was defined based on the pesticidal mode of action and similarity across the toxicity points of departure (below). The target organs of toxicity for the DENs (pinoxaden) were identified as the kidney, with effects related to kidney toxicity detected following 90 days of oral exposures in rats and mice, but not dogs. Pinoxaden was not associated with genotoxicity or carcinogenicity. All endpoints for risk assessment were based on maternal toxicity from the rabbit developmental toxicity studies (including acute dietary POD for females 13-49). For the DIMs, the liver was the target organ of toxicity across all compounds, with effects related to liver toxicity reported following 90 days of oral exposure. Other target organs were the hematopoietic system and kidneys. DIMs were not associated with genotoxicity. There was no clear evidence of carcinogenicity with the DIM compounds and the reference dose approach was used for all compounds with suggestive evidence of carcinogenicity. For acute dietary endpoints, the key studies were acute neurotoxicity studies or rat/rabbit developmental toxicity studies. For the chronic dietary endpoints, the adverse effects were all related to liver toxicity and the key studies were the chronic dog, chronic toxicity/carcinogenicity in rats, or carcinogenicity in mice. For the FOPs, the liver and kidney were the target organs of toxicity, with effects related to liver and kidney toxicity detected following 28/90 days of oral exposures. Peroxisome proliferation in the liver was evaluated and reported for all but one compound. The FOPs were not associated with genotoxicity. There was no clear evidence of a carcinogenic effect with the FOPs. For acute dietary endpoints, the FOPs contained compounds with no appropriate endpoint for setting an acute point of departure for risk assessment and compounds that had acute dietary endpoints set for the general population/females 13-48 based on acute neurotoxicity studies or rat developmental toxicity studies. In chronic studies, all compounds were associated with liver or kidney effects based on rat chronic carcinogenicity, mouse carcinogenicity, or 2 generation rat reproductive studies.

For the TAs/TADs, the target organs were the adrenal glands, liver, thyroid, and testes, with effects in the target organs detected following 90 days of oral exposure (Table A 0.1.). The TAs/TADs were not associated with genotoxicity. Spirodiclofen was the only compound classified as likely to be carcinogenic to humans with a Q1* assigned. All other compounds were classified as not likely to be carcinogenic to humans. Two compounds are not associated with any acute adverse effects and two compounds have acute endpoints for the general population assigned based on the acute neurotoxicity study or the 28-day oral toxicity study in dogs. For chronic dietary risk assessment, two compounds shared testicular effects in chronic oral toxicity in dogs or the chronic toxicity/carcinogenicity study in rats as the basis for the point of departure, the adverse effects in another study were based on decreased pup body weight in the 2generation rat reproduction study, and one compound was associated with thymus involution in dogs as the critical effect (Table A 0.2.).

Table A 0.1. Target organs for toxicity

Compound	Target Organs
DENs	
Pinoxaden	Kidney and general toxicity; ADME data indicate excretion is mainly renal

DIMs	
Clethodim	Liver and general toxicity
Cycloxydim	Liver and anaemia (short term); Body weight reduction and decrease in triglycerides (long term)
Sethoxydim	Liver
Tepraloxydim	Liver, spleen/hematopoietic system and reproductive system
Tralkoxydim	Liver, blood
FOPs	
Clodinafop-propargyl	Liver
Cyhalofop-butyl	Kidney
Diclofop-methyl	Liver
Fenoxaprop-P-ethyl	Liver and kidney
Fluazifop-P-butyl	Liver and kidney
Propaquizafop	Liver, systemic toxicity
Quizalofop-P-ethyl	Liver, ovaries, and testes
TAs/TADs	
Spirodiclofen	Adrenal glands, testes
Spiromesifen	Adrenal glands, thyroid, liver, spleen
Spirotetramat	Thyroid, thymus gland, testes
Spiropidion	Body weights, reductions in cholesterol and triglycerides in rodents; severe clinical signs indicative of systemic neurotoxicity in dogs.

Table A 0.2. Summary of POD and cPADs for read-across

Compound	cPAD	UF	POD (NOAEL)	LOAEL	Effect
DENs					
Pinoxaden	0.3	UF_A = 10X UF_H = 10X FQPA SF = 1X	30	100	Developmental Toxicity– Rabbit Based on morbid condition in one rabbit (mortality), abortion, decreased body weights, body-weight gains and food consumption.
DIMs					
Clethodim	0.3	UF_A = 10X UF_H = 10X FQPA SF = 1X	30	150	Carcinogenicity - Mice Based on reduced survival; decreased red cell mass; and increased incidences of bile duct hyperplasia, of pigmentation of the liver, and of foci of amphophilic macrophages in the lung
Cycloxydim[a]	0.07	UF_A = 10X UF_H = 10X	7	28	Carcinogenicity - Rat Increased liver weight with clinical chemistry changes
Sethoxydim	0.14	UF_A = 10X UF_H = 10X FQPA SF = 1X	14	41	Carcinogenicity - Mouse Based on liver hypertrophy and fatty degeneration
Tepraloxydim	0.05	UF_A = 10X UF_H = 10X FQPA SF = 1X	5	30	Carcinogenicity - Rat Based on male liver microscopic lesions (eosinophilic foci)
Tralkoxydim	0.005	UF_A = 10X UF_H = 10X	0.5	5	Chronic Toxicity – Dog Based on changes in liver morphology and function
FOPs					
Clodinafop-propargyl	0.0032	UF_A = 10X UF_H = 10X FQPA SF = 1X	0.32	10.2	Chronic/Carcinogenicity - Rat Based on toxicity in the liver (increased weight, clinical chemistry, and histopathology) and kidneys (increased weight, nephropathy, and tubular pigmentation) in both sexes
Cyhalofop-butyl	0.01	UF_A = 10X UF_H = 10X FQPA SF = 1X	1	10	Carcinogenicity – Mouse Based on effects on the kidney including tubular dilatation, chronic glomerulonephritis and hyaline casts in females as well as hyperplasia of the stomach mucosal epithelium in males

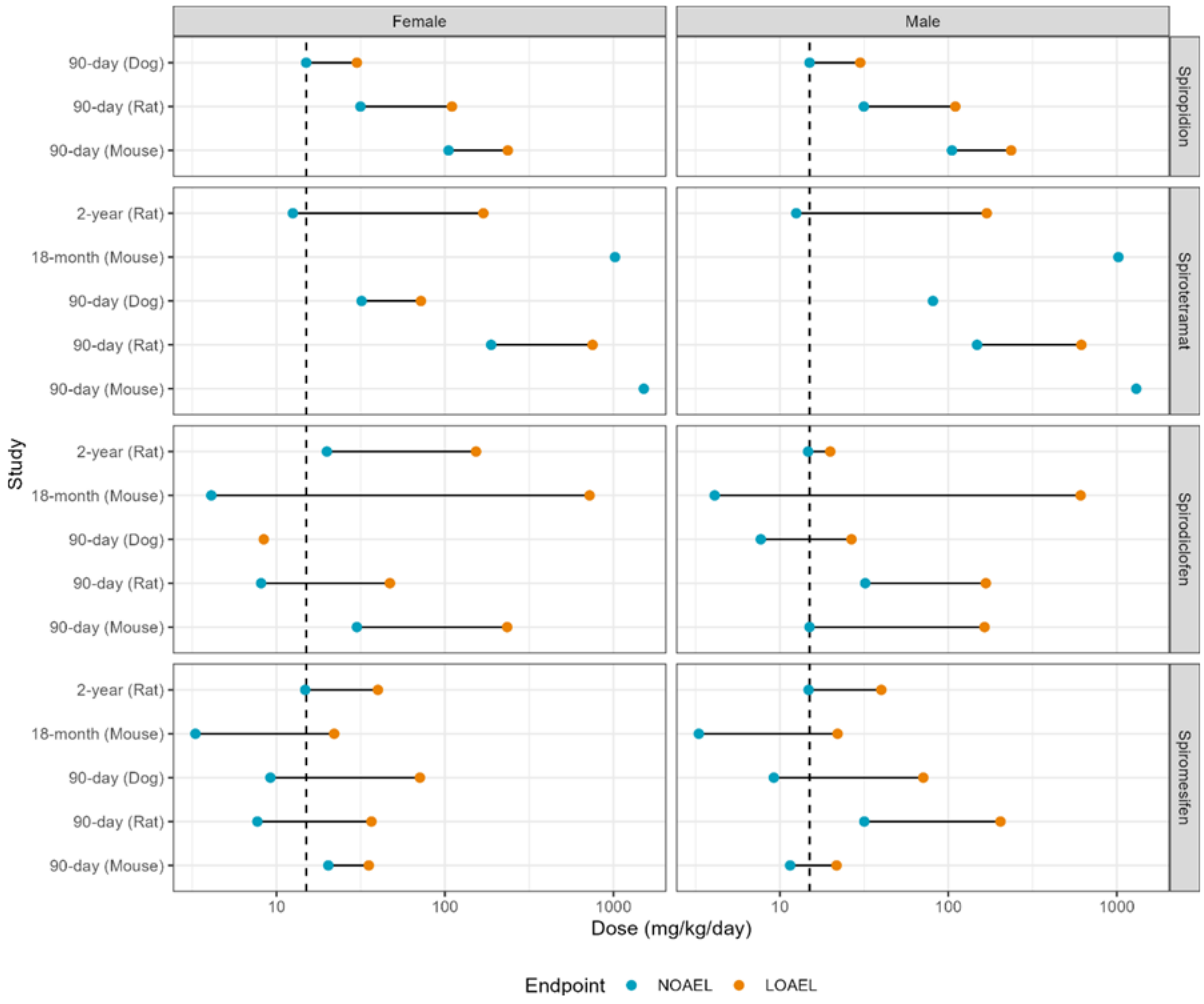
UF = uncertainty factor; UFA = extrapolation from animal to human (interspecies); UFH = potential variation in sensitivity among members of the human population (intraspecies); UF_L = use of a LOAEL to extrapolate a NOAEL; UF_{DB} = to account for the absence of key data (i.e., lack of a critical study); FQPA SF = FQPA Safety Factor

[a] Evaluated by EFSA and reported as ADI = acceptable daily intake and FQPA UF not evaluated

[b] Evaluated by JMPR and reported as ADI and FQPA UF not evaluated

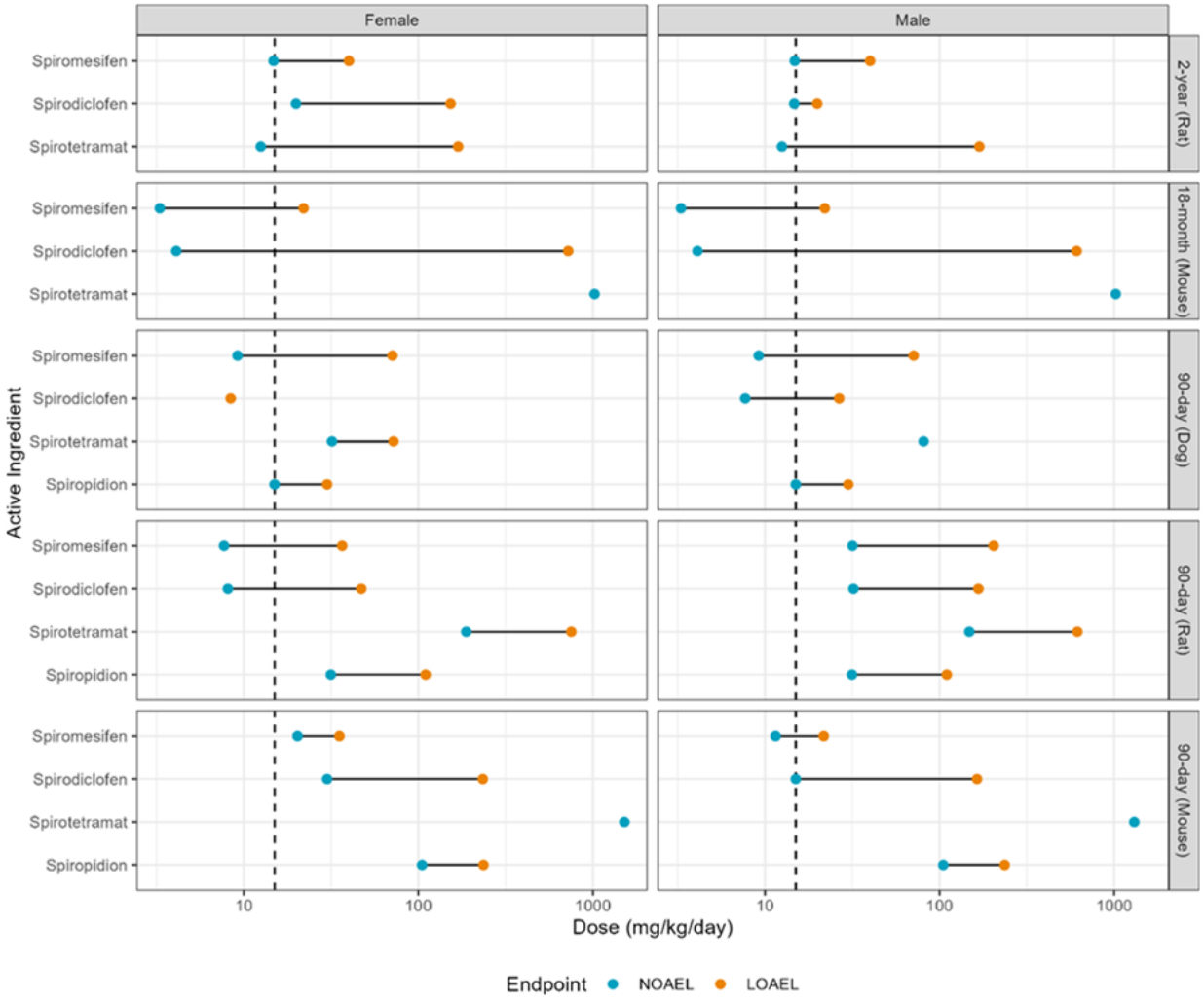
As summarized above, the HRAC and IRAC classification schemes were used to identify molecules known to inhibit ACCase. For these molecules, structural similarity was defined based on ToxPrints, a selection of 729 binary chemical fingerprints broadly associated with toxicity. ToxPrints are generally available for many molecules in the CompTox Chemicals Dashboard, and they can also be generated using the Chemotyper application. ToxPrints were gathered for all 23 ACCase inhibitors, and among the 729 defined fingerprints, 96 were present in one or more ACCase inhibitors. The other ToxPrints were excluded, as they did not provide additional information to compare structural similarity. A principal coordinate analysis was conducted based on the binary distance matrix between each ACCase inhibitor (Figure A 0.1.). Additionally, a clustering analysis was conducted (Figure A 0.2.). Both approaches clearly indicate grouping of the ACCase inhibitor classes, with spiropidion most similar to other TAs/TADs and distinct from other ACCase inhibitor classes.

Figure A 0.1. Chemical clustering based on pesticidal mode of action



Dashed line represents the Point of Departure proposed for the chronic risk assessment of spiropidion (NOAEL based on 90-day oral (capsule) toxicity study in dog).

Figure A 0.2. Chemical clustering based on toxicity endpoints



Dashed line represents Dashed line represents the Point of Departure proposed for the chronic risk assessment of spiropidion (NOAEL based on 90-day oral (capsule) toxicity study in dog).
The names and structures of the TAs/TADs included for read-across are presented in Table A 0.3. The sources for the data are also included in Table A 0.3.

The names and structures of the TAs/TADs included for read-across are presented in Table A 0.3. The sources for the data are also included in Table A 0.3.

Table A 0.3. Tetronic and tetramic acid derivatives read-across compounds

Chemical Subclass	Chemical Name	CAS Number	Structure	Data References
Tetronic and Tetramic acid	Spirodiclofen	148477-71-8		USEPA(2009) USEPA(2011)

derivatives (TAs/TADs)				USEPA(2013)
	Spiromesifen	283594-90-1		USEPA(2013) USEPA(2015a) USEPA(2015b) USEPA(2018) USEPA(2020a) USEPA(2020b)
	Spirotetramat	203313-25-1		USEPA(2008) USEPA(2013) USEPA(2017)
	Spiropidion	1229023-00-0		USEPA(2022) JMPR(2021)

A.3. Pesticidal Modes of Activity

ACCase is a biotin-containing enzyme that catalyses the ATP-dependent carboxylation of acetyl- CoA to form malonyl-CoA. This reaction is the first step in the synthesis of fatty acids: malonyl- CoA is the first building element of fatty acids.

Two forms of ACCase occur in plants, prokaryotic and eukaryotic forms. The prokaryotic form is insensitive to these ACCase-inhibiting herbicides and is found only in the plastids of dicotyledonous plants. The eukaryotic form is found in the cytoplasm of all plants and also in the plastids of grasses, in which the plastid-encoded prokaryotic form was lost and replaced by the eukaryotic, nuclear-encoded form with a transit peptide to target it for the plastid. The eukaryotic form is sensitive to ACCase-inhibiting herbicides.

Similarly, the pMoA is through the inhibition of fatty acid synthesis.

A.4. TAs/TADs Toxicokinetics Profiles

Table A 0.4 below presents the available toxicokinetic data for the ACCase-inhibiting herbicides and insecticides including available dermal absorption data.

From the available information, TAs/TADs insecticides, the oral absorption values. The oral absorptions were reported as 57-62% in males and 74% in females for spirodiclofen, 6-7% for spiromesifen, 89-98% for spirotetramat, and 72-77% for spiropidion. Excretion was rapid with >90% of the TAs/TADs excreted within 48 hours. Excretion was mainly via urine, followed by faeces and bile, except for spiromesifen (Low dose 34-40% urine; 53- 57% faeces [bile 6.7%]; high dose 6.5-9% urine; 90-93% faeces). Metabolism for spirodiclofen, spiromesifen and spirotetramat was to the enol-metabolite. The tissue distribution was reported to be highest in the liver, gastrointestinal tract and kidney for spiromesifen, spirotetramat, and spiropidion. Sex differences were reported for spirodiclofen (oral absorption greater in females than males) and spirotetramat (at low dose (2 mg/kg-bw single or repeated dosing), the AUC for males was about 60% higher than females and much greater at the high dose). Dermal absorption values ranged from 2-10%.

Table A 0.4. Toxicokinetic results for TAs/TADs read-across compounds

	Oral Absorption	Excretion	Metabolism	Distribution	Sex difference	Dermal absorption
TAs/TADs						
Spirodiclofen	Females absorbed a greater fraction of approximately 74% versus 57-62% estimated for males.	56-75% urine; 22-31% faeces (bile = 11%); 90% eliminated within 48 hours	Female rats metabolized spirodiclofen to a urinary enol metabolite more extensively than did males (39.65-52.42% versus 4.88-5.41% of the administered dose, respectively). The parent compound was detected only in small quantities in the faeces (probably reflecting unabsorbed compound) and was not detected in urine or bile.	Liver, kidneys, plasma, GI-tract, skin. No tissue accumulation. In females, organ and tissue levels were about 5-15 times lower than in males. Compared to single-dosed males, tissue levels in males treated for 15 days were 4 times lower.	Yes	2%
Spiromesifen	Low 6-7%	Low dose 34-40% urine; 53-57% faeces (Bile 6.7%) High dose 6.5-9% urine; 90-93% faeces For both low and high dose, 88 to 90% of the dose was eliminated within the first 24 hours	Metabolized to the keto-enol by loss of the dimethylbutyric acid moiety. Both the phenyl and cyclopentyl rings were hydroxylated and the methyl groups on the phenyl ring were ultimately oxidized to a carboxylic acid. These metabolites were largely recovered in the bile and urine. The predominant moiety recovered in the faeces was the unmetabolized test material. No conjugation with either glucuronic acid or sulphate was observed.	Liver, gastrointestinal tract, fat, skin, and blood	ND	3.3%
TAs/TADs						

Spirotetramat	89-98%	88-95% urine; 2-11% faeces by 48 hours	The main metabolic reaction was cleavage of the ester group, which resulted in formation of the major metabolite BYI 08330-enol (53-87% of the administered dose). All other identified metabolites could be derived from the primary enol metabolite. Male rats exhibited much higher rates of demethylation of BYI 08330-enol to BYI 08330- desmethyl-enol (25-37%) when compared to female rats (5-10%).	Less than 0.2% of the administered dose was detected in the body at sacrifice and the highest concentrations were detected at similar levels in the liver and kidney.	At 2 mg/kg-bw (single or repeated dosing), the AUC for males was about 60% higher than females and much greater at the high dose.	10% (*in vivo* rat)
Spiropidion	72–77% in male and female rats	>94 % of the administered dose was excreted within 48 h with excretion complete by 168 h; the major route of excretion was via urine (65%), followed by faeces (~15%) and bile (~10%) and <0.7% of dose recovered in the carcass	Extensively metabolized in rat via ester hydrolysis to form SYN547305 and subsequent loss of the methoxy moiety in the piperidine ring to form SYN548430. SYN548430 and an oxidized moiety of SYN548430, individually accounted for >10% of the administered dose in excreta. The major components (>10% of total radioactivity AUC) identified in plasma were SYN547305 and SYN548430	Widely distributed, with the highest concentration s of radioactivity observed in the GI tract, liver, and kidney	No	ND

ND = No Data

A.5. Acute Toxicity – TAs/TADs

The ACCase inhibitor compounds used for read-across are primarily of low acute toxicity and low irritation potential (Table A 0.5.).

The TAs/TADs are generally of low acute toxicity via oral, dermal, and inhalation routes of exposure and low irritation potential (Categories III and IV), except for spirotetramat which is a Category II for eye irritation. All three TAs/TADs were skin sensitizers. None of the remaining chemicals had a reported EPA toxicity category less than III.

Table A 0.5. Acute toxicity results for read-across compounds (EPA toxicity category)

Compound	Acute Oral (rat) LD50mg/kg-bw	Acute Dermal(rat) LD50 mg/kg-bw	Acute Inhalation (rat)LC50mg/L	Skin Irritation	Eye Irritation	Skin Sensitisation
TAs/TADs						
Spirodiclofen	≥2,000 (III)	≥2,000 (III)	≥5.03 (IV)	Not irritating (IV)	Not irritating (IV)	Positive
Spiromesifen	≥2,000 (III)	>2,000 (III)	≥4.87 (IV)	Not irritating (IV)	Not irritating (IV)	Positive
Spirotetramat	>2,000 (III)	>2,000 (III)	>4.2 (IV)	Not irritating (IV)	Irritating (II)	Positive
Spiropidion	>2,000 (III)	>5,000 (IV)	>1.12 (IV)	Not irritating (IV)	Mildly irritating (IV)	Positive

A.6. Short-term Toxicity Read-across

Results for the TAs/TADs read-across compounds in rat, mouse, and dog 28-day studies are shown in Table A 0.6, and 90-day studies are shown in Table A 0.7. Results for 28-day dermal and inhalation studies in rats are shown in Table A 0.8. The NOAELs, LOAELs, and effects at the LOAEL were obtained from the regulatory documentation. The adversity of these effects under current EPA policy and practices may be different from that reported in the documentation.

A.7. Sub-acute Oral Toxicity Read-across

Limited reporting of 28-day oral toxicity studies was available. For the TAs/TADs, the NOAEL for spiromesifen in rats was 53.4 mg/kg/day with a LOAEL of 536.3 based on clinical signs, decreased gain and food consumption, haematology, clinical chemistry, liver enzyme, increased spleen and lymph node

cell proliferation, organ weights, gross pathology, and microscopic findings and the NOAEL is mice was <202.6/269.6 mg/kg/day (M/F) with a LOAEL of 202.6/269.6 mg/kg/day (M/F) based on decreased body weight gain (25% in males and 41% in females). The NOAEL for spirotetramat in rats was 501.8 mg/kg/day with no adverse effects reported at the highest dose level tested, in mice the NOAEL was 1415 mg/kg/day with no adverse effects reported at the highest dose level tested, and in dogs the NOAEL was 42/70 mg/kg/day (M/F) with a LOAEL of 104/127 mg/kg/day (M/F) based on decreased thymus size and weight and decreased body weight and food consumption, which resulted in emaciation.

Table A 0.6. Overview of 28-day sub-acute toxicity of read-across compounds

Compound	28-DayRat (mg/kg/day)	28-DayMouse (mg/kg/day)	28-DayDog (mg/kg/day)
TAs/TADs			
Spirodiclofen	ND	ND	ND
Spiromesifen	NOAEL 53.4 LOAEL 536.3 Based on clinical signs, decreased BW gain and food consumption, haematology, clinical chemistry, liver enzyme, increased spleen and lymph node cell proliferation, organ weights, gross pathology, and microscopic findings.	NOAEL not established LOAEL 202.6/269.6 (M/F) Based on decreased BW gain (25% in males and 41% in females)	ND
Spirotetramat	NOAEL 501.8 (F) LOAEL >501.8 (F) No adverse effects up to the highest dose tested	NOAEL 1,415 (M) LOAEL >1,415 (M) No adverse effects up to the highest dose tested	NOAEL 42/70 (M/F) LOAEL 104/127 (M/F) Based on decreased thymus size and weight as well as decreased BW and food consumption, which resulted in emaciation.
Spiropidion	NOAEL 31.5/36.1 (M/F) LOAEL 159/110 (M/F) Both sexes: ↓foot splay, cholesterol, triglyceride, total protein and albumin levels; ↑white blood cell count, alanine aminotransferase; microscopic pathology findings of diffuse follicular cell hypertrophy and colloid contraction in the thyroid glands at >110. Females: ↓BW gain and food utilization	NOAEL 117/126 (M/F) LOAEL 449/465 (M/F) Premature deaths, ↓ body weights	NOAEL 30 LOAEL 65/100 (M/F) Based on clinical signs and mortality.

ND = No Data

A.8. Sub-chronic Oral Toxicity Read-across

For the TAs/TADs, liver and kidney toxicity was seen in rats and dogs. Other targets of toxicity were thyroid, clinical biochemistry, and general systemic toxicity effects (body weight/food consumption) in rats, mice and dogs. In rats, NOAELs ranged from 6.2 mg/kg/day (males and females) for spiropidion to 188 mg/kg/day (females) for spirotetramat; In mice, NOAELs ranged from 15 mg/kg/day (males) for spirodiclofen to 1,515 mg/kg/day (females) for spirotetramat; and in dogs, which was the most sensitive species, NOAELs ranged from 1.9 mg/kg/day (females) for spiromesifen to 81 mg/kg/day (males) for spirotetramat.

Table A 0.7. Overview of 90-day sub-chronic oral toxicity of read-across compounds

Compound	90-DayRat (mg/kg/day)	90-DayMouse (mg/kg/day)	90-DayDog (mg/kg/day)
TAs/TADs			
Spirodiclofen	NOAEL = 32.1/8.1 (M/F) LOAEL = 166.9/41.7 (M/F) Based on increased incidence and severity of cytoplasmic vacuolation in the cortex of adrenal glands, decreased cholesterol, decreased triglycerides in males, increased incidence of small cytoplasmic vacuolation in the cortex of adrenal glands in females.	NOAEL = 15/30 (M/F) LOAEL = 164/234 (M/F) Based on an increased incidence of hypertrophic Leydig cells in the testes in males, and an increased incidence of cytoplasmic vacuolation of the adrenal cortex in females.	NOAEL = 7.7/≤8.4 (M/F) LOAEL = 26.6/8.4 (M/F) Based on decreased BW gains, increased liver and adrenal weights, decreased prostate weights, and histopathology findings in the adrenal glands, testes, epididymis, thymus, and prostates in males, and increased adrenal gland weight which coincided with histopathology findings (cytoplasmic vacuoles in the Zona fasciculata of the adrenal glands).
Spiromesifen	NOAEL = 31.7/7.7 (M/F) LOAEL = 204/36.6 (M/F) Based on thyroid, kidney, and liver effects in males and females.	NOAEL not established LOAEL = 21.7/35.3 (M/F) Based on gross and microscopic findings, and in the presence of cytoplasmic eosinophilia in zona changes in the adrenal glands in both males and females.	NOAEL = 1.9 LOAEL = 9.2 Based on decreased thyroxine (T4) levels in females.
Spirotetramat	NOAEL = 148/188 (M/F) LOAEL = 616/752 (M/F) Based on decreased body weight, abnormal spermatozoa and hypospermia in the epididymis, decreased testicular weight, testicular degeneration and vacuolation in males, and alveolar macrophages in both sexes.	NOAEL = 1305/1515 (M/F) LOAEL > 1,305/1,515 No adverse effects at the highest dose tested.	NOAEL = 81 (HDT)/32 (M/F) LOAEL = 72 (F) Based on decreased food consumption, depressed RBC parameters (red blood cell count, haemoglobin level. and haematocrit), and thymus atrophy.
Spiropidion	NOAEL = 6.2 LOAEL = 31.5 Based on reduced cholesterol (M/F) and reduced triglycerides (F).	NOAEL = 105 LOAEL = 236 Based on reduced BW (F), and reduced BW gain, reduced food utilization, and	NOAEL = 15 LOAEL = 30 (F) Based on mortality and clinical signs in females. Capsule administration

		changes in clinical biochemistry parameters (M/F).	

A.9. Dermal/Inhalation Repeated Dose Toxicity Read-across

In the dermal toxicity studies, no adverse effects were seen at the highest doses tested for the TAs/TADs.

Only a single inhalation toxicity study was available with spiromesifen (TAs/TADs) with no adverse effects reported up to 0.1 mg/L (equivalent to 21.4 mg/kg/day). The human equivalent concentration (HEC) was greater than the NOAEL reported in the subchronic oral toxicity studies.

Table A 0.8. Overview of dermal and inhalation sub-chronic toxicity of read-across compounds

Compound	28-DayDermalRat(mg/kg/day)	28-DayInhalationRat(mg/L)
TAs/TADs		
Spirodiclofen	NOAEL = 1000 LOAEL > 1000 No adverse effects at the highest dose tested	Waived
Spiromesifen	NOAEL = 1000 LOAEL > 1000 No adverse effects at the highest dose tested	NOAEL = 0.1 mg/L (21.4 mg/kg/day) LOAEL > 0.1 mg/L (21.4 mg/kg/day) No adverse effects at the highest dose tested
Spirotetramat	NOAEL = 1000 LOAEL > 1000 No adverse effects at the highest dose tested	Waived
Spiropidion	NOAEL = M/F: 300 LOAEL = M/F: 1000 In males and females:↓BW gain in females: minimal follicular cell hypertrophy in thyroid gland	ND

ND = No Data

A.10 Genetic Toxicity – Read-Across

The TAs/TADs used for read-across have been reported as non-genotoxic (Table A 0.9).

Table A 0.9. Genotoxicity results for read-across compounds

Compound	*In vitro*				*In vivo*
	Gene mutation assay in bacterial Cells (Ames)	Chromosomal aberration assay	Mammalian cell gene mutation assay	Unscheduled DNA Synthesis	Micronucleus/ Chromosomal aberration

TAs/TADs					
Spirodiclofen	Negative (+S9/-S9)	Negative (+S9/-S9)	Negative (+S9/-S9)	ND	Negative (Mouse; 800 mg/kg)
Spiromesifen	Negative	Negative	Negative	ND	Negative (Mouse; 0, 100, 200, 400 mg/kg)
Spirotetramat	Negative (+S9/-S9)	Weakly clastogenic at cytotoxic concentrations only	Negative (+S9/-S9)	Negative	Negative (Mouse; 0, 125, 250, 500 mg/kg)
Spiropidion	Negative (+S9/-S9)	Negative (+S9/-S9)	Negative (+S9/-S9)	ND	Negative

ND = No Data

A.11. Chronic Toxicity/Carcinogenicity

Chronic and carcinogenicity toxicity data for ACCase inhibitor read-across compounds are presented in Table A 0.10. The cancer classifications of the read-across compounds are presented in Table A 0.11.

For TAs/TADs, spirodiclofen was classified by EPA as “Likely to be Carcinogenic to Humans” based on increased liver tumours in mice and increased testes Leydig cell adenoma in males and increased adenoma/adenocarcinoma in females. Of note, the subchronic toxicity studies with spirodiclofen showed hypertrophy/hyperplasia of Leydig cells, interstitial cell degeneration of the testes, atrophy of the testes, epididymides, prostate, and seminal vesicles in the rats, mice, and dogs. These findings were not recorded in the subchronic toxicity studies with the other TAs/TADs. Spiromesifen and spirotetramat were classified by the US EPA or the JMPR as “not likely” or “unlikely” to be carcinogenic (Table A 0.11.). Overall, there is no genotoxic concern for the TAs/TADs.

In an 80-week mouse dietary carcinogenicity study, administration of spiropidion to Crl:CD-1 mice at 0, 50, 250 or 500 ppm, for a period of up to 80 weeks was associated with lower mean body weight and body weight gain at 250 ppm or 500 ppm. There was no evidence of any differences in the survival patterns seen for the treated groups in either sex, with the exception of females that received 250 ppm which had a statistically significantly lower mortality rate. There were no adverse neoplastic or non-neoplastic findings that were associated with spiropidion (Kan-King-Yu, 2020). A dose-related increase in the incidence of mesenteric lymph node erythrophagocytosis was observed in animals at >50 ppm in males (statistically significant) and >250 ppm in females (not statistically significant) when compared with controls. This finding correlated with the dark/dark red discoloration observed at necropsy. The significance of the increased incidence in mesenteric lymph node erythrophagocytosis in this study is uncertain due to the potential for this change to occur from agonal or procedural processes and due to the absence of any concurrent regional tissue haemorrhage or hemosiderin. In the absence of any deleterious effect on the morphology or function of the lymph node, the observation was considered not to be adverse. An increased incidence and severity of calculi (gallstones) in the gallbladder was noted in males at □ 50 ppm (statistically significant at 500 ppm) and at >250 ppm in females (not statistically significant). These findings were considered to be test substance-related but were not considered to be adverse, due to the absence of accompanying inflammatory/epithelial changes or evidence of a deleterious effect on gallbladder function. Based on the findings in this study, the NOAEL is established at 50 ppm, which is equivalent to 6.4 mg/kg/day in males and 7.0 mg/kg/day in females.

In a 104-week rat dietary carcinogenicity study combined with a 52-week chronic toxicity study, administration of spiropidion to Crl:WI (Han) rats at 0, 50, 100 or 500 ppm for males and 50, 100 or 300 ppm for females for a period of up to 104 weeks resulted in an initial lower mean body weight and body weight gain, which showed some recovery towards the end of treatment. Also observed were lower food utilisation and lower plasma cholesterol levels in males given 500 ppm and lower albumin and total protein levels at Weeks 27 and 52 in males given 100 or 500 ppm. Histopathologically, there was an increased incidence of luminal dilatation and inflammation of the bile duct in males at 500 ppm, at week 104. No test substance-related neoplastic findings were noted. In a summary of the histological findings observed in the toxicity and carcinogenicity study, neoplastic findings recorded in the brain included the incidence of two malignant ependymomas at the high dose of 500 ppm, in 2 of the 64 males in this treatment group (12 animals from the chronic toxicity study, 52 animals from the carcinogenicity study). This slightly higher incidence of malignant ependymoma in the brain was not statistically significant in either the Fisher's Exact test [p=0.49] or the Peto trend test [p=0.12]. Based on the ACCase mode of action and read-across of structurally similar chemicals; lack of statistical significance, precursor lesions in both sexes, or genotoxic potential; absence of benign or malignant ependymomas in females, or any other indications of tumorigenesis in tissues that could be linked in terms of underlying anatomy, this slight increase in ependymomas in male rats in the carcinogenicity study with spiropidion is considered to be spontaneous in origin, incidental and not related to treatment. (Goetz and McInnes, 2020).

Based on the findings in this study, a NOAEL is established at 100 ppm for males, based on lower body weight and body weight gain and increased incidence of luminal dilatation and inflammation in the bile duct observed in males at 500 ppm. No adverse effects were observed in females. The NOAEL is established at 300 ppm for females. This is equivalent to 5.5 (52 weeks) or 4.7 (104 weeks) mg/kg/day in males and 20.5 (52 weeks) or 18.7 (104 weeks) mg/kg/day in females.

In conclusion, spiropidion was not carcinogenic in the mouse or rat and clear NOAELs can be established for non-neoplastic findings.

Table A 0.10. Read-across chronic/carcinogenicity oral toxicity summaries

Compound	Chronic Toxicity/Carcinogenicity Rat(mg/kg/day)	Carcinogenicity Mouse (mg/kg/day)	Chronic Dog(mg/kg/day)
TAs/TADs			
Spirodiclofen	NOAEL = 14.7/19.9 (M/F) LOAEL=110.1/152.9(M/F) Based on decreased body weights, decreased body-weight gain, increased APh levels, decreased cholesterol and triglyceride levels, increased vacuolated jejunum enterocytes, and increased incidences of Leydig cell hyperplasia for males, and decreased body weights, decreased body-weight gain, increased APh levels, increased TSH, uterus nodules and increased vacuolated jejunum enterocytes in females. Increase in tested Leydig cell adenoma in males, increase in uterine adenoma and/or adenocarcinoma in females.	NOAEL = 4.1/5.1 (M/F) LOAEL=610/722(M/F) Based on increased absolute and relative liver and adrenal weights, decreased absolute and relative kidney weight, enlarged adrenal gland, discoloured testis, adrenal gland vacuolization, interstitial cell degeneration of the testes in males, and increased absolute and relative adrenal weight, decreased absolute and relative kidney weight, increased incidences of adrenal gland pigmentation, and adrenal vacuolization in females. Increase in hepatocellular adenoma and carcinoma.	NOAEL=1.38/1.52(M/F) LOAEL=4.33/4.74 (M/F) Based on increased relative adrenal weights in both sexes, increased relative testis weight in males and histopathology findings in the adrenal gland of both sexes.
Spiromesifen	NOAEL=14.8/19.5(M/F)	NOAEL=3.3/3.8(M/F) LOAEL =	NOAEL=11.5/10.8(M/F)

	LOAEL = 40/53.5 (M/F) Based on clinical signs, gross necropsy, histopathology, and increased TSH in females. *No evidence of carcinogenicity.*	22/30 (M/F) Based on gross and microscopic changes. *No evidence of carcinogenicity.*	LOAEL = 109/117 (M/F) Based on an increase in alkaline phosphatase and liver histopathology.
Spirotetramat	NOAEL=12.5/16.8(M/F) LOAEL = 169/229 (M/F) Based on decreased kidney weight and renal tubular dilation. *No evidence of carcinogenicity.*	NOAEL=1022/1319 (M/F) LOAEL > 1022/1319 No adverse effects at the highest dose tested. *No evidence of carcinogenicity.*	NOAEL=6/48(M/F) LOAEL = 20 (M) Based on thymus involution in males.
Spiropidion	NOAEL = 4.7/18.7 (M/F) LOAEL = 24.0/>18.7 (M/F) Based on ↓BW and BW gain, ↑incidence luminal dilatation and inflammation of bile duct at 500 ppm in males. No adverse effect in females. No treatment-related increases in tumour incidence were observed.	NOAEL = 6.7/7.0 (M/F) LOAEL = 31.8/36.8 (M/F) Based on ↓BW, ↓BW gain. No treatment-related increases in tumour incidence were observed.	NOAEL = 10 (M/F) LOAEL = 30 (M/F) Based on two animals euthanised due to adverse clinical observations (dosing at 30 mg/kg/day stopped on Day 14).

Table A 0.11. Read-across cancer classification and tumour types

Compound	Cancer Classification/Tumour Types
TAs/TADs	
Spirodiclofen	"Likely to be Carcinogenic to Humans"
Spiromesifen	"Not Likely to Be Carcinogenic to Humans" based on the absence of treatment-related tumours in two adequate rodent carcinogenicity studies.
Spirotetramat	"Not likely to be carcinogenic to humans" based on lack of evidence of carcinogenicity in rats and mice.

A.12. Reproductive and Developmental Toxicity – Read-across

Rat reproductive and developmental toxicity studies in rats and rabbits for the read-across chemicals are presented in (Table A 0.12.). For TAs/TADs, an increased incidence of slight dilatation of the renal pelvis was observed in foetuses at the limit dose (1000 mg/kg/day) in the absence of maternal toxicity was seen in the rat developmental toxicity study for spirodiclofen. EPA (2022) noted that concern is low since the effect was slight, only seen at the limit dose, occurred without statistical significance, and did not display a clear dose-response. In the rabbit developmental study, there were no developmental effects and maternal toxicity was evidenced by body weight decrements. In the two-generation reproduction toxicity study, offspring effects (body weight decrements) occurred in the presence of parental effects, and there were additional effects observed at higher doses (i.e., delayed sexual maturation, increased severity of ovarian luteal cell vacuolation/degeneration, decreased testicular spermatid and epididymal sperm counts, and atrophy of the testes, epididymides, prostate, and seminal vesicles). For spiromesifen, EPA concluded that there was no evidence of increased quantitative or qualitative foetal susceptibility in the developmental

studies in rats and rabbits. In the reproductive study in rats, there was evidence of increased quantitative offspring susceptibility. The offspring effects were observed at a lower dose than parental effects. Reproductive toxicity in females (increased primordial follicles, decreased growing follicles, and decreased corpora lutea) was observed at the same dose causing parental effects. For spirotetramat, there was evidence of increased qualitative susceptibility in the rat developmental study with reduced foetal weight and increased incidences of malformations and skeletal deviations observed at the limit dose, while maternal effects at this dose consisted of only body-weight decrements. EPA concluded that there was no evidence of increased quantitative or qualitative susceptibility to offspring following pre- or post-natal exposure to spirotetramat in the rabbit developmental or two-generation reproduction studies. For spiropidion, there were no developmental effects in rats, and an increase in skeletal effects in rabbits at a higher dose than maternal toxicity (body weight stasis and decreased body weight gain). There were no reproductive or offspring effects in the reproductive study and an increased incidence of thyroid follicular hypertrophy in females at the highest dose tested. The JMPR concluded that spiropidion is not teratogenic.

Table A 0.12. Reproductive and developmental toxicity for read-across compounds

Compound	2-GenerationReproduction (mg/kg/day)	Developmental Rat (mg/kg/day)	Developmental Rabbit (mg/kg/day)
TAs/TADs			
Spirodiclofen	Parental/systemic NOAEL = 5.2-6.4/5.5-7.0 (M/F) LOAEL = 26.2-30.2/27.6-34.4 (M/F) Based on decreased body weight in F_0 males; decreased absolute and relative liver weight in F_0 ales; decreased cholesterol and triglycerides in F_1 males; and increased severity of adrenal cortical vacuolation in F_1 males, and decreased unesterified fatty acids in F_1 females, and increased severity of adrenal cortical vacuolation in F_0 and F_1 females. Reproductive NOAEL = 26.2-30.2/27.6-34.4 (M/F) LOAEL = 134.8-177.6/139.2-192.7 (M/F) Based on delayed sexual maturation, decreased testicular spermatid and epididymal sperm counts (oligospermia), and atrophy of the testes, epididymides, prostate and seminal vesicles in males, and increased severity of ovarian luteal cell vacuolation/degeneration in females. Offspring NOAEL = 5.2-6.4/5.5-7.0 (M/F) LOAEL = 26.2-30.2/27.6-34.4 (M/F) Based on decreased body weight and body weight gain in F_1 male and female pups.	Maternal NOAEL =1000 LOAEL > 1000 No adverse effects at the highest dose tested. Developmental NOAEL = 300 LOAEL = 1000 Based on an increased incidence of slight dilatation of the renal pelvis.	Maternal NOAEL = 100 LOAEL = 300 Based on body weight loss and decreased food consumption. Developmental NOAEL = 1000 LOAEL > 1000 No adverse effects at the highest dose tested.
Spiromesifen	Parental/systemic NOAEL = 8.8/14.2 (M/F) LOAEL = 37/64 (M/F) Based on decreased body weight in lactating P females, significantly decreased spleen weight (absolute in P females and F1 males and relative in P females), and histopathological findings in the liver, thyroid gland, and adrenal gland. Reproductive NOAEL = 37/14.2 (M/F) LOAEL = not observed/64 (M/F)	Maternal NOAEL = 70 LOAEL = 500 Based on clinical signs (convulsions). Developmental NOAEL = 500 LOAEL > 500	Maternal NOAEL = 35 LOAEL = 250 Based on an increased incidence of abortions (late) and total resorptions (late). Developmental NOAEL = 35 LOAEL = 250

	Based on increased primordial follicles, decreased growing follicles, and decreased corpora lutea in females. Offspring NOAEL = 3.8 LOAEL = 14.2 Based on decreased pup body weight.	No adverse effects at the highest dose tested.	Based on an increased incidence of abortions (late) and total resorptions (late).
Spirotetramat	Parental/Systemic NOAEL = 70.7/82.5 (M/F) LOAEL = 419.3/484.7 (M/F) Based on decreases in body weight (F1 males and females), food consumption during lactation (P- and F1 females), and kidney histopathology and decreased kidney weights (F1 males and females). Reproductive NOAEL = 70.7/484.7 (M/F) LOAEL = 419.3 (M) Based on abnormal sperm cells and decreased reproductive performance in the F1 males. Offspring NOAEL = 70.7/82.5 (M/F) LOAEL = 419.3/484.7 (M/F) Based on decreased body weight during lactation in both F1 and F2 generations. Male reproductive Toxicity -- 1000 mg/kg/day (3, 10, 21, or 41 days - Primary testicular effects on or after day 10 were degeneration of round and elongating spermatids (stage 7-8 and 9-14, respectively), decreased sperm count, and increased numbers of aberrant/abnormal spermatozoa in the epididymis.	Maternal NOAEL = 140 LOAEL = 1000 Based on impaired food consumption, transient body weight loss, and reduced final body weight. Developmental NOAEL = 140 LOAEL = 1000 Based on reduced foetal weight and increased incidences of malformations and skeletal variations.	Maternal NOAEL = 40 LOAEL = 160 Based on sacrifice of animals in moribund condition and clinical signs. Developmental NOAEL = 160 LOAEL > 160 No adverse effects at the highest dose tested.
Spiropidion	Parental NOAEL = 7.8 LOAEL = 23 (F) Based on increased incidence of thyroid follicular hypertrophy in females. Reproductive	Maternal NOAEL = 30 LOAEL = 100 Based on body weight loss, reduced body weight gain and food consumption.	Maternal NOAEL = 10 LOAEL = 30 Based on body weight stasis in the first six days of treatment and decreased body weight gain

	NOAEL = 23 LOAEL > 23 Based on no adverse effects at the highest dose tested. <u>Offspring</u> NOAEL = 23 LOAEL > 23 Based on no adverse effects at the highest dose tested.	<u>Developmental</u> NOAEL = 100 NOAEL > 100 Based on no adverse effects at the highest dose tested.	thereafter. <u>Developmental</u> NOAEL = 30 NOAEL = 60 Based on increased incidence of skeletal effects (unossified pelvic girdle and incomplete xyphoid cartilage).

A.13. Evidence of Hormone Perturbation – Read-across: Oestrogen and Androgen ToxCast Pathway Models

ToxCast pathway model data are available for the ACCase inhibitors included in this assessment.

For the estrogenic and androgenic pathways, data available for the oestrogen receptor (ER) ToxCast Pathway Model (AUC) are presented in Table A 0.13. and data for the androgen receptor (AR) are presented in Table A 0.14. Overall, none of the ACCase inhibitors were predicted to interact with the oestrogen or androgen pathway. The highest AUC reported was 0.000117 (equivalent to an AC50 of 117,000 µM) for the ER antagonist pathway or 0.0647 (equivalent to an AC50 of 6470 µM) for the AR antagonist pathways. An AUC of 0.1 (equivalent to 100 µM) is considered to be weakly active and an AUC of 1 is considered to be active in this model (Browne et al., 2015).

Table A 0.13. Summary of oestrogen receptor ToxCast Pathway Model

Compound	ER Agonist (AUC)	ER Antagonist AUC
TAs/TADs		
Spirodiclofen	0	0
Spiromesifen	0	0
Spirotetramat	ND	
Spiropidion	ND	

Table A 0.14. Summary of the androgen receptor ToxCast Pathway Model

Compound	AR Agonist (AUC)	AR Antagonist (AUC)
TAs/TADs		
Spirodiclofen	0	0
Spiromesifen	0	0
Spirotetramat	ND	
Spiropidion	ND	

A.14. Thyroid Toxicity

Three of the four TAs/TAD compounds were associated with thyroid effects.

With spiromesifen, the thyroid gland was a target organ of toxicity in the subchronic rat studies. The incidence and severity of thyroid follicular cell hypertrophy was increased in the ≥ 500 ppm females and the 3000 ppm males while the incidence of colloidal alteration was increased in the ≥ 500 ppm males and in the 3000 ppm females. In the 500 ppm females and the 3000 ppm males and females, thyroid stimulating hormone (TSH) and thyroxine binding capacity (TBC) were increased. Decreased triiodothyronine (T3) and thyroxine (T4) were observed in the 3000 ppm males. Decreased T4 was reported in dogs, and EPA has placed an additional 10X database uncertainty factor until a comparative metabolism study is provided.

For spirotetramat, in subchronic dog studies, lower circulating levels of the thyroid hormones T3 and T4 were seen in both sexes. Males exhibited the effect at a lower dose than females. These differences are supported by data in the toxicokinetic studies showing that males achieve higher systemic exposures than their female counterparts do. Decreases in T4 were also observed in a one-year study in dogs. The only histopathological change in the thyroid was a reduction in follicle size in the high-dose group, a possible indication of a reduced amount of colloid. There were no correlative changes in thyroid weight, thyroid histopathology, or TSH in either sex.

For spiropidion, thyroid follicular cell hypertrophy and/or colloid contraction were observed in the short-term and subchronic toxicity studies in the rat with spiropidion. In the 28-day oral (dietary) toxicity study in rats minimal follicular cell hypertrophy was observed in the thyroid glands of animals that received 2000 or 3000 ppm (177 or 259 mg/kg bw/day, respectively). In the 90-day oral (dietary) toxicity study in rats, microscopic findings of diffuse follicular cell hypertrophy and colloid contraction were observed in the thyroid glands only in the high-dose groups (2500 and 1500 ppm, equivalent to 159 and 110 mg/kg bw/day in males and females, respectively). There was no increase in thyroid weight in any treatment group of these studies in the rat. There was no change in thyroid weight or histopathological changes in the mouse or dog. It was noted that effects observed in the thyroid occurred only at dose levels exceeding the maximum tolerated dose as noted by the significant decrease in body weights, body weight gains, and clinical pathological parameters.

Follicular epithelial hypertrophy was observed in the thyroid glands of females at the highest dose tested in both the parental (P) and first filial (F1) generation of the multigeneration reproductive toxicity study. There was no increase in thyroid weight in any treatment group of this study in the rat.

An overview of the findings noted in the liver and thyroid across short-term, subchronic, chronic, and carcinogenicity studies in the Han Wistar rat are detailed below in Table A 0.15.

Table A 0.15. Summary of Liver and Thyroid Data from Short-Term Toxicity to Carcinogenicity studies in the Rat

	Study (weight: g)	**Males**				**Females**			
28 days dermal, rat	Dose Level (mg/kg bw/day)	0	100	300	1000	0	100	300	1000
	Thyroid FC hypertrophy, minimal	0	0	0	0	0	0	0	6
	Thyroid + parathyroid wt, relative	0.0046	0.0055	0.0046	0.0054	0.0074	0.0059	0.0065	0.0066
	Liver weight, relative	3.77	3.44	3.39	3.54	3.72	3.70	3.86	3.81
28-day dietary, rat	Dose Level (ppm)	0	500	2000	3000	0	500	2000	3000
	Thyroid FC hypertrophy, diffuse, min.	0	0	**4***	**4***	0	0	**4***	**4***
	Liver weight, relative	4.19	3.61	4.02	3.85	3.62	3.86	4.09	4.24
90-day dietary, rat	Dose Level (ppm)	0	100	500	2500	0	100	500	1500
	Thyroid FC hypertrophy, diffuse, min	0	--	0	1	0	--	0	4
	Thyroid FC hypertrophy, diffuse, mild	0	--	0	3	0	--	0	3
	Thyroid contraction colloid min., mild	0/0	--	0/0	3/5	0/0	--	0/0	1/3
	Thyroid weight, relative	0.00501	0.00453	0.00571	0.00571	0.00723	0.00689	0.00719	0.00702
	Liver weight, relative	3.15	2.98	3.06	3.07	3.26	3.10	3.23	3.37
104-week, carcinogenicity, rat	Dose Level (ppm)	0	50	100	500	0	50	100	300
	Thyroid FC adenoma/carcinoma	3/0	3/0	2/0	5/0	3/0	1/0	4/0	1/1
	Thyroid weight, relative	0.0082	0.00850	0.0075	0.0085	0.0098	0.0089	0.0173	0.0100
	Liver weight, relative	2.73	2.77	2.89	2.69	2.87	2.77	2.82	2.76
Multigeneration Reproductive Toxicity, rat	Dose Level (ppm)	0	50	100	500	0	50	100	300
	P: Thyroid, follicular epithelium, min.	0	0	0	0	0	0	0	9
	P: thyroid weight, relative	0.0058	0.0053	0.0049	0.0058	0.0064	0.0070	0.0068	0.0075
	P: liver weight, relative	3.05	2.97	3.04	2.95	4.75	4.70	4.54	4.60
	F1: Thyroid follicular epithelium, min	0	0	0	0	0	0	0	5
	F1: thyroid weight, relative	0.0055	0.0056	0.0054	0.0053	0.0062	0.0060	0.0054	0.0060

	F1: liver weight, relative	3.11	3.17	3.11	3.02	4.83	4.54	4.84	4.75
90-day UDPGT, rat T4 glucuronidation	Dose Levels (ppm)	0	--	--	2500	0	--	--	1500
	pmol/min/mg	1.531	--	--	**3.86*** **	1.140	--	--	**2.490*** **
	pmol/min/g liver	22.523	--	--	**85.42*** **	15.986	--	--	**32.65*** **
	pmol/min/total liver weight	282.552	--	--	**171.58*** **	123.946	--	--	**237.15*** **
	pmol/min/relative liver weight	708.288	--	--	**2608.74*** **	506.502	--	--	**1101.72*** **

Bold: statistically significant from control: * p<0.05, ** p<0.01, *** p<0.001. UDPGT data generated from liver sampled from 90-day dietary toxicity study in rats. FC = follicular cell; min. = minimal; -- not measured; liver histopathology not included as there were no significant findings.

Additional mechanistic studies were conducted to further understand the mode of action in the rat (Table A 0.16.). A study to assess the potential induction of uridine diphosphate glucuronosyltransferase (UDP-GT) enzymes (UGT) in liver samples from Han Wistar rats was conducted following oral administration of spiropidion by diet at inclusion levels of 2500 and 1500 ppm (males and females, respectively) for 13 weeks. Statistically significant increases in the rate of thyroxine glucuronidation were observed (Table A 0.17.). It was concluded that spiropidion is an inducer of hepatic UDP-GT activity in both male and female Han Wistar rats. Uridine diphosphate glucuronosyltransferase (UDP-GT) activity was increased at the dose levels that caused effects on the thyroid, thus effects on the thyroid following exposure to spiropidion is considered to be secondary to liver enzyme induction.

An *in vitro* study was conducted to evaluate the effect of spiropidion and its principal metabolite SYN547305 on rat (Han Wistar) thyroid peroxidase (TPO) activity (Table A 0.18. and Table A 0.19.). Treatment had no significant effect on rat TPO activity at any concentration tested. It was concluded that spiropidion and its principal metabolite SYN547305 were not inhibitors of rat thyroid peroxidase activity *in vitro*. Thyroid peroxidase inhibition as a MOA for thyroid effects was found to be inactive for spiropidion. Overall, it was considered there is no concern for a direct effect of spiropidion on the thyroid gland.

Table A 0.16. Summary of mechanistic studies performed with spiropidion and metabolite SYN547305

Type of test organism, tested doses (ppm) mg/kg bw per day, or M	Critical effects	Purity (%) Batch No.	Reference
Spiropidion only UDP-glucuronosyltransferase induction, rat liver samples, diet (0, 2500/1500 ppm males/females) 0, 159/110 mg/kg bw per day males/females	↑rate in T4 glucuronidation (2.5/2-fold ↑ males/females). spiropidion is inducer of hepatic UGT activity	98.4 CSH001-002-001	Madden (2014)
Spiropidion and SYN547305 Rat thyroid peroxidase activity, microsomal preparation 0, 0.05, 0.5, 2, 5 and 10 µM	No significant effects on rat thyroid peroxidase activity. spiropidion and SYN547305 are not inhibitors of rat thyroid peroxidase activity *in vitro*.	98.4 CSH001-002-001	Lake (2014)

N/A: not applicable

Table A 0.17. Mean rate of [^{125}I]-thyroxine glucuronidation in hepatic microsomes

	Dose concentration (ppm)			
	Males		Females	
	0	2500	0	1500
Mean rate of Thyroxine Glucuronidation (pmol/min/mg protein)	1.531 ± 0.430	3.857 ± 0.760	1.140 ± 0.471	2.490 ± 0.519
p value	-	0.000004	-	0.00002
Mean hepatic [^{125}I]-Thyroxine Glucuronyl Transferase Activity (pmol/min/mg liver)	22.523 ± 8.590	85.418 ± 21.619	15.986 ± 7.568	32.648 ± 13.307
p value		0.000004		0.004
Mean hepatic [^{125}I]-Thyroxine Glucuronyl Transferase Activity	282.552	932.609	123.946	237.147

(pmol/min/total liver weight) p value	± 117.386	± 171.579 0.000001	± 64.613	± 86.043 0.005
Mean hepatic [^{125}I]-Thyroxine Glucuronyl Transferase Activity (pmol/min/relative liver weight) p value	708.288 ± 270.034	2608.740 ± 602.454 0.000003	506.502 ± 239.810	1101.723 ± 416.819 0.002

Table A 0.18. Effect of spiropidion (SYN546330) and PTU on rat thyroid peroxidase activity

Addition	Thyroid peroxidase activity (nmol/minute/mg protein)
Control (DMSO only)	4.34 ± 0.186 (100)
SYN546330 0.05 µM	4.22 ± 0.050 (97)
SYN546330 0.5 µM	4.27 ± 0.029 (98)
SYN546330 2 µM	4.27 ± 0.080 (98)
SYN546330 5 µM	4.33 ± 0.334 (100)
SYN546330 10 µM	4.37 ± 0.176 (101)
PTU 10 µM	0.003 ± 0.0006** (0.07)

Values in parentheses are percentage of control levels

** Statistically significant difference from relevant control value (P<0.01)

Table A 0.19. Effect of SYN547305 and PTU on rat thyroid peroxidase activity

Addition	Thyroid peroxidase activity (nmol/minute/mg protein)
Control (DMSO only)	5.15 ± 0.220 (100)
SYN547305 0.05 µM	4.93 ± 0.367 (96)
SYN547305 0.5 µM	4.78 ± 0.648 (93)
SYN547305 2 µM	4.97 ± 0.191 (97)
SYN547305 5 µM	4.71 ± 0.419 (91)
SYN547305 10 µM	4.94 ± 0.230 (96)
PTU 10 µM	0.002 ± 0.0006** (0.04)

Values in parentheses are percentage of control levels

** Statistically significant difference from relevant control value (P<0.01)

In addition to the MOA described, alternative modes of action for the induction of thyroid tumours exist. One such alternative MOA is genotoxicity. This MOA can be excluded as spiropidion has been demonstrated to be negative for genotoxicity in a comprehensive package of *in vitro* and *in vivo* assays for genotoxicity (Bohnenberger 2015, Dunton 2015 and 2018, Naumann 2018, Sokolowski 2014, Thompson 2018, Whitwell 2018).

Adult male rats have higher serum TSH levels than female rats (Chen, 1984), and they are often more sensitive to stimulation of thyroid growth and carcinogenesis.

A second alternative MOA is the direct inhibition of thyroid hormone synthesis. Organification of iodine via monoiodination of L-tyrosine is the first step in the synthesis of T3 and T4 and is catalysed by the enzyme TPO. Inhibition of TPO, to reduce circulating T3/T4, by compounds such as PTU is exploited as a treatment for hyperthyroidism in humans (e.g., Graves' disease). PTU has also been shown to induce thyroid follicular cell adenomas in rats (IARC, 2001). This MOA can be excluded for spiropidion as it was found not to be an inhibitor of male rat thyroid-derived TPO *in vitro*, whereas PTU was shown to be a potent inhibitor (Lake, 2014).

The available data for spiropidion support a proposed MOA in rats involving:

- Induction of hepatic UDP-GT, resulting in increased conjugation and excretion of triiodothyronine (T3) and thyroxine (T4),
- A decrease in serum T4 levels,
- A compensating increase in thyroid stimulating hormone (TSH) levels secreted via the hypothalamic-pituitary-thyroid (HPT) axis; and
- Under the chronic proliferative stimulus of TSH, thyroid follicular cells undergo hypertrophy, and eventually hyperplasia and progress to form follicular cell adenomas and/or carcinomas.

One factor that may play a role in interspecies quantitative sensitivity to thyroid stimulation deals with the influence of protein carriers of thyroid hormones in the blood (Table A A.8).

Both humans and rodents have nonspecific low-affinity protein carriers of thyroid hormones (e.g., albumin). However, in humans, other primates, and dogs there is a high-affinity binding protein, thyroxine-binding globulin, which binds T4 (and T3 to a lesser degree); this protein is missing in rodents and lower vertebrates. As a result, more T4 remains bound to proteins with lower affinity in the rodent and is more susceptible to removal from the blood, metabolism, and excretion from the body. In keeping with this finding, the serum half-life of T4 is much shorter in rats (less than 1 day) than in humans (5 to 9 days); this difference in T4 half-life results in a 10-fold greater requirement for exogenous T4 in the rat with a non-functioning thyroid than in the adult human (Döhler et al., 1979). Serum T3 levels also show a species difference; the half-life in rats is about 6 hr while that in humans is about 24 hr (Oppenheimer, 1979; Larsen, 1982).

There is a morphological consequence to these hormone differences.

High thyroid hormone synthetic activity is demonstrated in follicles in rodents: they are relatively small, surrounded often by cuboidal epithelium. Follicles in primates demonstrate less activity and are large with abundant colloid, and follicular cells are relatively flattened (low cuboidal) (McClain, 1992).

The available data also demonstrate that a threshold exists for the induction of the key events in this MOA. This mode of action is not relevant for human hazard/risk assessment purposes due to qualitative and quantitative differences in response to UDP-GT induction and increased T3/T4 clearance between rats and humans. In summary, the toxicity data supports the conclusion that spiropidion does not pose a carcinogenic hazard to humans.

A.15. EDSP Screening Data

None of the read-across compounds were included in the US EDSP Tier 1 Screening Assessment (US EPA, 2015c).

A.16. Evidence of Immune Suppression –Read-across

Immunotoxicity was not considered a target for any of the read-across chemicals or compound groups. In all available studies, immunotoxic effects were not reported at the highest dose level tested (Table A 0.20.).

Table A 0.20. Read-across immunotoxicity summaries

Compound	Immunotoxicity(mg/kg/day)
Tas/TADs	
Spirodiclofen (Mouse)	NOAEL = 1216, highest dose tested LOAEL > 1216
Spiromesifen (Rat, Mouse)	(Rat) NOAEL = 52.8/45.7 (M/F) LOAEL = 291.6/28.6 (M/F) Based on mortality (females), clinical signs (both sexes), and decreased body weights and body weight gains (both sexes). Treatment did not significantly alter the IgM antibody-forming cell response to the T cell-dependent antigen, sheep erythrocytes.
	(Mouse) NOAEL = 162.5/278.7 (M/F) LOAEL = 1226.6/1510.2 (M/F) Based on decreased body weights and body weight gains in males and decreased water consumpt and spleen weights in both sexes. Under the conditions of this study, no immunotoxicity (as detecte plaque forming cells) was observed
Spirotetramat (Rat)	Functional immunotoxicity NOAEL = 795 LOAEL > 795 Systemic toxicity NOAEL = 164 (M) LOAEL = 795 (M) Based on decreased body weights, lowered food consumption, lower thymus weight, and observations of atrophic thymus.
Spiropidion	"No evidence of immunotoxicity was reported in routine toxicological studies"

A.17. Evidence of Neurotoxicity – Read-across

The nervous system was not considered to be a primary target of toxicity for the read-across ACCase inhibitor compounds (Table A 0.21). While the read-across compounds had no notable neurotoxic effects, the potential for neurotoxicity was addressed in the US EPAs review of spiropidion (2022) establishing tolerance levels. In the acute neurotoxicity study (I) in female rats and in the subchronic and chronic dog studies clinical signs were observed indicative of potential neurotoxicity. The US EPA (2022) noted that the concern is low because (1) the effects observed in the acute neurotoxicity study were observed at higher dose levels than systemic toxicity; (2) clear NOAELs were identified for the neurotoxic effects; and (3) the points of departure chosen for risk assessment are protective of any potential neurotoxicity observed in the database.

The JMPR report included an acute neurotoxicity study for spiropidion, which indicated convulsions in females at the highest dose tested, but no histopathological changes in the nervous system. The JMPR concluded that "although there were no indications of neuropathological effects due to spiropidion", it "may cause, acute neurobehavioral effects at high doses." The acute neurotoxicity study was the basis for the acute dietary point of departure for spirotetramat.

Table A 0.21. Read-across neurotoxicity summaries

Compound	Acute Neurotoxicity- Rat (mg/kg bw)	Subchronic Neurotoxicity–Rat (mg/kg/day)	Developmental Neurotoxicity – Rat (mg/kg/day)
TAs/TADs			
Spirodiclofen	NOAEL = 2000 No neurotoxicity observed.	NOAEL=70.3/87.3 (M/F) LOAEL = 1088.8/1306.5 (M/F) Based on decreased body weights, food consumption, and increased urine staining in both sexes and decreased motor and locomotor activity (week 4) in females only.	Maternal NOAEL = 135.9/273.8 LOAEL = Not established.
			Offspring NOAEL = Not established. LOAEL = 6.5/14.0 Based on effects in memory phase of the water maze test in PND 60 females.
Spirodiclofen	NOAEL = 2000 LOAEL not established	NOAEL = 31.8/38.3(M/F) LOAEL = 122.7/149.3(M/F) Based on decreased body weight and food consumption.	ND
Spiromesifen	NOAEL = 100 LOAEL = 200 Based on clinical signs (males and females) and decreased motor activity (males).	NOAEL = 585/707 (M/F) LOAEL = not established	ND
Spiropidion	Neurotoxicity NOAEL = 150 LOAEL = 500 (F) Based on clinical signs (convulsions) in females.	ND	ND
	Systemic NOAEL = 150 LOAEL = 500 (M) Based on body weight loss in males.		

ND = No Data

A.18. Special Studies and Endpoints – Read across Target Organs of Toxicity

Target organs of toxicity are included in Table A 0.22.

For the TAs/TADs, the adrenal glands were identified as the target organ for spirodiclofen and spiromesifen. Thyroid toxicity was identified as a target organ for spiromesifen and spirotetramat. Testicular effects were identified with spirodiclofen and spirotetramat. The liver was identified as a target organ for spiromesifen and spiropidion. Only spiropidion was associated with neurotoxicity in dogs.

Table A 0.22. Target organs for toxicity

Compound	Target Organs
TAs/TADs	
Spirodiclofen	Adrenal glands and testes
Spiromesifen	Adrenal glands, thyroid, liver, and spleen
Spirotetramat	Thyroid, thymus gland, and testes
Spiropidion	Body weights, reductions in cholesterol and triglycerides in rodents; liver and thyroid in rats, severe clinical signs indicative of systemic neurotoxicity in dogs.

A.19. Proposed Points of Departure (PODs) – Read-across : *Endpoints for Chronic Dietary Risk Assessment*

Table A 0.23. summaries the endpoints for chronic dietary risk assessment. For the TAs/TADs, the chronic reference doses (cRfDs) ranged from 0–0038 - 0.2 mg/kg/day. Total UF were 100X for all compounds except for spiromesifen which has the FQPA UF retained as SF/UFDB for the lack of a comparative metabolism study. The key study for spirodiclofen and spirotetramat was the chronic toxicity studies in dogs, the key study for spiromesifen was the 2-generation rat reproduction study, and the key study for spiropidion was the rat chronic toxicity/carcinogenicity study.

Table A 0.23. Summary of POD and cRfDs for read-across

Compound	cRfD	UF	POD (NOAEL)	LOAEL	Effect
TAs/TADs					
Spirodiclofen	0.014	UF = 100X FQPA SF = 1X	1.38	4.7	Chronic Toxicity - Dog Based on increased relative adrenal weights in both sexes, increased relative testis weight in males and histopathology findings in the adrenal gland of both sexes.
Spiromesifen	0.0038	UF_A = 10X UF_H = 10X FQPA SF/UF_{DB} = 10X	3.8	14.2	Two-Generation Reproductive Toxicity - Rat Based on decreased pup body weight.
Spirotetramat	0.05	UF_A = 10X UF_H = 10X FQPA SF = 1X	5	20	Chronic Toxicity - Dog Based on thymus involution in males.

UF = uncertainty factor; UFA = extrapolation from animal to human (interspecies); UFH = potential variation in sensitivity among members of the human population (intraspecies); UF_L = use of a LOAEL to extrapolate a NOAEL; UF_{DB} = to account for the absence of key data (i.e., lack of a critical study); FQPA SF = FQPA Safety Factor

A.20. Conclusions

The target organs of toxicity for the TAs/TADs, were the adrenal glands, liver, thyroid, and testes, with effects in the target organs detected following 90 days of oral exposure. The TAs/TADs were not associated with genotoxicity. Spirodiclofen was the only compound classified as likely to be carcinogenic to humans with a Q1* assigned. All other compounds were classified as not likely to be carcinogenic to humans. Two compounds are not associated with any acute adverse effects and two compounds have acute endpoints for the general population assigned based on the acute neurotoxicity study or the 28-day oral toxicity study in dogs. For chronic dietary risk assessment, two compounds shared testicular effects in chronic oral toxicity in dogs or the chronic toxicity/carcinogenicity study in rats as the basis for the point of departure, the adverse effects in another study were based on decreased pup body weight in the 2-generation rat reproduction study, and one compound was associated with thymus involution in dogs as the critical effect.

A.21. References

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Annex C. Lessons Learned and Next Steps for use of the IATA to support a WoE-based carcinogenicity assessment

By the nature of the OECD IATA Case Study Project (CSP), there is a well-structured timeline and associated milestones to receive feedback from OECD members and observers. What is unique to this framework is that the initial case study (Annex A) was submitted as a pilot to receive OECD feedback to help inform the refinement of a framework. The hope was that a more overarching framework could be used to support a WoE-based assessment for carcinogenicity, such that a series of case studies could be submitted to demonstrate 1) the applicability of the framework; 2) to identify gaps; and 3) start to align on the sufficiency of data within the WoE-based approach for chronic safety evaluations.

In taking the 'pilot' approach to the OECD IATA CSP, the authors were fortunate to have opportunities to receive several rounds of review whereby key lessons were learned during the development and application of this framework, from contextualizing the tools and methods used, to considerations for reporting a clear rationale for the decisions made throughout the WoE-based assessment. Valuable recommendations were collected during the OECD review of the two case study examples provided in this IATA publication Annex A and Annex B). The major areas for feedback included:

- Framework application: the framework should be globally applicable in structuring a WoE-based carcinogenicity assessment.
- Read-across assessment: provide a sufficient level of detail in the read-across assessment, including the criterion for analogue inclusion or exclusion.
- Uncertainty analysis: adequately captures and reports uncertainties related to the quality of information supporting the WoE.

Ultimately, the case studies submitted through the OECD CSP can be used to evaluate the sufficiency of data to support the development of guidance for WoE-based carcinogenicity assessment. Thus, the goal of sharing these lessons is to improve the acceptance of this framework through preparing stronger case studies in the future.

Herein, a summary of key learnings is shared as they relate to 1) framework application; 2) read-across assessment; and 3) uncertainty analysis.

1. Framework application

- The framework provides a starting place to structure evidence for the WoE assessment. It is not prescriptive, rather it is a tool to aid in organizing and presenting information.
- The framework is not regional, it is designed to be global. The WoE should consider how the individual regions interpret and assess the hazard and risk of chemicals.
- Authors should provide a narrative explaining the processes used for each line of evidence; from the cited references used and location of the data sources collected to the approach used in conducting the vulnerability assessment of each study report for its reliability in reference to the current test guidelines.
- When considering the appropriate tools and models in the WoE, it is the author's responsibility to decide what are the best tools/resources that are available to conduct the necessary measurements for each aspect of the assessment.
    - For example: the selection of tools to assess the structural similarity of chemicals, methods in any mechanistic research, or approach to conducting a literature search and review will rely on the purpose of the analysis.
- Authors are encouraged to articulate the objective of the work that was done, the rationale for the tools selected, and an explanation of the interpretations made with the resulting information.
- The two example case studies published in this OECD IATA used the regulatory reviews and interpretations of the studies from one regulatory Authority based on the availability of data and consistency in the practice used in characterising hazard and assessing the human safety risk. In future case studies, a comparison of all available Agency data reviews will expand on the hazard assessment and report the concerns and uncertainties for each chemical in greater detail.
- When citing a study, be clear on the version and year of the regulatory testing guideline used (note: the results of a study that has used a previous version of the test guidelines are still credible, and documentation should be provided if a new guideline is available).
- Provide a vulnerability assessment and describe how the interpretation of the study may have changed based on the updates to the test guideline. A vulnerability assessment of a study compares the version of the guideline used in the conduct of the study with the current guideline published. This serves to identify, quantify, and prioritise the vulnerabilities i.e., uncertainties, data gaps, and the interpretation of the results by the current practice used by the Authorities. This assessment will reduce the uncertainty and increase the reliability of the data used in the WoE assessment.

2. Read-across assessment

- Clearly define the process and criteria for the analogue selection (inclusion and exclusion).
    - Report if biological or structural groups were used as a starting search for analogue inclusions.
    - The authors note that when the target substance belongs to a large chemical class, or a chemical class with a clearly established pMOA and/or MOA, these could serve as a helpful starting point to determine inclusion – especially if the read-across is used to propose a biological MOA.

    - The reliance on chemical class and MOA may be different for substances that are not agrichemical, where data are not well-developed, such that structural similarities will likely be the more appropriate starting point to search for analogues.
- Report the tools used to conduct the read-across assessment and explain how the tools were used.
- Report the similarity index used, where applicable, and report the cutoff values for analogue inclusion/exclusion.
- Explain the uncertainty of structural similarity and justify when structural similarity should be considered over the bioactivity or pesticidal MOA when selecting analogues.

For the analogues selected for read-across assessment, evaluate potential regional differences in interpretations of the toxicity profile across publicly available data, including regulatory documents. This is to ensure that the endpoints used in the read-across assessment are in compliance with the regional risk assessment paradigm.

3. Uncertainty analysis

- A thorough uncertainty analysis is critical to identify strengths and weaknesses within the WoE. Clearly report data gaps and/or limitations found in using the WoE framework, tools, and selected approaches during the WoE assessment.
- Test guidelines and guidance documents should be reported (including their publisher and publication date), where available. The year in which the guidance was used should also be reported.
    - For example: If data are used within the WoE from an older version of a guideline, but a newer version of the guideline is available, discuss possible changes in interpretation. Explaining the differences will reduce uncertainty and increase the reliability of the data used. Note, *that the study does not need to be repeated*, this is just to illustrate potential differences in data generation and interpretation.
- If the WoE is conducted for a regional assessment only, report uncertainties that may arise due to the targeted application of the WoE-based assessment.
- Report uncertainty that can arise in the WoE by lack of data quality and/or lack of data access (E.g., limited or no availability of study reports for chemical analogues). Identify areas of uncertainty with respect to the relevance and reliability of the data and how this may change the interpretation of the data.
- Report uncertainty that can arise by regional differences in regulatory data needs.
    - For example: endocrine disruption data requirements are different in Europe compared to other regions like the US or Canada. There may be data gaps depending on regional regulatory submissions.

4. Next steps

The case studies in this IATA serve as examples to illustrate the potential use of the framework. These case studies are a starting point to help identify knowledge gaps and data needs for the successful use of this framework in supporting regulatory decisions. To build confidence in this approach, additional case studies will help to illustrate the applicability and context of use for this framework in supporting a WoE-based carcinogenicity assessment, without the rodent

cancer bioassay(s). To fully test this framework, it would also be helpful to assess additional active ingredient agrichemical substances for those that are carcinogenic, and those that are clearly non-carcinogenic; as well as case studies demonstrating how to fulfil data requirements in a spectrum of regulatory jurisdictions. Having a variety of WoE carcinogenicity case studies would increase the confidence in implementing the framework in different use-case scenarios. Case studies could also be developed to examine the use of this framework to non-agrichemical substances such as industrial chemicals.

As more case studies are developed, this will demonstrate how the framework could be applied to support WoE-based carcinogenicity assessment. A series of case studies could be used to facilitate a workshop to identify remaining data gaps, as well as the sufficiency of data, which could ultimately be used to support the development of OECD guidance. Thus, the learnings shared in this document offer insight to reduce uncertainty in future case studies, with the goal of bettering the subsequent cases to get one step closer to defining a globally accepted WoE-based approach to fulfilling carcinogenicity data needs, without relying on the rodent cancer bioassays.

Case Study on the Use of Integrated Approaches for Testing and Assessment (IATA) for Chronic Toxicity and Carcinogenicity of Agrichemicals with Exemplar Case Studies - Ninth Review Cycle (2023)

Series on Testing and Assessment No. 402

The objective of the Integrated Approaches for Testing and Assessment (IATA) Case Studies Project is to increase experience with the use of IATA by developing case studies which constitute examples of predictions that are fit for regulatory use. The aim of this project is to create common understanding of using novel methodologies and the generation of considerations/guidance stemming from these case studies. This case study was developed by the International Council on Animal Protection in OECD Programmes (ICAPO) to illustrate practical uses of IATA, and was submitted to the 2023 review cycle of the IATA Case Studies Project. The case study provides a framework to fulfil an IATA for chronic toxicity and carcinogenicity assessment through a weight of evidence (WoE)-based approach, in the absence of rodent cancer bioassays. The purpose of this IATA is to illustrate the use of the Rethinking Carcinogenicity Assessment for Agrichemicals Project (ReCAAP) framework, which is a scientific, WoE-based approach that allows the estimation of a Point of Departure (POD) for use in agrochemical risk assessment. To illustrate the use of the ReCAAP framework, two examples are presented in this IATA.