ReCAAP: A reporting framework to support a weight of evidence safety assessment *without* long-term rodent bioassays

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Outline

Method Description

ReCAAP framework overview

Context of Use

Regulatory implications

Biological Relevance

Application of ReCAAP Framework

Technical Characterization

Scientific Validity

Method Description

ReCAAP Framework

Method description

Rethinking Carcinogenicity Assessment for Agrochemicals Project (ReCAAP)

Weight of evidence (WoE)-based assessment to estimate a health-protective point of departure (POD) for chronic risk assessment.

EPA. 2013. Guiding principles for data requirements
EPA. 2016. Weight of evidence in ecological assessment
EFSA. 2017. Guidance on the use of the weight of evidence approach in scientific assessments
HC. 2018. Weight of evidence: General principles and current applications at Health Canada
SHEER. 2018. Memorandum on weight of evidence and uncertainties
OECD. 2019. Guiding principles and key elements for establishing a weight of evidence for chemical assessment

Method description

US EPA guidelines to assess health effects for agrochemicals

Series 870 - Health Effects Test Guidelines

The final Health Effects Test Guidelines are generally intended to meet testing requirements for human health impacts of chemical substances under FIFRA and TSCA.

Supplemental Guidance

Test Guidelines/Acute Toxicity - Acute Oral Toxicity Up-And-Down-Procedure
Guidance for Waiving or Bridging of Mammalian Acute Toxicity Tests for Pesticides and Pesticide Products
Guidance for Neurotoxicity Battery, Subchronic Inhalation, Subchronic Dermal and Immunotoxicity Studies
Genetic Toxicology: Integration of in vivo Testing into Standard Repeat Dose Studies
Use of an Alternate Testing Framework for Classification of Eye Irritation Potential of EPA Pesticide Products
Update on the Use of the Local Lymph Node Assay for End Use Pesticide Products and Adoption of the Reduced Dose Protocol for LLNA (rLLNA)

Group A – Acute Toxicity Test Guidelines

870.1000 - Acute Toxicity Testing--Background (December 2002)
870.1100 - Acute Oral Toxicity (December 2002)
870.1200 - Acute Dermal Toxicity (August 1998)
870.1300 - Acute Inhalation Toxicity (August 1998)
870.2400 - Acute Eye Irritation (August 1998)
870.2500 - Acute Dermal Irritation (August 1998)
870.2600 - Skin Sensitization (March 2003)

Group B – Subchronic Toxicity Test Guidelines

870.3050 - Repeated Dose 28-Day Oral Toxicity Study in Rodents (July 2000)
870.3100 - 90-Day Oral Toxicity in Rodents (August 1998)
870.3150 - 90-Day Oral Toxicity in Nonrodents (August 1998)
870.3200 - 21/28-Day Dermal Toxicity (August 1998)
870.3250 - 90-Day Dermal Toxicity (August 1998
870.3465 - 90-Day Inhalation Toxicity (August 1998)
870.3550 - Reproduction/Developmental Toxicity Screening Test (July 2000)
870.3650 - Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (July 2000)
870.3700 - Prenatal Developmental Toxicity Study (August 1998)
870.3800 - Reproduction and Fertility Effects (August 1998)

Group C – Chronic Toxicity Test Guidelines

870.4100 - Chronic Toxicity (August 1998)
870.4200 - Carcinogenicity (August 1998)
870.4300 - Combined Chronic Toxicity/Carcinogenicity (August 1998)

Group D – Genetic Toxicity Test Guidelines

870.5100 - Bacterial Reverse Mutation Test (August 1998)
870.5140 - Gene Mutation in *Aspergillus nidulans* (August 1998)
870.5195 - Mouse Biochemical Specific Locus Test (August 1998)
870.5200 - Mouse Visible Specific Locus Test (August 1998)
870.5250 - Gene Mutation in *Neurospora crassa* (August 1998)
870.5275 - Sex-linked Recessive Lethal Test in *Drosophila melanogaster* (August 1998)
870.5300 - *In vitro* Mammalian Cell Gene Mutation Test (August 1998)
870.5375 - *In vitro* Mammalian Chromosome Aberration Test (August 1998)
870.5380 - Mammalian Spermatogonial Chromosomal Aberration Test (August 1998))
870.5385 - Mammalian Bone Marrow Chromosomal Aberration Test (August 1998)
870.5395 - Mammalian Erythrocyte Micronucleus Test (August 1998)
870.5450 - Rodent Dominant Lethal Assay (August 1998)
870.5460 - Rodent Heritable Translocation Assays (August 1998)
870.5500 - Bacterial DNA Damage or Repair Tests (August 1998)
870.5550 - Unscheduled DNA Synthesis in Mammalian Cells in Culture (August 1998)
870.5575 - Mitotic Gene Conversion in *Saccharomyces cerevisiae* (August 1998)
870.5900 - *In vitro* Sister Chromatid Exchange Assay (August 1998)
870.5915 - *In vivo* Sister Chromatid Exchange Assay (August 1998)

Group E – Neurotoxicity Test Guidelines

870.6100 - Acute and 28-Day Delayed Neurotoxicity of Organophosphorus Substances (August 1998)
870.6200 - Neurotoxicity Screening Battery (August 1998)
870.6300 - Developmental Neurotoxicity Study (August 1998)
870.6500 - Schedule-Controlled Operant Behavior (August 1998)
870.6850 - Peripheral Nerve Function (August 1998)
870.6855 - Neurophysiology Sensory Evoked Potentials (August 1998)

Group F – Special Studies Test Guidelines

870.7200 - Companion Animal Safety (August 1998)
870.7485 - Metabolism and Pharmacokinetics (August 1998)
870.7600 - Dermal Penetration (August 1998)
870.7800 - Immunotoxicity (August 1998)

Group G – Health Effects Chemical-Specific Test Guidelines

870.8355 - Combined Chronic Toxicity/Carcinogenicity Testing of Respirable Fibrous Particles (July 2001)

https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-870-health-effects-test-guidelines

Data integration

Rethinking Carcinogenicity Assessment for Agrochemicals Project (ReCAAP)

ICH S1B addendum – pharmaceuticals

- Knowledge of intended drug target
- Genetic toxicology
- Subchronic
- Metabolic profile
- Hormone perturbation
- Immune suppression
- Special studies
- **Non-rodent chronic**
- **Transgenic mouse**

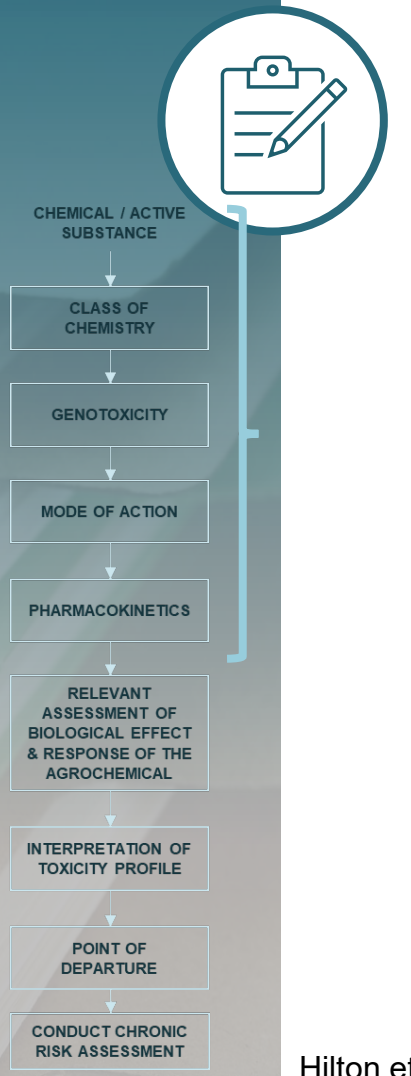
ReCAAP – agrochemicals

- **Read-across**
- Genetic toxicology
- ADME
- Toxicity (subchronic)
- Hormone perturbation
- Immunotoxicity
- Special studies (MOA)
- **Intended use**
- **Exposure**
- **Risk estimates (POD)**

S1B(R1) Addendum to S1B Testing for Carcinogenicity of Pharmaceuticals Guidance for Industry
https://www.fda.gov/regulatory-information/search-fda-guidance-documents/s1br1-addendum-s1b-testing-carcinogenicity-pharmaceuticals

Hilton, et al, 2022, Rethinking chronic toxicity and carcinogenicity assessment for agrochemicals project (ReCAAP): A reporting framework to support a weight of eviden
without long-term rodent bioassays
https://pubmed.ncbi.nlm.nih.gov/353116

Method workflow



ReCAAP Framework to support WoE carcinogenicity assessment

CHEMICAL / ACTIVE SUBSTANCE

CLASS OF CHEMISTRY

- ↔ Structural analogs; categorical or 1:1 read across assessment
- ↔ QSAR → Structural alerts for (i) genotoxicity, (ii) systemic toxicity for active substance and analogs

GENOTOXICITY

- ↔ Mutagenic, aneugenic, and/or clastogenic → Standard risk assessment for genotoxic chemicals

MODE OF ACTION

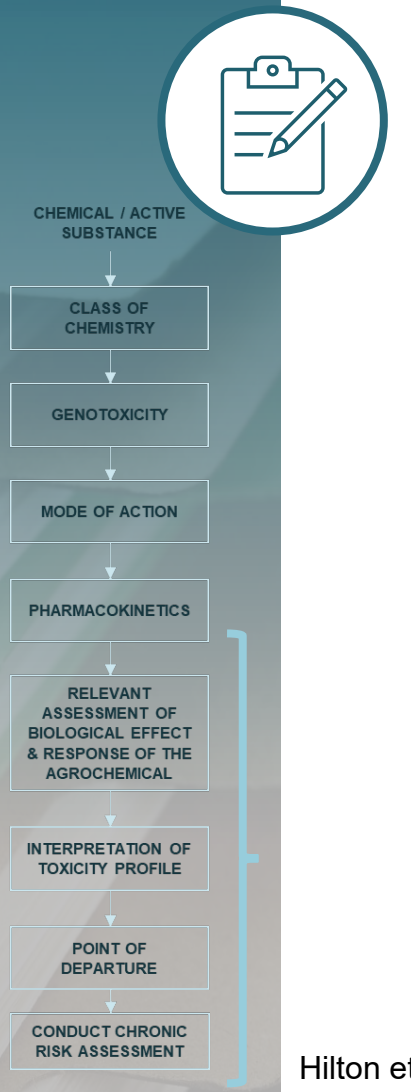
- ↔ Analogs' effects can forecast toxicity and influence safety testing strategy

PHARMACOKINETICS

- ↔ ADME profile with TK analysis and PBK modelling. Identifies systemic exposure, clearance rates, and assesses dose proportional exposure. Read Across with analogs.
- ↔ Physical Chemistry characteristics e.g., is it lipophilic? Read-across with analogs.

Hilton et al., 2024, OECD IATA Case Study, Approved *(OECD declassification in progress)*

Method workflow (continued)



ReCAAP Framework to support WoE carcinogenicity assessment

RELEVANT ASSESSMENT OF BIOLOGICAL EFFECT & RESPONSE OF THE AGRO CHEMICAL

↔ Tiered approach:
In silico modelling e.g., differences in binding?
In vitro screening e.g., comparison of metabolism profiles across species
Acute toxicity i.e., oral, dermal, inhalation, skin sensitization
Systemic Target Organ(s) of Toxicity; short-term and subchronic exposures

INTERPRETATION OF TOXICITY PROFILE

↔ Mode of Action research, assess for human relevance

↔ Identify and assess potential for chronic toxicity, based on WOE

POINT OF DEPARTURE

↔ Use the safety profile of the chemical to characterize the hazard and define the human health protective threshold i.e., dose level for no biological effect(s)

↔ WOE supported by input from relevant structural analog chemicals

CONDUCT CHRONIC RISK ASSESSMENT

↔ Uncertainty Factors

↔ Use patterns & Exposure scenarios

Hilton et al., 2024, OECD IATA Case Study, Approved *(OECD declassification in progress)*

Context of Use

Regulatory Implications

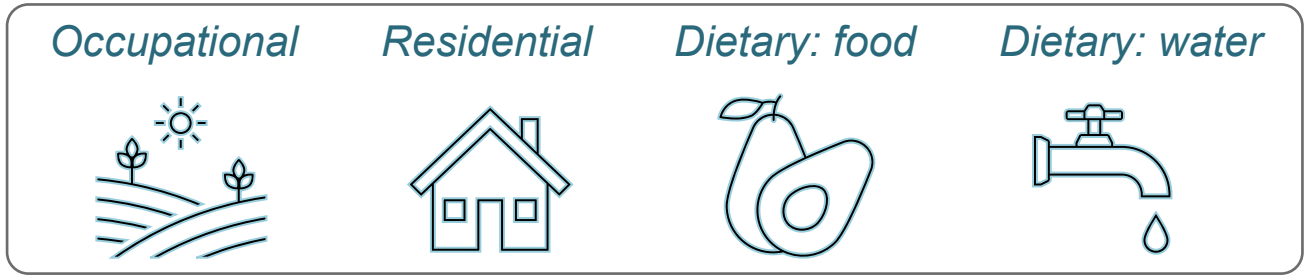
Context of use: risk assessment

Step 1 - Hazard Identification

Step 2 - Dose-Response Assessment

Step 3 – Exposure Assessment

Step 4 – Risk Characterization



Chronic dietary risk assessment

The **chronic population adjusted dose (cPAD)** is the dose at which a person could be exposed over the course of a lifetime, with no expected adverse health effects.

https://www.epa.gov/risk/human-health-risk-assessment

Estimating POD for chronic risk

Regulatory Application

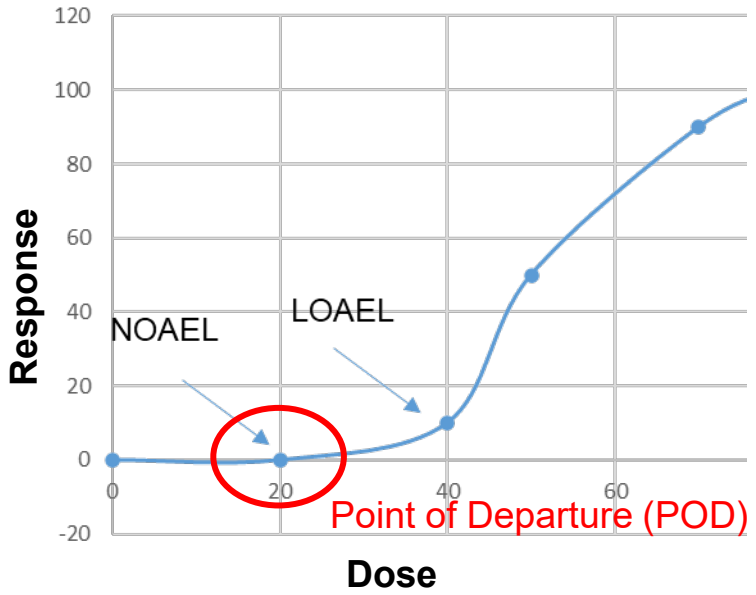
Title 40 / Chapter I / Subchapter E / Part 158 / Subpart F — Previous / Next / Top

Table of Contents
Details
Print/PDF
Display Options
Subscribe
Timeline
Go to Date
Compare Dates
Published Edition
Developer Tools

Expand Table

Estimating chronic risk

Guideline Number	Data Requirements	Use Pattern		Test substance to support		Test Note No.
		Food	Nonfood	MP	EP	
870.3100	90-day Oral - rodent	R	CR	TGAI	TGAI	8, 9
870.3150	90-day Oral - non-rodent	R	CR	TGAI	TGAI	36
870.3200	21/28-day Dermal	R	NR	TGAI	TGAI and EP	10, 11
870.3250	90-day Dermal	CR	R	TGAI	TGAI and EP	11, 12
870.3465	90-day Inhalation - rat	CR	CR	TGAI	TGAI	13, 14
870.6100	28-day Delayed neurotoxicity-hen	CR	CR	TGAI	TGAI	6, 15
870.6200	90-day Neurotoxicity - rat	R	R	TGAI	TGAI	7, 16
Chronic Testing						
870.4100	Chronic oral - rodent	R	CR	TGAI	TGAI	17, 18, 19
870.4200	Carcinogenicity - two rodent species - rat and mouse preferred	R	CR	TGAI	TGAI	9, 17, 18, 19, 20, 21



$$cPAD = \frac{NOAEL}{\text{Uncertainty Factor (UF)}}$$

https://www.ecfr.gov/current/title-40/chapter-I/subchapter-E/part-158/subpart-F

Chronic risk assessment

$$cPAD = \frac{4.6\ mg/kg/day}{10X\ (x)\ 10X} = \frac{0.046}{mg/kg/day}$$

4.5.4 Summary of Points of Departure and Toxicity Endpoints Used in Human Risk Assessment

Table 4.5.4.1. Summary of Toxicological Doses and Endpoints for Saflufenacil for Use in Dietary Human-Health Risk Assessments.

Exposure Scenario	Point of Departure	UFs/ FQPA SF	RfD and PAD	Study and Toxicological Effects
Acute Dietary (General Population, including Infants and Children)	NOAEL = 500 mg/kg bw	UF_A = 10X UF_H = 10X FQPA SF = 1X	**aRfD** = 5.0 mg/kg **aPAD** = 5.0 mg/kg	Acute Neurotoxicity Study - rats NOAEL = 500 mg/kg bw. LOAEL = 2000 mg/kg bw based on decreased motor activity representing mild and transient systemic toxicity in males.
Chronic Dietary (All Populations)	NOAEL = 4.6 mg/kg/day	UF_A = 10X UF_H = 10X FQPA SF = 1X	**cRfD** = 0.046 mg/kg/day **cPAD** = 0.046 mg/kg/day	Chronic/Carcinogenicity (mouse) NOAEL = 4.6 mg/kg bw/day. LOAEL = 13.8 mg/kg bw/d based on decreased red blood cells, hemoglobin, hematocrit, and porphyria observed in the satellite group.
Cancer (oral, dermal, inhalation)	Classification: Not likely carcinogenic to humans based on the lack of tumors in the mouse and rat carcinogenicity studies and lack of mutagenicity.			

= lowest-observed adverse-effect level. UF = uncertainty
erspecies). UF_H = potential variation in sensitivity among
= margin of exposure. LOC = level of concern. FQPA SF
se (a = acute, c = chronic). RfD = reference dose.

NOAEL = no-observed adverse-effect level. LOAEL =
factor. UF_A = extrapolation from animal to human (int
members of the human population (intraspecies). MOE
= FQPA Safety Factor. PAD = population-adjusted do

https://www.regulations.gov/document/EPA-HQ-OPP-2013-0008-0015

EPA's Level of Concern

Fulfill the chronic risk estimation with a WoE assessment

Population Subgroup	cPAD (mg/kg/day)[a]	Chronic Dietary Exposure (mg/kg/day)[b]	Total Exposure % cPAD
General U.S. Population	0.046	0.004223	9.2%
All Infants (<1 year old)	0.046	0.009099	20%
Children 1-2 years old	0.046	0.008368	18%
Children 3-5 years old	0.046	0.006993	15%
Children 6-12 years old	0.046	0.004872	10%
Youth 13-19 years old	0.046	0.003409	7.4%
Adults 20-49 years old	0.046	0.003946	8.6%
Adults 50-99 years old	0.046	0.003679	8%
Females 13-49 years old	0.046	0.003759	8.2%

[a]cPAD is based on the NOAEL from a carcinogenicity mouse study (4.6 mg/kg/day) and a total 100X uncertainty factor, to extrapolate to chronic exposures to human.
[b]Chronic Dietary Exposure was estimated using the agency's Dietary Exposure Evaluation Model (DEEM).

Total Exposure %cPAD = Chronic Dietary Exposure (mg/kg/day) ÷ cPAD

%cPAD < 100 is under the EPA's level of concern

Integrating lines of evidence

ReCAAP cPAD estimate is more conservative than the original estimation using a carcinogenicity study

Population Subgroup	cPAD (mg/kg/day)	Chronic Dietary Exposure (mg/kg/day)	Total Exposure % cPAD
All Infants (<1 year old)	0.0105	0.009099	87
All Infants (<1 year old)	0.046	0.009099	20

ReCAAP estimated cPAD

EPA estimated cPAD

Exposure

Read-across

Mechanistic

Genotoxicity

Metabolism

Both < EPA's level of concern (100%)

Carcinogenicity

Opportunity to use WoE

Regulatory Application

	Existing Guidance	Pre-submission Opportunity
Australian Pesticides and Veterinary Medicines Authority (APVMA)	Agricultural data guidelines: 3.1.1. Submission (2017) https://apvma.gov.au/node/1036	Pre-application assistance https://apvma.gov.au/node/106
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-developing-applications-data-codes-parts-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

Biological Relevance

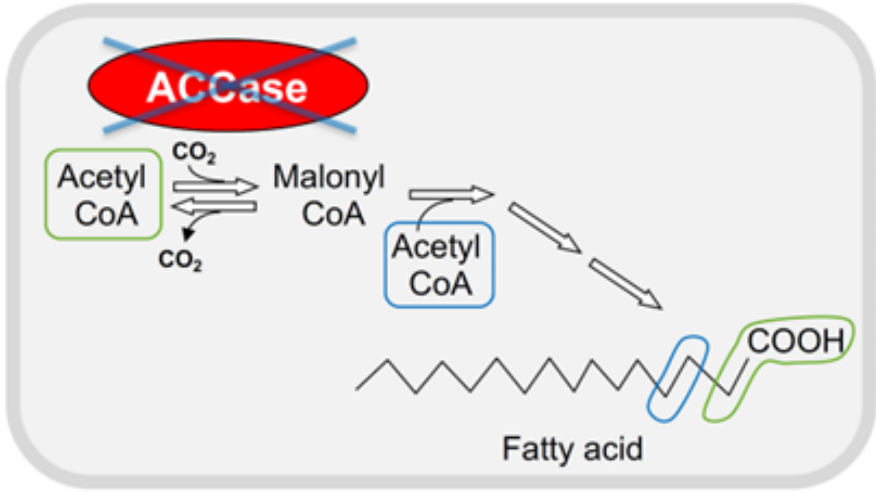
Application of ReCAAP Framework

Biological Relevance

ReCAAP case study example

Key contributions to the WoE assessment

1. Toxicological relevance of ACCase inhibition to mammalian safety profile
2. Mode of Action research
3. Reliability of read-across analogues
    i. Structural similarity
    ii. Biological similarity
    iii. Mechanistic understanding

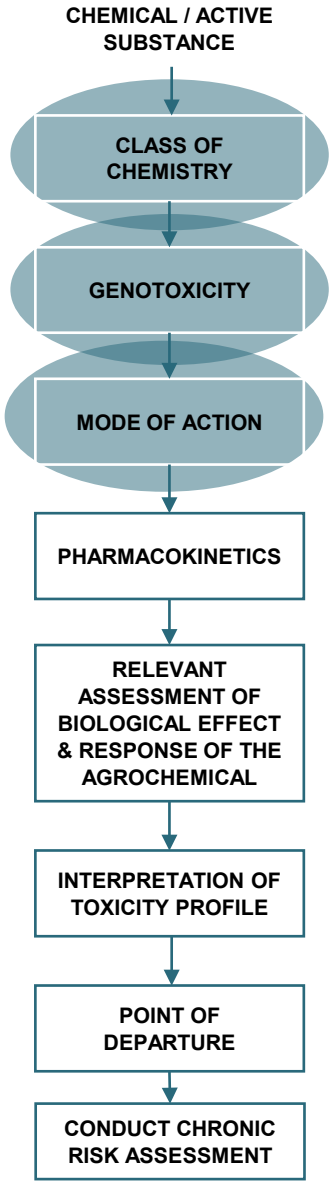


ACCase inhibitors

Target and disrupt growth and development

- ACCase (acetyl CoA carboxylase) catalyzes the first and rate-limiting step of fatty acid biosynthesis.
- ACCase inhibitors prevent biosynthesis of fats needed for growth and development resulting in incomplete molts and desiccation of the insect.

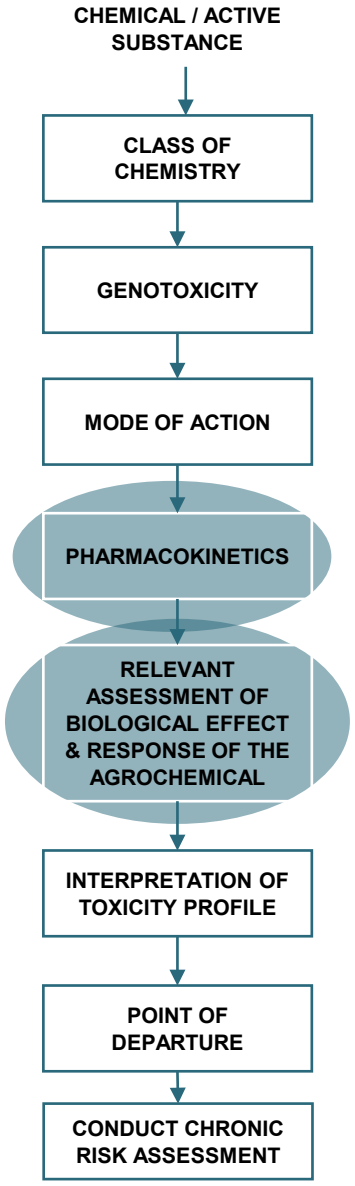
Evaluation of Data



- Spiropidion is an ACCase inhibitor, member of the tetramic and tetronic acid ACCase class of insecticides (IRAC Group 23).
- Spiropidion is non-genotoxic.
- Toxicological mode of action (MoA) studies addressed the quantitative non-human relevance of effects recorded on the thyroid.
- Results of the toxicological data support that hormone perturbation and immune suppression MoAs are not relevant to the chronic toxicity / carcinogenicity in humans.

Strengthen the WoE assessment by providing 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.

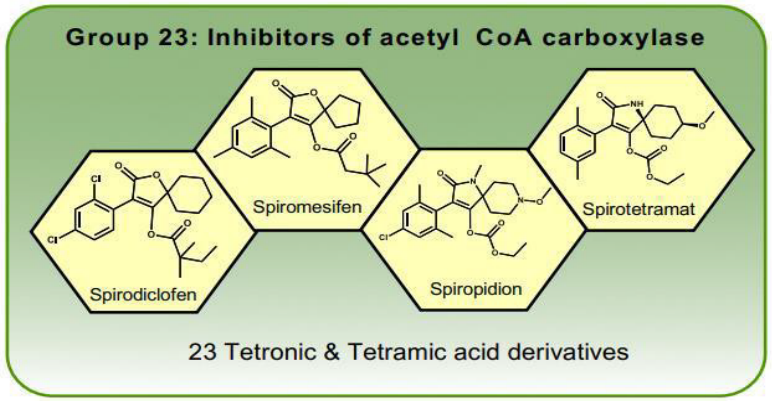
Evaluation of Data (continued)

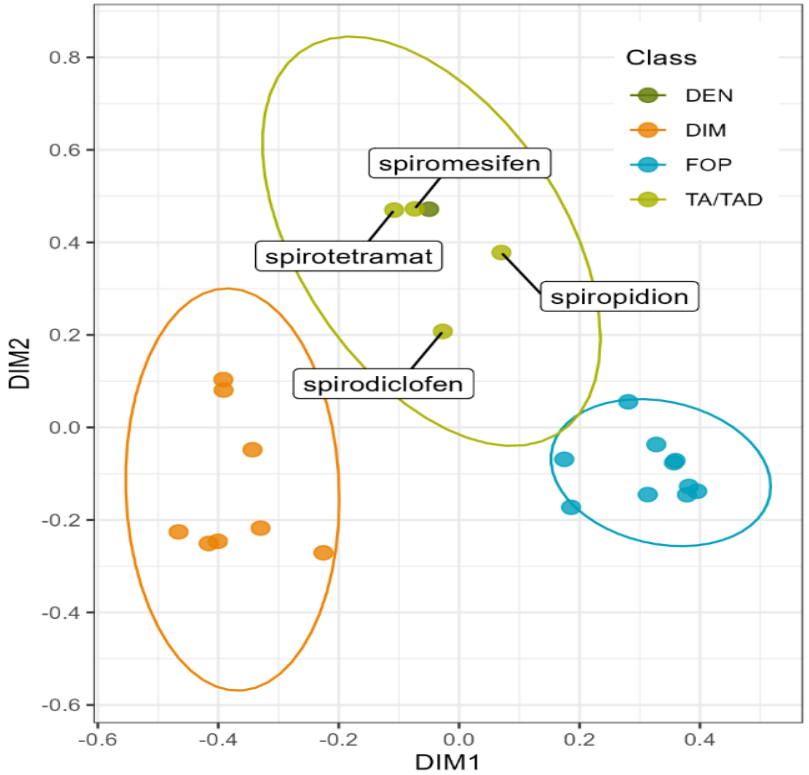


- Spiropidion demonstrates extensive metabolism and rapid excretion supporting lack of increased toxicity over time.
  - Additional dosing will not increase systemic exposure.
  - TA/TADs also demonstrate extensive metabolites and rapid clearance
- Key target organs and effects of spiropidion included effects in the liver for mice, liver and thyroid for rats, and clinical effects in dogs.
  - TAs/TADs target organs were liver, thyroid, adrenal glands, and testes.
- Selection of relevant source analogues for of read-across.
  - Structural and biological similarity were factored into the selection of analogues.
  - All target organs of toxicity and precursor effects from analogues were evaluated in the read-across analysis; one of the source analogues had carcinogenic effects.

When conducting read-across, clearly define the process and criteria for the analogue selection (inclusion and exclusion). 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.

Selection of Source Analogues for Read-Across





Chemical clustering based on ToxPrint Chemotypes

Potential compounds for use in read-across were selected based on the ACCase inhibition pesticidal mode of action, defined by the HRAC and IRAC.

ACCase inhibitor herbicides included phenylpyrazolin (DENs), cyclohexanedione (DIMs), and aryloxyphenoxypropionate (FOPs) chemistries.

ACCase inhibitor insecticides included the tetronic and tetramic acid derivatives (TA/TADs).

Twenty-three herbicides and insecticides identified as ACCase inhibitor chemicals representing all the identified chemical groups (DENs, DIMs, FOPs, TA/TADs).

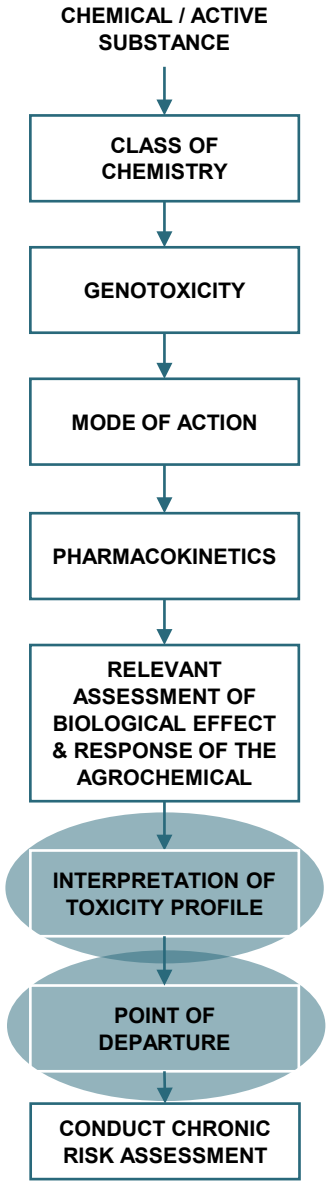
Seventeen of the 23 chemicals had regulatory-relevant data-rich toxicology data available for read-across assessment.

Read-across analysis accessed the published regulatory reviews from the US EPA, EFSA, and JMPR.

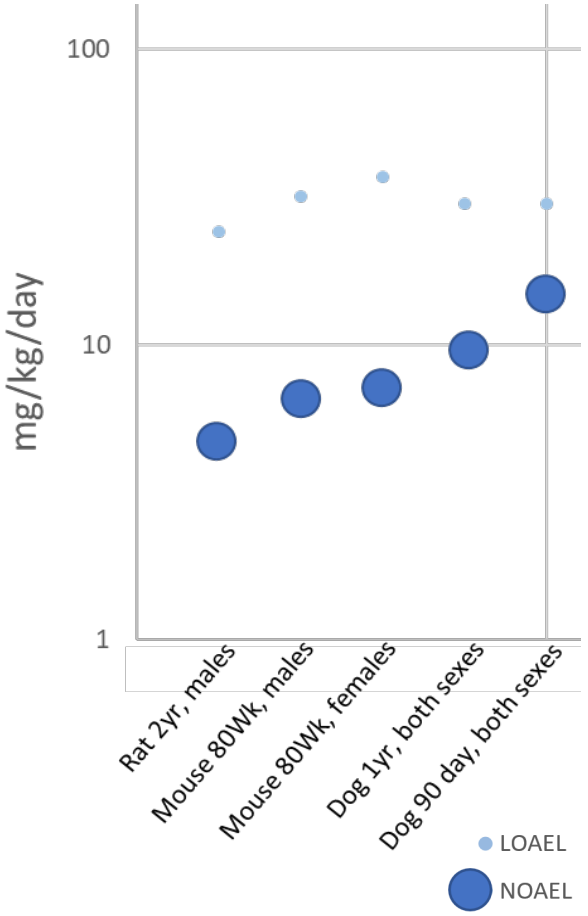
Based on review of the available data, read-across with the TA/TADs molecules was considered most relevant for the assessment of spiropidion. This conclusion was based on both structural and biological similarity.

Each of the TA/TADs have visual similarities in structure to the target chemical, namely the toxophore, the potent active principle responsible for the target site binding in insects.

Evaluation of Data (continued)



- Thyroid effects investigated; mechanistic studies support quantitative non-relevance to humans.
  - A threshold exists for the induction of key events in this MOA
- Weak alignment to toxicological profiles with TA/TADs.
  - Similar effects as expected for ACCase inhibiting compounds
  - Investigative, mechanistic research identified the differences
  - Analogues with carcinogenic effects; all potential precursor findings were evaluated in the target chemical
- POD selected from 90-day dog study (15 mg/kg/day).
  - POD is higher than other NOAELs; all LOAELs are similar
  - Process allows for POD derived from non-chronic studies, which would be protective of chronic/carcinogenic effects.



When considering the appropriate tools and models in the WoE, it is the author's responsibility to decide what are that are available to conduct the necessary measurements for each aspect of the assessment.

Integrating lines of evidence

ReCAAP cPAD estimate is more conservative than the original estimation using a chronic/carcinogenicity study

Population Subgroup	cPAD (mg/kg/day)	Chronic Dietary Exposure * (mg/kg/day)	Total Exposure % cPAD
Children (1-2 years old)	**0.015**	**0.002298**	**15.3**
Children (1-2 years old)	**0.15**	**0.010024**	**6.7**

* Chronic dietary (food only) exposures and risk assessment was conducted for the purposes of the import tolerance; this active substance is currently under review for domestic registration in the U.S.

ReCAAP estimated cPAD

EPA estimated cPAD

Exposure

Read-across

Mechanistic

Genotoxicity

Metabolism

Both < EPA's level of concern (100%)

Chronic toxicity

Carcinogenicity

Technical Characterization

Scientific Validity

Addressing the Uncertainties

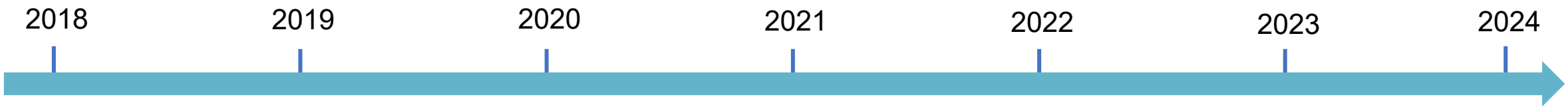
Sources of variability; quality of data sources

Robustness; durability of data package interpretation(s)

Reproducibility; consistency of framework for data-poor chemicals

Transferability; functionality under regional requirements

Technical Characterization



2018

Problem formulation

Chemical selection

Draft framework v1

2019

Phase 1: write CS waivers

Phase 1: regulatory review

Phase 1: revise framework v2

Phase 2: write CS waivers

2020

Phase 2: regulatory review

Phase 2: revise framework v3

Phase 3: write CS waivers

Phase 3: regulatory review

Phase 3: revise framework v4

2021

Publish ReCAAP framework

Discussions to develop case studies for OECD

2022

Develop pilot case study for OECD

Receive OECD feedback on pilot case

Develop additional case study for OECD

2023

Submit final case studies to OECD

Analyze OECD feedback to gauge sufficiency of data

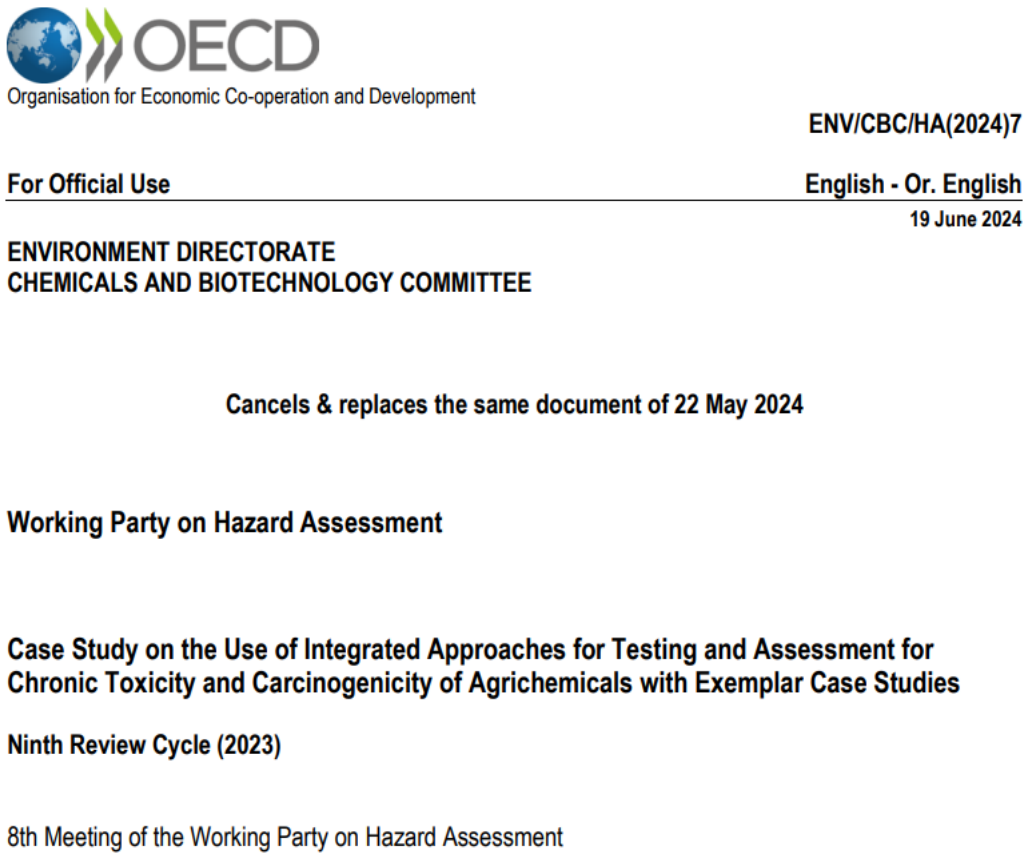
2024

OECD approval from WPHA

OECD publication *(pending declassification)*

Technical Characterization

OECD review of the ReCAAP Framework



OECD
Organisation for Economic Co-operation and Development

ENV/CBC/HA(2024)7

For Official Use — English - Or. English

19 June 2024

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Cancels & replaces the same document of 22 May 2024

Working Party on Hazard Assessment

Case Study on the Use of Integrated Approaches for Testing and Assessment for Chronic Toxicity and Carcinogenicity of Agrichemicals with Exemplar Case Studies

Ninth Review Cycle (2023)

8th Meeting of the Working Party on Hazard Assessment

ReCAAP Framework submitted to the OECD IATA Case Study Project (CSP)

- Reviewed by
  - Australia
  - Canada
  - EFSA
  - Germany
  - Japan
  - Italy
  - Netherlands
  - United States
- 500+ hours of regulatory review and feedback
- WPHA approval by member states in June 2024

https://www.oecd.org/en/topics/sub-issues/assessment-of-chemicals/integrated-approaches-to-testing-and-assessment.html

Thank you for your attention

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Retrospective Analysis

ReCAAP *(2020 WoE assessment)*

POD = 15 mg/kg/d from 90-day dog study.
Total UF = 1000X. Not likely to be carcinogenic.

JMPR *(2021 Report)*

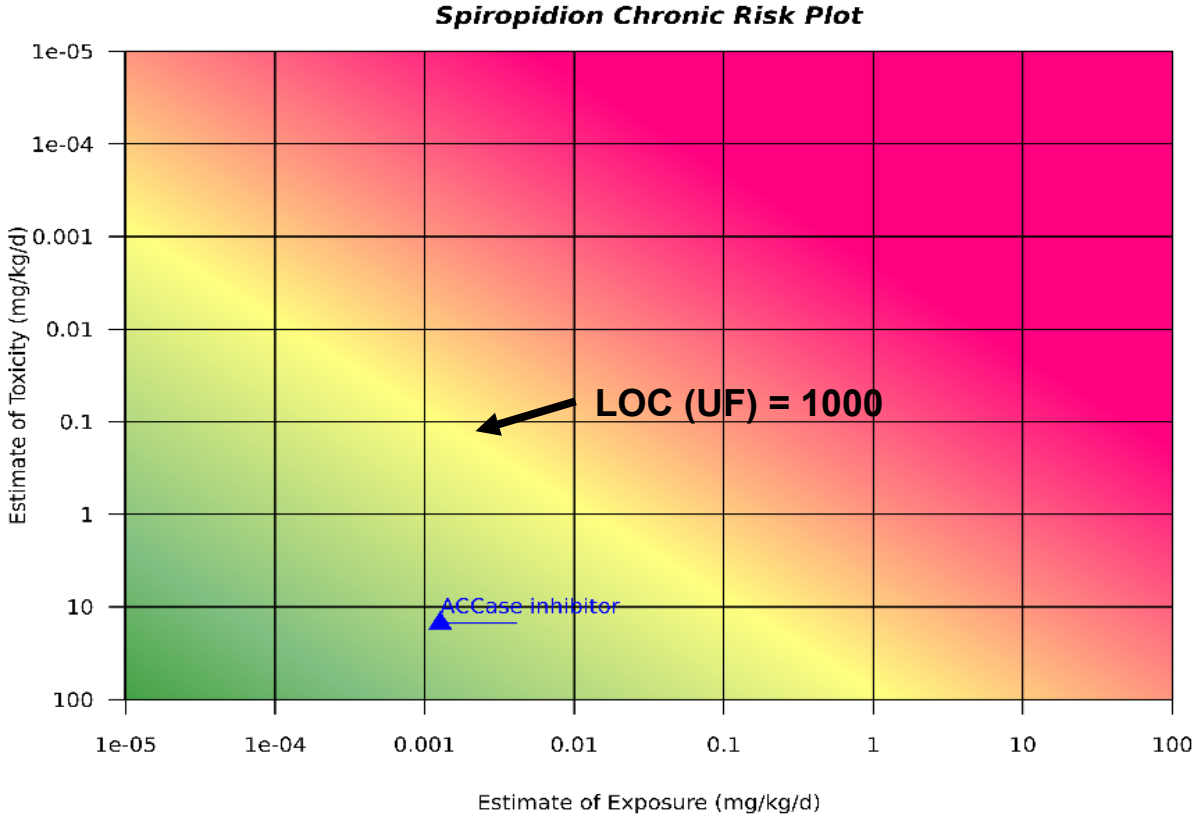
POD = 2.4 mg/kg/d from rat carcinogenicity study based on equivocal increase Leydig cell tumours. Spiropidion is unlikely to pose a carcinogenic risk to humans from the diet.

US EPA *(2022, 2023; Import Tolerance)*

POD = 15 mg/kg/d from 90-day dog study.
Total UF = 100X. Not likely to be carcinogenic.

Incidence of Leydig cell adenomas was not statistically significant, lacked a dose-response relationship, within the historical control data range for this age and strain of rat at the CRO Lab and 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 is considered not to be treatment related.

Risk21® graph for predicted spiropidion chronic exposure and risk assessment



The RISK21® graph and the risk assessment results demonstrate that the % cRfD values calculated from the 90-day dog NOAEL is below the EPA level of concern

The yellow line in this RISK21® tool represents the acceptable difference between the cPAD (as an estimate of risk) and the US EPA modeled exposure values (as estimates of exposure). The Health and Environmental Sciences Institute provide RISK21® tools: https://risk21.org/webtool/

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