European Commission

Methodology for evidence screening of chemicals developed in the context of an impact assessment on criteria to identify endocrine disruptors

Brussels, 6th November 2015

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Institute for Health & Consumer Protection

Joint Research Centre

GENERAL OUTLINE

OVERVIEW – Sharon Munn

DATA SOURCES AND DATASHEET POPULATION – Sander van der Linden

DATA ANALYSIS AND CHEMICAL SELECTION – Alfonso Lostia

Disclaimer

The screening methodology was developed in the context of an impact assessment and cannot replace the regulatory decision making process of determining the chemicals considered as having endocrine-disrupting properties.

The methodology aims at estimating which substances may fall under the different ED IA policy options.

The methodology is based on a screening of existing evidence (desk work). No additional experimental data, experimental screening or discussion in scientific committees is foreseen.

The screening does not substitute full evaluations of individual substances to be carried out in the context of chemical legislation. Therefore, the screening does not pre-empt the regulatory conclusions that may eventually be made on the basis of such evaluations.

Scope of the screening methodology

- To assess in a limited amount of time the potential endocrine disrupting properties of approximately 700 substances subject to:

  - Plant Protection Products Regulation (PPPR) (approx. 400)
  - Biocidal Products Regulation (BPR) (approx. 100)

  - REACH Regulation
  - Cosmetic Products Regulation
  - Water Framework Directive (WFD)

  } Sample of approx. 200 substances

- Apply the four policy options for criteria for identifying EDs in EC Roadmap based on available data
- http://ec.europa.eu/smart-regulation/impact/planned_ia/docs/2014_env_009_endocrine_disruptors_en.pdf

Option 1. No policy change.

Interim criteria set in the BPR and the PPPR to be applied.

Substances are or may be considered as EDs if they are or have to be classified as:

- CLP "carcinogenic category 2" and "toxic for reproduction category 2", OR
- CLP "toxic for reproduction category 2" and "toxic effects on the endocrine organs"

Substances not fulfilling above criteria will be considered not ED according to interim criteria

Option 2. EDs identified according to WHO/IPCS definition

An endocrine disrupter is an exogenous substance or mixture ***that alters function(s) of the endocrine system**** *and consequently causes* ***adverse health effects*** *in an intact organism, or its progeny, or (sub)populations" (IPCS/WHO, 2002).*

Two elements**: adversity** and ***endocrine disrupting mode of action**

Need evidence for both

Option 3: WHO definition and additional categories

Option 3 proposes two additional categories based on the strength of evidence for fulfilling the WHO/IPCS definition:

- Cat I (fulfils WHO definition, equivalent to option 2)
- Cat II (suspected ED) –evidence insufficient to place in Cat I
- Cat III (endocrine active substance) –evidence insufficient to place in Cat II

Substances not fulfilling any of these categories designated 'unclassified'

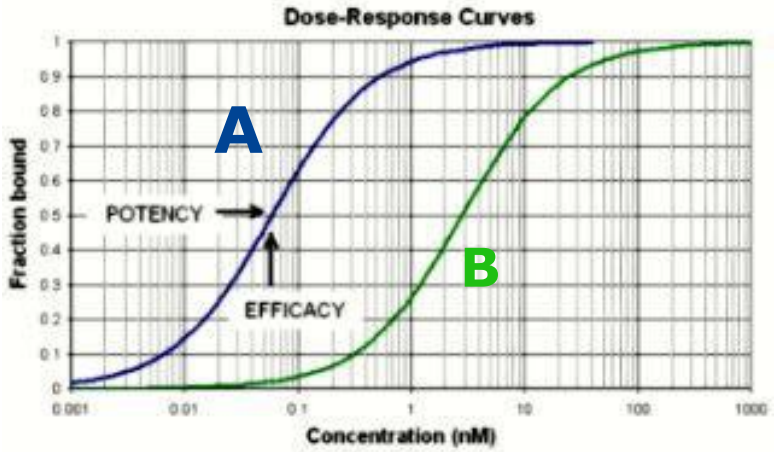
Option 4: WHO definition with the inclusion of potency

Potency refers to the amount of substance necessary to produce a certain effect. A substance A which produces an effect at 5 mg is 10 times more potent than a substance B which produces the same effect at 50 mg.

Applying a potency cut-off at 10 mg,

Substance A confirmed ED

Substance B not considered ED



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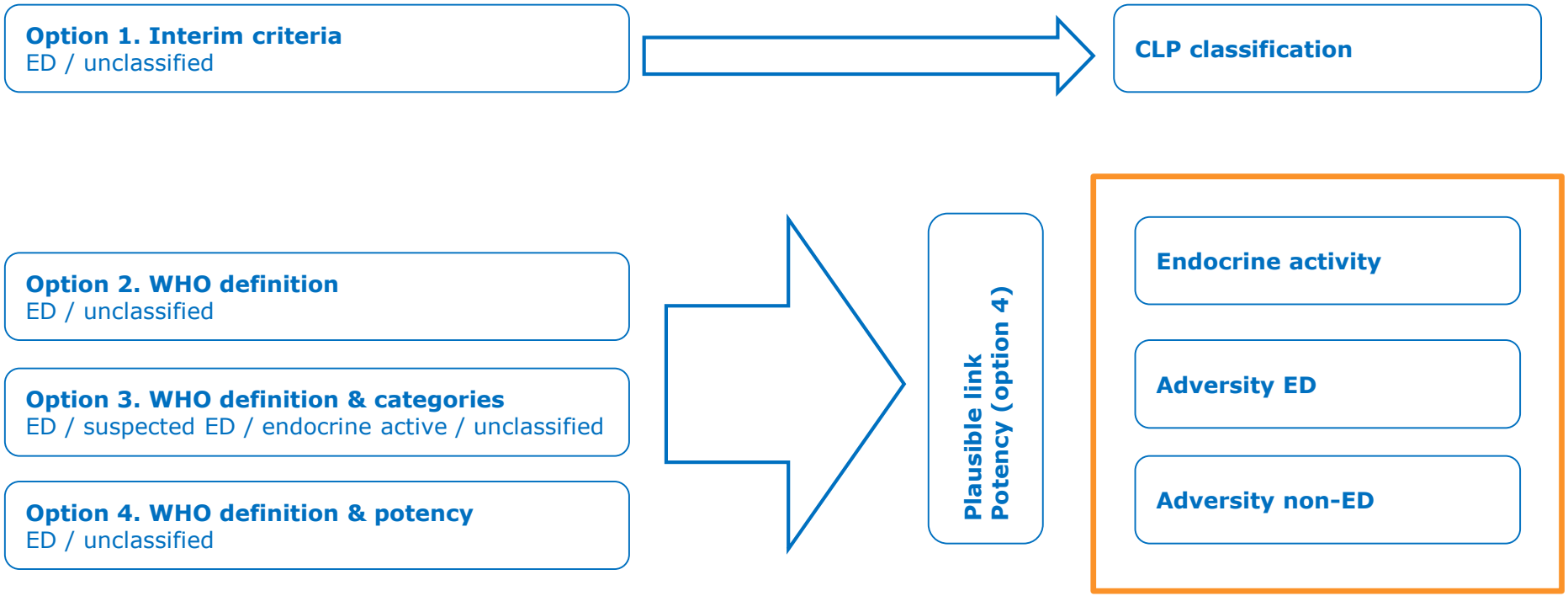
INFORMATION REQUIREMENTS AND DATA SOURCES

Information requirements



Focus

- Focus on EATS (Estrogens, Androgens, Thyroid, Steroidogenesis), so endocrine disruption via other modes of action not assessed
- Mammalian toxicity: reproductive toxicity, carcinogenicity and repeated dose toxicity
- Ecotoxicology focus on fish and amphibians, to a limited extent birds (not invertebrates)

OECD CONCEPTUAL FRAMEWORK

Level 1: Existing data and non-test information (incl. QSAR)

Level 2: ***In vitro*** assays providing data about selected endocrine mechanism(s)/pathway(s)

Level 3: ***In vivo*** assays providing data about selected endocrine mechanism(s)/pathway(s)

Level 4: ***In vivo*** assays providing data on adverse effects on endocrine-relevant endpoints

Level 5: ***In vivo*** assays providing more comprehensive data on adverse effects on endocrine-relevant endpoints (more extensive part of organism life cycle)

OECD Guidance Document 150

Data Gathering: sources

Rely on already existing readily accessible information

Primarily: evaluated data from the existing regulatory assessment reports***, including*** EFSA conclusions, MS Draft Assessment Reports, MS Competent Authority Reports, REACH restriction dossiers, Support documents for identification of SVHC, opinions of Scientific Committee on Consumer Safety (SCCS).

Supplemented by additional information: gathered from databases focusing on endocrine effects including non-regulatory studies such as JRC's Endocrine Active Substances Information System, TEDX, SIN list, ToxCast, EDSP WoE analyses and targeted literature searching

ASSUMPTIONS AND LIMITATIONS

Regarding data quality

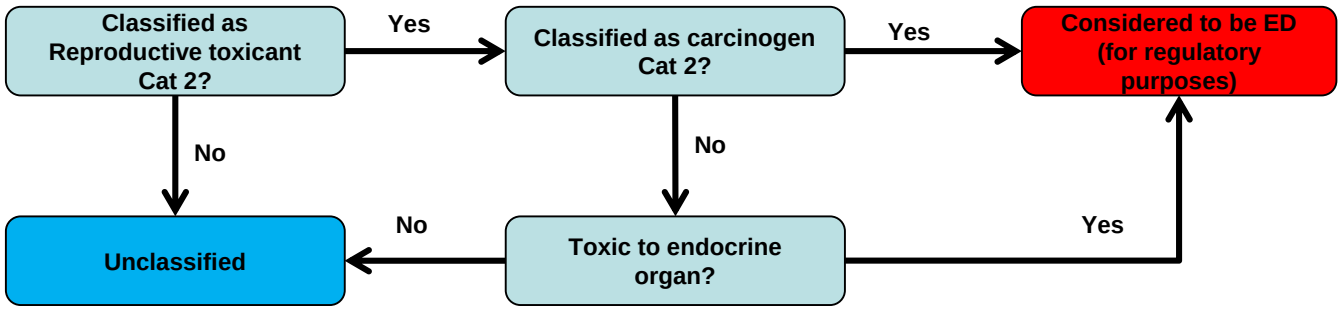
- All data in the regulatory documents are assessed (peer reviewed) and relevant by default
- Published scientific literature are reliable

Regarding data relevance

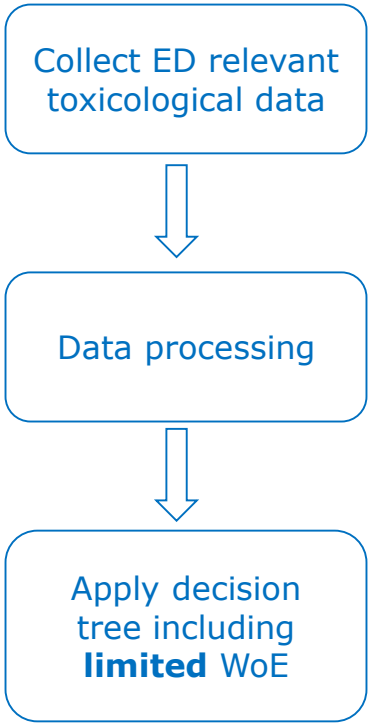
- All mammalian data are human relevant, unless specifically stated otherwise
- Understanding of the endocrine system of many invertebrate species is limited, the focus for ecotoxicological effects is on mammals, fish, amphibians and to a limited extent on birds

DECISION-MAKING WORKFLOW

Option 1 – Interim Criteria



Options 2 to 4 ED CAT I, II, III



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Data Processing for options 2 to 4

Category	Description
In vitro mechanistic	Scientific literature, ToxCast
In vivo mechanistic & in vivo hormone levels	OECD CF Level 3 assays plus hormone levels
Adversity – EATS specific	Endpoints that can be specific for Estrogen, Androgen, Steroidogenesis or Thyroid pathways
Non-specific adversity (may or may not be indicative of EATS)	Endpoints potentially sensitive to, but not specific for, EATS pathways
Adversity – General	Non EATS related effects, including food intake, systemic toxicity, body weight change etc.

Options 2 & 3
ED CAT I, II, III

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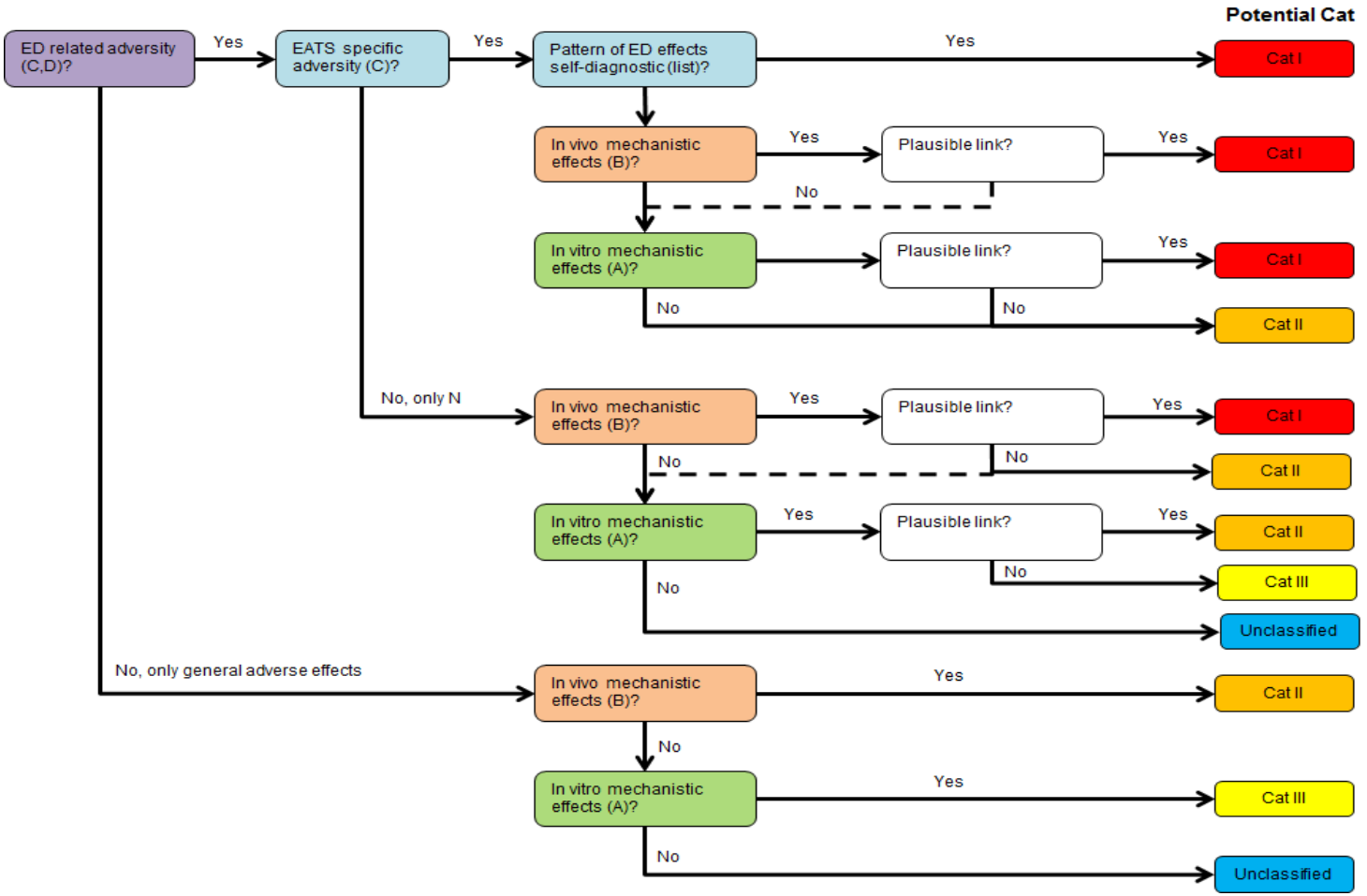
Apply decision tree including **limited** WoE



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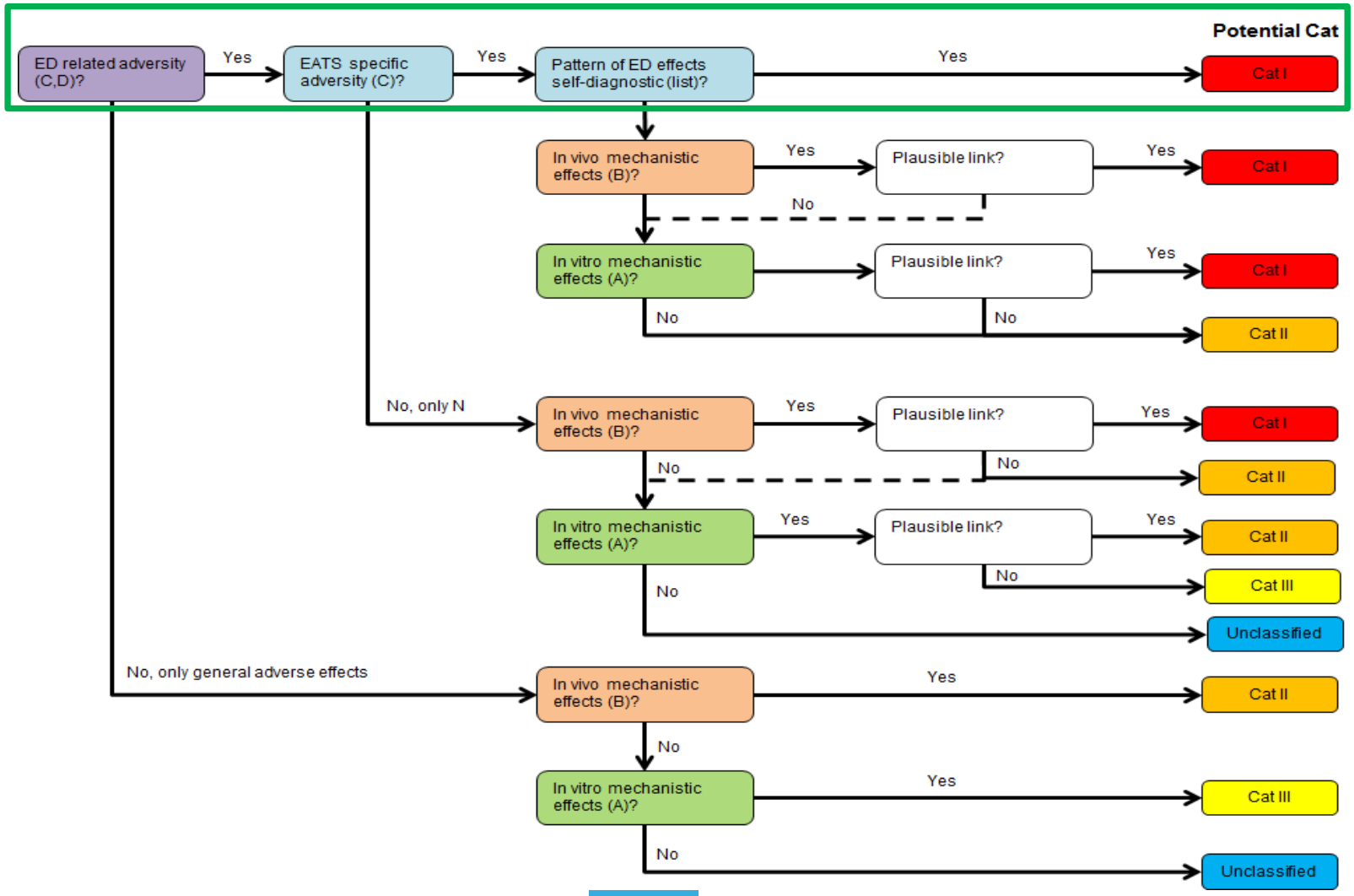
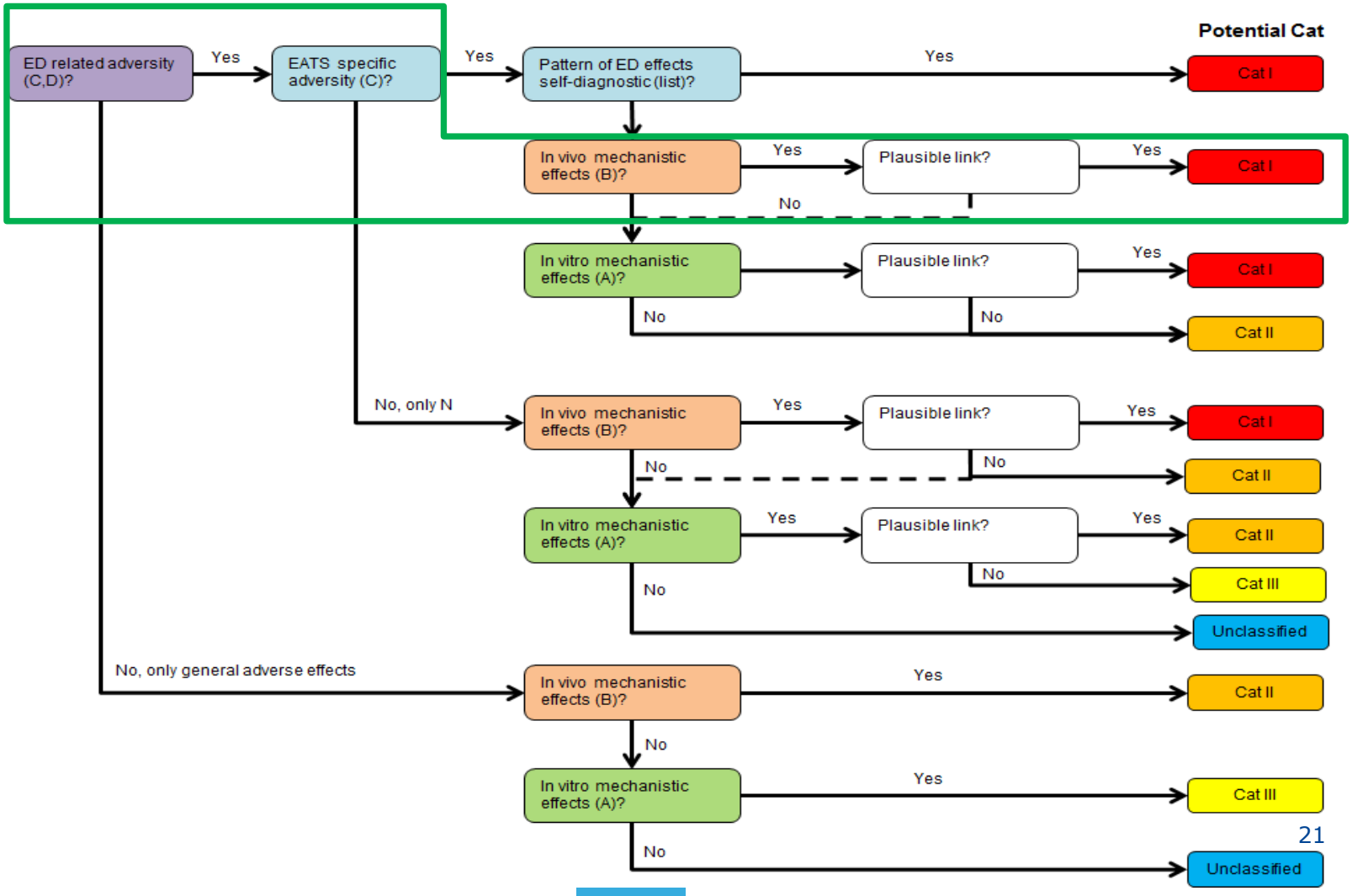
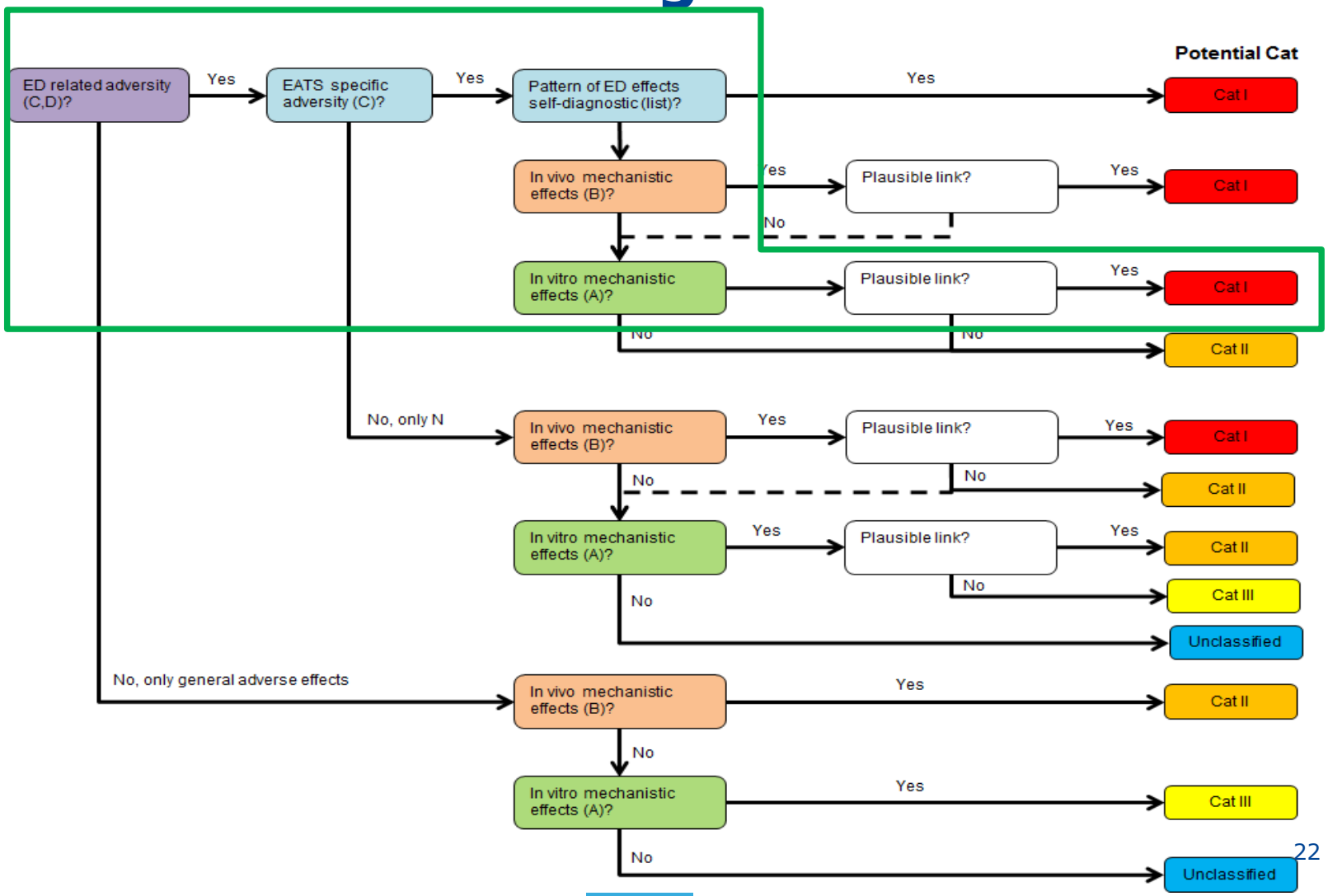
Path 1 leading to Cat I



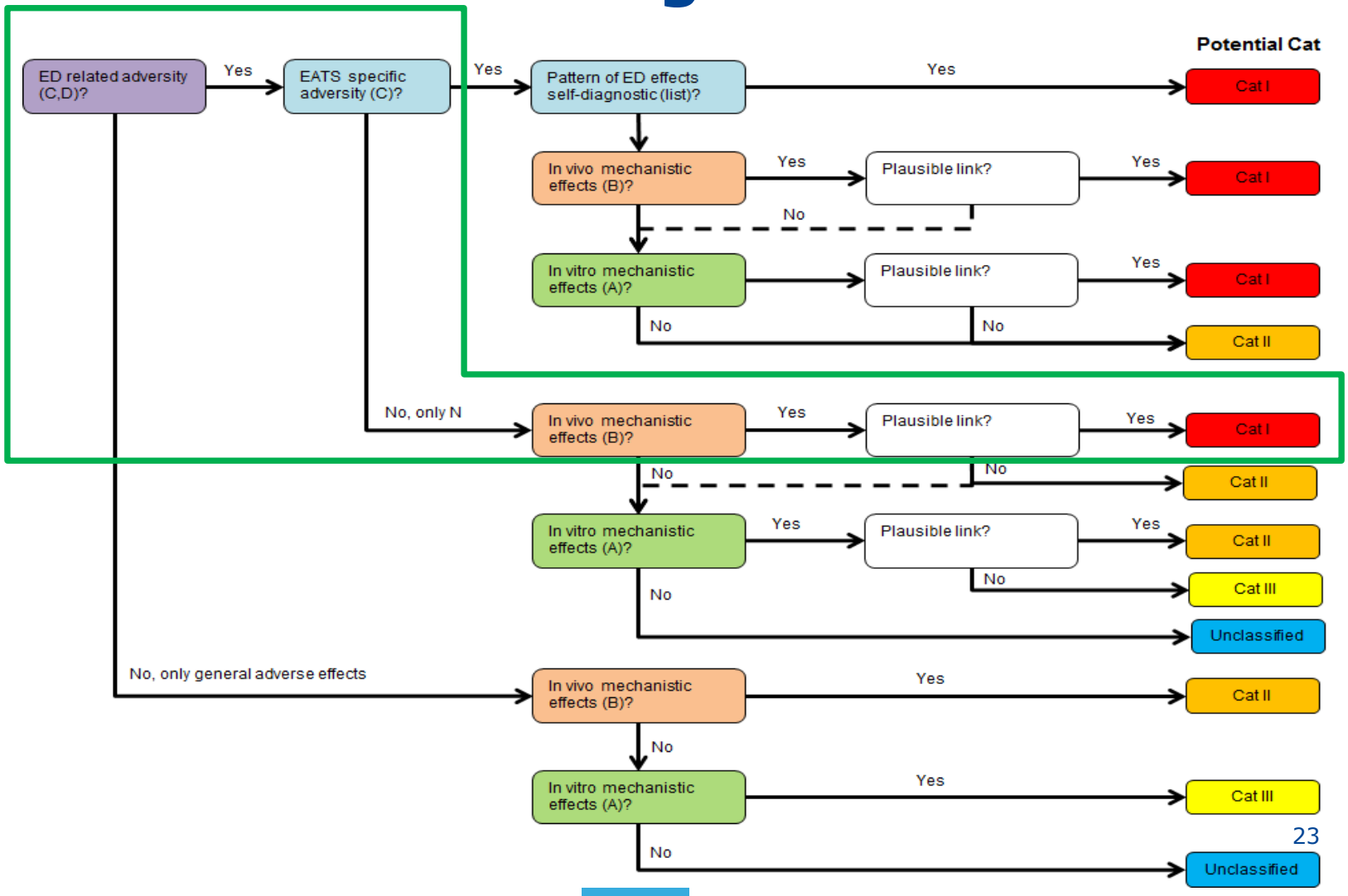
Path 2 leading to Cat I



Path 3 leading to Cat I



Path 4 leading to Cat I



Options 2 & 3 – ED Categories II & III

Cat II

- Specific *in vivo* effects, indicating endocrine specific effects (level 4 and 5) not secondary to generalised systemic toxicity, **but without** supporting mechanistic evidence (*in vivo*, *in vitro*), plausibly linking to observed adverse *in vivo* effects
- Positive mechanistic *in vivo* (level 3) evidence, without *in vivo* evidence of adversity from level 4 and 5 assays

Cat III

- No *in vivo* evidence indicating endocrine specific effects (level 4 and 5) but mechanistic evidence *in vitro.*

Unclassified

- No *in vivo* effects, indicating endocrine specific effects (level 4 and 5) and no mechanistic evidence (*in vivo*, *in vitro*).

Assessment under option 4 - potency

Potency-based STOT-RE Cat 1 & 2 trigger values (from CLP) proposed as cut-off criteria

Indicate for all EDs under option 2 whether there is an observed ED effect at or below the designated guidance value
If above guidance value not considered ED (unclassified) for purposes of IA.

RESULTS

OPTION 1			OPTION 2 & 3					OPTION 4	
			Mammalian			Ecotox			
Question	Answer (Yes/No	Reasoning	Question	Answer (Yes/No	Reasoning	Answer (Yes/No	Reasoning	Answer (Yes/No)	Reasoning
CLP-harmonised "carcinogenic category 2"			Is there evidence of adversity that may or may not be EATS-specific in an intact organism, or its progeny, or in a (sub)population?						
CLP-harmonised "toxic for reproduction category 2"			Is there evidence of Adversity – EATS specific in an intact organism, or its progeny, or in a (sub)population?						
toxic effects on the endocrine organs.			Is there evidence of in vivo mechanistic and/or in vivo hormone levels information?						
CLP-proposal "carcinogenic category 2"			Is there evidence of in vitro mechanistic information?						
CLP-proposal "toxic for reproduction category 2"			Is there evidence of a plausible link between in vitro/in vivo mechanistic information and the observed EATS-specific or non-specific adversity?						
Evaluation result									

Practical Implementation

- Draft Screening Methodology submitted to contractor, includes:-
  - data sources to be consulted
  - type of data to extract
  - template for recording and summarising data
  - decision trees to follow to apply options for criteria in a systematic manner to 700 substances,

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Data sources and datasheet population

Brussels, 6th November 2015

Sander van der Linden

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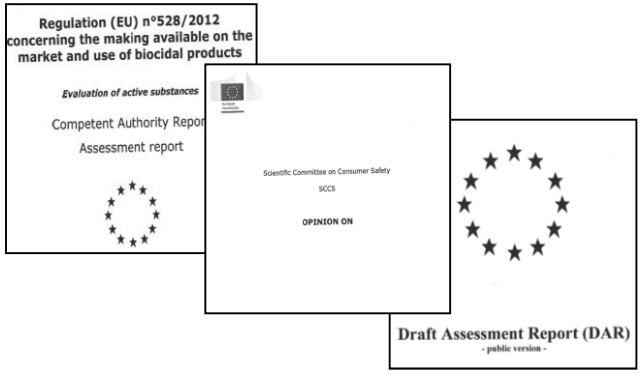
Workflow of screening methodology

Source Documents



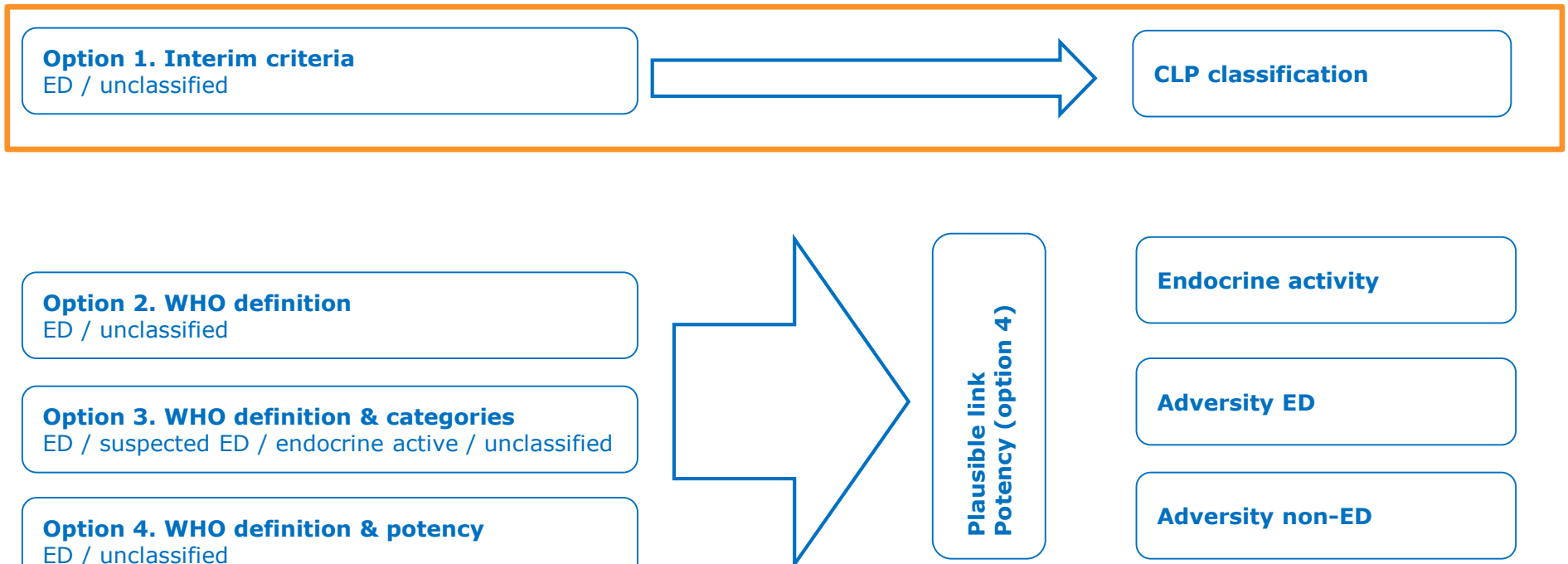
1. Data collection

- In vitro
- Mammalian toxicity
- Wildlife toxicity

Type of toxicity	Study principle	Study guideline (OECD/US EPA) or remarks	Reporting date	Species	Route of administration	Duration of exposure	Duration unit	Generation/Life stage	Sex (effect dose)	Lowest Effect dose	Effect type	Effect target
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 409	2005	Dog	Oral	1	Years	Adult	M	20	Organ histopathology	[Not in list]
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 409	2005	Dog	Oral	1	Years	Adult	M	20	Organ histopathology	Brain histopathology
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 409	2005	Dog	Oral	1	Years	Adult	F	19	Clinical chemistry	T3 and T4 level
Mammalian in vivo - Repeated dose	Repeated Dose 28-Day Oral Toxicity in rodent	OECD 407	2006	Rat	Oral	28	Days	Adult	M+F		No relevant effect observed	No relevant effects
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	In life observation	Growth
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	Organ Weight	Kidney weight
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	Organ Weight	Liver weight
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	M	189	Organ histopathology	Lung histopathology
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	Organ histopathology	Lung histopathology
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	In life observation	Systemic toxicity
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	M	189	Organ histopathology	Testis histopathology
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	M	373	Organ histopathology	Epididymis histopathology
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	M	169	Organ histopathology	Kidney histopathology
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	Organ histopathology	Kidney histopathology
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	M	169	Organ Weight	Kidney weight
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	Organ Weight	Kidney weight
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	M	169	Organ histopathology	Lung histopathology
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	Organ histopathology	Lung histopathology
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	In life observation	Systemic toxicity
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	M	373	Organ histopathology	Testis histopathology
Mammalian in vivo - Repeated dose	Carcinogenicity	OECD 451	2006	Mouse	Oral	18	Months	Adult	M		No relevant effect observed	No relevant effects
Mammalian in vivo - Repeated dose	Carcinogenicity	OECD 451	2006	Mouse	Oral	18	Months	Adult	F		No relevant effect observed	No relevant effects
Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0/F1)	M	419	In life observation	Body weight
Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0/F1)	F	485	In life observation	Body weight
Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0/F1)	M	419	In life observation	Food consumption
Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0/F1)	F	485	In life observation	Food consumption

2. Data classification

Information requirements



Classification as C or R (cat 2)

Harmonized classification

- Plant protection products (EU Pesticide Database)
- Biocidal products (C&L Inventory)
- Other (C&L Inventory)

Proposed classification (if newer)

- Plant protection products (DAR/EFSA conclusion)
- Biocidal products (CAR)
- Other (CLH report, ECHA website)

- If present in more than one category: all collected
- If no classification found, indicated in data sheet

For the purpose of impact assessment…

Endocrine organ

- Hormone secreting organs and their targets that are included in the OECD GD 150

This includes: mammary gland, accessory sex glands (e.g. Cowper's gland, seminal vesicles, prostate gland, bulbourethral glands, Glans penis), testis, epididymis, penis, cervix, uterus (endometrium), vagina, hypothalamus, pituitary, thyroid, adrenals, ovaries, placenta, Levator ani/bulbocavernosus muscles (LABC)

Information requirements

Option 1. Interim criteria
ED / unclassified

→ **CLP classification**

Option 2. WHO definition
ED / unclassified

Option 3. WHO definition & categories
ED / suspected ED / endocrine active / unclassified

Option 4. WHO definition & potency
ED / unclassified

→ **Plausible link**
Potency (option 4)

- **Endocrine activity**
- **Adversity ED**
- **Adversity non-ED**

Source documents for toxicological data

Rely on already existing readily accessible information

Primarily: evaluated data from the existing regulatory assessment reports***, including*** EFSA conclusions, MS Draft Assessment Reports, REACH restriction dossiers, Support documents for identification of SVHC, MS Competent Authority Report, opinions of Scientific Committee on Consumer Safety.


Scientific Committee on Consumer Products

SCCP

OPINION ON

Resorcinol

COLIPA n° A11

Scientific Committees

The SCCP adopted this opinion at its 19th plenary of 15 April 2008


Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State France for the existing active substance

FIPRONIL

of the second stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

Volume 1

January 2005

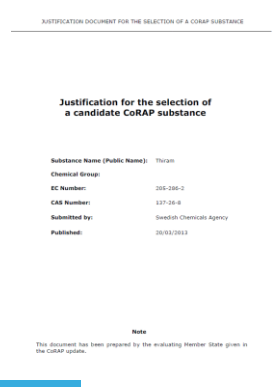

efsa

CONCLUSION ON PESTICIDE PEER REVIEW

Conclusion on the peer review of the pesticide risk assessment of the active substance iprovalicarb

European Food Safety Authority

ABSTRACT


JUSTIFICATION DOCUMENT FOR THE SELECTION OF A CORAP SUBSTANCE

Justification for the selection of a candidate CoRAP substance

Substance Name (Public Name): Thiram

Chemical Group:

EC Number: 205-286-2

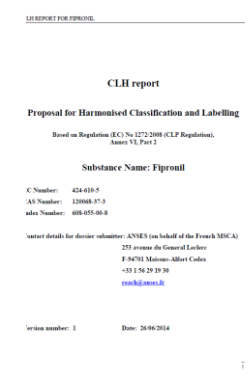
CAS Number: 137-26-8

Submitted by: Swedish Chemicals Agency

Published: 20/03/2013

Note

This document has been prepared by the evaluating Member State given in the CoRAP update.


LH REPORT FOR FIPRONIL

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: Fipronil

C Number: 424-610-5

AS Number: 120068-37-3

ndex Number: 608-055-00-8

ontact details for dossier submitter: ANSES (on behalf of the French MSCA)

253 avenue du General Leclerc

F-94701 Maisons-Alfort Cedex

+33 1 56 29 19 30

reach@anses.fr

ersion number: 1 Date: 26/06/2014

Supplemented by additional sources

Open literature

- For all compounds, a literature search is performed
  - SCOPUS: compound name & endocrine
  - SciFinder: concept "endocrine disruption" & substance identifier based on CAS

ToxCast (US EPA)

- US EPA database with ED relevant *in vitro* assay data

Endocrine Disruptor Screening Program (US EPA)

- WoE analysis (summarized data) of ED relevant *in vitro* and *in vivo* assays, focusing on estrogens, androgens, thyroid and steroidogenesis

ToxRefDB (US EPA)

- Database with ED relevant *in vivo* data.

Supplemented by additional sources

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ToxRefDB (US EPA)

- Database with ED relevant *in vivo* data.

Check for additional sources

The Endocrine Disruption Exchange (TEDX)

- List of chemicals that show the potential to affect the endocrine system (reference(s) provided)

Substitute It Now (SIN)

•List of substances identified by NGO ChemSec as Substances of Very High Concern, including ED criteria (reference(s) provided)

Public consultation

•List of references supplied by public consultation

Community Rolling Action Plan (ECHA)

- Flag presence of CoRAP if ED motivated

3.2 Grounds for concern

☐ (Suspected) CMR	☒ Wide dispers
☐ (Suspected) Sensitiser	☐ Consumer us
☐ (Suspected) PBT	☐ Exposure of
☒ Suspected endocrine disruptor	☐ Other (provid

Type of studies to be captured

Mammalian toxicity

- developmental toxicity, reproductive toxicity, carcinogenicity and (sub)chronic (repeated dose) toxicity

Ecotoxicology

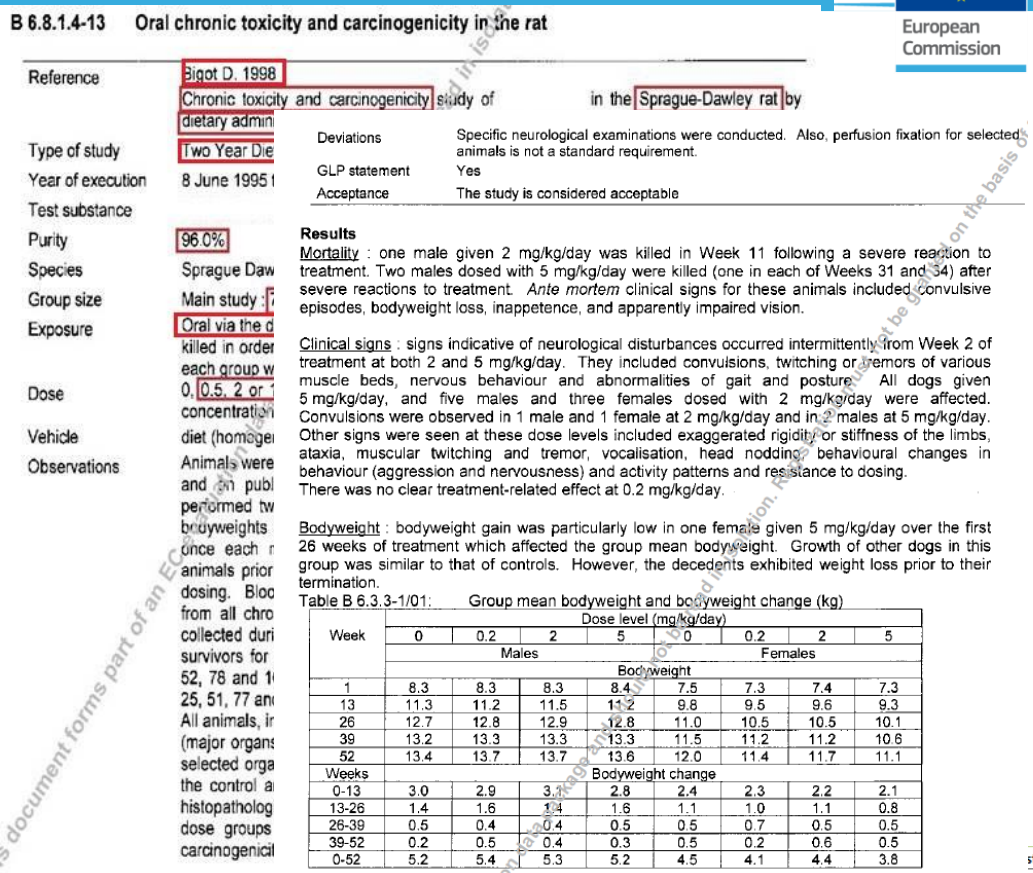
- non-acute toxicity, reproductive toxicity in fish and amphibians (and birds to a limited extent)

Focus on test methods specified within the OECD CF (OECD GD 150 TG or equivalent)

Types of effects to be captured

- Production/action of steroid hormones (estrogen, testosterone), impacts on reproduction (fertility, abnormalities in development, onset of puberty) and thyroid hormones (impact on growth and

Test guideline or other test method [Reference to interpretation table within this document]	Endpoints for estrogen-mediated activity		Endpoints for androgen-mediated activity		Endpoints for thyroid-related activity	Endpoints for steroidogenesis-related activity	Endpoints potentially sensitive to, but not diagnostic of, EATS modalities
	Agonistic	Antagonistic	Agonistic	Antagonistic			
OECD TG 416: 2-generation reproduction toxicity study (including guidance on OECD TG 415: 1-generation study)	Change in AGD in male and female pups. Changes in estrus cyclicity (P, F1). Decreased age at Vaginal opening (F1).	Studies using pure antagonists are lacking. However, changes may occur in the following: AGD in male and female pups. Estrus cyclicity (P, F1).	Studies using agonists are lacking. However, changes may occur in the following: Increased AGD in male pups, change in AGD in female pups.	Decreased AGD in male pups, change in AGD in female pups. Changes in estrus cyclicity (P, F1). Changes in age at vaginal opening (F1).	Increased thyroid weight. Possible liver weight increase (in combination with other thyroid-related endpoints). Histopathologic changes in thyroid	Possible effects on: AGD in male and female pups. Estrus cyclicity (P, F1). Age at Vaginal opening (F1). Age at preputial separation (F1).	Changes in : Weights of adrenals Time to mating Male fertility Female fertility Gestation length Dystocia



B 6.8.1.4-13 Oral chronic toxicity and carcinogenicity in the rat

Reference	Bigot D. 1998
	Chronic toxicity and carcinogenicity study of in the Sprague-Dawley rat by dietary admini
Type of study	Two Year Die
Year of execution	8 June 1995 t
Test substance	
Purity	96.0%
Species	Sprague Daw
Group size	Main study : 7
Exposure	Oral via the d killed in order each group w
Dose	0, 0.5, 2 or 1 concentration
Vehicle	diet (homoge
Observations	Animals were and (in publ performed tw bodyweights once each r animals prior dosing. Bloo from all chro collected duri survivors for 52, 78 and 1 25, 51, 77 an All animals, ir (major organs selected orga the control a histopatholog dose groups carcinogenici

Deviations	Specific neurological examinations were conducted. Also, perfusion fixation for selected animals is not a standard requirement.
GLP statement	Yes
Acceptance	The study is considered acceptable

Results

Mortality : one male given 2 mg/kg/day was killed in Week 11 following a severe reaction to treatment. Two males dosed with 5 mg/kg/day were killed (one in each of Weeks 31 and 34) after severe reactions to treatment. *Ante mortem* clinical signs for these animals included convulsive episodes, bodyweight loss, inappetence, and apparently impaired vision.

Clinical signs : signs indicative of neurological disturbances occurred intermittently from Week 2 of treatment at both 2 and 5 mg/kg/day. They included convulsions, twitching or tremors of various muscle beds, nervous behaviour and abnormalities of gait and posture. All dogs given 5 mg/kg/day, and five males and three females dosed with 2 mg/kg/day were affected. Convulsions were observed in 1 male and 1 female at 2 mg/kg/day and in 2 males at 5 mg/kg/day. Other signs were seen at these dose levels included exaggerated rigidity or stiffness of the limbs, ataxia, muscular twitching and tremor, vocalisation, head nodding, behavioural changes in behaviour (aggression and nervousness) and activity patterns and resistance to dosing. There was no clear treatment-related effect at 0.2 mg/kg/day.

Bodyweight : bodyweight gain was particularly low in one female given 5 mg/kg/day over the first 26 weeks of treatment which affected the group mean bodyweight. Growth of other dogs in this group was similar to that of controls. However, the decedents exhibited weight loss prior to their termination.

Table B 6.3.3-1/01: Group mean bodyweight and bodyweight change (kg)

Week	Dose level (mg/kg/day)							
	0	0.2	2	5	0	0.2	2	5
	Males				Females			
	Bodyweight							
1	8.3	8.3	8.3	8.4	7.5	7.3	7.4	7.3
13	11.3	11.2	11.5	11.2	9.8	9.5	9.6	9.3
26	12.7	12.8	12.9	12.8	11.0	10.5	10.5	10.1
39	13.2	13.3	13.3	13.3	11.5	11.2	11.2	10.6
52	13.4	13.7	13.7	13.6	12.0	11.4	11.7	11.1
Weeks	Bodyweight change							
0-13	3.0	2.9	3.1	2.8	2.4	2.3	2.2	2.1
13-26	1.4	1.6	1.4	1.6	1.1	1.0	1.1	0.8
26-39	0.5	0.4	0.4	0.5	0.5	0.7	0.5	0.5
39-52	0.2	0.5	0.4	0.3	0.5	0.2	0.6	0.5
0-52	5.2	5.4	5.3	5.2	4.5	4.1	4.4	3.8

- Species: rat
- Strain: Sprague-Dawley
- Number of animals per dose: 10
- Route of administration: oral
- Method of administration: feed
- Purity: 96 %
- Dose range
  - Male: 0.5, 2, 10 ppm
  - Female: 0.5, 2, 10/6 ppm

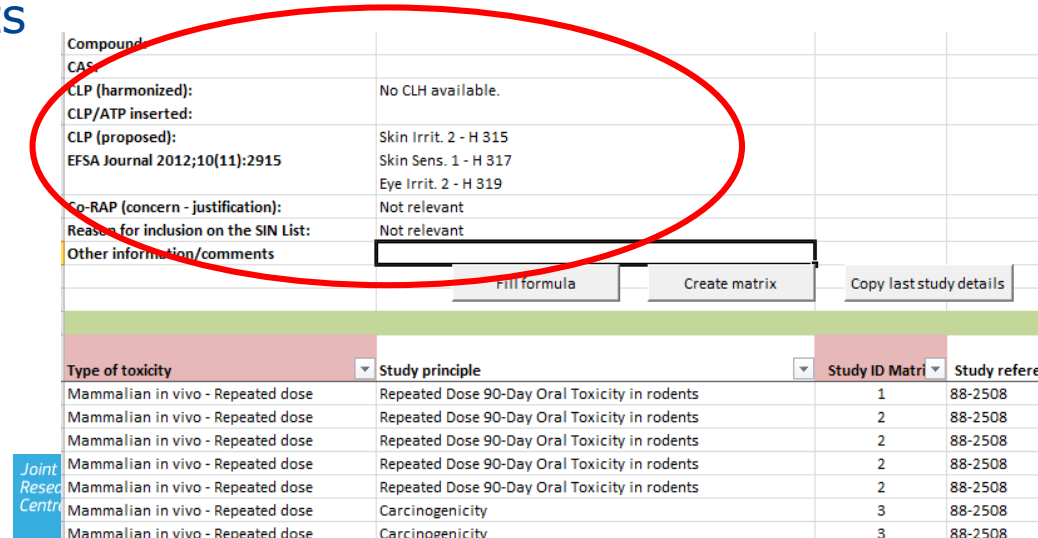
						stag	Sex (effect dose)	Lowest Effect dose	Effect type	Effect target
Mammalian in vivo - Repeated dose	Chronic toxicity		5	31665	Adult		F	117	Organ histopathology	Adrenals histopathology
Mammalian in vivo - Repeated dose	Chronic toxicity		5	31665	Adult		F	117	In life observation	Growth
Mammalian in vivo - Repeated dose	Chronic toxicity		5	31665	Adult		M	109	In life observation	Haematological parameters
Mammalian in vivo - Repeated dose	Chronic toxicity		5	31665	Adult		F	117	In life observation	Haematological parameters
Mammalian in vivo - Repeated dose	Chronic toxicity		5	31665	Adult		M	109	Organ histopathology	Liver histopathology
Mammalian in vivo - Repeated dose	Chronic toxicity		5	31665	Adult		F	117	Organ histopathology	Liver histopathology
Mammalian in vivo - Repeated dose	Chronic toxicity		5	31665	Adult		M	109	Clinical chemistry	T3 and T4 level
Mammalian in vivo - Repeated dose	Chronic toxicity		5	31665	Adult		F	117	Clinical chemistry	T3 and T4 level

Structure of data template

- Spreadsheet based (Excel): versatile, easy to use
- 40 columns for data details
- Structured template to capture/store diverse types of data from variety of sources (databases, scientific literature)
- All relevant effects captured from study (study ID)
  - Capture all ED relevant effects
  - Capture general toxicity effect at similar or lower dose (interpretation)
  - Each row describes one effect at single dose from one study

General substance information

- Compound name
- CAS number
- CLP (harmonized), including date of classification
- CLP (proposed), including date of classification
- Co-RAP (concern - justification)
- Reason for inclusion on the SIN List
- Other information/comments



Compound:	
CAS:	
CLP (harmonized):	No CLH available.
CLP/ATP inserted:	
CLP (proposed):	Skin Irrit. 2 - H 315
EFSA Journal 2012;10(11):2915	Skin Sens. 1 - H 317
	Eye Irrit. 2 - H 319
Co-RAP (concern - justification):	Not relevant
Reason for inclusion on the SIN List:	Not relevant
Other information/comments	

Fill formula | Create matrix | Copy last study details

Type of toxicity	Study principle	Study ID Matri	Study refere
Mammalian in vivo - Repeated dose	Repeated Dose 90-Day Oral Toxicity in rodents	1	88-2508
Mammalian in vivo - Repeated dose	Repeated Dose 90-Day Oral Toxicity in rodents	2	88-2508
Mammalian in vivo - Repeated dose	Repeated Dose 90-Day Oral Toxicity in rodents	2	88-2508
Mammalian in vivo - Repeated dose	Repeated Dose 90-Day Oral Toxicity in rodents	2	88-2508
Mammalian in vivo - Repeated dose	Repeated Dose 90-Day Oral Toxicity in rodents	2	88-2508
Mammalian in vivo - Repeated dose	Carcinogenicity	3	88-2508
Mammalian in vivo - Repeated dose	Carcinogenicity	3	88-2508

Data organisation

Study information	Study details	Effect	Indications
Guideline	No. animals	Generation	OECD CF level
Source	Purity	Sex	OECD 150
Reference	Route of administration	Lifestage	Comparable to OECD150
Date	Doses tested (+units)	Effect dose	Pathway
Species	Duration (+units)	Effect type	Human relevance
		Effect target	Adjusted effect dose
		Description	
		Direction	

Data Template

	A	B	C	D	E	F	G	H	I	J	K	L	M
6													
7													
8	Type of toxicity	Study principle	Study guideline (OECD/US EPA) or remarks	Reporting date	Species	Route of administration	Duration of exposure	Duration unit	Generation/Life stage	Sex (effect dose)	Lowest Effect dose	Effect type	Effect target
9	Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 409	2005	Dog	Oral	1	Years	Adult	M	20	Organ histopathology	[Not in list]
10	Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 409	2005	Dog	Oral	1	Years	Adult	M	20	Organ histopathology	Brain histopathology
11	Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 409	2005	Dog	Oral	1	Years	Adult	F	19	Clinical chemistry	T3 and T4 level
12	Mammalian in vivo - Repeated dose	Repeated Dose 28-Day Oral Toxicity in rodent	OECD 407	2006	Rat	Oral	28	Days	Adult	M+F		No relevant effect observed	No relevant effects
13	Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	In life observation	Growth
14	Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	Organ Weight	Kidney weight
15	Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	Organ Weight	Liver weight
16	Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	M	189	Organ histopathology	Lung histopathology
17	Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	Organ histopathology	Lung histopathology
18	Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	In life observation	Systemic toxicity
19	Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	M	189	Organ histopathology	Testis histopathology
20	Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity St	OECD 453	2006	Rat	Oral	2	Years	Adult	M	373	Organ histopathology	Epididymis histopathology
21	Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity St	OECD 453	2006	Rat	Oral	2	Years	Adult	M	169	Organ histopathology	Kidney histopathology
22	Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity St	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	Organ histopathology	Kidney histopathology
23	Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity St	OECD 453	2006	Rat	Oral	2	Years	Adult	M	169	Organ Weight	Kidney weight
24	Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity St	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	Organ Weight	Kidney weight
25	Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity St	OECD 453	2006	Rat	Oral	2	Years	Adult	M	169	Organ histopathology	Lung histopathology
26	Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity St	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	Organ histopathology	Lung histopathology
27	Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity St	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	In life observation	Systemic toxicity
28	Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity St	OECD 453	2006	Rat	Oral	2	Years	Adult	M	373	Organ histopathology	Testis histopathology
29	Mammalian in vivo - Repeated dose	Carcinogenicity	OECD 451	2006	Mouse	Oral	18	Months	Adult	M		No relevant effect observed	No relevant effects
30	Mammalian in vivo - Repeated dose	Carcinogenicity	OECD 451	2006	Mouse	Oral	18	Months	Adult	F		No relevant effect observed	No relevant effects
31	Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0+F1)	M	419	In life observation	Body weight
32	Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0+F1)	F	485	In life observation	Body weight
33	Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0+F1)	M	419	In life observation	Food consumption
34	Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0+F1)	F	485	In life observation	Food consumption
35	Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F1)	M	419	Organ histopathology	Kidney histopathology

Effect type

In life observation
Organ Weight
Organ histopathology
Reproductive
Developmental
Abnormalities
Clinical chemistry
No relevant effect observed
[Not in list]

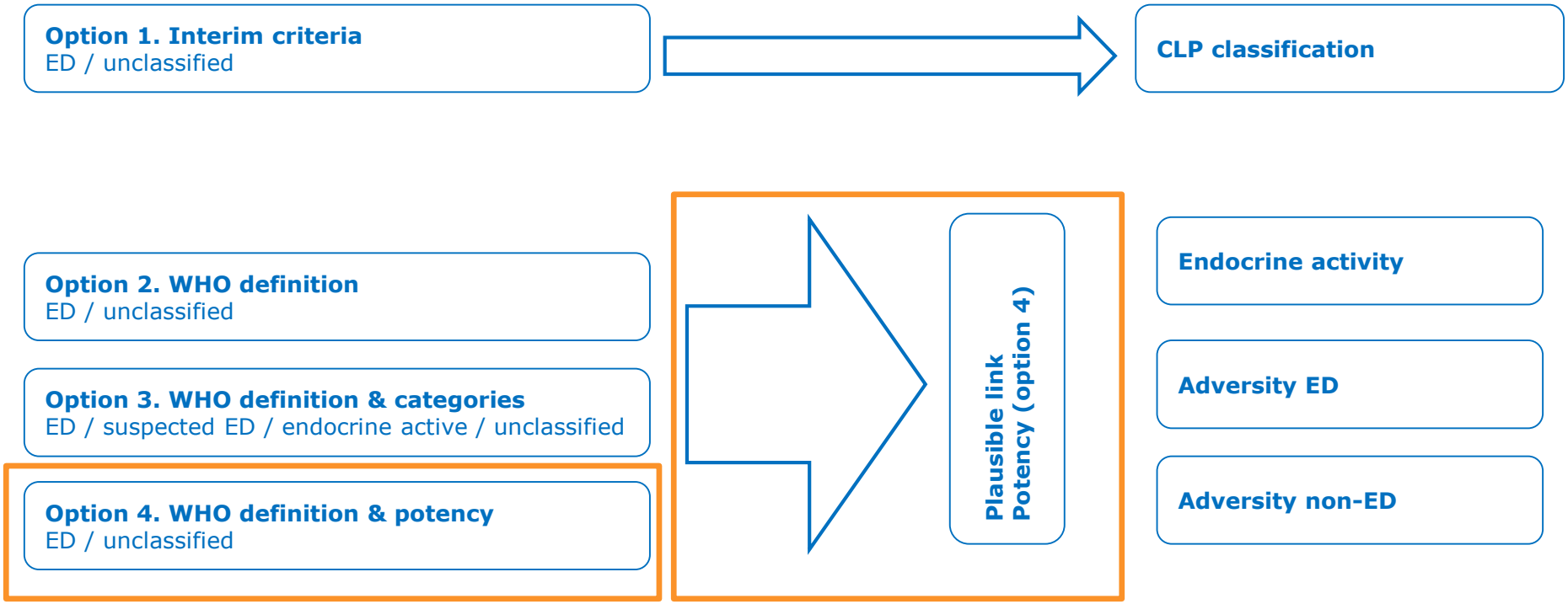
Effect target

Age at first estrus
Age at preputial separation
Age at Vaginal opening
Birth index
Dystocia
Estrus cyclicity
Fertility
Gestational interval
Gestation length
Gestation Index
Intercurrent deaths
Lactation index
Litter size
Litter viability
Number of implantations, corpora lutea

Categorisation of effects

Category	Description
In vitro mechanistic	Scientific literature, ToxCast
In vivo mechanistic & in vivo hormone levels	OECD CF Level 3 assays plus hormone levels
Adversity – EATS specific	Endpoints that can be specific for Estrogen, Androgen, Steroidogenesis or Thyroid pathways
Non-specific adversity (may or may not be indicative of EATS)	Endpoints potentially sensitive to, but not specific for, EATS pathways
Adversity – General	Non EATS related effects, including food intake, systemic toxicity, body weight change etc.

Information requirements



Categorisation under option 4

- No consensus on potency cut-off values
- STOT RE values proposed in literature

- Determine whether EATS specific effects still occur at or below this dose

Route of exposure	STOT-RE Cat 1	STOT-RE Cat 2
Oral	10 mg/kg bw/day	100 mg/kg bw/day
Dermal	20 mg/kg bw/day	200 mg/kg bw/day
Inhalation (vapour)	0.2 mg/l/6h/day	1.0 mg/l/6h/day
Inhalation (dust/mist/fume)	0.02 mg/l/6h/day	0.2 mg/l/6h/day

Note: these reference values refer to effects seen in a standard 90-day toxicity study in rats

Time adjustments of the guidance value

Following Haber's rule:

- for a 28-day study the guidance values above are increased by a factor of three
- for a 2-year study the guidance values are decreased by a factor of eight.

Allometric scaling and different life spans of species for Repeated Dose Toxicity not yet been integrated into the CLP guidance

The same guidance values for rat, mouse and dog studies have been used

Equivalent guidance values for 28-day and 90-day studies for rat

Study type	Unit	Category 1 90-day	Category 1 28-day	Category 2 90-day	Category 2 28-day
Oral	mg/kg bw/d	≤ 10	≤ 30	≤ 100	≤ 300
Dermal	mg/kg bw/d	≤ 20	≤ 60	≤ 200	≤ 600
Inhalation (gas)	ppmV/ 6 h/d	≤ 50	≤ 150	≤ 250	≤ 750
Inhalation (vapour)	mg/l/ 6 h/d	≤ 0.2	≤ 0.6	≤ 1	≤ 3
Inhalation (dust/mist/fume)	mg/l/ 6 h/d	≤ 0.02	≤ 0.06	≤ 0.2	≤ 0.6

European Commission

Data Analysis and Chemical Selection

Brussels, 6th November 2015

Alfonso Lostia

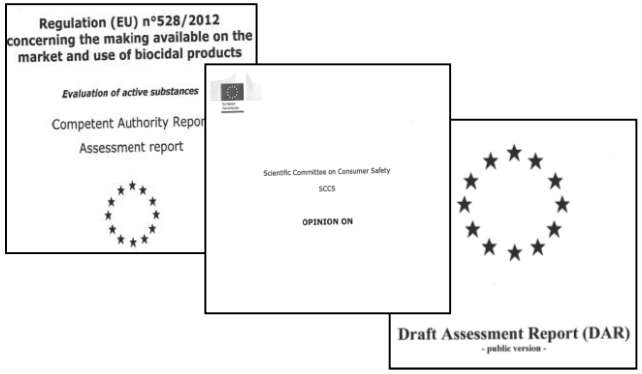
Systems Toxicology
Institute for Health & Consumer Protection

Joint Research Centre

Data Analysis

Workflow of screening methodology

Source Documents



Policy Options

1.
2.
3.
4.

1. Data collection

- In vitro
- Mammalian toxicity
- Wildlife toxicity

2. Data organisation

Type of toxicity	Study principle	Study guideline (OECD/US EPA) or remarks	Reporting date	Species	Route of administration	Duration of exposure	Duration unit	Generation/Life stage	Sex (effect dose)	Lowest Effect dose	Effect type	Effect target
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Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	Organ Weight	Kidney weight
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	Organ Weight	Liver weight
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	M	189	Organ histopathology	Lung histopathology
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Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	Organ histopathology	Kidney histopathology
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Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0/F1)	F	485	In life observation	Food consumption

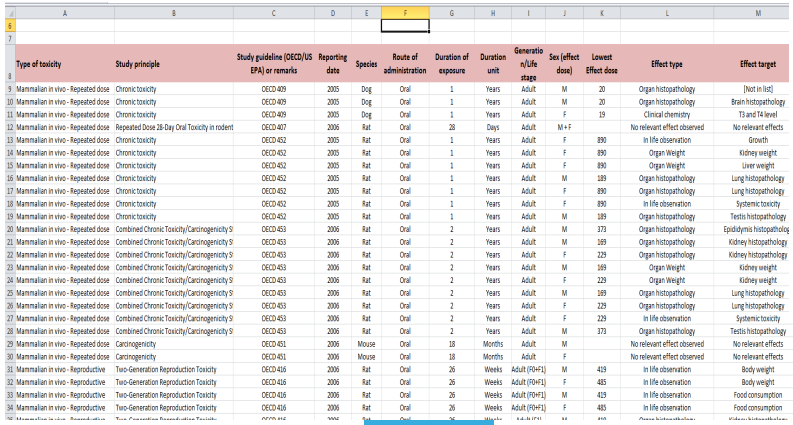
3. Data Analysis

Workflow of screening methodology

Policy Options

1.
2.
3.
4.

3. Data Analysis



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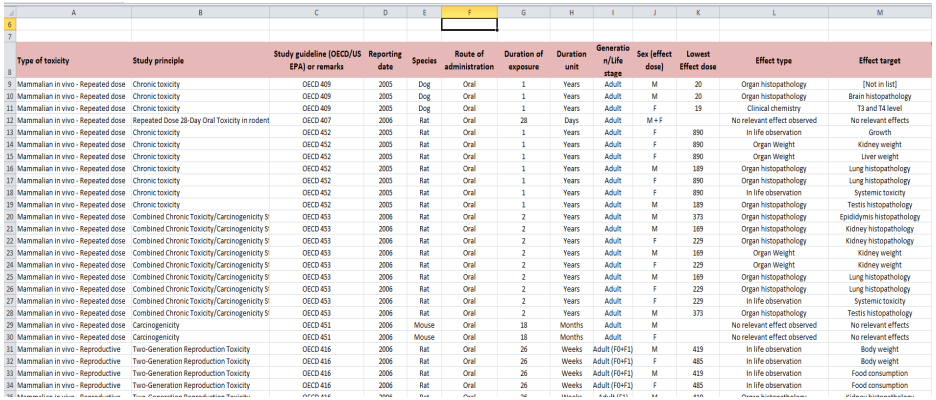
Stepwise approach to perform Data Analysis

Data collected in the template



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Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity St	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	Organ histopathology	Kidney histopathology
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity St	OECD 453	2006	Rat	Oral	2	Years	Adult	M	169	Organ Weight	Kidney weight
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity St	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	Organ Weight	Kidney weight
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity St	OECD 453	2006	Rat	Oral	2	Years	Adult	M	169	Organ histopathology	Lung histopathology
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity St	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	Organ histopathology	Lung histopathology
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity St	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	In life observation	Systemic toxicity
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity St	OECD 453	2006	Rat	Oral	2	Years	Adult	M	373	Organ histopathology	Testis histopathology
Mammalian in vivo - Repeated dose	Carcinogenicity	OECD 451	2006	Mouse	Oral	18	Months	Adult	M		No relevant effect observed	No relevant effects
Mammalian in vivo - Repeated dose	Carcinogenicity	OECD 451	2006	Mouse	Oral	18	Months	Adult	F		No relevant effect observed	No relevant effects
Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0+F1)	M	419	In life observation	Body weight
Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0+F1)	F	485	In life observation	Body weight
Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0+F1)	M	419	In life observation	Food consumption
Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0+F1)	F	485	In life observation	Food consumption

Stepwise approach to perform Data Analysis

Data collected in the template

Data processing

Category	Description
In vitro mechanistic	Scientific literature, ToxCast
In vivo mechanistic & in vivo hormone levels	OECD CF Level 3 assays plus hormone levels
Adversity – EATS specific	Endpoints that can be specific for Estrogen, Androgen, Steroidogenesis or Thyroid pathways
Non-specific adversity (may or may not be indicative of EATS)	Endpoints potentially sensitive to, but not specific for, EATS pathways
Adversity – General	Non EATS related effects, including food intake, systemic toxicity, body weight change etc.

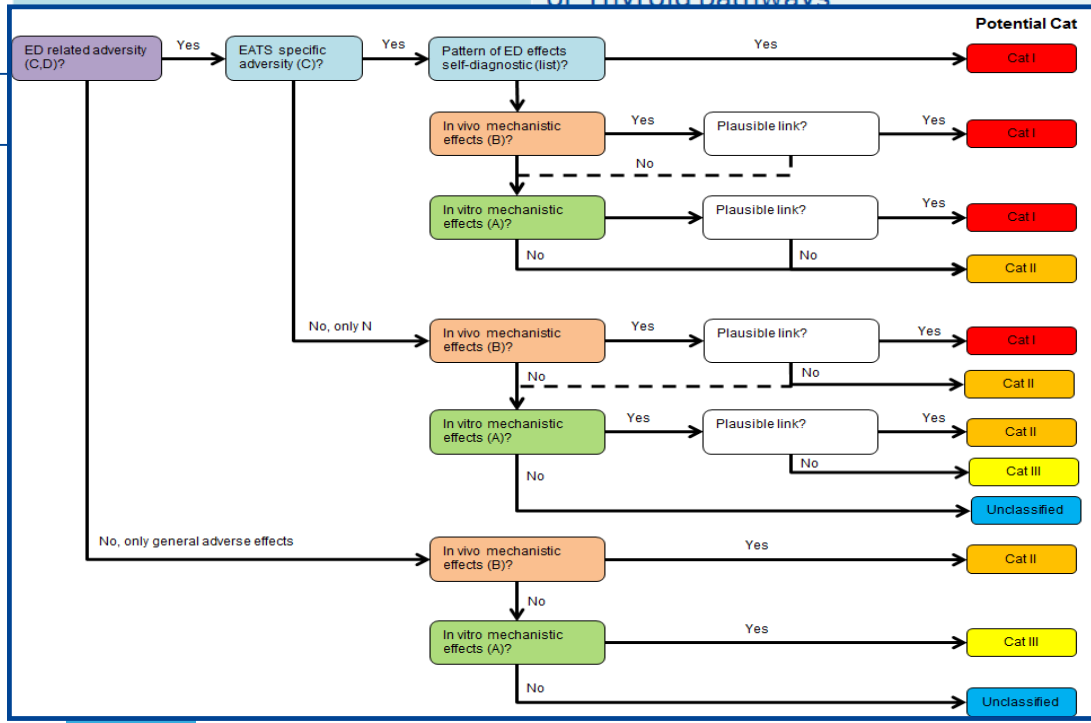
Stepwise approach to perform Data Analysis

Data collected in the template

Data processing

Apply decision tree with limited WoE

Category	Description
In vitro mechanistic	Scientific literature, ToxCast
In vivo mechanistic & in vivo hormone levels	OECD CF Level 3 assays plus hormone levels
Adversity – EATS specific	Endpoints that can be specific for Estrogen, Androgen, Steroidogenesis or Thyroid pathways



Stepwise approach to perform Data Analysis

Data collected in the template

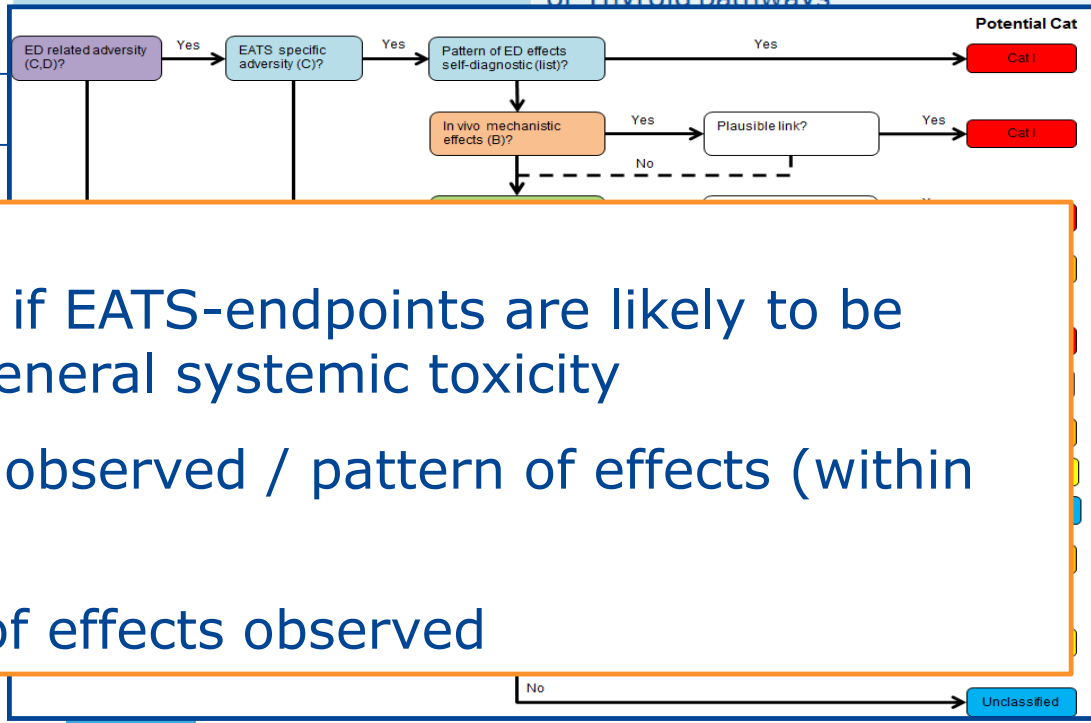
↓

Data processing

↓

Apply decision tree with limited WoE

Category	Description
In vitro mechanistic	Scientific literature, ToxCast
In vivo mechanistic & in vivo hormone levels	OECD CF Level 3 assays plus hormone levels
Adversity – EATS specific	Endpoints that can be specific for Estrogen, Androgen, Steroidogenesis or Thyroid pathways



WoE:

- Specificity: evaluating if EATS-endpoints are likely to be secondary effects of general systemic toxicity
- Consistency of effects observed / pattern of effects (within and between studies)
- Biological plausibility of effects observed

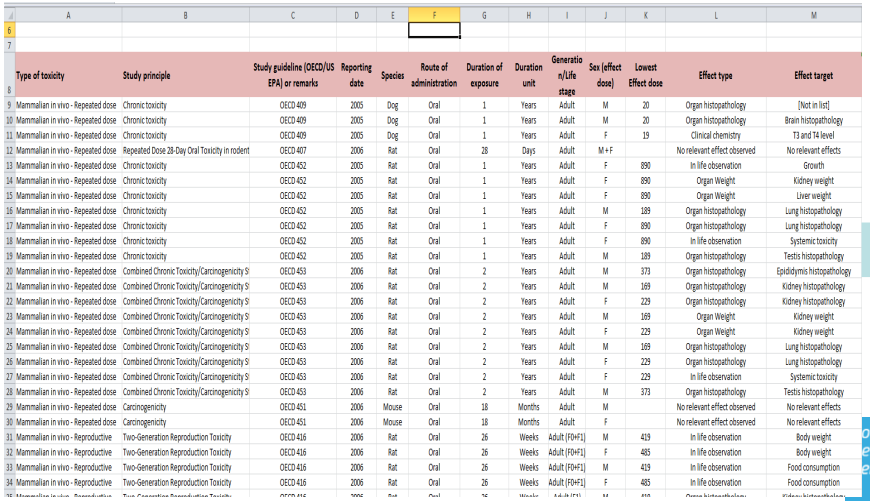
Complexity of Data Analysis

Going through all data captured in the template, to perform data-analysis, can be very complex and time-consuming.

In the template there are 40 columns and potentially hundreds of rows depending on substance.

There is the need to facilitate the data analysis by ensuring for substance evaluation:

- Usage of all data collected
- Transparency and traceabilty
- Medium-throughput (700 substances to be screened in a limited amount of time)



Type of toxicity	Study principle	Study guideline (OECD/US EPA) or remarks	Reporting date	Species	Route of administration	Duration of exposure	Duration unit	Generation/Life stage	Sex (effect dose)	Lowest Effect dose	Effect type	Effect target
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 409	2005	Dog	Oral	1	Years	Adult	M	20	Organ histopathology	[Not in list]
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 409	2005	Dog	Oral	1	Years	Adult	M	20	Organ histopathology	Brain histopathology
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 409	2005	Dog	Oral	1	Years	Adult	F	19	Clinical chemistry	T3 and T4 level
Mammalian in vivo - Repeated dose	Repeated Dose 28-Day Oral Toxicity in rodent	OECD 407	2006	Rat	Oral	28	Days	Adult	M+F		No relevant effect observed	No relevant effects
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	In life observation	Growth
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	Organ Weight	Kidney weight
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	Organ Weight	Liver weight
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	M	189	Organ histopathology	Lung histopathology
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	Organ histopathology	Lung histopathology
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	F	890	In life observation	Systemic toxicity
Mammalian in vivo - Repeated dose	Chronic toxicity	OECD 452	2005	Rat	Oral	1	Years	Adult	M	189	Organ histopathology	Testis histopathology
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	M	373	Organ histopathology	Epididymis histopathology
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	M	169	Organ histopathology	Kidney histopathology
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	Organ histopathology	Kidney histopathology
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	M	169	Organ Weight	Kidney weight
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	Organ Weight	Kidney weight
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	M	169	Organ histopathology	Lung histopathology
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	Organ histopathology	Lung histopathology
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	F	229	In life observation	Systemic toxicity
Mammalian in vivo - Repeated dose	Combined Chronic Toxicity/Carcinogenicity S	OECD 453	2006	Rat	Oral	2	Years	Adult	M	373	Organ histopathology	Testis histopathology
Mammalian in vivo - Repeated dose	Carcinogenicity	OECD 451	2006	Mouse	Oral	18	Months	Adult	M		No relevant effect observed	No relevant effects
Mammalian in vivo - Repeated dose	Carcinogenicity	OECD 451	2006	Mouse	Oral	18	Months	Adult	F		No relevant effect observed	No relevant effects
Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0+F1)	M	419	In life observation	Body weight
Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0+F1)	F	485	In life observation	Body weight
Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0+F1)	M	419	In life observation	Food consumption
Mammalian in vivo - Reproductive	Two-Generation Reproduction Toxicity	OECD 416	2006	Rat	Oral	26	Weeks	Adult (F0+F1)	F	485	In life observation	Food consumption

Can we find a simpler way to visualise all the data to facilitate data-analysis?

Data analysis: build a data-matrix

Type of toxicity	....	Study ID Matrix	....	Effect type	Effect target	....
Mammalian in vivo - Repeated dose		1		Organ Weight	Adrenals weight	
Mammalian in vivo - Repeated dose		1		Organ histopathology	Adrenals histopathology	
Mammalian in vivo - Repeated dose		1		Organ Weight	Kidney weight	
Mammalian in vivo - Repeated dose		1		Organ histopathology	Kidney histopathology	
Mammalian in vivo - Repeated dose		2		In life observation	Haematological parameters	
Mammalian in vivo - Repeated dose		2		In life observation	Haematological parameters	
Mammalian in vivo - Repeated dose		2		Organ Weight	Kidney weight	
Mammalian in vivo - Repeated dose		2		Organ histopathology	Spleen histopathology	
Mammalian in vivo - Repeated dose		2		In life observation	Systemic toxicity	
Mammalian in vivo - Repeated dose		2		In life observation	Systemic toxicity	
Mammalian in vivo - Repeated dose		3		In life observation	Haematological parameters	
Mammalian in vivo - Repeated dose		3		Organ histopathology	Kidney histopathology	
Mammalian in vivo - Repeated dose		3		Organ Weight	Kidney weight	
Mammalian in vivo - Repeated dose		4		Organ Weight	Adrenals weight	
Mammalian in vivo - Repeated dose		4		In life observation	Growth	
Mammalian in vivo - Repeated dose		4		In life observation	Haematological parameters	
Mammalian in vivo - Repeated dose		4		In life observation	Haematological parameters	
Mammalian in vivo - Repeated dose		4		Organ Weight	Liver weight	
Mammalian in vivo - Repeated dose		4		Organ Weight	Liver weight	
Mammalian in vivo - Repeated dose		4		Clinical chemistry	id stimulating hormone (TSH) level	
Mammalian in vivo - Repeated dose		5		No relevant effect observed	No relevant effects	

	Endpoint 1	Endpoint 2	Endpoint 3	Endpoint 4	Endpoint 5	Endpoint 6
Study 1						
Study 2						
Study 3						
Study 4						
Study 5						

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Data analysis: build a data-matrix

For each study, a bit string is constructed displaying all endpoints observed

Mammalian in vivo - Repeated dose	2		In life observation	Systemic toxicity
Mammalian in vivo - Repeated dose	2		In life observation	Systemic toxicity
Mammalian in vivo - Repeated dose	3		In life observation	Haematological parameters
Mammalian in vivo - Repeated dose	3		Organ histopathology	Kidney histopathology
Mammalian in vivo - Repeated dose	3		Organ Weight	Kidney weight
Mammalian in vivo - Repeated dose	4		Organ Weight	Adrenals weight
Mammalian in vivo - Repeated dose	4		In life observation	Growth
Mammalian in vivo - Repeated dose	4		In life observation	Haematological parameters
Mammalian in vivo - Repeated dose	4		In life observation	Haematological parameters
Mammalian in vivo - Repeated dose	4		Organ Weight	Liver weight
Mammalian in vivo - Repeated dose	4		Organ Weight	Liver weight
Mammalian in vivo - Repeated dose	4		Clinical chemistry	id stimulating hormone (TSH) level
Mammalian in vivo - Repeated dose	5		No relevant effect observed	No relevant effects

	Endpoint 1	Endpoint 2	Endpoint 3	Endpoint 4	Endpoint 5	Endpoint 6
Study 1						
Study 2						
Study 3						
Study 4						
Study 5						

Data matrix

Information from 4 different columns of the template is summarised in each box:

- Effect direction
- Effect dose
- Effect description
- Effect determination

Study design

Study	Source	Year	Type of toxicity	Species	Dose unit	Route of administration	Exposure	Additional remarks	In vitro mechanistic	In vivo mechanistic	EATS specific adversity	EATS specific adversity	Non-specific adversity (may or may not be indicative of EATS)	Non-specific adversity (may or may not be indicative of EATS)	Non-specific adversity (may or may not be indicative of EATS)	General adversity	General adversity	General adversity	General adversity
									Endpoint 1	Endpoint 2	Endpoint 3	Endpoint 4	Endpoint 5	Endpoint 6	Endpoint 7	Endpoint 8	Endpoint 9	Endpoint 10	Endpoint 11
1	DAR	1986	Mammalian in vivo -	Dog	mg/kg bw/day	Oral	1 Years			Decrease 500			Decrease 500				Decrease 500	Change 50	Increase 500
2	DAR	1981	Mammalian in vivo -	Mouse	mg/kg bw/day	Oral	2 Years				Change 100	Change 100					Decrease 100	Change 100	
3	TEDX	2013	In vitro	Mouse	µM		18 hours												
4	DAR	1978	Mammalian in vivo -	Rabbit	mg/kg bw/day	Oral	13 Days							Increase 55	Change 55	Decrease 55	Decrease 55		

Data matrix

The data matrix is automatically built from the template

									In vitro	In vivo	EATS	EATS	Non-specific adversity (may or	Non-specific adversity (may or	Non-specific adversity (may or	General	General	General	General
3	TEDX	2013	In vitro -	Mouse	µM		18 hours												
4	DAR	1978	Mammalian in vivo -	Rabbit	mg/kg bw/day	Oral	13 Days							Increase 55	Change 55	Decrease 55	Decrease 55		

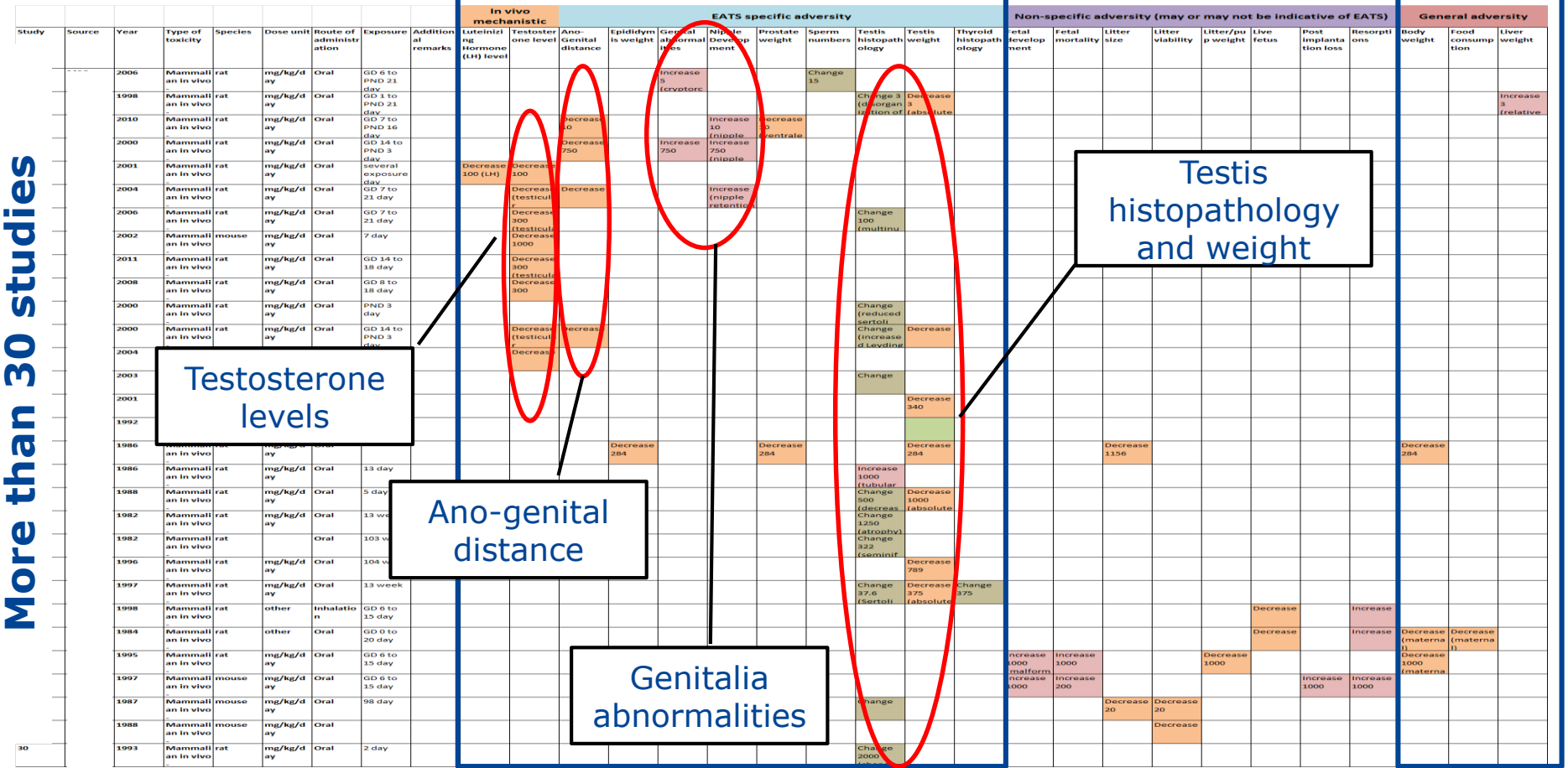
Examples Data Matrix

Example 1

Endpoints specific of EATS pathways

General adversity

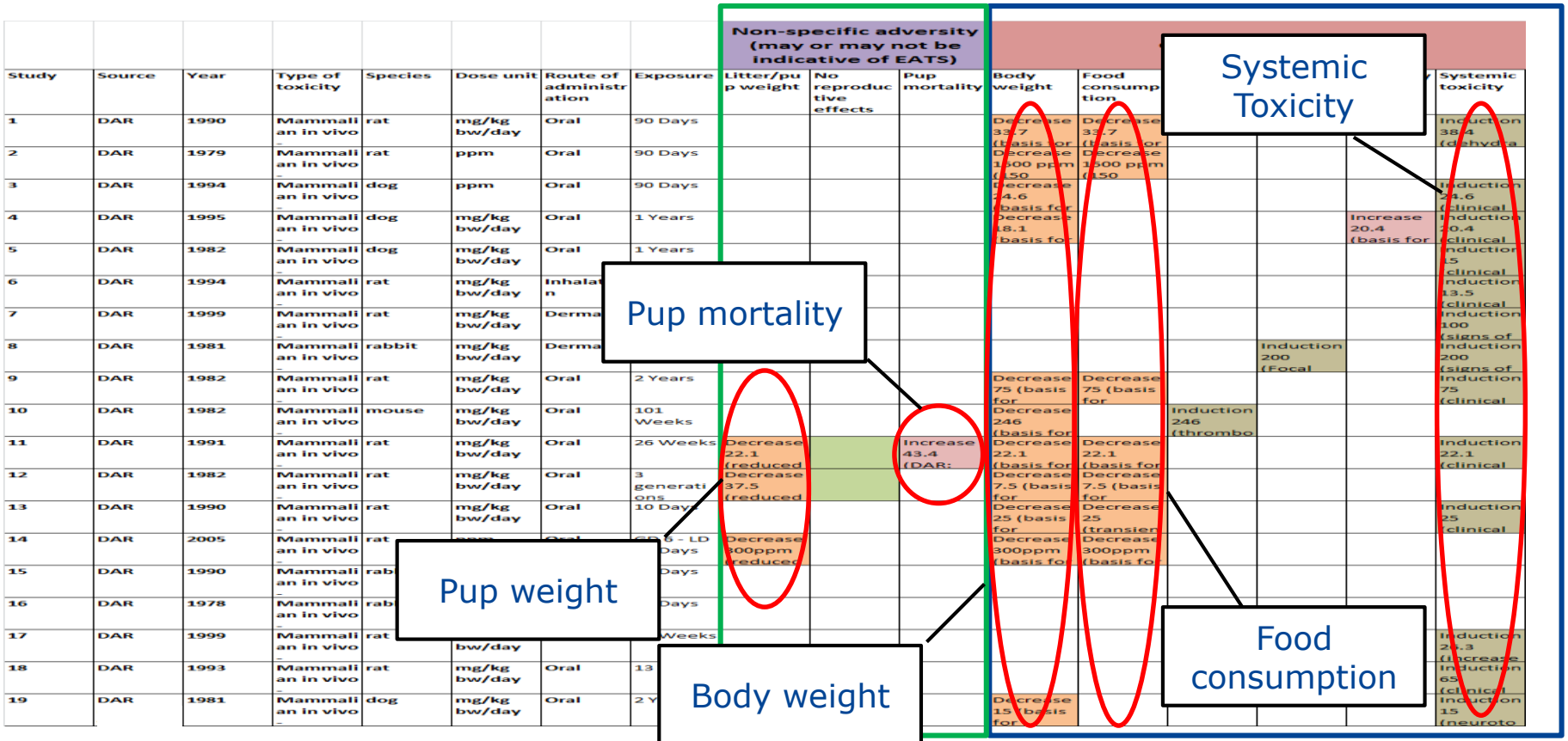
More than 30 studies



Example 2

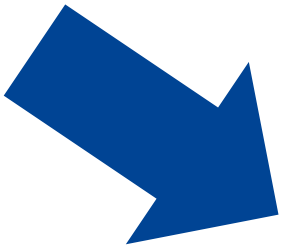
Few endpoints that may or may not indicative of EATS

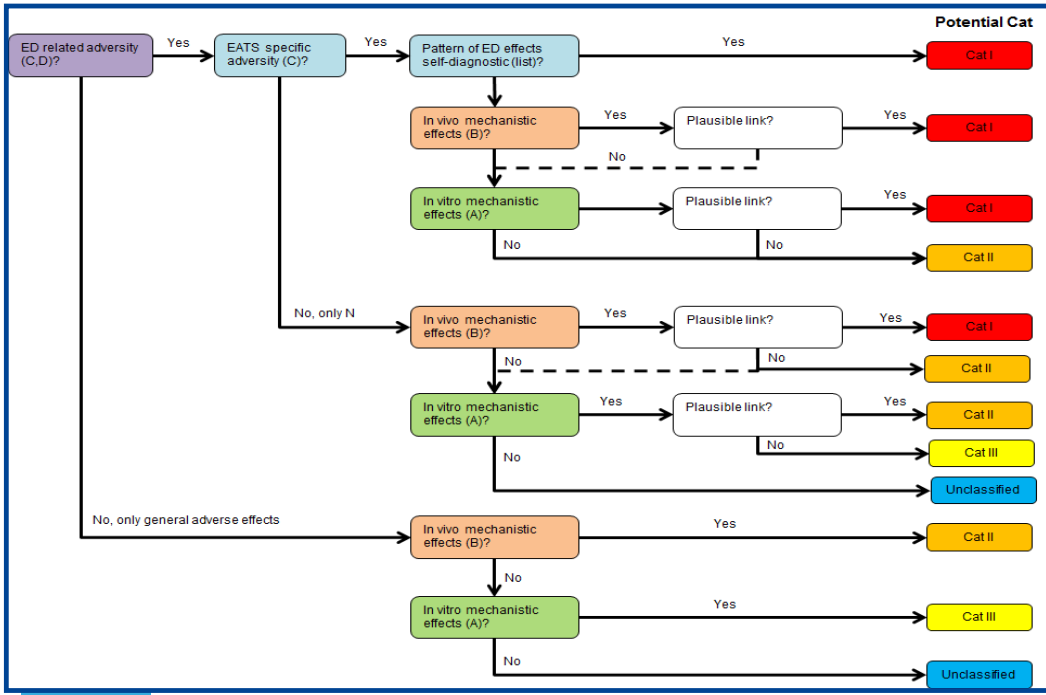
Most endpoints related to General adversity



Organising the data facilitates the analysis

Study	Source	Year	Type of toxicity	Species	Dose unit	Route of administration	Exposure	Additional remarks	Endpoint 1	Endpoint 2	Endpoint 3	Endpoint 4	Endpoint 5	Endpoint 6	Endpoint 7	Endpoint 8	Endpoint 9	Endpoint 10	Endpoint 11
1	DAR	1986	Mammalian in vivo -	Dog	mg/kg bw/day	Oral	1 Years			Decrease 500			Decrease 500				Decrease 500	Change 50	Increase 500
2	DAR	1981	Mammalian in vivo -	Mouse	mg/kg bw/day	Oral	2 Years				Change 100	Change 100					Decrease 100	Change 100	
3	TEDX	2013	In vitro	Mouse	µM		18 hours												
4	DAR	1978	Mammalian in vivo -	Rabbit	mg/kg bw/day	Oral	13 Days							Increase 55	Change 55	Decrease 55	Decrease 55		





Chemical Selection

Scope of Chemical Selection

1. Chemicals to be screened take in account the following EU legislations:

   - Plant Protection Products Regulation (PPPR)
   - Biocides Products Regulation (BPR)
   - REACH Regulation
   - Cosmetics Regulation
   - Water Framework Directive (WFD)

2. ED IA analysis to be performed on about 700 chemicals

3. For the selected chemicals, gather mechanistic-toxicological data to then apply the four policy options for identifying EDs.

Principles for Chemical Selection

The selection of the substances considered time constraints and efficient use of public money. It was based on the following principles:

- Transparency
- Objectivity, securing that all possible scenarios are covered to assess the impact of the various options for criteria at least on a qualitative basis
- Consideration of availability of data, which are crucial for the screening assessment of ED properties.
- The selection should (as far as possible) not lead to a bias in the assessment of the four options.

For PPP and BPs, all approved substances were considered, and then non relevant substances were taken out from the list.

For REACH and Cosmetics, the list was started with substances where information and concerns were already identified. If the list ends up being longer than the available resources, a selection would be done randomly.

The substances falling under the **WFD** were covered by the selection under REACH, Cosmetics, PPPs and BPs and not listed separately.

Chemical selection: strategy

1st Step:

Compile the list of all relevant chemicals from PPPR and BPR

2nd Step:

Expand the initial list, by adding chemicals from REACH regulation, Cosmetics Regulation and WFD

3rd Step:

Cross-check if these chemicals are also listed in other regulatory / toxicological / NGO databases that can be used to collect further available mechanistic & toxicological data for the ED IA

1st Step:
Selection of chemicals under PPPR and BPR

All approved chemical active substances from EU Pesticides database (DG SANTE) and all Biocidal Active Substances (ECHA)

The following substances are not included:

- Microorganisms (living organisms, NOT chemicals)
- Basic substances (being substances of no concern and no inherent capacity to cause endocrine disrupting effects, and where the approval procedures follow particular rules)
- Low risk substances (defined in Annex II to Regulation (EC) 1107/2009 as, among others properties, not deemed to be an endocrine disruptor)
- Natural extracts, mixtures, or repellents
- Attractants (pheromones) or plant hormones
- Some inert substances, salts, acids

European Commission

2nd Step:

Selection of substances under REACH Regulation

1. All substances on the Candidate List already identified as SVHCs because of ED concerns under Art. 57(f)

2. All substances for which an SVHC opinion on the identification of the substance as SVHC due to its endocrine disrupting properties was provided by the Member State Committee at ECHA

3. All substances on the Candidate list identified as SVHC because of reprotoxicity 1A/1B

4. All substances listed in Annex XVII for restrictions due to a ED concern or because of having a harmonised classification as reprotoxic 1A/1B

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European Commission

2nd Step:
Selection of substances under REACH Regulation

5. All substances placed on CoRAP due to ED concern

6. All substances discussed in the Endocrine Disruptor Expert Group

7. Substances flagged as SIN list substances because of ED concerns excluding those which are pesticides, biocides and non-registered substances

8. Substances flagged as Category 1 and 2 in the Commission's priority list of substances for further testing of their role in endocrine disruption (EASIS) excluding pesticides, biocides and non-registered substances

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2nd Step:
Selection of substances under Cosmetics Regulation

- Most substances for which an opinion of the Scientific Committee on Consumer Safety (SCCS) was provided, which contained a discussion on their endocrine disrupting potential
- Most substances for which an SCCS opinion was provided due to the their potential or de facto classification as CMR1A/1B or CMR2 under the CLP Regulation
- Most substances not classified as CMR but for which SCCS expressed some concern on toxicity endpoints
- Substances for which concern was raised by stakeholders / Member States on potential endocrine disrupting properties

3rd Step:

overlap of chemicals with other databases to collect additional data for ED IA

Cross-check if the chemicals selected from PPPR, BPR, REACH Regulation, Cosmetic Regulation and WFD are present in any of these databases/lists:

- US-EPA Endocrine Disruptors Screening Program
- ToxCast: database with in vitro data from US EPA
- EASIS
- Tedx: list of potential Eds
- SIN: list of potential EDs compiled by NGO ChemSec

Inventory of chemicals to be screened

Chemical Name	CAS	pesticides	biocides	cosmetics	REACH	WFD	EDSP	SIN ED label	Tedx	EASIS	ToxCast
Substance 1	xxxx	1	1						1	1	1
Substance 2	xxxx	1	1						1	1	1
Substance 3	xxxx	1	1						1	1	
Substance 4	xxxx	1	1						1	1	1
Substance 5	xxxx	1	1						1	1	1
Substance 6	xxxx	1	1						1	1	1
Substance 7	xxxx	1	1						1	1	1
Substance 8	xxxx	1	1						1	1	1
Substance 9	xxxx	1	1						1	1	1
Substance 10	xxxx	1	1			1			1	1	1
Substance 11	xxxx	1	1						1	1	
Substance 12	xxxx	1	1						1		

Indicate where to find relevant information to facilitate data-collection

Concluding Remarks

Concluding Remarks

- Contractor applied methodology to sample subset (35 substances) to test practical operability
- Fine tuning/adjustments were made according to feedback
- Methodology currently being applied in a phased manner to PPPs, Biocides and selection from REACH, cosmetic ingredients and priority substances under Water Framework Directive
- Strike appropriate balance between resources, time constraints and depth of analysis
- The JRC is continually supporting the contractor to ensure the faithful implementation of the methodology

Concluding Remarks

- To keep in mind:
  - The results of the screening do not constitute in any event a list of recognised endocrine disruptors.
  - Therefore, the results do not have any regulatory consequences, nor do they pre-empt any future decision regarding identification of a chemical substance as an endocrine disruptor.

Joint Research Centre (JRC)

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