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Valutazione dei prodotti con effetto sulla tiroide

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European Food Safety Authority

OUTLINE

- Regulation for pesticides
- Methodologies developed by the PPR Panel
- Establishment of cumulative assessment groups (CAGs) for effects on the thyroid
- Conclusions
- Recommendations
- Next steps

REGULATION 1107/2009

Regulation (EC) No 1107/2009 on the placing of plant protection products on the market

Art.4 (Approval criteria for active substances): The residues of plant protection products…shall not have any harmful effects on human health, including that of vulnerable groups,…***taking into account known cumulative and synergistic effects where the scientific methods accepted by the Authority to assess such effects are available,…***

Regulation (EC) No 396/2005 on maximum residue levels of pesticides in food and feed

Art.14 (Decision on applications concerning MRLs): «…account shall be taken of: (a) the scientific knowledge; (b) the possible presence of pesticide residues arising from other sources than current plant protection uses of active substances, ***and their known cumulative and synergistic effects, when the methods to assess such effects are avaiable****…»*

LEGAL CONTEXT FOR PESTICIDES

When should a cumulative risk assessment be conducted?

Pre marketing

- Applications for MRLs (Art. 14 of Regulation (EC) No 396/2005)
- Authorisation of plant protection products (Art. 4 of Regulation (EC) No 1107/2009)

Post marketing

- Analysis of official monitoring results regarding acute and chronic risks to the health of consumers

OUTPUTS OF THE PPR PANEL

2007: Suitability of existing methodologies and, if appropriate, the identification of new approaches to assess cumulative and synergistic risks from pesticides to human health with a view to set MRLs for those pesticides in the frame of Regulation (EC) 396/2005

http://www.efsa.europa.eu/en/efsajournal/pub/705

2008: Risk Assessment for a Selected Group of Pesticides from the Triazole Group to Test Possible Methodologies to Assess Cumulative Effects from Exposure through Food from these Pesticides on Human Health

http://www.efsa.europa.eu/en/efsajournal/pub/1167

2012: Guidance on the Use of Probabilistic Methodology for Modelling Dietary Exposure to Pesticide Residues

http://www.efsa.europa.eu/en/efsajournal/pub/2839

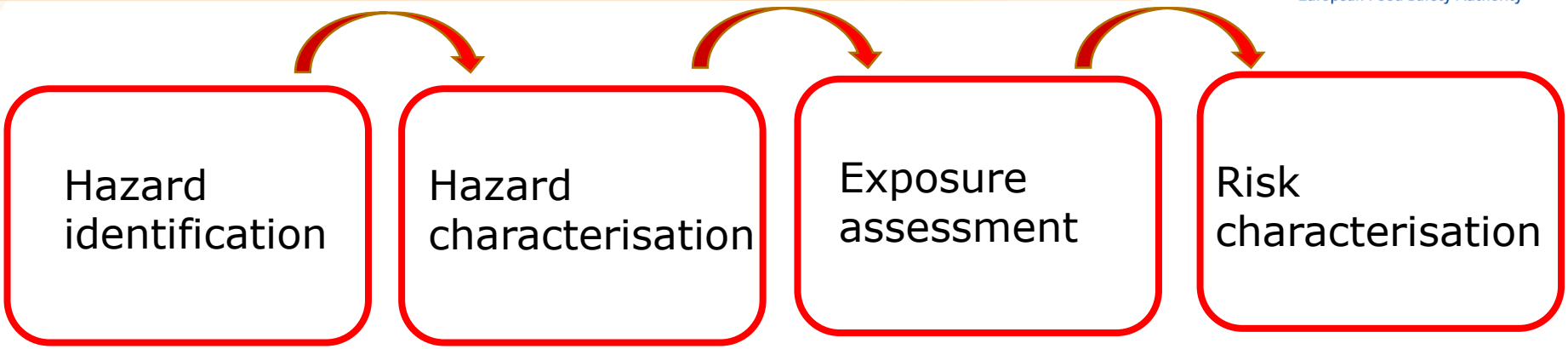
2013: Identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile

http://www.efsa.europa.eu/en/efsajournal/pub/3293

2013: Relevance of dissimilar mode of action and its appropriate application for cumulative risk assessment of pesticides residues in food

http://www.efsa.europa.eu/en/efsajournal/pub/3472

IMPLEMENTATION OF CRA



Hazard assessment

Establishment of cumulative assessment groups of pesticides for their effects on the thyroid https://www.efsa.europa.eu/en/efsajournal/pub/5801

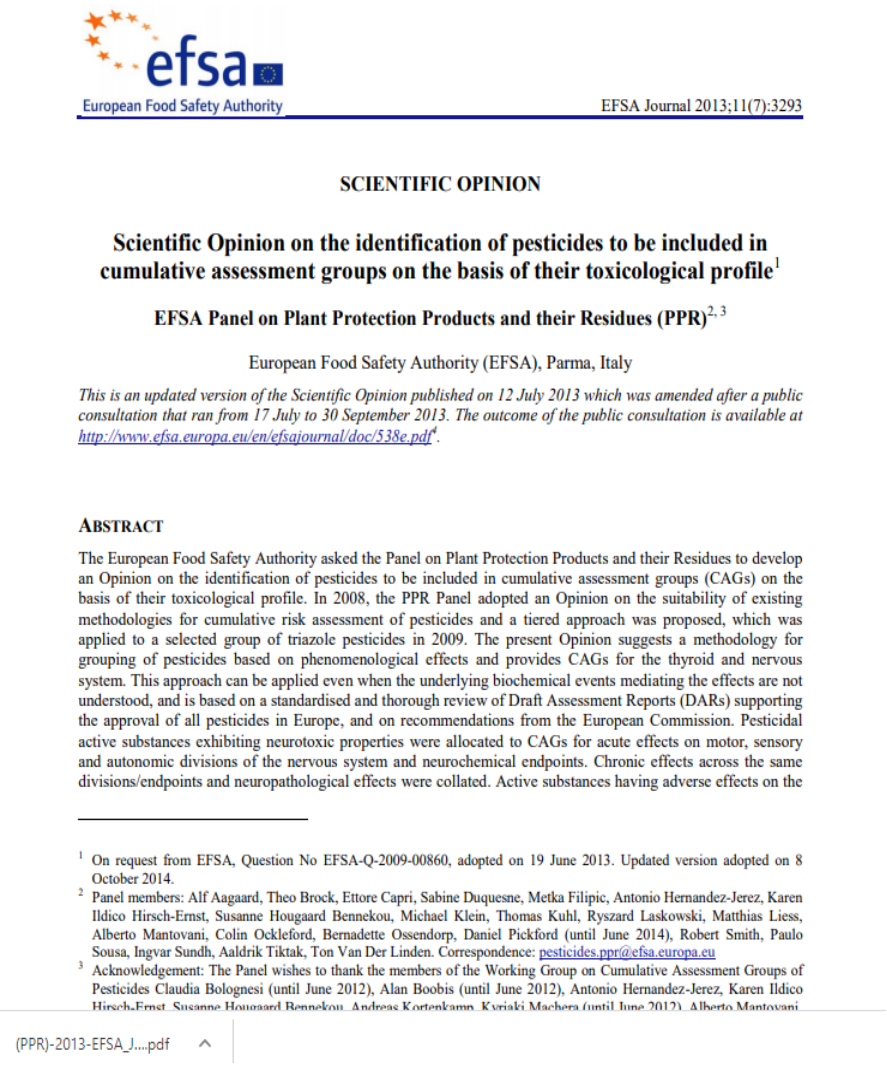
Cumulative dietary exposure assessment of pesticides that have chronic effects on the thyroid using SAS software https://www.efsa.europa.eu/it/efsajournal/pub/5763

Cumulative dietary exposure assessment of pesticides that have chronic effects on the thyroid using MCRA software https://www.efsa.europa.eu/en/supporting/pub/en-1707

Cumulative dietary risk characterisation of pesticides that have chronic effects on the thyroid (in progress, public consultation in Sept 2019, publication foreseen for March 2020)

CRA FOR PESTICIDES

- A cumulative assessment group is a set of pesticides that could plausibly act in combination and cause a specific toxicological effect
- All pesticides causing a common specific (adverse) effect are included within a same cumulative assessment group (assumption of dose addition)
- Why not similarity of chemical structure or mode of action?
  - Often modes/mechanism of action are unknown
  - Different modes/mechanisms of action may contribute to a same adverse effect
  - Consultation with EU Commission – precautionary principle



European Food Safety Authority

EFSA Journal 2013;11(7):3293

SCIENTIFIC OPINION

Scientific Opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile[1]

EFSA Panel on Plant Protection Products and their Residues (PPR)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This is an updated version of the Scientific Opinion published on 12 July 2013 which was amended after a public consultation that ran from 17 July to 30 September 2013. The outcome of the public consultation is available at http://www.efsa.europa.eu/en/efsajournal/doc/538e.pdf[4].

ABSTRACT

The European Food Safety Authority asked the Panel on Plant Protection Products and their Residues to develop an Opinion on the identification of pesticides to be included in cumulative assessment groups (CAGs) on the basis of their toxicological profile. In 2008, the PPR Panel adopted an Opinion on the suitability of existing methodologies for cumulative risk assessment of pesticides and a tiered approach was proposed, which was applied to a selected group of triazole pesticides in 2009. The present Opinion suggests a methodology for grouping of pesticides based on phenomenological effects and provides CAGs for the thyroid and nervous system. This approach can be applied even when the underlying biochemical events mediating the effects are not understood, and is based on a standardised and thorough review of Draft Assessment Reports (DARs) supporting the approval of all pesticides in Europe, and on recommendations from the European Commission. Pesticidal active substances exhibiting neurotoxic properties were allocated to CAGs for acute effects on motor, sensory and autonomic divisions of the nervous system and neurochemical endpoints. Chronic effects across the same divisions/endpoints and neuropathological effects were collated. Active substances having adverse effects on the

[1] On request from EFSA, Question No EFSA-Q-2009-00860, adopted on 19 June 2013. Updated version adopted on 8 October 2014.

[2] Panel members: Alf Aagaard, Theo Brock, Ettore Capri, Sabine Duquesne, Metka Filipic, Antonio Hernandez-Jerez, Karen Ildico Hirsch-Ernst, Susanne Hougaard Bennekou, Michael Klein, Thomas Kuhl, Ryszard Laskowski, Matthias Liess, Alberto Mantovani, Colin Ockleford, Bernadette Ossendorp, Daniel Pickford (until June 2014), Robert Smith, Paulo Sousa, Ingvar Sundh, Aaldrik Tiktak, Ton Van Der Linden. Correspondence: pesticides.ppr@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Cumulative Assessment Groups of Pesticides Claudia Bolognesi (until June 2012), Alan Boobis (until June 2012), Antonio Hernandez-Jerez, Karen Ildico Hirsch-Ernst, Susanne Hougaard Bennekou, Andreas Kortenkamp, Kyriaki Machera (until June 2012), Alberto Mantovani

(PPR)-2013-EFSA_J....pdf

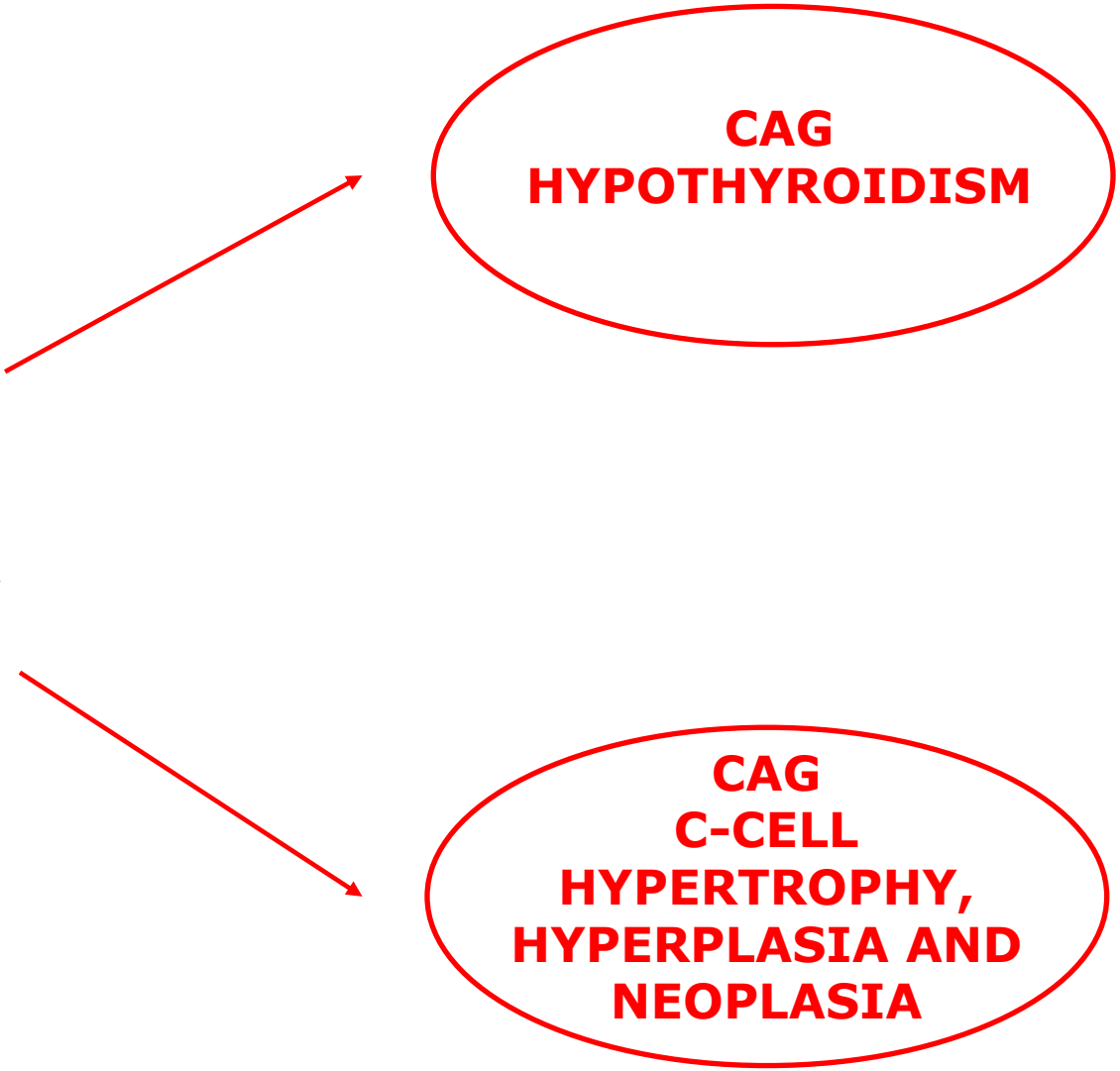
ESTABLISHMENT OF CUMULATIVE ASSESSMENT GROUPS - PROCESS

1. Identification of specific effects which qualify for cumulative risk assessment
2. Characterisation of specific effects
3. Data collection
4. Grouping of pesticides into CAGs
5. Selection of the Index Compound (IC) and of the relative potency factor (RPF)
6. Analysis of uncertainties

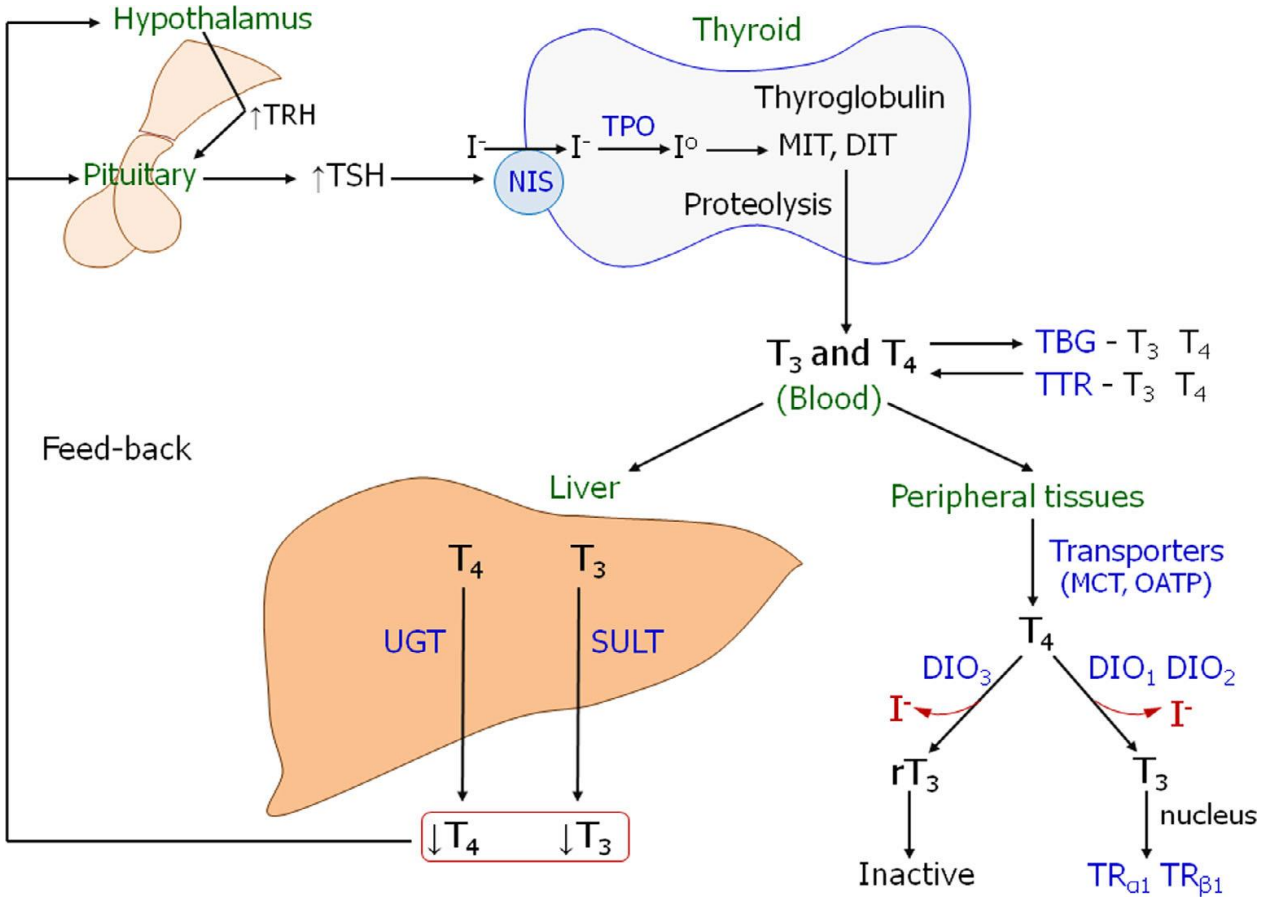
1. IDENTIFICATION OF THE SPECIFIC EFFECT FOR THE THYROID

Hypothyroidism

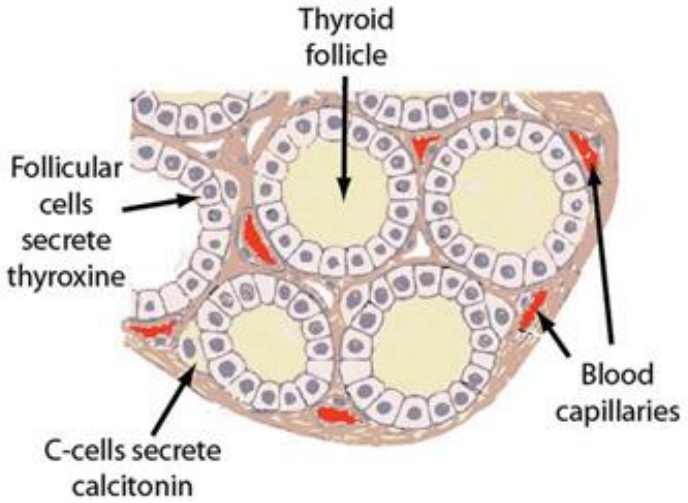
C-cell hypertrophy, hyperplasia and neoplasia



Altered function of the thyroid gland resulting in follicular cell hypertrophy, hyperplasia and neoplasia



C-CELL HYPERTROPHY, HYPERPLASIA AND NEOPLASIA



C-cells secrete hormone calcitonin involved in calcium homeostasis and regulation of bone marrow formation

- C-cell hypertrophy, hyperplasia and neoplasia are observed after repeated exposure to certain pesticides
- Sustained C-cell stimulation leading to hyperplasia is expected to play a promoting role in further progression to neoplasia
- These effects are considered relevant for humans

2. CHARACTERISATION OF THE SPECIFIC EFFECT

Definition of the descriptors (indicators) in toxicological studies suggesting that an active substance causes the specific effect

- Hypothyroidism:
  - Decreased circulating T3 level, decreased circulating T4 level
  - Increased circulating TSH levels
  - Increased relative thyroid weight
  - Follicular cell hypertrophy
  - Follicular cell hyperplasia
  - Follicular cell neoplasia, follicular cell adenoma, follicular cell carcinoma
  - Evidence of a MoA in direct relation to hypothyroidism
- Effects on C-cells:
  - C-cell hypertrophy
  - C-cell hyperplasia
  - C-cell tumours: C-cell adenoma, C-cell carcinoma
  - Evidence of MoA in direct relation with C-cell hypertrophy, hyperplasia and neoplasia

3. DATA COLLECTION FOR EFFECTS ON THE THYROID

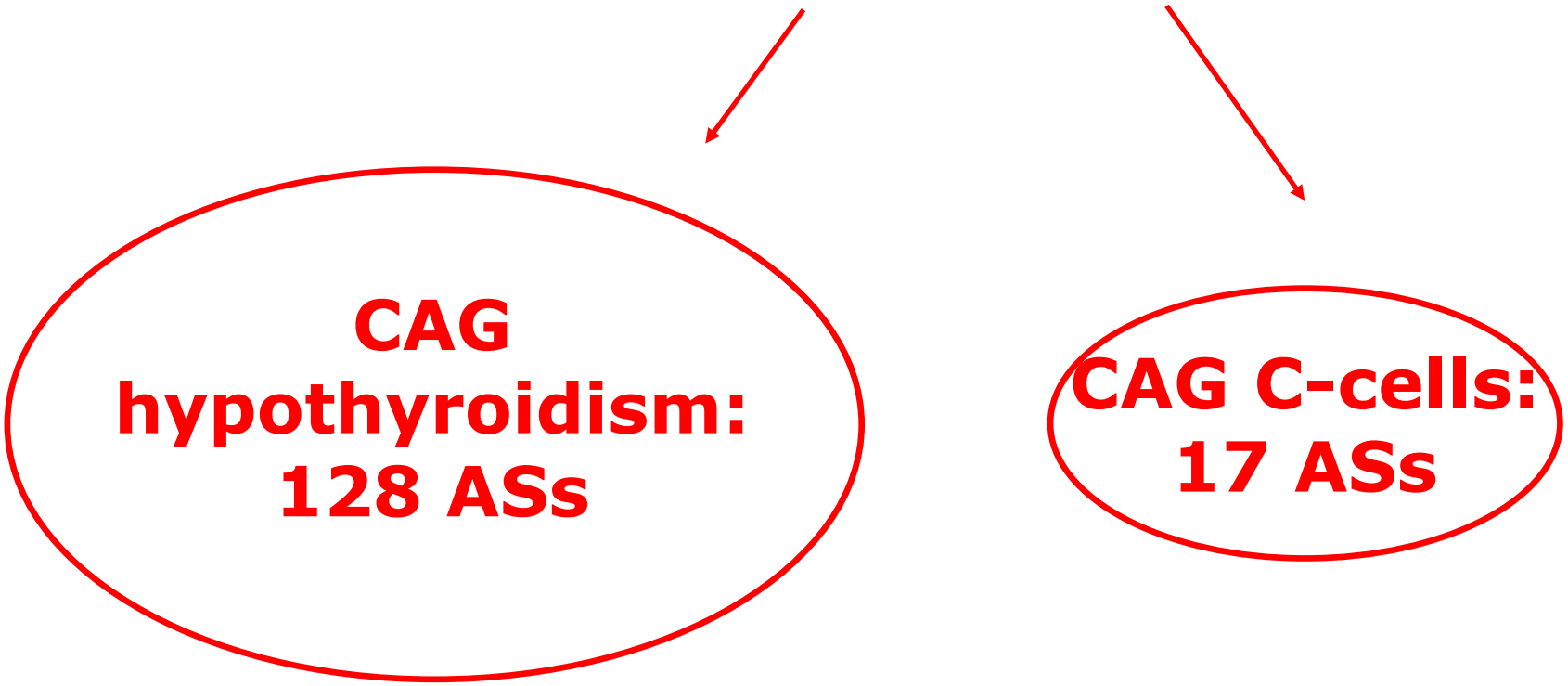
- Two data collections available:
    1. Consortium RIVM, ICPS and ANSES https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2016.EN-999
    2. EFSA

422 Active Substances (ASs) screened

- Studies rated as "acceptable" or "supportive" were considered from different animal species (mainly rat, mouse and dog) and any limitation which could have had an impact on the acceptability of the study were flagged
- The lowest no observed adverse effect level (NOAEL)/lowest adverse effect level (LOAEL) for a specific effect observed in the most sensitive sex
- When more than one specific effects was observed for an AS in one study, each of them was collected under a separate entry
- When several indicators of a specific effect have been observed in one study, the most sensitive one(s) has(have) been indicated in the column "Endpoint of a specific effect"
- Information on the potential MoA was collected

4. GROUPING OF PESTICIDES INTO CAGs

An AS was included in the CAG if at least one of the respective indicators was observed at statistically significant or biologically relevant level in at least one toxicological study assessed as 'acceptable' in the DAR, RAR or equivalent documents



CAG HYPOTHYROIDISM

Active substance	Indicator of specific effect	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Study	Remark	MoA
2,4-D	Increased relative thyroid weight	5	15	2-year rat [redacted]	Source: DAR 1997 and addendum EFSA (2014) Observation of increased relative thyroid weight with NOAEL at 1 mg/kg in 90-day rat [redacted] outweighed by 2 other more recent 90-day studies with higher NOAELs	Interference with transthyretin (van den Berg, 1991; Neal et al., 2017)
8-Hydroxyquinoline (incl. Oxyquinoleine)	Increased relative thyroid weight	10	50	90-day dog study [redacted]	Source: DAR 2009	Unknown
Aclonifen	Follicular cell hypertrophy	8.1	66.9	2-year rat (Kirsch, 1989)	Source: DAR 2006 EFSA (2008) 2-year rat [redacted], 90-day rat studies [redacted] combined	Unknown
Amisulbrom	Follicular cell hypertrophy	129	697	2-year rat [redacted]	Source: DAR 2012	Unknown
Amitrole	Increased relative thyroid weight, follicular cell hyperplasia	0.3	13	1-year dog [redacted]	Source: DAR 1996 EFSA (2014)	TPO inhibition (IPCS, 1998), NIS inhibition (hypothesised) (Hongmei et al., 2011), alteration of TTR, DIO1, DIO2, and TR-α gene expression (hypothesised) (Li et al., 2009)
Anthraquinone	Increased relative thyroid weight	12.58	20	90-day rat [redacted]	Source: DAR 2006	Unknown
Azadirachtin	Follicular cell hypertrophy	36 (12)	135	90-day rat [redacted]	Source: DAR 2007 EFSA Scientific Committee (2018) The NOAEL in the 90-day rat study is 36 mg/kg, but an additional SF of 3 needs to be applied due to the lack of long-term studies	Deiodinases inhibition (hypothesised) (Panda and Kar, 2000)

5. INDEX COMPOUND (IC) AND RELATIVE POTENCY FACTOR (RPF)

- IC and RPF are needed to perform cumulative exposure/risk assessments (to normalise the toxicity of all ASs in each CAG to the IC)
- IC selected between ASs of high potency and with highly convincing evidence that it causes the specific effect using the following criteria:
  - Quality of the study (considered acceptable, statistical robustness of findings)
  - Strength of the specific effect (NOAEL, number of indicators of the specific effect observed)
  - Evidence of dose-response relationship
  - Consistency in the occurrence of the specific effect across genders, species and studies

ICs selected: Ioynil for CAG hypothyroidisms and fenbuconazole for CAG on C-cells

$$RPF = \frac{\text{NOAEL of the IC}}{\text{NOAEL of the AS}}$$

6. UNCERTAINTY ANALYSIS

Regulation 1107/2009: Member States 'shall not be prevented from applying the precautionary principle where there is scientific uncertainty as to the risks with regard to human and animal health' and 'shall take into consideration possible elements of uncertainty in the information in order to ensure that the chances of failing to detect adverse effects or of underestimating their importance are reduced to a minimum'

Uncertainty analysis evaluating the potential of –under or overestimation of the actual risk for consumers

Sources of uncertainties:

- Method used to collect and assess toxicological data
- Limitations in the available data and scientific knowledge
- Composition of the CAGs
- Toxicological characterisation of the ASs
- Slope and shape of dose-response relationship
- Contribution of metabolites and degradation products
- Adequacy of dose addition
- Inter and intra-species differences in toxicological sensitivity

ANALYSIS OF UNCERTAINTIES

The CAGs established in this report were used to carry out cumulative exposure and risk assessments following the methodology developed by the PPR Panel. This methodology assumes that all ASs included in a CAG combine their effects by **dose addition**.

Question 1:

How sure is it that the CAG contains all the active substance causing the respective specific effect and only the AS causing this effect?

a) whether all ASs causing hypothyroidism are well included in the CAG (the outcome of the RA might be underestimated)

b) whether ASs not causing the effect are included in the CAG (the outcome of the RA might be overestimated)

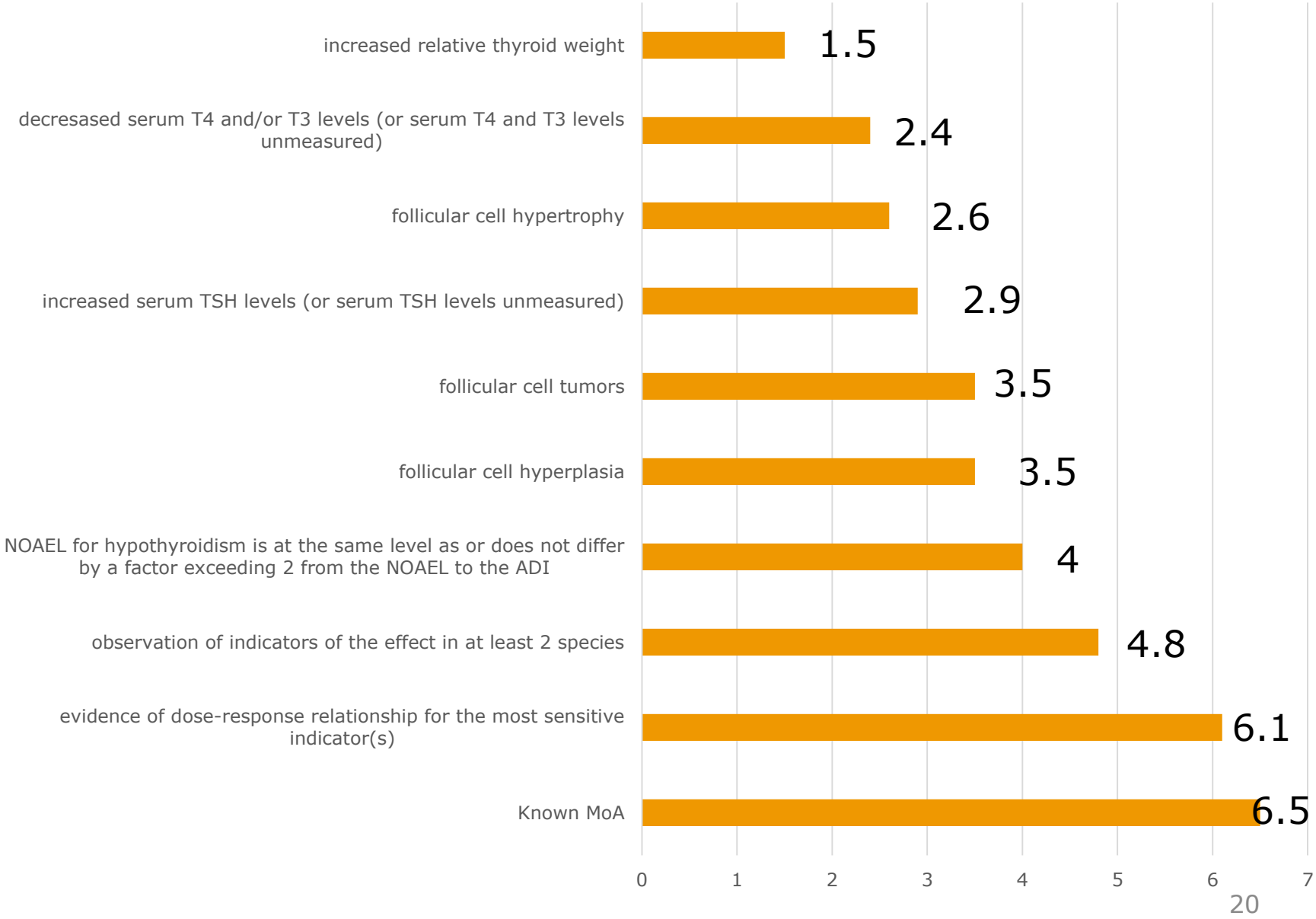
Question 2:

How sure is it that these ASs combine their individual toxicities according to the dose-addition model at their actual dietary exposure level?

QUESTION 1b - WEIGHT OF EVIDENCE AND EXPERT KNOWLEDGE ELICITATION

- Assess the probability that each substance actually causes the specific effect
- Development of a structured procedure which combines techniques for weight of evidence assessment and expert knowledge elicitation by:
  - Identifying lines of evidence that are important for assessing whether the AS causes the effect
  - Rating the weight of each line of evidence (score from 1 to 10)
  - Reviewing the evidence for each AS
  - Integrating the lines of evidence by multiplying all coefficients corresponding to the lines of evidence for each AS
  - Clustering the ASs in different groups of similar weight of evidence on the basis of their score
  - Assessing how many of the ASs in each subgroup actually cause the specific effect (by mean of expert knowledge elicitation-"Sheffield" protocol)
  - The elicited distributions for the subgroups were combined by 1D Monte Carlo simulation to calculate a probability distribution of ASs that actually cause the specific effect

LINES OF EVIDENCE FOR HYPOTHYROIDISM



INTEGRATION OF THE LINES OF EVIDENCE

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Active substance	Evidence assessment: 0=no/1=yes													Indicat
2		Decreased serum T4 and/or T3 levels (or serum T4 and T3 levels unmeasured)	Increased serum TSH levels (or serum TSH levels unmeasured)	Follicular cell hypertrophy	Follicular cell hyperplasia	Increased relative thyroid weight	Follicular cell tumours	Evidence of dose/response relationship for the most sensitive indicator(s)	Observation of indicators of the effect in at least 2 species	Evidenced MoA	NOAEL for hypothyroidism is at the same level (not differing by a factor exceeding 2) as the NOAEL for the ADI	Subgroup	Score	Ratios	T34=1
3		2.40	2.90	2.60	3.50	1.50	3.50	6.10	4.80	6.50	4.00			>1.25	125
4	Mancozeb	1	1	1	1	1	1	1	1	1	1	1	253136.26	2.60	
5	ETU (ethylenethiourea) (metabolite)	1	1	0	1	1	1	1	1	1	1	1	97360.10	1.35	
6	Ioxynil	1	1	1	0	1	1	1	1	1	1	1	72324.65	1.00	
7	Metiram	1	1	1	1	1	0	1	1	1	1	1	72324.65	1.14	
8	Amitrole	1	1	1	1	1	1	1	1	1	0	1	63284.06	1.63	
9	Clofentezine	1	1	1	1	1	1	1	1	0	1	1	38944.04	1.00	
10	Fluopyram	1	1	1	1	1	1	1	1	0	1	1	38944.04	1.00	
11	Proquinazid	1	1	1	1	1	1	1	1	0	1	1	38944.04	1.00	
12	Topramezone	1	1	1	1	1	1	1	1	0	1	1	38944.04	1.00	
13	Thiabendazole	1	1	1	1	1	1	1	1	0	1	1	38944.04	1.40	
14	Maneb	1	1	0	1	1	0	1	1	1	1	1	27817.17	1.07	
15	Isoxaflutole	1	1	1	1	0	1	1	1	0	1	1	25962.69	1.00	
16	Thiacloprid	1	1	1	1	0	1	1	1	0	1	1	25962.69	1.26	
17	2,4-D	1	1	1	0	1	0	1	1	1	1	1	20664.18	1.00	
18	Ziram	1	1	1	0	1	0	1	1	1	1	1	20664.18	1.37	
19	PTU (propylenethiourea) (metabolite)	1	1	1	0	1	1	1	0	1	1	2	15067.63	1.35	
20	Bixafen	1	1	1	1	1	0	1	1	0	1	2	11126.87	1.00	
21	Buprofezin	1	1	1	1	1	0	1	1	0	1	2	11126.87	1.00	
22	Chlorpropham	1	1	1	1	1	0	1	1	0	1	2	11126.87	1.00	
23	Desmedipham	1	1	1	1	1	0	1	1	0	1	2	11126.87	1.00	
24	Flufenacet	1	1	1	1	1	0	1	1	0	1	2	11126.87	1.00	
25	Thiophanate-methyl	1	1	1	0	1	1	1	1	0	1	2	11126.87	1.11	
26	Benthiavalicarb	1	1	0	1	0	1	1	1	0	1	2	9985.65	1.03	
27	Boscalid	1	1	1	1	1	1	1	1	0	0	2	9736.01	1.00	
28	Carbetamide	1	1	1	1	1	1	1	1	0	0	2	9736.01	1.00	
29	Pendimethalin	1	1	1	1	1	1	1	1	0	0	2	9736.01	1.20	
30	Fenpyrazamine	1	1	1	1	1	1	1	0	0	1	2	8113.34	1.00	
31	Fluxapyroxad	1	1	1	1	1	1	1	0	0	1	2	8113.34	1.00	
32	Pyrethrins	1	1	1	1	1	1	1	0	0	1	2	8113.34	1.17	
33	Propineb	1	1	0	0	1	1	1	1	1	0	2	6954.29	1.29	
34	Propyzamide	1	1	1	1	0	1	1	0	0	1	3	5408.89	1.00	
35	Quintozene	1	1	1	1	0	1	1	0	0	1	3	5408.89	1.26	

Appendix C | Sub-grouping of pesticides

EXPERT KNOWLEDGE ELICITATION

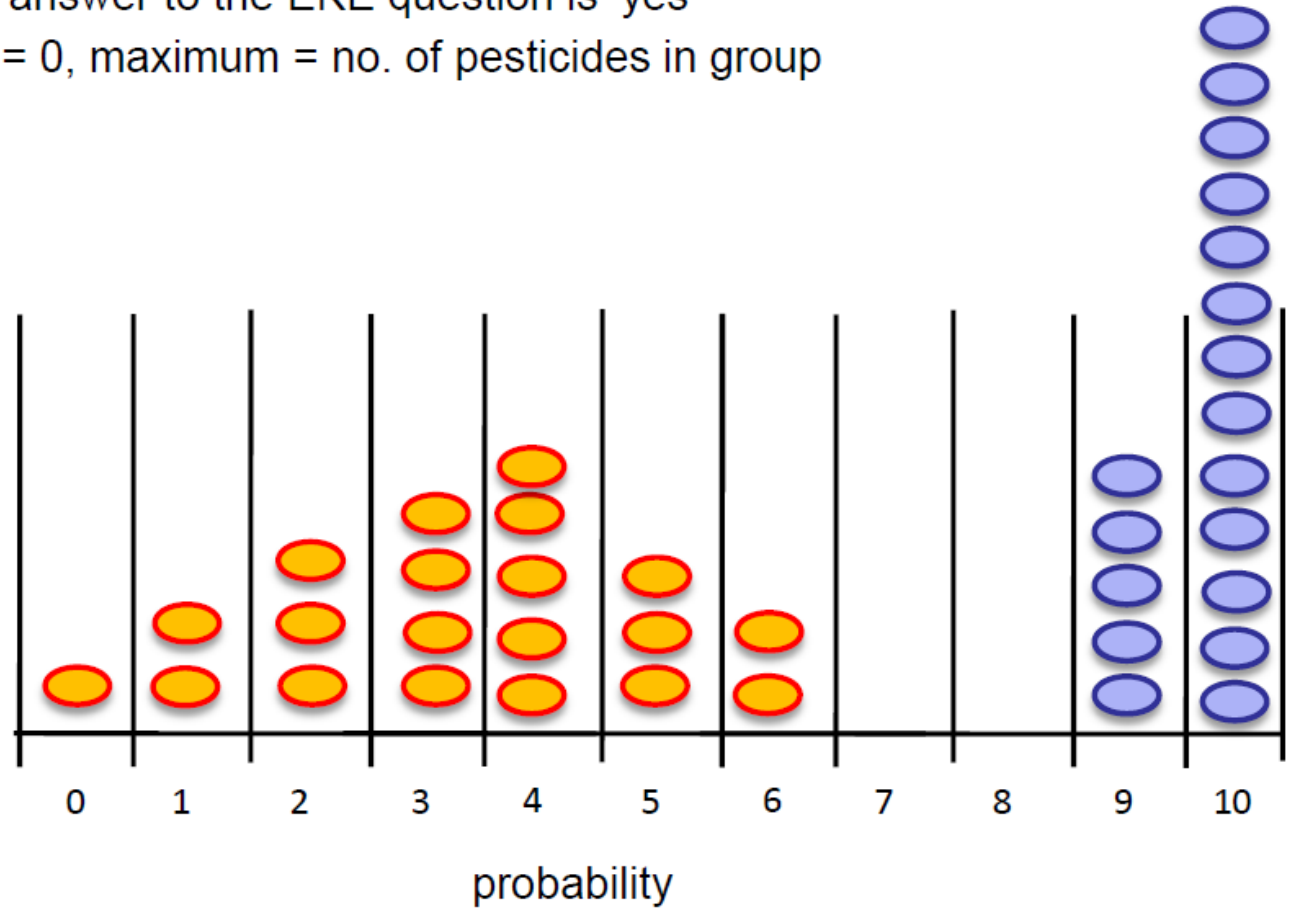
The assessment of the number of ASs in each subgroup actually causing hypothyroidism was conducted individually by three toxicologists addressing the following question:

How many pesticides in this group cause hypothyroidism, defined as a dose-related increase of any size in incidence and/or severity of hypertrophy and/or hyperplasia and/or neoplasm over any dose range in thyroid follicular cells of one or more laboratory mammal species?

This was followed by a facilitated discussion of the individual assessments, leading to agreement on a consensus distribution and reasoning for each subgroup

ROULETTE METHOD

- A grid comprising a number of columns
- These represent the possible values of X, the true number of pesticides in the group for which the answer to the EKE question is 'yes'
- Minimum = 0, maximum = no. of pesticides in group



1D MONTE CARLO SIMULATION

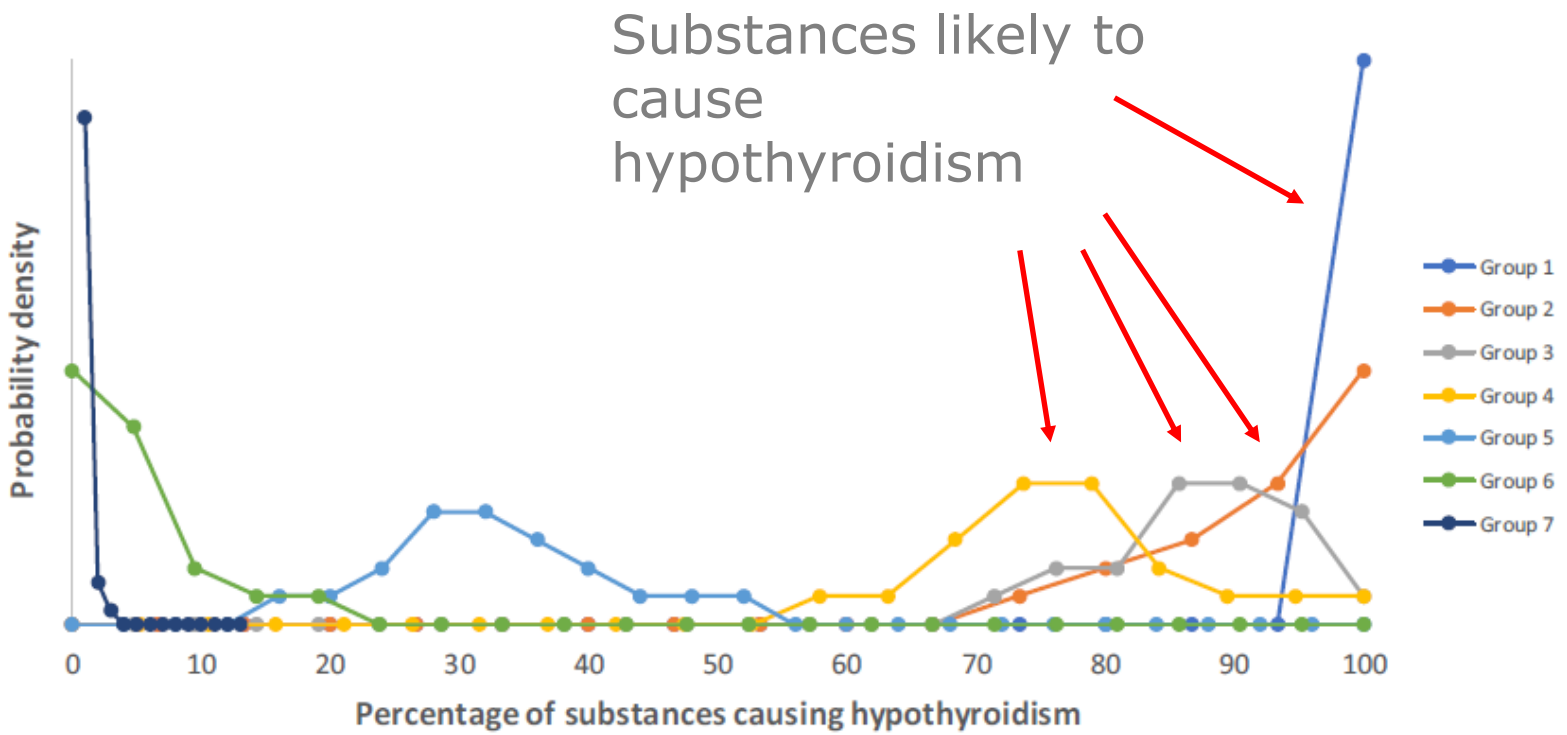
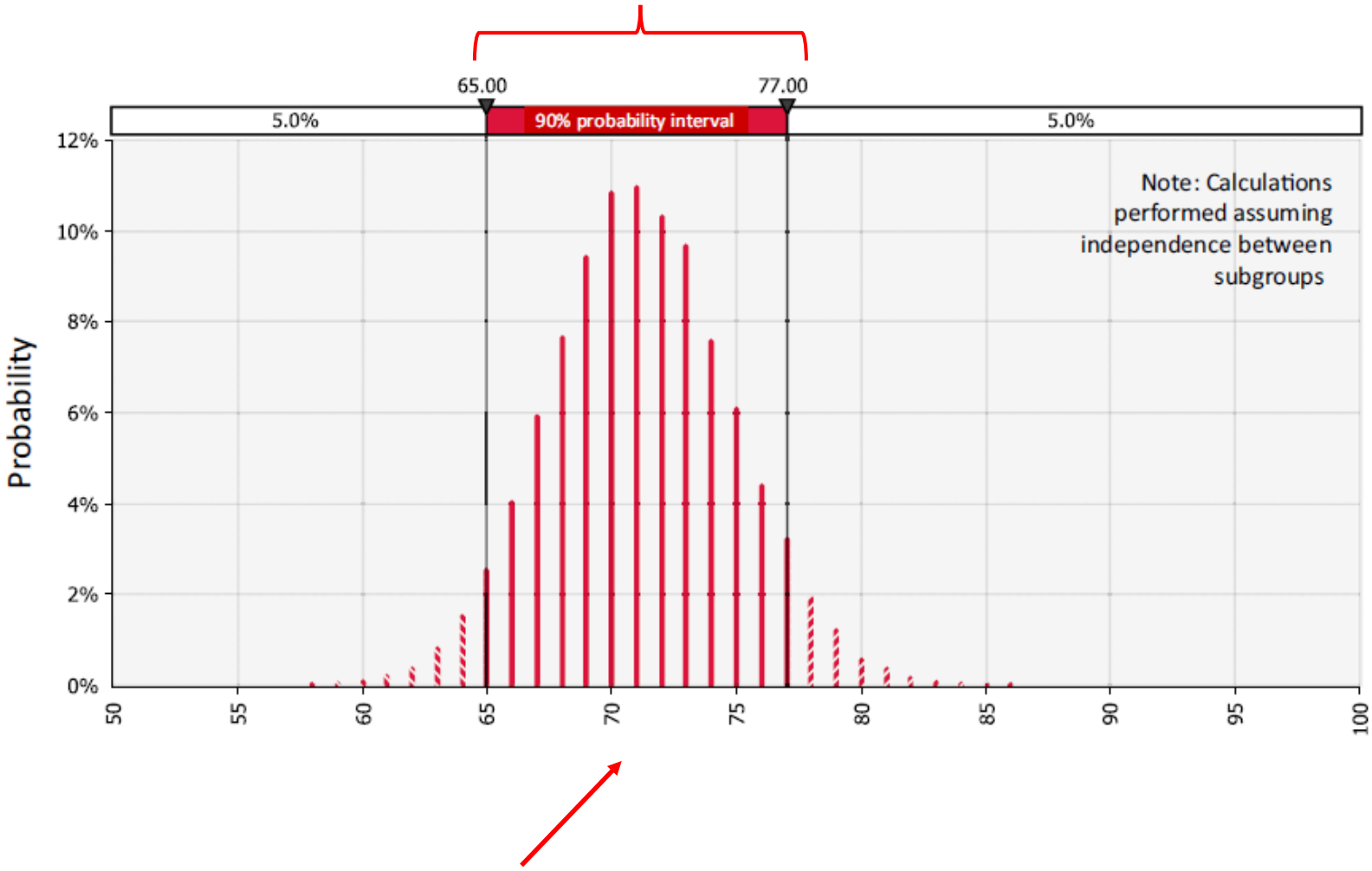


Figure 1: Distributions quantifying uncertainty about the percentage of substances in each subgroup that cause hypothyroidism. The vertical axis (probability density) quantifies the experts' judgement of the likelihood of different proportions of substances causing hypothyroidism within each subgroup

NUMBER OF SUBSTANCES CAUSING HYPOTHYROIDISM

Total number of substances really causing hypothyroidism



Median estimate: 71 substances

CONCLUSIONS

- CAGs for effects of pesticides on the thyroid were established by the PPR Panel in 2013
- Two specific effects relevant for CRA were confirmed: hypothyroidism and C-cell hypertrophy, hyperplasia and neoplasia
- CAGs were updated on the basis of additional information collected
- NOAELs were defined to characterise the ASs included in the CAGs for the respective specific effect; ICs were proposed to enable cumulative exposure and RA with methods using RPFs
- The assessment of cumulative risks of pesticides for the thyroid should be focused on hypothyroidism because the highest risks are expected to be observed for this effect
- Sources of uncertainties resulting from the methodological approach and from the limitations in available data and scientific knowledge have been identified and considered

RECOMMENDATIONS

- Uncertainties should be integrated into the CRA: by incorporating the probabilities of CAG membership into a probabilistic calculation or by sensitivity analyses
- Liver enzyme induction should be considered as a relevant effect for CRA when CAGs for the effects of pesticides on the liver will be established
- OECD TG 443 requires measurement of TH levels; however, many compounds have not been tested with these current guidelines yet. It is recommended, when missing, to require the measurement of such hormones: they would also provide the basis to establish CAGs and characterise ASs with respect to thyroid-mediated neurodevelopment
- The CAGs established should be regularly updated

NEXT STEPS

- The EFSA Scientific Committee is currently working on a Guidance which should provide specific criteria for grouping of ASs into CAGs
- EFSA will then proceed with the establishment of CAGs for effects on other organs/systems (the liver, adrenals, eye, repro and development)
- EFSA will propose a plan for systematic update of the already established CAGs and for the possible need to collect further data on other organs/organ systems (e.g. hematopoietic system, kidney, testes)

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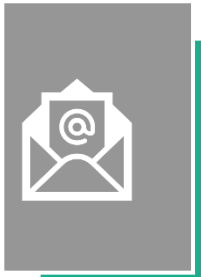

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BACK UP SLIDES

COMPARISON BETWEEN THE CAG ON HYPOTHYROIDISM AND THE CAG ON C-CELLS

- The CAG for hypothyroidism includes 7 times as many ASs as the CAG for C-cell hypertrophy, hyperplasia and neoplasia
- 12 out of the 17 ASs included in the CAG for C-cell hypertrophy, hyperplasia and neoplasia are also included in the CAG for hypothyroidism
- Only 2 ASs present in the CAG for C-cell hypertrophy, hyperplasia and neoplasia only have a NOAEL below of 2 mg/kg bw per day; in contrast, 25 ASs and metabolites present on the CAG for hypothyroidism have a NOAEL below 2 mg/kg bw per day

↓

The C-cells are usually less sensitive to pesticides than follicular cells or that effect on C-cells are more difficult to be detected

↓

It should be sufficient to perform a CRA with the CAG on hypothyroidism, assuming that similar protection goals would apply to hypothyroidism and C-cell hypertrophy, hyperplasia and neoplasia