SCIENTIFIC REPORT

Establishment of cumulative assessment groups of pesticides for their effects on the thyroid

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Abstract

Cumulative assessment groups of pesticides have been established for two specific effects on the thyroid: firstly hypothyroidism, and secondly parafollicular cell (C-cell) hypertrophy, hyperplasia and neoplasia. Sources of uncertainties resulting from the methodological approach and from the limitations in available data and scientific knowledge have been identified and considered. This report supports the publication of a scientific report on cumulative risk assessment to pesticides affecting the thyroid, in which all uncertainties identified for either the exposure assessment or the establishment of the cumulative assessment groups are incorporated into a consolidated risk characterisation.

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Summary

From all possible effects of pesticides on the thyroid, two were found to be meeting the criteria established by the EFSA Panel on Plant Protection Products and their Residues (PPR Panel) and specific for consideration in cumulative risk assessment (CRA) (EFSA, 2013). These specific effects were hypothyroidism and parafollicular cell (C-cell) hypertrophy, hyperplasia and neoplasia. There was insufficient information to address the combined effects of pesticides with respect to thyroid-mediated impaired neurodevelopment.

A cumulative assessment group (CAG) was established for each specific effect and more than 400 active substances (ASs) were screened for possible inclusion in these CAGs. Any AS exhibiting selected indicators (toxicological endpoints) reflecting the specific effect in regulatory toxicological studies was included in the respective CAG.

In total, 128 ASs were included in the CAG for hypothyroidism and 17 in the CAG for C-cell hypertrophy, hyperplasia and neoplasia. All ASs included in the CAGs were characterised by no observed adverse effect levels (NOAELs) for long-term cumulative exposure/risk assessment, derived from the most sensitive indicator, using all available information across studies, species and sexes. Index compounds (ICs) have been proposed to enable cumulative exposure and risk assessments with methods using relative potency factors (RPFs).

Based on the number and NOAELs of ASs in each CAG, performing CRAs with the CAG for hypothyroidism is expected to cover the combined effects of pesticides associated to C-cell hypertrophy, hyperplasia and neoplasia.

The number and the identity of the ASs included in the CAGs, as well as the allocated NOAELs, are subject to uncertainties. Sources of uncertainty resulting from the methods used to collect and assess toxicological data and from the limitations in the available data and scientific knowledge were therefore identified for appropriate consideration during the CRA conducted with these CAGs. The identified sources of uncertainty were related to the composition of the CAGs, the toxicological characterisation of the ASs, the slope and shape of the dose–response relationship, the contribution of metabolites and degradation products, the adequacy of the dose-addition model and the inter- and intra-species differences in toxicological sensitivity.

With respect to the composition of the CAGs, the uncertainty about the total number of ASs in the CAG for hypothyroidism that actually cause the effect was thoroughly addressed using weight of evidence and expert knowledge elicitation (EKE) techniques. In this process, ASs were allocated in subgroups of varying levels of evidence and a median estimate of 71 was derived for the number of ASs actually causing hypothyroidism. A similar exercise was not conducted with the CAG for C-cell hypertrophy, hyperplasia and neoplasia because the cumulative risk of C-cell hypertrophy, hyperplasia and neoplasia is very likely lower than the cumulative risk of hypothyroidism.

A mechanism for periodic update of the CAGs established in the present report will be put in place by EFSA in order to make use of all relevant new information. It is also recommended in the future, to characterise the ASs included in the CAGs using, as reference points, lower confidence limits of a benchmark dose (BMDL) suitable for regulatory purpose to remediate to the uncertainty resulting from the use of NOAELs.

This report should be read in conjunction with the EFSA scientific report on the cumulative dietary exposure assessment to pesticides that have chronic effects on the thyroid using SAS® software (EFSA, 2019a), the National Institute for Public Health and the Environment (RIVM) scientific report on cumulative dietary exposure assessment of pesticides that have chronic effects on the thyroid using MCRA software (Van Klaveren, 2019) and the EFSA scientific report on the cumulative dietary risk characterisation of pesticides that have chronic effects on the thyroid (EFSA, 2019b).

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1. Introduction

Regulation (EC) No. 396/2005 on maximum residue levels (MRLs) of pesticides in or on food and feed states that cumulative and synergistic effects of pesticides should be taken into account for dietary risk assessment when appropriate methodologies are available. Regulation (EC) No. 1107/2009 concerning the placing of plant protection products on the market also states that the residues of the plant protection products shall not have any harmful effects on human health, taking into account known cumulative and synergistic effects where the scientific methods accepted by EFSA to assess such effects are available.

In view of this legal context, the European Food Safety Authority (EFSA) and the Panel on Plant Protection Products and their Residues (PPR Panel) started in 2007 the development of the necessary methodologies to carry out cumulative risk assessment (CRA) of pesticide residues. This methodological development included a procedure to establish cumulative assessment groups (CAGs) of pesticides on the basis of their toxicological profile (EFSA PPR Panel, 2013a).

1.1. Background and Terms of Reference

In 2014, EFSA started a programme of activities aimed at implementing the CRA of pesticides, using the methodologies developed by the PPR Panel. As part of this program, the Pesticides Unit (nowadays Pesticides Residues and Pesticides Peer Review units) has been requested by EFSA to prepare a scientific report on CAGs of pesticides for their effects on the thyroid.

1.2. Purpose of this scientific report

The EFSA implementation plan for CRA also requested the Pesticides Unit to carry out retrospective CRAs for the effects of pesticides on the thyroid, using the results of official controls conducted by Member States under the annual monitoring programmes foreseen by Regulation (EC) No 396/2005.

The CAGs established in the present report are the result of the hazard assessment step of this process. They define and characterise the compounds of relevance for risk assessment of combined effects of pesticide residues on the thyroid. They have been used to perform CRAs, presented in a separate report (EFSA, 2019a), dealing with the following assessment questions:

- What is the chronic cumulative risk of hypothyroidism resulting from combined dietary exposure to pesticide residues?
- What is the chronic cumulative risk of parafollicular cell (C-cell) hypertrophy, hyperplasia and neoplasia resulting from combined dietary exposure to pesticide residues?

These CRAs will be conducted under the assumption of dose addition (EFSA, 2008). In 2015, the European Commission (EC) informed EFSA that the Standing Committee on Plants, Animals, Food and Feed (PAFF Committee) agreed on the use of the combined margin of exposure (MOET, also known as total margin of exposure) concept as the mode of expression of cumulative risks (see Section 2.2.3 for details on the calculation method). The CAGs established in the present report are compatible with this concept.

1.3. Precautionary principle and uncertainties

Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market does not prescribe precisely how CRA of pesticides should be performed. However it provides that 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'. These provisions are valid for the assessment of cumulative effects of pesticides and have been prevailing in the elaboration of principles to establish CAGs of pesticides by the PPR panel (EFSA PPR Panel, 2013a,b), in view of the large areas of uncertainty related to the combined toxicity of chemicals on human health. This might contribute to explain differences with approaches developed under other jurisdictions with respect to the grouping strategy.

In this context, the CRAs performed with the CAGs established in the present report and under the assumption of dose addition have been accompanied by an uncertainty analysis evaluating the potential of under- or overestimation of the actual risk for consumers. As a preliminary step to this uncertainty analysis, this report has considered how sure it is that the CAG contains all the active

substances (ASs) causing the respective specific effect and only the ASs causing this effect and how sure it is that these ASs combine their individual toxicities according to the dose-addition model at their actual level in food.

2. Data and methodologies

2.1. Data

Two data collections were carried out to retrieve information supporting the establishment of CAGs of ASs of plant protection products for their effects on the thyroid. Only chemical ASs were considered in these data collections.

The sources of these data collections were in first instance the official documents produced during the approval of ASs under Regulation (EC) No 1107/2009: Draft Assessment Reports (DARs), Renewal Assessment Reports (RARs), as well as the respective addenda, evaluation and discussion tables, EFSA conclusions and European Commission review reports. If necessary, original study reports were consulted for more details. When an EU evaluation was not available or outdated, assessment reports from recognised international bodies (e.g. Joint Meeting on Pesticide Residues (JMPR), United States Environmental Protection Agency (US-EPA) etc.) were scrutinised. All repeated dose (short-term and long-term) toxicological studies based on oral administration (diet, gavage, capsule) were considered. *In vitro* studies were also used for information on mode of actions (MoAs).

A first data collection was conducted by EFSA in two steps resulting in two data collection spreadsheets. The first step covered the ASs approved until 31 May 2009, while the second step covered those approved between 1 June 2009 and 31 December 2011.

A second data collection (RIVM, ICPS, ANSES, 2016) was outsourced to a consortium of the Netherlands National Institute for Public Health and the Environment (RIVM), the International Centre for Pesticides and Health Risk Prevention in Italy (ICPS) and the French Agency for Food, Environmental and Occupational Health & Safety (ANSES). It covered all ASs approved after 1 January 2012 and until 31 May 2013, a number of new ASs pending approval at that time, and an additional list of non-approved ASs present in the diet of EU consumers as identified in the 2011 Annual report on the Rapid Alert System for Food and Feed (European Commission, 2011) and in the 2010 Annual Report on Pesticide Residues in Food (EFSA, 2013). This data collection was organised in accordance with the specific effects identified for the thyroid by the PPR Panel (EFSA PPR Panel, 2013a) and their respective indicators. For each AS covered by this data collection, the main principles followed by the contractor were as follows:

- All studies rated as 'acceptable' or 'supportive' from all animal species reported in the regulatory documents with observations of specific effects were considered (mainly rat, mouse and dog).
- When more than one specific effect was observed for an AS in one study, each of them was collected under a separate entry.
- No observed adverse effect levels (NOAELs)/lowest observed adverse effect levels (LOAELs) for a same indicator of specific effect that were overlapping in two or more studies of the same duration in the same species were not combined and were reported in separate entries.
- The lowest NOAEL/LOAEL for a specific effect observed in the most sensitive sex in the study has been reported.
- 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', and the others have been reported in the column 'Remarks about the effect'.
- NOAELs/LOAELs for a specific effect have been collected regardless of the respective reference values (acceptable daily intake (ADI)/acute reference dose (ARfD)).
- Cases where age-related changes were not clearly separated from treatment-related effects were flagged.
- Information on the potential MoA was collected.
- Information on study statistics was collected.
- Any limitation which could have had an impact on the acceptability of the study and the evaluation/occurrence of the specific effect were flagged.

Further details of the data collection can be found in the external scientific report (RIVM, ICPS, ANSES, 2016) and the resulting data collection spreadsheet. The collected information slightly evolved

over time with the growing experience about the exact information needed to establish CAGs. It is acknowledged that the second data collection was performed with higher quality standards and that some relevant information might have been omitted in the first one.

The complete list of ASs (422 in total) covered by these data collections is given in Appendix A.

2.2. Methodologies

The establishment of CAGs followed a sequence of tasks comprising the identification of the specific effects on the system or organ considered (in this case the thyroid), the definition of the hazard characterisation principles of these specific effects, the establishment of CAGs, the selection of an index compound (IC) and an analysis of uncertainties about the adequacy of the CAG with respect to the specific effect.

2.2.1. Identification of the specific effects

From all the effects of pesticides observed on the system or organ considered (in this case the thyroid), this step consisted in identifying those which should be considered in CRA. Such effects, which can result from a combined action of pesticides, are generically designated as '**specific effects**' in this report. This identification was based on information analysis and expert judgement aimed at:

- Excluding local effects: Local effects, not being produced by the potentially absorbed dose, are excluded. Furthermore, they do not form the basis of reference values in regulatory dietary risk assessment.
- Excluding non-adverse effects: Non-adverse effects are not used as basis for setting a toxicological reference value and are therefore also not considered as relevant for CRA. In discriminating between an adverse and a non-adverse effect, consideration is given to its adaptive nature, its transient or persistent nature, its magnitude, its association with other alterations, whether it is a precursor to a more relevant effect, and its impact on the overall function of the organism (Lewis et al., 2001; EFSA PPR Panel, 2013a).
- Excluding effects not relevant to humans: Effects not considered as relevant for human are not relevant for the CRA.
- Evaluating the unambiguous nature of the effect: A specific effect needs to be unambiguous and well-defined in terms of site and nature.

These criteria were developed by the PPR Panel in 2013 (EFSA PPR Panel, 2013a) and resulted in CAGs of pesticides causing either a common phenomenological effect, or, in some cases where underlying MoAs are known, a common biochemical effect.

2.2.2. Characterisation of the specific effects

This step established the hazard characterisation principles applicable to the identified specific effects. In practice, this meant defining the **indicators** of specific effects (endpoints) observed in toxicological studies building evidence that an AS causes the specific effect and deciding how NOAELs are derived to characterise the AS for this specific effect. This was done based on the information available in application of the regulatory data requirements, following the respective study guidelines and in a way to ensure equal treatment of all ASs.

2.2.3. Establishment of CAGs and selection of ICs

For each specific effect identified in the first step of the process, a CAG was established.

The population of each CAG by the appropriate ASs was based on a critical analysis of the information collected as described in Section 2.1. For each specific effect, the criteria used to perform this critical analysis were described with sufficient details to enable an independent assessor to repeat it.

Once CAGs were populated, one of the ASs was selected as the IC. The approach used to select the IC was defined on an ad-hoc basis for each specific effect, as explained in Sections 3.3.1 and 3.3.2.

It needs, however, to be highlighted that any of the ASs of a CAG can be used as IC without any impact on the MOET, and that a MOET can also be calculated without any IC.

Indeed, two options are possible to calculate a MOET:

Directly, by calculating the reciprocal of the sum of the reciprocals of individual MOEs to each chemical contributing to the risk (EFSA, 2008):

$\frac{1}{\text{MOET}} = \frac{1}{\text{MOE}_1} + \frac{1}{\text{MOE}_2} + \frac{1}{\text{MOE}_3} \cdots + \frac{1}{\text{MOE}_n}$, where MOE_i is the margin of exposure for the ith chemical,

$\text{MOE}_i = \frac{\text{RfP}_i}{\text{E}_i}$ and RfP_i is the toxicological reference point (e.g. NOAEL, Lower confidence limit of a benchmark dose (BMDL)) for chemical i and E_i its exposure.

<u>Indirectly</u>, by determining the sum of potency-normalised individual exposures as total IC equivalents and translating the IC equivalents into the MOET to the reference point of the IC. This approach however requires additional work to select an IC and calculate a relative potency factor (RPF_i) for each chemical.

$\text{RPF}_i = \frac{\text{RfP}_{\text{IC}}}{\text{RfP}_i}$ where RfP_{IC} and RFP_i are the reference points for the IC and chemical i,

$\text{MOET} = \frac{\text{RfP}_{\text{IC}}}{\sum_i \text{E}_i \times \text{RPF}_i}$ where the denominator sums over all chemicals including the IC.

The present report is elaborated in such a way to make both options possible. In particular, it will include the selection of ICs for each established CAG. It should be noted that direct or indirect calculations lead exactly to the same results. This is demonstrated as follows:

$\frac{1}{\text{MOET}} = \frac{\sum_i \text{E}_i \times \frac{\text{RfP}_{\text{IC}}}{\text{RfP}_i}}{\text{RfP}_{\text{IC}}}$ inverting the previous equation and substituting for RfP_i

$\frac{1}{\text{MOET}} = \frac{\sum_i \text{E}_i \times \frac{\text{RfP}_{\cancel{\text{IC}}}}{\text{RfP}_i}}{\text{RfP}_{\cancel{\text{IC}}}}$ cancelling out RfP_{IC} in numerator and denominator

So: $\frac{1}{\text{MOET}} = \sum_i \frac{\text{E}_i}{\text{RfP}_i} = \frac{1}{\text{MOE}_1} + \frac{1}{\text{MOE}_2} + \frac{1}{\text{MOE}_3} \cdots + \frac{1}{\text{MOE}_n}$ as in the direct calculation above.

An important consequence of this is that the choice of the IC has no influence at all on the result of the assessment, nor on the uncertainties affecting the MOET. This is because any change in RfP_{IC}, e.g. through choosing a different IC or errors in the RfP of the IC, affects both the numerator and denominator of the equation and cancels out, as shown above.

In order to perform the CRAs mentioned in Section 1.2 of the present report, cumulative exposure assessments were performed, either using ICs (Van Klaveren, 2019) or not (EFSA, 2019a).

2.2.4. 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. To inform on whether the results tend to either over- or underestimate the actual risks, uncertainties relating to two questions have been considered.

Question 1

How sure is it that the CAG contains all the ASs causing the specific effect and only ASs causing this effect?

If the CAG does not contain all ASs contributing to the specific effect, the results of the assessment will tend to underestimate the risk. If, in contrast, it includes ASs not contributing to the effect, the results of the assessment will tend to overestimate the risk.

Question 2

How sure is it that these ASs combine their individual toxicities according to the dose-addition model at the actual dietary exposure level? Where possible, clusters of ASs for which dose addition is virtually certain should be defined.

The rationale of using dose addition to perform CRA of pesticide residues was given in the Scientific Opinions of the PPR Panel on the identification of pesticides to be included in CAGs on the basis of their toxicological profile (EFSA PPR Panel, 2013a) and on the relevance of dissimilar MoA and its appropriate application for CRA of pesticides residues in food (EFSA PPR Panel, 2013b).

Although dose addition is expected in principle when chemicals in a mixture act by the same MoA, and differ only in their potencies, its use is recommended by the PPR Panel (EFSA PPR Panel, 2013b) to assess the cumulative effects of chemicals eliciting the same adverse effect by different MoAs. Similarly, the EFSA Scientific Committee recommends adoption of the mixture assessment concept of

dose addition as a pragmatic and precautious default assumption, unless there are indications that the alternative concept of response addition is more appropriate (EFSA Scientific Committee, 2019).

For one of the CAGs established in the present report (hypothyroidism), Question 1 was addressed using a combination of weight of evidence and expert knowledge elicitation techniques, described in the following section. With respect to Question 2, this report reviewed the available information regarding MoAs leading to hypothyroidism, but a full assessment, relying on expert judgement, was only possible during the respective CRA after identification of the precise ASs driving the risk (EFSA, 2019a).

For the other CAG (C-cell hypertrophy, hyperplasia and neoplasia), a similar exercise was not done, because this effect is less prevalent than hypothyroidism in terms of cumulative risk (see Section 3.3.2).

2.2.5. Weight of evidence and expert knowledge elicitation techniques

The amount, reliability, relevance and consistency of evidence for causing effects on the thyroid vary between ASs. This makes it uncertain which substances should be included in a given CAG, with some substances being more likely to belong than others. This can be quantified by assessing the probability that each substance actually causes the specific effect. This could be done separately for each substance but, due to the large number of substances involved, it was more practical to form subgroups of substances for which the weight of evidence is similar, and then assess what proportion of chemicals in each subgroup cause the effect. This was done by developing a structured procedure which combines techniques for weight of evidence assessment (EFSA Scientific Committee, 2017) and expert knowledge elicitation (EFSA, 2014). This procedure comprises the following sequence of tasks:

1) Defining in precise terms the specific effect that is to be assessed.
2) Identifying lines of evidence that are important for assessing whether the AS causes the effect: lines of evidence typically include the indicators as defined in Section 2.2.2 but are not necessarily restricted to these indicators. Depending on the specific effect, additional factors contributing to the evidence may be defined.
3) Rating the weight of each line of evidence: the lines of evidence are assessed with respect to their reliability and relevance to the assessment question. This assessment is conducted by expert discussion and results in the allocation of a coefficient or weight to each line of evidence, varying from 1 to 10 and which is a relative measure of the contribution that positive findings for each line of evidence would make to increase the probability of a chemical causing the effect.
4) Reviewing the evidence for each AS included in the CAG in order to identify which lines of evidence are positive.
5) Integration of the lines of evidence by multiplying all coefficients corresponding to the lines of evidence for each AS. This gives a score to each AS which is proportionate to the number and strength of the positive lines of evidence and reflects the overall weight of evidence on whether the AS is causing the effect. The individual and aggregated scores for every substance were recorded and colour coded in a large table, to facilitate their use by the experts in the following steps.
6) Clustering the ASs in different groups of similar weight of evidence on the basis of their score. This was done by ordering the ASs in decreasing order of the calculated scores, identifying points in the ranked list where there are large changes in score, and using this to inform decisions about how to divide the list into subgroups. These decisions were made by expert discussion, balancing the need for a practical number of subgroups against the homogeneity of scores and lines of evidence within each subgroup.
7) Assessing how many of the ASs in each subgroup actually cause the specific effect. This was done by a structured expert knowledge elicitation (EKE) procedure, using a modified version of the 'Sheffield' EKE protocol described by EFSA (2014) to elicit a discrete probability distribution quantifying the experts' uncertainty about the number of substances in each subgroup that actually cause the effect. For each subgroup, experts first worked individually, reviewing the evidence and making their own judgements. This was not based simply upon the weight of evidence scores, but on evaluation of all relevant considerations (e.g. information on the MoA) using expert judgement. This was followed by a facilitated discussion of the individual distributions and reasoning, leading to agreement on a

consensus distribution and reasoning for each subgroup. Both the individual and consensus distributions were elicited using the 'roulette' method (EFSA 2014, pp. 169–170), as this is well suited to eliciting a discrete distribution and the experts found it easy to use when making their judgements. Finally, results for all the subgroups were displayed together for the experts to review and, where necessary, adjusted.

8) The elicited distributions for the subgroups were combined by 1D Monte Carlo simulation (EFSA Scientific Committee, 2018) to calculate a probability distribution for the total number of ASs that actually cause the specific effect. This was done twice, first assuming independence between subgroups and then assuming perfect positive dependence, to explore the potential impact of dependency on the results.

Steps 2–8 were carried out twice: once to generate the results that were presented in the draft report submitted to public consultation, and then a second time, to generate the results in this report, after verification of the database and consideration of comments received in the public consultation (EFSA, 2019c).

The results of this procedure comprised (a) a probability distribution for the number of ASs in each subgroup that cause the specific effect, each with accompanying rationale, and (b) two probability distributions for the total number of ASs causing the effect, one assuming independence between subgroups, and the other assuming positive dependence.

Additional sources of uncertainties will be considered in a subsequent report when assessing overall uncertainty in the CRA.

3. Assessment

3.1. Identification of the specific effects

On the basis of the results of a project commissioned by EFSA to the Danish Technical University (DTU) (Nielsen et al., 2012) and of the internal data collections performed by EFSA, the two specific effects of pesticides on the thyroid identified by the PPR Panel (EFSA PPR Panel, 2013a) were as follows:

- Hypothyroidism: defined here as an altered function of the thyroid gland resulting in follicular cell hypertrophy, hyperplasia and neoplasia.

  Rationale: The thyroid follicular cells produce the iodine-containing hormones called iodothyronines (thyroid hormones, TH), of which triiodothyronine (T3) and thyroxine (T4) are the most important. The decrease in circulating TH levels in the absence of elevated serum thyroid-stimulating hormone (TSH) is regarded as a physiological alteration or a secondary adaptive change. Conversely, when low TH levels lead to increased TSH levels as a compensatory response of the hypothalamic–pituitary–thyroid (HPT) axis, this condition is referred to as 'hypothyroidism'. If this stimulation is sustained over time, it usually ends up with morphological and/or histopathological changes in the thyroid (hypertrophy and/or hyperplasia). These changes may ultimately progress into follicular cell adenoma and carcinoma (Botts et al., 1991), a prolonged hyperplasia due to stimulation of follicular cells by TSH acting as a promoting factor for tumour formation. As alterations of T3/T4 and TSH levels, follicular cells hypertrophy and hyperplasia, increased relative thyroid weight and follicular cells tumours occur as an adverse continuum, they cannot be treated separately in CRA and will be addressed as one single effect (hypothyroidism).

  Pregnant women in particular may be susceptible to variations in TH levels, and it is well established that such fluctuations can have adverse effects on offspring. Increased and decreased levels of maternal free T4 have been associated with decreased IQ and decreased cortex volume (Korevaar et al., 2016).

  By its nature, hypothyroidism is adverse and can be triggered by repeated (subchronic or chronic) exposure and is therefore subject to a long-term CRA.

  It is noted that fluctuations in TH levels may be less pronounced in humans (Nielsen et al., 2012), and that humans are quantitatively less susceptible to chemically induced follicular cell hyperplasia and tumours than rats (Dellarco et al., 2006) because of a decreased sensitivity of the human thyroid pituitary axis (Crofton, 2008). However, this specific effect is adverse and relevant for humans.

The ECHA/EFSA Guidance for the identification of endocrine disruptors (ED) in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 reflects specifically in its Appendix A on the human relevance of effects on the thyroid (ECHA and EFSA, 2018). Here it is noted that the thyroid physiology is highly conserved across species and therefore that environmental factors affecting thyroid signalling or function in non-human species might be relevant for humans. It is recognised that there are notable quantitative species-specific differences between rats and humans in the regulation of the thyroid system. However, the guidance states the following:

'Using the current understanding of thyroid physiology and toxicology (Brunel University London and DTU National Food Institute, 2017), it is proposed that the following be applied when interpreting data from experimental animals:

1) Substances inducing histopathological changes (i.e. follicular cell hypertrophy and/or hyperplasia and/or neoplasia) in the thyroid, with or without changes in the circulating levels of THs, would pose a hazard for human thyroid hormone insufficiency in adults as well as pre- and post-natal neurological development of offspring.

2) Substances that alter the circulating levels of T3 and/or T4 without histopathological findings would still present a potential concern for neurodevelopment.

3) In the absence of substance-specific data which provide proof of the contrary, humans and rodents are considered to be equally sensitive to thyroid-disruption (including cases where liver enzyme induction is responsible for increased TH clearance)'.

On the other hand, serum levels of TH and TSH, thyroid weight, and thyroid histopathology are separate indicators of thyroid toxicity. Thus, where a chemically induced reduction in serum T4 is not followed by an increase in serum TSH, thyroid weight and histopathology remain unchanged. This accounts for TSH being the main driver of altered thyroid weight and histopathology endpoints (US EPA, 2018).

- C-cell hypertrophy, hyperplasia and neoplasia
  Rationale: In humans, it is important to distinguish physiological hyperplasia of C-cells from C-cell hyperplasia associated with neoplastic growth. Hypertrophy and hyperplasia of C-cells can occur in a physiological form associated with hypercalcaemia and other conditions. However, C-cell hyperplasia may also occur in association with medullary carcinoma and multiple endocrine neoplasia. C-cell hypertrophy, hyperplasia and neoplasia are observed following repeated exposure to certain pesticides, and this effect is considered relevant for humans. Sustained C-cell stimulation leading to hyperplasia is expected to play a promoting role in further progression to neoplasia. It is, therefore, considered that C-cell hypertrophy, hyperplasia and neoplasia are interrelated and form one single specific effect in the context of CRA. Although it is known that C-cells produce the hormone calcitonin which is involved in calcium homeostasis and regulation of bone formation, information on serum levels of calcitonin is generally not available in regulatory toxicological studies. Therefore, it is not possible to define a CAG of pesticides in explicit relation to alterations of calcium homeostasis, which is further regulated by the parathyroid hormone (PTH), secreted by the parathyroid glands and representing the functional counterpart to calcitonin. Indeed, both PTH and calcitonin play important roles in calcium homeostasis through their actions on osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells), respectively (Carter and Schipani, 2006).
  By its nature, this effect is also triggered by repeated (subchronic or chronic) exposure and is therefore subject to long-term CRA.

Additional information on these specific effects can be found in the scientific opinion of the PPR Panel on the identification of pesticides to be included in CAGs on the basis of their toxicological profile (EFSA PPR Panel, 2013a).

Interdependencies

No interdependency between the two specific effects is expected because they represent alterations of different biological functions relying on totally independent mechanisms. There is no evidence that a common molecular initiating event (MIE)/key event (KE) is shared by the toxicity pathways leading to these two adverse effects.

Effects not leading to CAGs

Other effects of pesticides on the thyroid, after due consideration by the PPR Panel (EFSA PPR Panel, 2013a) and further review in the context of this report, were not selected as specific effects relevant for CRA. In particular:

- Alterations of TH levels as such were not retained as a specific effect in the context of this report since they were not consistently measured in regulatory toxicology studies scrutinised for the data collection.
- While hyperthyroidism (namely, an inappropriately high synthesis and secretion of TH by the thyroid) has been associated with pesticide exposure in human epidemiological studies (Shrestha et al., 2018), this thyroid condition has not been retained as specific effect in the sense of the present report. A first reason is that the prevalence of hyperthyroidism is lower than that of hypothyroidism and the latter has been more often reported to be associated with pesticide exposure in humans (Shrestha et al., 2018). A second reason is that hyperthyroidism is less frequently reported in regulatory studies on experimental animals. Out of the 422 ASs covered by the data collections reported in Section 2.1, 21 ASs were reported to cause increased levels of serum TH levels. These were aclonifen, amitrole, clofentezine, etridiazole, flubendiamide, fluopyram, fluxapyroxad, ioxynil, metiram, metribuzin, oxadiargyl, pendimethalin, propineb, proquinazid, prothioconazole, pymetrozine, spirodiclofen, spiromesifen, thiacloprid, thiophanate-methyl and ziram. All these ASs but one (oxadiargyl) are in the CAG for hypothyroidism. Moreover, most of them have convincing evidence of causing hypothyroidism as 16 of these ASs are in subgroups 1 (8 ASs), 2 (4 ASs) or 3 (4 ASs) of the CAG on hypothyroidism (Section 4.2.1). The fact that increases in circulating TH are preferably observed with ASs causing hypothyroidism is problematic and cannot be easily explained. In an 'idealized view' of the thyroid system, decreases in circulating TH are compensated by increases in TSH in a feedback loop to restore serum TH to their original levels. However, this view is not consistently observed in experimental studies as sometimes decreases in T4 following chemical exposures are not always accompanied by decreases in T3 or increases in serum TSH. There are examples where continued suppression of T4 after exposure to certain chemicals resulted in unchanged, or even decreased, serum TSH. Thus, the data sets of serum TH measurements contained in many chemical dossiers are difficult to interpret. This picture is further complicated by evidence of autonomous regulation of TH action at the tissue level, without involvement of the HPT axis and corresponding changes in serum TH (Brunel University London and DTU National Food Institute, 2017).
- Inflammation of the thyroid gland/lymphocytic thyroiditis, resulting in follicular cell degeneration, was not regarded as a specific effect relevant for CRA. However, when AS-related inflammatory disorders led to an alteration of the thyroid function, observed through the indicators of hypothyroidism listed later, the ASs acting through this route were included in the CAG on hypothyroidism.
- Treatment-related pigmentation of follicular cells was not regarded as a specific effect on its own, since it is not considered to be adverse if observed in isolation. Pigment deposition adversely affecting the thyroid function is expected to be accompanied by follicular cell degeneration and/or changes in T3/T4 or TSH levels. In such case, ASs causing pigmentation of follicular cells would also be included in the CAG on hypothyroidism.
- Additional histopathological changes reported in DARs and listed by the DTU report (Nielsen et al., 2012), such as increased/decreased amount of colloids, small/large follicles, different shapes of follicular cells, increased vascularisation, increased vacuolisation, follicular cysts, follicular atrophy or necrosis of follicular cells would also become relevant in the case they result in an alteration of the thyroid function with observed signs of hypothyroidism. As such, in the absence of any consistent causality and histological pattern, these effects are however lacking specificity and not deemed appropriate to be considered as specific effects in view of CRAs.
- Thyroid amyloidosis observed in the mouse is considered as a systemic disorder and not specifically as organ toxicity. Congenital effects (thyroglossal duct cysts or ultimobranchial cysts resulting from persistence of embryonic structures (Frith et al., 2000; Nielsen et al., 2012) should be envisaged as developmental effects.

- Thyroid-mediated impaired neurodevelopment: owing to the lack of sufficient data in the pesticide dossiers, this effect has not been considered in this report and should be considered when effects of relevance for CRA in the area of developmental toxicity are defined.
- Effects regarded as age related (e.g. mineralisation within follicular lumina) were also considered not appropriate as specific effects for CRA.
- Effects associated with non-monotonic dose–response relationships were not considered because the current design of regulatory toxicity studies does not allow for their investigation and a generic approach would be desirable to explore these properties in a harmonised way.

3.2. Characterisation of the specific effects

All indicators of effects of pesticides on the thyroid were reviewed in view of characterising the two specific effects (i.e. hypothyroidism, and C-cell hypertrophy, hyperplasia and neoplasia).

3.2.1. Hypothyroidism

The specific indicators of toxicity observable in toxicological studies contributing to the evidence that an AS causes hypothyroidism are:

- Changes in serum thyroid hormone (T3/T4) levels: decreased circulating T3 level, decreased circulating T4 level
- Changes in circulating TSH: increased circulating TSH level
- Increased relative thyroid weight
- Follicular cell hypertrophy
- Follicular cell hyperplasia
- Follicular cell tumours: follicular cell adenoma, follicular cell carcinoma
- Evidence of a MoA in direct relation with hypothyroidism.

3.2.2. C-cell hypertrophy, hyperplasia and neoplasia

The specific indicators of toxicity observable in toxicological studies contributing to the evidence that an AS causes C-cell hypertrophy, hyperplasia and neoplasia are:

- C-cell hypertrophy
- C-cell hyperplasia
- C-cell tumours: C-cell adenoma, C-cell carcinoma
- Evidence of a MoA in direct relation with C-cell hypertrophy, hyperplasia and neoplasia.

3.3. Establishment of CAGs, setting of NOAELs and selection of ICs

3.3.1. General provisions

Establishment of CAGs:

Based on the two data collections (RIVM, ICPS, ANSES, 2016; internal EFSA data collection) referred to in Section 2.1 and indicators listed in Section 3.2, CAGs were elaborated for the two specific effects of pesticides on the thyroid.

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

- this observation was clearly non-specific, e.g. age related or occurring at or above the maximum tolerated dose (MTD), or,
- Consideration of the dose–response relationship showed that the observation was not treatment related.[1]

Although data from supportive studies were included in the data collection, studies assessed as 'supportive' or 'unacceptable' in the final DAR or RAR were not considered in any stage of the elaboration and characterisation of the CAGs. In addition, any limitation in acceptable studies identified

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[1] In other words, dose-response relationship was established when the effect was observed in at least 2 dose levels (not only at the top dose) and the comparison of the responses at the different dose levels did not exclude their relationship with the treatment

during the data collection (Section 2.1) and flagged as impacting the value of the study was also considered.

Additional specific conditions, applicable on an ad-hoc basis to specific effects may have been defined and, in such case, have been mentioned in the respective following sections.

When a metabolite or degradation product present in food had been investigated by regulatory studies and found to meet the above conditions, it was also included in the CAG.

Setting of NOAELs:

The data collection spreadsheets mentioned in Section 2.1 were used to characterise each AS included in a CAG for the respective specific effect. A NOAEL for long-term cumulative exposure/risk assessment was derived for each AS from the most sensitive indicator, using all available information across studies, species and sexes.

In case only a LOAEL was available for a certain indicator, a default NOAEL was determined from this LOAEL by applying an additional uncertainty factor (UF), as recommended by the guidance of EFSA on default values to be used in the absence of measured data (EFSA Scientific Committee, 2012). In the present report, the value of this additional UF was however not defined on a case-by-case basis, but, instead, it was set at 10 in all cases.

All indicators listed in Section 3.2 are equally valid for the setting of NOAELs, with the exception of changes in hormones levels, which were not considered. This is because hormone levels are not systematically available and using them would have introduced a bias resulting in unequal treatment between ASs. This needs to be considered in the overall uncertainty analysis.

Only NOAELs established in studies assessed as 'acceptable' in the DAR or RAR or equivalent regulatory documents were considered in this exercise.

In case two or more studies of similar design within the same species investigated the same indicators of a specific effect, they were combined to derive the respective NOAEL on the basis of the whole information.

EFSA conclusions on the peer review of the pesticide risk assessment in the context of Regulation (EC) No 1107/2009 finalised until end 2018 and dealing with ASs included in the CAGs were considered to retrieve any element of expert judgement regarding their effects on the thyroid. For ASs not reviewed by EFSA, the scientific evaluations conducted by the body constituting the main source of the data collection were also considered (e.g. 1993 JMPR evaluations of dithiocarbamates).

Human studies were never used for the establishment of CAGs, as the provisions of Commission Regulation (EU) No 283/2013 authorising their use (scientific validity, ethical generation and leading to lower regulatory limit values compared to animal studies) were never met.

Selection of ICs:

To enable risk assessors to perform cumulative exposure/risk assessments using an IC and RPFs, an IC was proposed from the ASs included in the CAG. The IC was preferably 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 (study meeting the requirements of regulation (EC) No 1107/2009, 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

In CRAs using ICs, RPFs need to be calculated to normalise the toxicity of all ASs in each CAG to the IC, by dividing the NOAEL of the IC by the NOAEL of the AS.

3.3.2. Cumulative assessment groups

This section presents the CAGs proposed to be used for future CRAs. They differ to some extent from those elaborated by the PPR Panel and published in 2013 (EFSA PPR Panel, 2013a), because only the first step of the EFSA internal data collection used in this report was available to the Panel when it adopted its opinion.

Hypothyroidism:

The general provisions applicable to the establishment of CAGs as described in Section 3.3.1 were applied. However, ASs for which the increased relative thyroid weight was the only indicator of hypothyroidism were not included when this observation was concurrent with reduced body weight gain.

The CAG related to hypothyroidism included 128 ASs, metabolites or degradation products as follows: 2,4-D, 8-hydroxyquinoline, aclonifen, amisulbrom, amitrole, anthraquinone, azadirachtin, beflubutamid, benalaxyl, benalaxyl-M, benfluralin, benthiavalicarb, bitertanol, bixafen, boscalid, bromide ion, bromopropylate, bromoxynil, bromuconazole, bupirimate, buprofezine, carbaryl, carbetamide, carbosulfan, chlordane, chlorpropham, clethodim, clodinafop, clofentezine, cyantraniliprole, cycloxydim, cyflufenamid, cyproconazole, cyprodinil, dazomet, desmedipham, diclofop, dicloran, diethofencarb, dinocap, dithianon, ethylenethiourea (ETU), etofenprox, etridiazole, fenamidone, fenarimol, fenbuconazole, fenoxycarb, fenpyrazamine, fipronil, flazasulfuron, flubendiamide, flufenacet, flumioxazin, fluopicolide, fluopyram, fluoxastrobin, fluquinconazole, flutolanil, fluxapyroxad, folpet, fuberidazole, haloxyfop-P (haloxyfop-R), heptachlor, hymexazol, imazosulfuron, ioxynil, isoxaflutol, lufenuron, maleic hydrazide, mancozeb, maneb, MCPA (metabolite of MCPB), meptyldinocap, metam (incl. -potassium and -sodium), metiram, metribuzin, myclobutanil, orthosulfamuron, oryzalin, oxadiazon, oxyfluorfen, pendimethalin, penflufen, penthiopyrad, pethoxamid, phoxim, picolinafen, propaquizafop, propineb, propyzamide, proquinazid, propylenthiourea (PTU), prothioconazole, pymetrozine, pyrethrins, pyridalyl, pyridate, pyrimethanil, pyriofenone, quinmerac, quinoclamine, quintozene, quizalofop-P-tefuryl, sedaxane, silthiofam, spinosad, spirodiclofen, spiromesifen, sulfoxaflor, tembotrione, tepraloxydim, terbuthylazine, tetraconazole, thiabendazole, thiacloprid, thiamethoxam, thiencarbazone, thiophanate-methyl, tolylfluanid, topramezone, triadimefon, trifluralin, tritosulfuron, valifenalate, vinclozolin, ziram, zoxamide.

In this CAG, the IC is proposed as ioxynil, considering that follicular cell hypertrophy/hyperplasia was a recurrent finding in different animal species (rat, dog and mouse) and reported in conjunction with thyroid hormone changes and in some instances also with tumours.

C-cell hypertrophy, hyperplasia and neoplasia:

The general provisions applicable to the establishment of CAGs as described in Section 3.3.1 were applied.

The CAG related to C-cell hypertrophy, hyperplasia and neoplasia included 17 ASs as follows: 2,4-DB, 2,4-D, amitrole, buprofezin, desmedipham, fenamidone, fenbuconazole, flazasulfuron, folpet, hexythiazox, imidacloprid, ioxynil, ipconazole, oryzalin, oxyfluorfen, thiram, ziram.

In this CAG, the following substances were envisaged as IC: fenbuconazole, fenamidone, desmedipham, ziram, amitrole, ioxynil and ipconazole. The selected IC was fenbuconazole since it was the only substance for which the same effect (C-cell adenoma) was consistently observed in two different studies.

A comparison of this CAG with the CAG for hypothyroidism indicated that:

- 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. The NOAEL for hypothyroidism is lower than the NOAEL for C-cell hypertrophy, hyperplasia and neoplasia in 5 cases (2,4-D, buprofezin, folpet, ioxynil and ziram), and higher in 3 cases (fenamidone, flazasulfuron, oryzalin). In 4 cases (amitrole, desmedipham, fenbuconazole, oxyfluorfen), the NOAELs are the same for the two effects.
- Only 2 ASs present in the CAG for C-cell hypertrophy, hyperplasia and neoplasia only have a NOAEL below 2 mg/kg body weight (bw) per day (ipconazole and thiram). In contrast, 25 ASs and metabolites (chlordane, clofentezine, dazomet, ETU, fipronil, flufenacet, fluopyram, fluoxastrobine, fluquinconazole, haloxyfop-P, heptachlor, isoxaflutole, lufenuron, MCPA, metribuzine, oxyfluorfen, propineb, proquinazid, PTU, quintozene, quizalofop-P, tembotrione, tetraconazole, thiacloprid, topramezone) present in the CAG for hypothyroidism have a NOAEL below 2 mg/kg bw per day.

This comparison strongly suggested that C-cells are usually less sensitive to pesticides than follicular cells, or that effects on C-cells are more difficult to detect. It was therefore anticipated that cumulative risks for C-cell hypertrophy, hyperplasia and neoplasia resulting from the exposure to pesticide residues

would be assessed at lower levels than the cumulative risks for hypothyroidism. This was confirmed by the results of the CRAs for the 2 CAGs (EFSA, 2019b).

Two tables (see Appendix B) were prepared to support long-term cumulative exposure/risk assessments for hypothyroidism and for C-cell hypertrophy, hyperplasia and neoplasia. For each AS included in the CAG, these tables indicate which indicator of the specific effect is used for hazard characterisation, the respective reference point (NOAEL/LOAEL) and the reference of the study from which this reference point was retrieved. They also mention the source of information (e.g. DAR 2011, JMPR 1993), the EFSA conclusions considered (e.g. EFSA (2008)) and the available information on MoAs. In accordance with Article 63 of Regulation (EC) No 1107/2009, the names of persons involved in these studies are confidential and hidden in the study reference details.

3.3.3. Use of the CAGs to assess consumer safety

As indicated in Section 3.3.2, in order to assess the combined effects of pesticide residues on the thyroid, 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.

In conducting these CRAs, the potential contribution of metabolites and degradation products to the specific effects should be taken into account. It should be considered whether the residue definition for risk assessment established with respect to the critical effect(s) (e.g. effect(s) on which the ADI and/or ARfD are based) can be used. If this is not appropriate, another residue definition should be considered on a case-by-case basis and in a manner consistent with the respective specific effect. In doing so, it is recommended to use the guidance of the PPR Panel on the establishment of the residue definition for dietary risk assessment (EFSA PPR panel, 2016).

As a rule, when a metabolite associated to the parent AS is included in a residue definition for risk assessment, it is considered to have the same toxicity as the parent compound.

In some cases, however, toxicological studies have been conducted on metabolites or degradation products, allowing their own toxicological characterisation for their effects on the thyroid. This is in particular the case of bromide ion (degradation product of methyl bromide), ETU (common metabolite of ethylene-bis-dithiocarbamates (EBDCs) fungicides such as maneb, mancozeb, and metiram and zineb) and PTU (metabolite of propineb). These metabolites were specifically reviewed by JMPR in 1988 and 1993 (FAO, 1993) and specific NOAELs for hypothyroidism could be derived from these reviews. For this reason, bromide ion, ETU and PTU have been included in the table related to hypothyroidism in Appendix B.

4. Uncertainty analysis

4.1. General considerations

The actual and first-hand information supporting the establishment of CAGs lies in the original studies submitted by the applicants for approval of ASs. For reasons of resources, these studies have only occasionally been consulted for the purpose of the present exercise. Instead, regulatory documents, where information from the original studies is reported in a condensed form have been used as the primary source of information. Information of relevance for the establishment of CAGs might not have been captured when these regulatory documents were drafted, as their main purpose is to establish the reference values of the ASs (ADI and ARfD). This constitutes a general source of uncertainty which may result in some underestimation of the actual risk, because the most common issue with these regulatory documents is likely to be the omission to report effects at doses exceeding the overall NOAEL of the respective study.

Also, the transfer of the information reported in the regulatory document to the three data collection spreadsheets used by EFSA to prepare this scientific report, may have suffered from occasional inaccuracies, which can result in either underestimations or overestimations of the actual risks.

In addition, for a number of ASs, especially for ASs which are no longer approved in the EU, the quality of the toxicological dossier does not conform to the current standards and causes an additional source of uncertainty. This also leads to some possible over- or underestimation of the contribution of the respective ASs to the actual cumulative risk.

Specific sources of uncertainties related to the CAG for hypothyroidism are addressed in detail in the Sections 4.2 and 4.3 deals briefly with sources of uncertainties related to the CAG for C-cell

hypertrophy, hyperplasia and neoplasia. In Section 4.4, recommendations are given about the overall sources of uncertainties to be systematically reviewed when CRAs are conducted with the CAGs established in the present report.

4.2. CAG for hypothyroidism

4.2.1. Question 1: Does the CAG for hypothyroidism contain all ASs contributing to this effect and only ASs contributing to this effect?

Question 1 implies providing responses to two discrete questions.

The first one is *whether all ASs causing the effect are well included* in the CAG. To evaluate the chance of omitting ASs contributing to the effect, the reader should refer to the first bullet point of Section 4.4 (Overall uncertainty analysis).

The second one is *whether ASs not causing the effect are included* in the CAG. The possibility of including ASs not contributing to hypothyroidism has been addressed by weight of evidence assessments and EKE techniques, following the method described in Section 2.2.5. The process was conducted as follows:

a) A key step in EKE is specification of the question to be addressed in a well-defined manner and, if possible, such that the answer to the question is potentially observable, at least in principle (EFSA, 2014). The question of interest for the establishment of the CAG for hypothyroidism is, for each AS: *Does this chemical 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?*

b) With respect to the question of interest, 10 lines of evidence were identified. Their respective weights were rated from 1 to 10 independently by four experts and the four estimated coefficients were later averaged. The lines of evidence of hypothyroidism and their respective weighted coefficients are:

- Known MoA: 6.5.
- Evidence of dose–response relationship[2] for the most sensitive indicator(s): 6.1
- Observation of indicators of the effect in at least 2 species: 4.8
- NOAEL for hypothyroidism is at the same level as or does not differ by a factor exceeding 2 from the NOAEL leading to the ADI[3]: 4
- Follicular cell hyperplasia: 3.5
- Follicular cell tumours: 3.5
- Increased serum TSH levels (or serum TSH levels unmeasured): 2.9
- Follicular cell hypertrophy: 2.6
- Decreased serum T4 and/or T3 levels (or serum T4 and T3 levels unmeasured): 2.4
- Increased relative thyroid weight: 1.5.

The most convincing line of evidence of hypothyroidism is the knowledge/demonstration of a MoA of direct relevance (average score: 6.5) as this shows an inherent capability of the AS to produce the effect. The second most relevant line of evidence was the dose–response relationship (average score: 6.1) because this is what establishes in the most convincing way that an effect is treatment related. Observation of indicators of the effect in at least two species, as this results from fully independent experimentations, was also considered as a strong line of evidence (average score: 4.8). The fact that the NOAEL for hypothyroidism is established at a similar level as the NOAEL leading to the ADI (average score: 4) brings significant contribution to the evidence that an AS causes hypothyroidism as a primary effect. The experts also allocated medium weights to follicular cell hyperplasia and neoplasia (average scores: 3.5 for both) because these histopathological indicators are highly specific of hypothyroidism. Increased TSH levels (average score: 2.9) were considered as more reliable

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[2] The effect is observed in at least two dose levels (not only at the highest tested dose) and the comparison of the responses at the different dose levels does not invalidate their relationship with the treatment.
[3] The ADI referred to is the ADI established by EFSA in conclusions on the peer review of the pesticide risk assessment of active substances until 31 December 2018, or when the AS has not been reviewed by EFSA, the ADI indicated in the EU pesticides database on 31 December 2018 (http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN).

indicator of a functional alteration of the thyroid than decreased serum TH levels (average score: 2.4). Weak lines of evidence, because less specific, were follicular cell hypertrophy and increased relative thyroid weight (average scores: 2.6 and 1.4, respectively).

c) Based on the lines of evidence, scores were calculated for all ASs of the CAG. Considering the high number of ASs, and the difficulty to address the question of interest for each AS individually, these scores were used to distribute the ASs into seven subgroups of decreasing scores. The compositions of these seven subgroups are as follows:

- Subgroup 1 (15 ASs): 2,4-D, amitrole, clofentezine, ETU, fluopyram, ioxynil, isoxaflutole, mancozeb, maneb, metiram, proquinazid, thiabendazole, thiacloprid, topramezone, ziram
- Subgroup 2 (15 ASs): benthiavalicarb, bixafen, boscalid, buprofezin, carbetamide, chlorpropham, desmedipham, fenpyrazamine, flufenacet, fluxapyroxad, pendimethalin, propineb, PTU, pyrethrins, thiophanate-methyl
- Subgroup 3 (21 ASs): aclonifen, benfluralin, bupirimate, cyflufenamid, etridiazole, fenamidone, fenbuconazole, fenoxycarb, fipronil, flubendiamide, hymexazol, imazosulfuron, MCPA, metribuzin, oryzalin, penflufen, penthiopyrad, propyzamide, quintozene, sedaxane, tolylfluanid
- Subgroup 4 (19 ASs): azadirachtin, benalaxyl-M, bromoxynil, cyantraniliprole, cyprodinil, etofenprox, fuberidazole, heptachlor, lufenuron, orthosulfamuron, pethoxamid, picolinafen, quizalofop-P-tefuryl, silthiofam, spiromesifen, tembotrione, tetraconazole, trifluralin, valifenalate
- Subgroup 5 (25 ASs): 8-hydroxyquinoline, amisulbrom, anthraquinone, benalaxyl, bromide ion, bromopropylate, chlordane, cycloxydim, cyproconazole, diclofop, dicloran, diethofencarb, fluoxastrobin, fluquinconazole, haloxyfop-P (haloxyfop-R), propaquizafop, prothioconazole, pymetrozine, pyridalyl, pyrimethanil, spinosad, tepraloxydim, thiencarbazone, triadimefon, tritosulfuron
- Subgroup 6 (21 ASs): beflubutamid, carbaryl, carbosulfan, clodinafop, dazomet, dinocap, flumioxazin, fluopicolide, flutolanil, folpet, maleic hydrazide, meptyldinocap, metam, myclobutanil, oxadiazon, oxyfluorfen, quinoclamine, sulfoxaflor, terbuthylazine, thiamethoxam, zoxamide
- Subgroup 7 (12 ASs): bitertanol, bromuconazole, clethodim, dithianon, fenarimol, flazasulfuron, phoxim, pyridate, pyriofenone, quinmerac, spirodiclofen, vinclozolin.

Appendix C provides details on the evidence collected for each AS, its score and the subgroup it belongs to.

d) The question of interest under a) was reworded to make it compatible with the grouping of ASs in subgroups of similar evidence. 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.

e) The EKE exercise for subgroups 1–7 concluded that:

In subgroup 1 (15 ASs):

- Eight ASs have a known MoA for hypothyroidism.
- All seven ASs without a known MoA for hypothyroidism have dose–response relationship and show indicators in two or more species. All show a progressive nature in the effect (all three histopathological indicators (hypertrophy, hyperplasia and tumours) observed).
- All the substances with weaker evidence for histopathological indicators have a known MoA for hypothyroidism.
- It was concluded that it is almost certain that all these ASs cause hypothyroidism.

In subgroup 2 (15 ASs):

- Two ASs have a known MoA for hypothyroidism (PTU and propineb).
- All 15 ASs have evidence of dose–response relationship.
- All ASs but one (propineb) show at least two of the histopathological indicators.
- In 11 cases, indicators are observed in two or more species.

- In 11 cases, the NOAEL for hypothyroidism is close to the NOAEL for the critical endpoint for the ADI.
- It is therefore likely that 14 or 15 ASs cause hypothyroidism, with 5% chance that only 11 cause hypothyroidism.

In subgroup 3 (21 ASs):

- All but five (aclonifen, etridiazole, MCPA, sedaxane, flubendiamide) of these ASs have high probability of causing hypothyroidism based on evidence of more than one histopathological indicators plus other lines of evidence.
- Tolylfluanid is the only substance in this group with known MoA and the only one that lacks a dose–response relationship.
- Aclonifen, sedaxane, flubendiamide have only the milder histopathological effect (hypertrophy) but do show a dose–response relationship, and effects observed in a second species, and a NOAEL for hypothyroidism close to the NOAEL for the ADI.
- It is therefore likely that 18 to 20 ASs cause hypothyroidism, with a possible range from 15 to 21.

In subgroup 4 (19 ASs):

- All these ASs show at least one histopathological indicator.
- Dose–response relationship is seen for all ASs except one (pethoxamid) where the effect is progressive and seen in two or more species.
- Of the 19 chemicals, 12 have either indicators in at least two species (3 ASs), a known MoA (heptachlor, bromoxynil) or NOAEL for hypothyroidism close to the NOAEL for the ADI (7 ASs); only one of these 12 lacks evidence to show a dose–response relationship.
- The probability of causing hypothyroidism is close to certain for the two ASs with known MoA. All others have a moderate to high probability of causing the effect. The probability is the lowest for seven ASs showing hypertrophy but not hyperplasia or tumours (cyantraniliprole, cyprodinil, valifenalate, spiromesifen, azadirachtin, lufenuron, quizalofop-P-tefuryl).
- Overall, it is judged likely that about 13–16 ASs cause the effect, with a possible range from 11 to 19.

In subgroup 5 (25 ASs):

- Bromide is considered likely to cause the effect, based on a known MoA. None of the other substances in this group have a known MOA for hypothyroidism.
- Seven ASs (thiencarbazone, tritosulfuron, 8-hydroxyquinoline, cycloxydim, fluoxastrobin, spinosad, prothioconazole) have effects on thyroid weight with a dose–response relationship, but no histopathological indicators, and are considered unlikely to cause hypothyroidism.
- Seventeen chemicals (bromopropylate, pyrimethanil, triadimefon, pymetrozine, anthraquinone, haloxyfop-P, pyridalyl, tepraloxydim, benalaxyl, chlordane, diclofop, amisulbrom, cyproconazole, dicloran, propaquizafop, fluquinconazole, diethofencarb) are more likely to cause hypothyroidism, based on evidence of histopathological indicators and of either indicators in at least two species or dose–response relationship or NOAEL for hypothyroidism close to the NOAEL for the ADI or progressive nature of the effect.
- Overall, it is judged likely that about 6–10 ASs cause the effect, with a possible range from 4 to 13.

In subgroup 6 (21 ASs):

- Dose–response relationship is observed only for the ASs with increased relative thyroid weight but without histopathological indicators.
- In all but two (carbaryl and terbuthylazine) of the ASs with histopathological indicators, only one indicator is seen (but without a dose–response relationship).
- In only one case (quinoclamine) are there indicators in more than one species, and in this case histopathological indicators are missing.
- In one case (dazomet), the NOAEL for hypothyroidism is in the same range as the critical NOAEL and in this case again histopathological indicators are missing.

- It is likely that none or one AS only of this subgroup causes hypothyroidism, with a small probability that up to four ASs cause the effect.

In subgroup 7 (12 ASs):

- One AS (spirodiclofen) has evidence of hyperplasia and increased TSH but no decrease in T3/T4 and no other supporting evidence so there is a small chance that this AS causes hypothyroidism.
- All other substances in this group have at most weak evidence of hypothyroidism: increased relative thyroid weight without any additional evidence. They are therefore very unlikely to cause hypothyroidism.
- Therefore, it is very likely that none of the ASs of this subgroup causes hypothyroidism.

The probability terms used in the above considerations are recommended in the EFSA guidance on communication of uncertainty (EFSA, 2019d).

f) Assessing the total number of ASs causing hypothyroidism:

- The elicited distributions for the seven subgroups were combined by 1D Monte Carlo simulation, assuming independence between subgroups. This produced a combined distribution for the total number of ASs in the CAG that actually cause hypothyroidism (Figure 1). Thus, it was elicited that the compounds belonging to subgroups 1–4 are likely to cause hypothyroidism while the compounds belonging to subgroups 5–7 were much less likely to cause hypothyroidism. The median estimate was 71 ASs (55% of the ASs in the CAG), with a 90% confidence interval of 65–77 ASs (51–60%, see Figure 2).
- A second Monte Carlo simulation was conducted assuming perfect positive dependence between subgroups. This produced an alternative distribution for the total number of ASs in the CAG that actually cause hypothyroidism. The median estimate was 70 ASs (55%), but with a 90% confidence interval of 56–84 ASs (44–66%, see Figure 3).
- The experts considered that there will be some, but less than perfect, positive dependence between their judgements for the different subgroups. The distributions in Figures 2 and 3 therefore provide a lower and upper bound for the impact of this dependence on the width of the combined distribution. This will be considered as part of overall uncertainty analysis when CRA is performed.
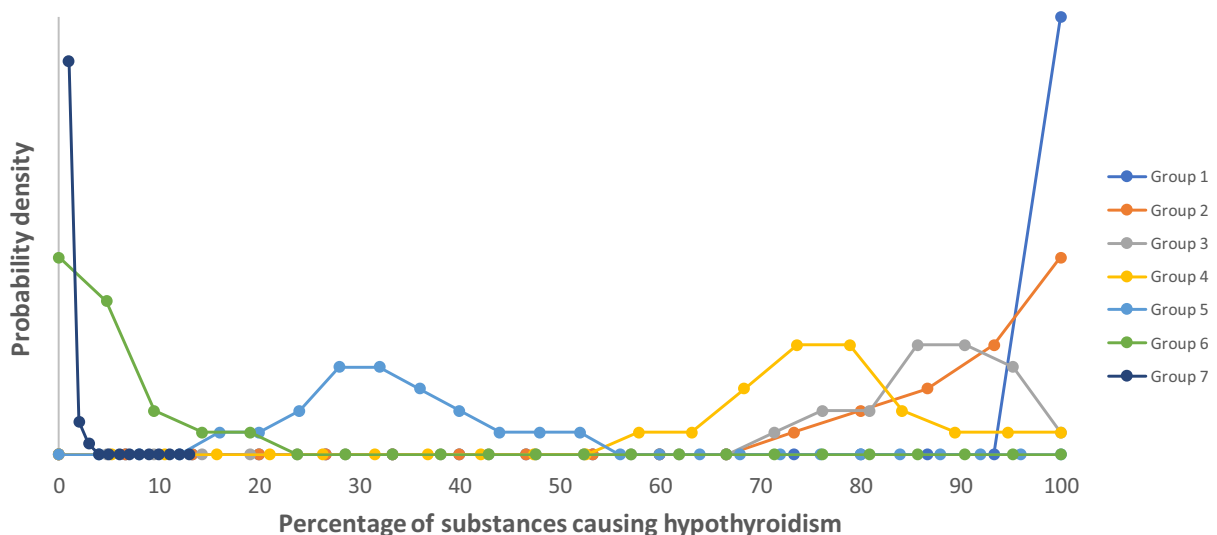
Figure 1: Distributions quantifying uncertainty about the percentage of substances in each subgroup that cause hypothyroidism. The vertical axis (probability density) quantifies the experts' judgement of the likelihood of different proportions of substances causing hypothyroidism within each subgroup
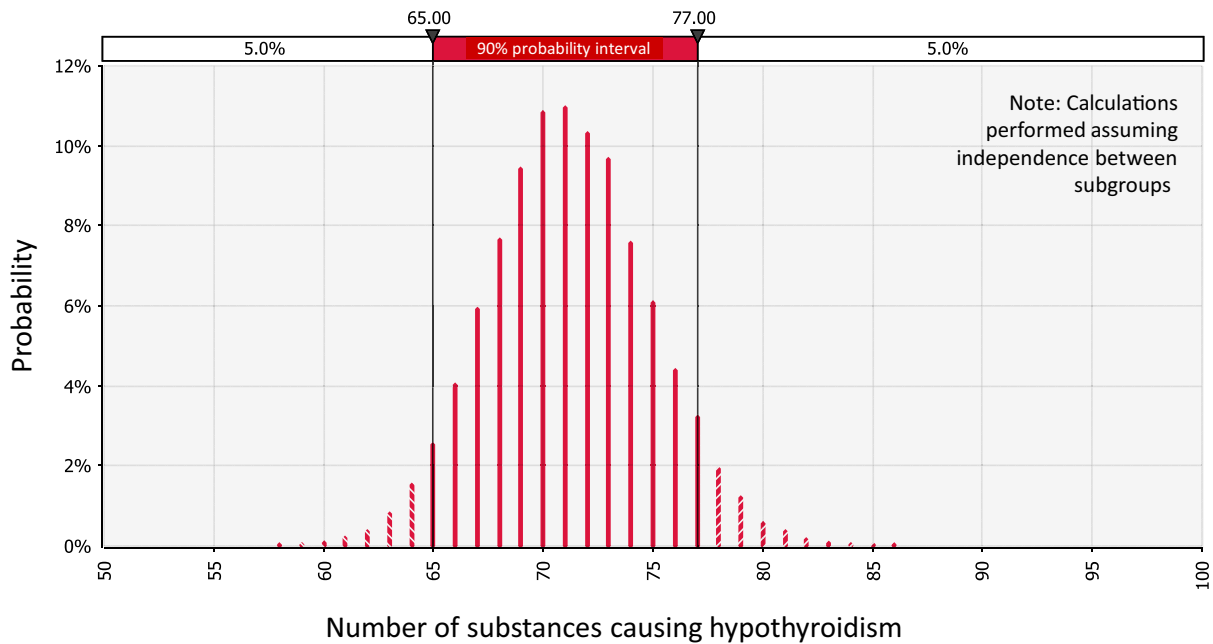
Figure 2: Distribution quantifying uncertainty about the total number of substances from subgroups 1 to 7 that cause hypothyroidism, obtained using Monte Carlo simulation assuming that the elicited distributions for the seven subgroups are independent
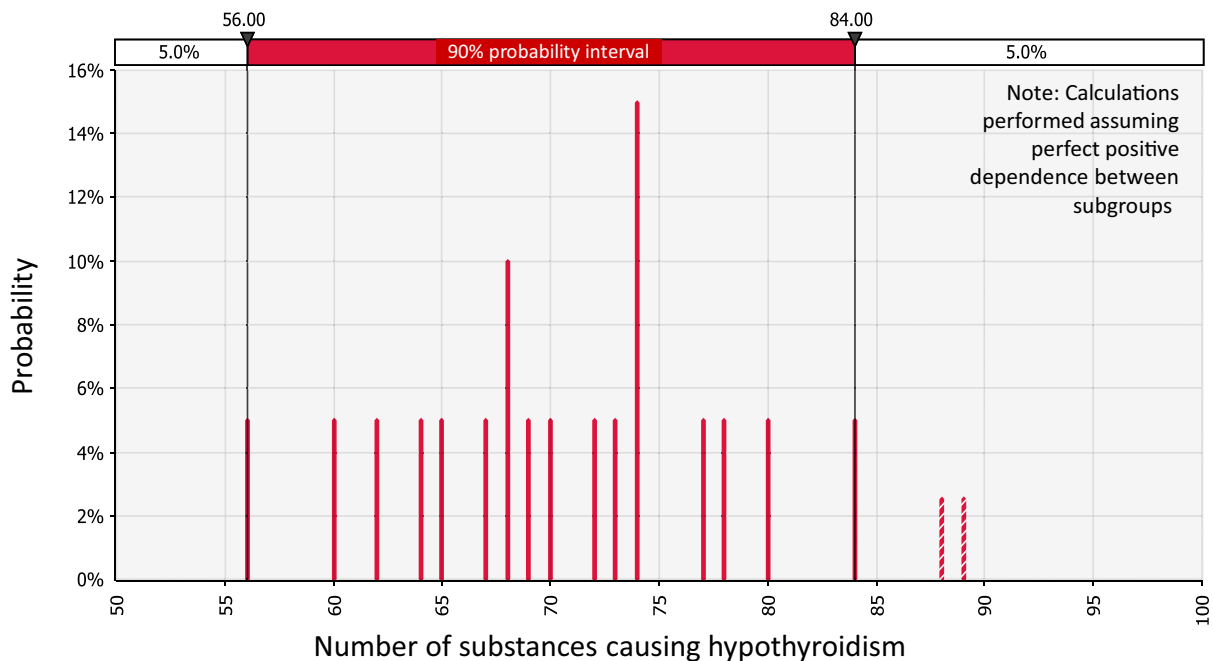
Figure 3: Distribution quantifying uncertainty about the total number of substances from subgroups 1 to 7 that cause hypothyroidism, obtained using Monte Carlo simulation assuming perfect positive dependence between the elicited distributions for the seven subgroups

The results summarised above were obtained in the final EKE session, after verification of the database and consideration of comments received in the public consultation of February to March 2019 (EFSA, 2019c).

4.2.2. Question 2: How sure is it that these ASs combine their individual toxicities according to the dose addition model at their actual level in food?

Question 2 was ultimately addressed as part of the CRA for the CAG on hypothyroidism, on the basis of the observed risk-drivers (EFSA, 2019b). This was facilitated by the information given in the present section which reviews and sorts out the ASs in the CAG for hypothyroidism according to their MoA.

Potential MoAs of chemicals leading to thyroid toxicity

Many chemical substances can impair the thyroid function by disturbing several pathways of thyroid hormone regulation. The biological targets and/or molecular mechanisms involved are diverse and the most prominent based on the current knowledge include the following (Bianco and Kim, 2006, Crofton, 2008; Miller et al., 2009; Chemicals Regulation Directorate 2013; Dong and Wade, 2017; Groeneweg et al., 2017; Lerro et al., 2018; Mughal et al., 2018):

- Blockage of or competition for the iodide uptake into follicular cells of the thyroid gland via sodium iodide symporter (NIS), which is the initial step of thyroid hormone biosynthesis. Several anions are blockers or competitive inhibitors of NIS, such as perchlorate and thiocyanate.
- Inhibition of thyroperoxidase (TPO), the follicular cell enzyme involved in the catalysis of the first two steps in thyroid hormones synthesis. These steps consist of oxidation of iodide to iodine, iodination of tyrosine residues on the thyroglobulin (TG) molecule, and further coupling of iodothyronine residues in the presence of hydrogen peroxide. As a result of TPO inhibition, thyroid hormone (T4 and T3) production is decreased. TPO can be inhibited by thionamides (e.g., PTU and ETU), anilines and substituted phenols.
- Interference with thyroid hormone binding proteins, which are transport proteins that facilitate the distribution of the thyroid hormones through the blood and further delivery to their target tissues. There are three principal plasma thyroid hormone-binding proteins: transthyretin (TTR, originally called prealbumin), thyroxine-binding globulin (TBG) and albumin.
- Liver enzyme induction resulting in increased thyroid hormones clearance and disruption of the thyroid–pituitary axis (TPA). Hepatic glucuronidation is the rate-limiting step for biliary excretion of T4, whereas hepatic sulfation (primarily phenol sulfotransferase) is the rate limiting step for biliary excretion of T3. Activation of nuclear receptors/xenosensors (such as AhR, CAR, PXR and PPARα) induces the expression of phase II liver enzymes (i.e., uridine 5′-diphospho-glucuronosyltransferase (UDPGT or UGT) and sulfotransferase (SULT)). These enzymes increase the rate of hepatic catabolism and excretion of thyroid hormones, leading to increased TSH secretion from the pituitary and further compensatory hyperplasia in the thyroid. Hepatocellular hypertrophy, as an indicator of an adaptive liver response, may also be observed. While the induction of UGT has been associated with thyroid tumours in rodents, in humans the increase in metabolism would initially be compensated for by the reservoir of thyroid hormone bound to TBG.
- Reduced uptake into target cells by interference with T4- and T3-membrane bound transporters, such as monocarboxylate transporter (MCT) 8, MCT 10 and organic anion transporting polypeptide (OATP) 1C1. In particular, MCT8 and OATP1C1 are important for the regulation of local TH activity in the brain and thus for brain development.
- Inhibition of iodothyronine deiodinases (DIOs) in peripheral tissues. Once inside the cell, T4 can be activated via conversion to T3 by the DIOs, such that the cytoplasmic pool of T3 includes both T3 from the plasma and T3 generated by DIOs. The different DIOs isoforms, e.g. DIO1 (expressed mainly in liver) and DIO2 (present in most thyroid hormone target tissues), catalyse the conversion (deiodination) of intracellular T4 (inactive prohormone) into T3, the most metabolically active thyroid hormone. Then, T3 is transferred to the nucleus and binds to its receptors initiating downstream gene expression responses. Hence, DIOs play a pivotal role in regulating circulation of peripheral thyroid hormones levels and maintaining the ratios of T4 and T3. Where DIO1 and DIO2 are inhibited, T4 levels increase and T3 is reduced. Conversely, DIO3 (normally expressed in the central nervous system, the placenta and foetal tissues) is the principal inactivating enzyme that degrades T4 to reverse (r)T3, thus playing a role in the inactivation/turnover of T4. Reduced T3 formation in peripheral tissues following DIO1 and DIO2 inhibition is followed by an increased TSH production to compensate for a

decrease in serum T3. This MoA is relevant for humans, because, although excess T4 is buffered by TBG, there may be effects on the negative feedback system in the pituitary that will cause thyroid stimulation.

- Direct binding to TH nuclear receptors (TRα1, TRβ1 and TRβ2) in peripheral tissues resulting in induction or suppression of the transcription of TH-responsive genes. These genes are involved in a variety of regulatory functions in development and metabolism.
- Altered gene expression (RNA transcription levels) of thyrotropin-releasing hormone receptor-2 (TRHR2), thyroid-stimulating hormone receptor (TSHR), sodium-iodide symporter (NIS), thyroid peroxidase (TPO), TTR, deiodinases 1 and 2 (DIO1 and DIO2) and thyroid hormone receptors-alpha and -beta (TRα and β) in different tissues.

These mechanisms are shown graphically in Figure 4:
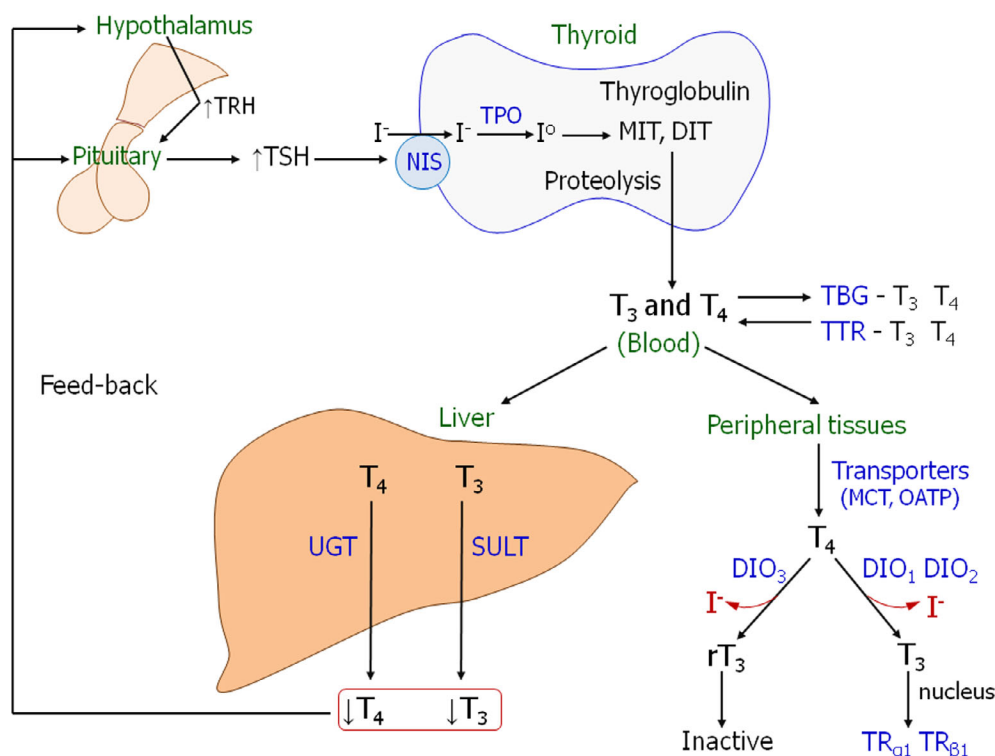
Figure 4: Mechanisms of toxic action of pesticidal active substances disrupting the thyroid hormone homeostasis (potential targets are shown in blue colour. NIS: Sodium/iodide symporter; TPO: thyroperoxidase; MIT, DIT: monoiodothyronine, diiodothyronine; TBG: thyroid-binding globulin; TTR: TTR; DIO: deiodinases; MCT: monocarboxylate transporter; OATP: organic anion transporting polypeptide; TRα1 TRβ1: thyroid receptors α and β; UGT: uridine 5'-diphospho-glucuronosyltransferases; SULT: sulfotransferases; TSH: thyroid-stimulating hormone; TRH: thyrotropin-releasing hormone)

Sorting the ASs in the CAG for hypothyroidism per MoA

Open peer-reviewed scientific literature and regulatory evaluations (EFSA conclusions, JMPR evaluations, US-EPA Federal Register, RAC opinions for pesticides by ECHA) have been consulted to collect information on the possible MoA of all ASs included in the CAG for hypothyroidism (see Table B1 in Appendix B).

For several ASs, there is strong evidence for a MoA-causing hypothyroidism. These ASs are:

- NIS inhibition: bromide ion
- TPO inhibition: amitrole, ETU, heptachlor, mancozeb, maneb, metiram, propineb, PTU, tolylfluanid, ziram

- Interference with TTR: 2,4-D, bromoxynil, ioxynil

For most of the ASs, varying levels of evidence are available to support a hypothesised[4] MoA causing hypothyroidism. The sources of information used in this report to hypothesise a MoA for the ASs listed below can be found in Appendix B.

- NIS inhibition: amitrole
- TPO inhibition: thiophanate-methyl
- Liver enzyme induction: bitertanol, bixafen, boscalid, bupirimate, buprofezin, carbetamide, chlordane, chlorpropham, clofentezine, cyantraniliprole, cyflufenamid, cyproconazole, cyprodinil, diethofencarb, dithianon, etofenprox, etridiazole, fenarimol, fenbuconazole, fipronil, fenpyrazamine, flubendiamide, flufenacet, fluopicolide, fluopyram, fluoxastrobin, fluquinconazole, fluxapyroxad, isoxaflutole, metribuzin, myclobutanil, orthosulfamuron, pendimethalin, penflufen, penthiopyrad, pethoxamid, propyzamide, proquinazid, prothioconazole, pymetrozine, pyrethrins, pyrimethanil, pyriofenone, quinmerac, quintozene, quizalofop-P-tefuryl, sedaxane, silthiofam, spiromesifen, sulfoxaflor, tembotrione, terbuthylazine, tetraconazole, thiabendazole, thiacloprid, thiamethoxam, thiophanate-methyl, topramezone, triadimefon, trifluralin, vinclozolin
- Deiodinases inhibition: azadirachtin
- Antagonism of T3 or T4 receptors: carbaryl, etofenprox
- Altered gene expression (RNA transcription levels) of proteins/receptors/enzymes involved in thyroid hormone homeostasis: amitrole, flutolanil, ioxynil

No information on possible MoA for hypothyroidism has been found for the following compounds: 8-hydroxyquinoline, aclonifen, amisulbrom, anthraquinone, beflubutamid, benalaxyl, benalaxyl-M, benfluralin, benthiavalicarb, bromopropylate, bromuconazole, carbosulfan, clethodim, clodinafop, cycloxydim, dazomet, desmedipham, diclofop, dicloran, dinocap, fenamidone, fenoxycarb, flazasulfuron, flumioxazin, flutonalil, folpet, fuberidazole, haloxyfop-P, hymexazol, imazosulfuron, lufenuron, maleic hydrazide, MCPA, meptyldinocap, metam, oryzalin, oxadiazon, oxyfluorfen, phoxim, picolinafen, propaquizafop, pyridalyl, quinoclamine, spinosad, spirodiclofen, tepraloxydim, thiencarbazone, tritosulfuron, valifenalate, zoxamide.

Liver enzyme induction

As explained above, thyroid toxicity can be mediated through a number of toxicity pathways and MoAs. Increased thyroid hormones clearance can occur through liver enzyme induction of phase 2 enzymes and phase 3 transporters. This is an important MoA to understand in order to assess whether observed signs of hypothyroidism might be secondary to increased metabolism of the thyroid hormones. Indeed, in such case, it would be conceivable that the dose-addition model would not apply to ASs acting through this MoA as long as the dietary level of exposure to these ASs is below a threshold triggering significant liver enzyme induction and thus the increased metabolism of the thyroid hormones would not occur.

However, observed liver toxicity in the presence of signs of hypothyroidism does not necessarily mean that liver enzyme induction is the responsible MoA. Several MoAs could be occurring simultaneously. In this respect, the Guidance on identification of EDs (ECHA and EFSA, 2018) makes the following recommendations:

'To investigate whether liver enzyme induction is responsible for the effects seen on TH levels and/or thyroid histopathology and weight, as well as whether the effect is or not likely to be human relevant, the following three pieces of information are needed:

1) Results of analysis of serum/plasma samples (if available) for TSH, T3 and T4 in the existing repeated dose toxicity studies. If unavailable, a specifically designed *in vivo* toxicity study should be considered. In this study, TSH, T3 and T4 should be measured, and where possible, additional data on liver enzyme induction (e.g. measurement of UDPGT) should be included.

2) Comparative studies of enzyme activity induced by the test substance in liver *in vitro* systems in both the relevant test species (e.g. rat, mouse and dog) and humans. The *in vitro* metabolism of the specific substance absorption, distribution, metabolism, excretion

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[4] In relation with liver enzyme induction, "hypothesised MoA" should be understood as a MoA which is not has not been assessed against or is not meeting the 3 conditions listed in the ECHA/EFSA guidance to demonstrate that it is responsible for the effects seen on TH levels and/or thyroid histopathology and weight.

(ADME properties) in both test species and humans, and the activity of possible metabolites must be considered when this comparison is conducted.

3) The presence of other possible thyroid-disrupting modes of action such as interference with TH synthesis should also be excluded, e.g. by evaluating *in vitro* the potential for inhibition of the sodium–iodide symporter (NIS) (Cianchetta et al., 2010; Kogai and Brent, 2012; Hallinger et al., 2017) and thyroid peroxidase (TPO) (Kambe and Seo, 1997; Paul et al., 2014; Paul Friedman et al., 2016; Wu et al., 2016). It must, however, be acknowledged that substances may interfere with the thyroid hormone system through many different mechanisms of action, and that currently validated/standardised *in vitro* assays do not exist to investigate all these different pathways and a reasonable effort is anticipated, based on available tools and current understanding of thyroid physiology'.

For the majority of the ASs included in the CAG for hypothyroidism for which liver enzyme induction is hypothesised to be the operating MoA, the above evidence has not been assessed and is probably not totally available. Should this evidence be available in the future, it would be reasonable to assume that one would be protected from effects on the thyroid system if the health-based guidance value had been established based on levels triggering the enhanced hepatic metabolism of thyroid hormones.

Conclusion

There is a wide variety of MoAs known or hypothesised to be of relevance for hypothyroidism. It is virtually certain that ASs sharing the same MoA combine their effects by dose addition.

Between AS acting with dissimilar MoA, the uncertainty about how closely combined effects conform to those predicted by dose addition was addressed in EFSA (2019b) considering the empirical information on the respective combined toxicity if available. The extent to which this uncertainty impacted the risk assessment depended on various considerations, including the knowledge of the precise ASs driving the risk.

4.3. CAG for C-cell hypertrophy, hyperplasia and neoplasia

4.3.1. Question 1: Does the CAG for C-cell hypertrophy, hyperplasia and neoplasia contain all ASs contributing to this effect and only ASs contributing to this effect?

As stated above, question 1 implies providing responses to two discrete questions.

As to *whether all ASs causing the effect are well included* in the CAG, the reader should refer to the first bullet point of Section 4.4 (overall uncertainty analysis).

As to *whether ASs not causing the effect are included* in the CAG, an EKE session has not been conducted because the cumulative risk of C-cell hypertrophy, hyperplasia and neoplasia is very likely lower than the cumulative risk of hypothyroidism (see Section 3.3.2).

4.3.2. Question 2: How sure is it that these ASs combine their individual toxicities according to the dose addition model at their actual level in food?

Question 2 was addressed when the CRA for C-cell hypertrophy, hyperplasia and neoplasia was performed in the light of the observed risk drivers (EFSA, 2019b). Although a number of MoAs are known to cause these effects (Rosol et al., 2013; Cote et al., 2015), they were not investigated for any of the ASs included in the CAG.

4.4. Overall uncertainty analysis

In subsequent CRAs performed with the CAGs established in the present report, an evaluation of all uncertainties affecting these assessments will be conducted. To address the uncertainties resulting from the composition of the CAG and from the assumption that ASs in the CAG combine their effects by dose addition, it is recommended to consider systematically all relevant sources of uncertainties, including the following:

- Uncertainty related to the composition of the CAG:

  — How certain is it that the CAG includes all the substances contributing to the specific effect of interest? If the CAG does not contain ASs contributing to the risk, the outcome of the risk assessment might be underestimated. The assessors should consider the probability that ASs causing the specific effect might have not been identified during the data collection procedure (possibility that information of relevance in original toxicological studies is omitted or misreported in summary documents used as source of information) or omitted due to the methodology and criteria used to populate CAGs, and evaluate their potential contribution to the risk.

  — How certain is it that the CAG includes only ASs contributing to the specific effect of interest? If the CAG contains ASs not contributing to the risk, the outcome of the risk assessment might be overestimated. This needs to be considered in the light of probabilities of CAG membership assessed in Section 4.2.1 (CAG for hypothyroidism) and of the individual contribution of each AS to the risk. For the CAGs related to C-cell hypertrophy, hyperplasia and neoplasia, this source of uncertainty should be addressed based on appropriate lines of evidence.

- Uncertainty related to the characterisation of ASs included in the CAG: Can NOAELs be either under- or overestimated?. This question needs to be evaluated in the light of the data collection procedure and of the principles used to establish NOAELs. In this respect, the assessors will at least consider the adequacy of the data collection procedure to the principles adopted for the hazard characterisation (including the adopted indicators), the quality and exhaustiveness of the toxicological dossiers of the ASs included in the CAG (availability of the ad hoc studies for the hazard characterisation, e.g. 2-year rodent and 1-year dog studies).
- Uncertainty regarding relative contribution of ASs to the cumulative risk resulting from the use of NOAELs rather than BMDLs.
- Uncertainty regarding the slope of the dose–response relationship and consequently regarding the effect size at the actual levels of exposure.
- Uncertainty about the contribution of metabolites and degradation products to the cumulative risk: Not only ASs, but also their metabolites and degradation products may contribute to the specific effect (e.g., ETU and PTU in the case of dithiocarbamate fungicides). If this contribution is not considered, this needs to be treated as a source of uncertainty when a CRA is performed for any of the CAG related to the effects of pesticides on the thyroid.
- Uncertainty about the adequacy of the dose addition model: How closely will the actual risks for the specific effect of interest conform to those predicted by dose addition? It is recommended to focus on the observed combinations of ASs at the percentiles of the exposure distribution of interest for the risk managers. The evaluation will consider whether risk drivers have similar or dissimilar MoAs. Empirical information on their combined effects in peer-reviewed scientific literature should be considered if available. The distribution of ASs according to their known or hypothesised MoAs in Section 4.2.2 might be useful in the evaluation of this source of uncertainty.
- Uncertainty about the inter- and intra-species variability in toxicological sensitivity. This source of uncertainty concerns the adequacy for human risk assessment of the toxicological characterisation of ASs on the basis of animal data, and in particular the human relevance of thyroid effects resulting from liver enzyme induction.
- Uncertainties resulting from the use of chronic exposure calculation model not necessarily reflecting the actual time course of toxicokinetic and toxicodynamic processes in humans.

5. Conclusions

Cumulative assessment groups for the effects of pesticides on the thyroid were previously established by the PPR Panel in 2013. The two specific effects that are of relevance for CRA have been confirmed: hypothyroidism and C-cell hypertrophy, hyperplasia and neoplasia. The CAGs have been updated based on additional information collected from more recent data collections.

NOAELs have been defined to characterise the ASs included in the CAGs for the respective specific effects. ICs have been proposed to enable cumulative exposure and risk assessments with methods using RPFs.

For an efficient use of resources, the assessment of the cumulative risks of pesticides residues for the thyroid should be focussed 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 in accordance with the anticipated assessment question which will govern CRA conducted with these CAGs.

6.   Recommendations

If the outcome of CRAs conducted with these CAGs exceeds regulatory thresholds of acceptance, empirical research is recommended on how ASs driving the risk combine their effects at the anticipated dietary exposure levels, especially if they act by dissimilar MoAs, and on the extent to which this combination of effects deviates from dose addition. The question of synergistic effects of pesticide residues has been considered in an opinion of the PPR Panel (EFSA, 2008). Although interactions from pesticide residues in food cannot be ruled out, there is no empirical evidence for their occurrence at the expected levels of exposure from pesticide residues in food.

If the outcome of CRAs conducted with these CAGs, as currently characterised by NOAELs, exceeds regulatory thresholds of acceptance, an alternative cumulative exposure/risk assessment should be considered with BMDLs used as reference points, after agreement on benchmark dose levels suitable for regulatory purposes. This is not likely to change significantly the outcome of the assessment but would make it independent from the dose selection in toxicological studies and better reflecting the actual relative potencies of ASs in the CAG.

The approaches developed in the present report to evaluate uncertainties should be integrated into the CRA which follows. This could be done by incorporating the probabilities of CAG membership into a probabilistic calculation of cumulative risk and taking account of other uncertainties (including those identified in this report and any others arising in the risk assessment) when assessing the overall uncertainty by expert judgement. A simpler alternative would be to do sensitivity analyses, starting with all subgroups of substances included and removing them one at a time in order to increase probability of CAG membership, and use the results of this to inform expert judgement of the contribution of CAG membership uncertainty to overall uncertainty.

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. A threshold level of adversity for this effect should be defined, in order to specifically protect consumers against this effect and prevent its contribution to other adverse effects of pesticides. This would allow excluding from the CAG on hypothyroidism the ASs causing hypothyroidism secondary to liver enzyme induction in rats.

It is noted that current OECD test guidelines for repeated dose toxicological studies and TG 443 (extended one-generation reproductive toxicity study; OECD, 2011) require measurements of thyroid hormone levels. However, many compounds have not been tested with these current guidelines yet. Therefore, it is recommended that, when missing, such data on thyroid hormone levels in serum (total and free T3/T4 and TSH) are required, in particular during critical windows of nervous system development. These measurements would make CAGs more functional rather than histopathological and closer to the clinical settings for humans. They would also provide the basis to establish CAGs and characterise ASs with respect to thyroid-mediated impaired neurodevelopment and contribute to an adequate protection against this effect.

The CAGs established in this report should be regularly updated in the light of the toxicological information provided to EFSA in the context of its regulatory activities.

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Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, excretion
ANSES	French Agency for Food, Environmental and Occupational Health and Safety
ARfD	acute reference dose
AS	active substance
BMDL	lower confidence limit of the benchmark dose
bw	body weight
CAG	cumulative assessment group
CRA	cumulative risk assessment
DAR	Draft Assessment Report
DIO	iodothyronine deiodinase
RAR	Renewal Assessment Report
DTU	Technical University of Denmark
EBDC	ethylene-bis-dithiocarbamate
ECHA	European Chemicals Agency
ED	Endocrine Disruptor
EKE	expert knowledge elicitation
ETU	ethylenethiourea
HPT	hypothalamic–pituitary–thyroid
IC	index compound
ICPS	International Centre for Pesticides and Health Risk Prevention
JMPR	Joint Meeting on Pesticides Residues
KE	key event

LOAEL	lowest observed adverse effect level
LOEL	lowest observed effect level
MCT	monocarboxylate transporter
MoA	mode of action
MIE	molecular initiating event
MOE	margin of exposure
MOET	combined margin of exposure
MRL	maximum residue level
MTD	maximum tolerated dose
NIS	sodium iodide symporter
NOAEL	no observed adverse effect level
NOEL	no observed effect level
OATP	organic anion transporting polypeptide
OECD	Organisation for Economic Co-operation and Development
PPR	EFSA Panel on Plant Protection Products and their Residues
PTH	parathyroid hormone
PTU	propylenethiourea
RIVM	National Institute for Public Health and the Environment
RPF	relative potency factor
RfP	(toxicological) reference point
SULT	sulfotransferase
T3	triiodothyronine
T4	thyroxine
TG	thyroglobulin
TBG	thyroxine-binding globulin
TH	thyroid hormone
TPA	thyroid–pituitary axis
TPO	thyroperoxidase
TRHR2	thyrotropin-releasing hormone receptor-2
TSH	thyroid-stimulating hormone
TSHR	thyroid-stimulating hormone receptor
TTR	transthyretin
$TR\alpha 1$, $TR\beta 1$, $TR\beta 2$	thyroid receptors
UDPGT/UGT	5'-diphospho-glucuronosyltransferase
UF	uncertainty factor
US-EPA	United States Environmental Protection Agency

Appendix A − List of active substances considered in view of establishing CAGs for effects of pesticides on the thyroid

Active substances covered by the first and second steps of the EFSA internal data collection:

1-Methylcyclopropene	Cyazofamid	Fluazifop-P	Mesosulfuron	Pyridate
1-Naphthylacetamide (1-NAD)	Cyclanilide	Fluazinam	Mesotrione	Pyrimethanil
1-Naphthylacetic acid (1-NAA)	Cycloxydim	Fludioxonil	Metalaxyl-M	Pyriproxyfen
2,4-D	Cyflufenamid	Flufenacet (formerly fluthiamide)	Metaldehyde	Quinmerac
2,4-DB (metabolised to 2,4-D)	Cyfluthrin	Flumioxazin	Metamitron	Quinoclamine
2-Phenylphenol (incl. sodium salt orthophenyl phenol)	Cyhalofop-butyl	Fluometuron	Metazachlor	Quinoxyfen
6-Benzyladenine	Cymoxanil	Fluopicolide	Metconazole	Quizalofop-P-tefuryl
Abamectin (aka avermectin)	Cypermethrin	Fluoxastrobin	Methiocarb (aka mercaptodimethur)	Rimsulfuron (aka renriduron)
Acetamiprid	Cyproconazole	Flupyrsulfuron-methyl (DPX KE 459)	Methomyl	Silthiofam
Acibenzolar-S-methyl (benzothiadiazole)	Cyprodinil	Fluquinconazole	Methoxyfenozide	Sintofen (aka Cintofen)
Aclonifen	Cyromazine	Flurochloridone	Metiram	S-Metolachlor
alpha-Cypermethrin (aka alphamethrin)	Daminozide	Fluroxypyr	Metosulam	Sodium 5-nitroguaiacolate
Aluminium ammonium sulfate	Dazomet	Flurtamone	Metrafenone	Sodium hypochlorite
Aluminium phosphide	Deltamethrin	Flusilazole	Metribuzin	Sodium o-nitrophenolate
Amidosulfuron	Desmedipham	Flutolanil	Metsulfuron-methyl	Sodium p-nitrophenolate
Amitrole (aminotriazole)	Dicamba	Flutriafol	Milbemectin	Spinosad
Azimsulfuron	Dichlorprop-P	Folpet	Molinate	Spirodiclofen
Azoxystrobin	Diclofop	Foramsulfuron	Myclobutanil	Spiroxamine
Beflubutamid	Diethofencarb	Forchlorfenuron	Napropamide	Sulcotrione
Benalaxyl	Difenoconazole	Formetanate	Nicosulfuron	Sulfosulfuron
Benfluralin	Diflubenzuron	Fosetyl	Omethoate	Sulfuryl fluoride
Bensulfuron	Diflufenican	Fosthiazate	Oryzalin	tau-Fluvalinate
Bentazone	Dimethachlor	Fuberidazole	Oxadiargyl	Tebuconazole
Benthiavalicarb	Dimethenamid-P	Gibberellin	Oxadiazon	Tebufenozide
Benzoic acid	Dimethoate	Glufosinate	Oxamyl	Tebufenpyrad
beta-Cyfluthrin	Dimethomorph	Glyphosate (incl. trimesium aka sulfosate)	Oxasulfuron	Teflubenzuron
Bifenazate	Dimoxystrobin	Haloxyfop-P/R	Oxyfluorfen	Tefluthrin
Bifenox	Dinocap	Hexythiazox	Paclobutrazol	Tepraloxydim
Bispyribac	Diquat (dibromide)	Hymexazol	Penconazole	Terbuthylazine
Boscalid	Dithianon	Imazalil (aka enilconazole)	Pencycuron	Tetraconazole
Bromadiolone	Diuron	Imazamox	Pendimethalin	Thiabendazole
Bromoxynil	Dodemorph	Imazaquin	Penoxsulam	Thiacloprid
Bromuconazole	Dodine	Imazosulfuron	Pethoxamid	Thiamethoxam
Bupirimate	Epoxiconazole	Imidacloprid	Phenmedipham	Thifensulfuron-methyl
Buprofezin	Esfenvalerate	Indoxacarb	Phosmet	Thiophanate-methyl

Calcium phosphide	Ethephon	Iodosulfuron	Picloram	Thiram
Captan	Ethofumesate	Ioxynil	Picolinafen	Tolclofos-methyl
Carbendazim	Ethoprophos	Iprodione	Picoxystrobin	Tolylfluanid
Carbetamide	Ethoxysulfuron	Iprovalicarb	Pirimicarb	Tralkoxydim
Carboxin	Etofenprox	Isoproturon	Pirimiphos-methyl	Triadimenol
Carfentrazone-ethyl	Etoxazole	Isoxaben	Prochloraz	Tri-allate
Carvone	Etridiazole	Isoxaflutole	Profoxydim (aka Clefoxydim)	Triasulfuron
Chloridazon (aka pyrazone)	Famoxadone	Kresoxim-methyl	Prohexadione (incl. Prohexadione-calcium)	Triazoxide
Chlormequat (chloride)	Fenamidone	Lambda-Cyhalothrin	Propamocarb	Tribenuron (aka metometuron)
Chlorothalonil	Fenamiphos (aka phenamiphos)	Lenacil	Propaquizafop	Triclopyr
Chlorotoluron	Fenazaquin	Linuron	Propiconazole	Trifloxystrobin
Chlorpropham	Fenbuconazole	Lufenuron	Propineb	Triflumizole
Chlorpyrifos	Fenbutatin oxide	Magnesium phosphide	Propoxycarbazone	Triflumuron
Chlorpyrifos-methyl	Fenhexamid	Malathion	Propyzamide	Triflusulfuron
Chlorsulfuron	Fenoxaprop-P	Maleic hydrazide	Proquinazid	Trinexapac (aka cimetacarb ethyl)
Cinidon ethyl	Fenoxycarb	Mancozeb	Prosulfocarb	Triticonazole
Clethodim	Fenpropidin	Maneb	Prosulfuron	Tritosulfuron
Clodinafop	Fenpropimorph	MCPA	Prothioconazole	zeta-Cypermethrin
Clofentezine	Fenpyroximate	MCPB	Pymetrozine	Zinc phosphide
Clomazone	Fipronil	Mecoprop	Pyraclostrobin	Ziram (incl. impurity TMTU)
Clopyralid	Flazasulfuron	Mecoprop-P	Pyraflufen-ethyl	Zoxamide
Clothianidin	Flonicamid (IKI-220)	Mepanipyrim	Pyrethrins	
Copper compounds	Florasulam	Mepiquat	Pyridaben	

Active substances covered by the outsourced data collection (RIVM, ICPS, ANSES, 2016):

2-chloroethanol	Carbaryl	Ethametsulfuron	Mandipropamid	Prothiofos
8-Hydroxyquinoline incl. oxyquinoleine	Carbofuran	Ethion (aka diethion)	Meptyldinocap	Pyrazophos
Acephate	Carbosulfan	Ethylene oxide	Metaflumizone	Pyridalyl
Acequinocyl	Chlorantraniliprole	Fenarimol	Metalaxyl	Pyriofenone
Acrinathrin	Chlordane	Fenitrothion	Metam (incl. -potassium and -sodium)	Pyroxsulam
Aldicarb	Chlorfenapyr	Fenpropathrin	Methamidophos	Quintozene
Aluminium sulfate	Chlorfenvinphos	Fenpyrazamine	Methidathion	Resmethrin
Ametoctradin	Chlorobenzilate	Fenthion	Methoxychlor	Sedaxane
Aminopyralid	Chromafenozide	Fenvalerate	Metobromuron	Spinetoram
Amisulbrom	Cyantraniliprole	Ferric phosphate	Monocrotophos	Spiromesifen
Amitraz	Cyflumetofen	Fluazifop	Nicotine	Spirotetramat
Anthraquinone	DDT	Flubendiamide	Orthosulfamuron	Sulfoxaflor
Azadirachtin	Diazinon	Flufenoxuron	Oxadixyl	Tecnazene
Azinphos-ethyl	Dichlofluanid	Fluopyram	Oxydemeton-methyl	Tembotrione
Azinphos-methyl	Dichlorvos	Fluxapyroxad	Parathion	Tetradifon

Benalaxyl-M	Dicloran	Fonofos	Parathion-methyl	Tetramethrin
Benfuracarb	Dicofol	Halosulfuron methyl	Penflufen	Thiencarbazone
Benomyl	Dicrotophos	HCH	Penthiopyrad	Thiodicarb
Benzalkonium chloride	Didecyldimethylammonium chloride	Heptachlor	Permethrin	Tolfenpyrad
beta-cypermethrin	Dieldrin	Hexachlorobenzene	Phenthoate	Topramezone
Bifenthrin	Dinotefuran	Hexaconazole	Phosalone	Triadimefon
Bitertanol	Diphenylamine	Indolylbutyric acid	Phosphane	Triazophos
Bixafen	Dithiocarbamates	Ipconazole	Phoxim	Trichlorfon
Bromide ion	Emamectin benzoate	Iron sulfate	Pinoxaden	Trifluralin
Bromopropylate	Endosulfan	Isoprocarb	Procymidone	Valifenalate
Cadusafos (aka ebufos)	Endrin	Isopyrazam	Profenofos	Vinclozolin
Camphechlor	EPN	Lindane	Propargite	

Appendix B – Tables supporting Cumulative Risk Assessments using the CAGs for effects of pesticides on the thyroid

Note 1: In the following tables, the names of persons involved in testing on vertebrate animals are confidential and not shown in the study reference details.

Table B.1: CAG on hypothyroidism: toxicological characterisation of ASs to be considered in long-term cumulative exposure/risk assessments

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 ▮▮	Source: DAR 1997 and addendum EFSA (2014) Observation of increased relative thyroid weight with NOAEL at 1 mg/kg in 90-day rat ▮▮ 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 ▮▮	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 ▮▮, 90-day rat studies ▮▮ combined	Unknown
Amisulbrom	Follicular cell hypertrophy	129	697	2-year rat ▮▮	Source: DAR 2012	Unknown
Amitrole	Increased relative thyroid weight, follicular cell hyperplasia	0.3	13	1-year dog ▮▮	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 ▮▮	Source: DAR 2006	Unknown
Azadirachtin	Follicular cell hypertrophy	36 (12)	135	90-day rat ▮▮	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)

Active substance	Indicator of specific effect	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Study	Remark	MoA
Beflubutamid	Increased relative thyroid weight/ follicular cell adenoma	17.7	150	2-year rat ▮ ▮	Source: DAR 2002	Unknown
Benalaxyl	Follicular cell hypertrophy, hyperplasia	100	800	5-week rat ▮ ▮	Source: DAR 2000	Unknown
Benalaxyl-M	Follicular cell hypertrophy	30.1	100	90-day rat ▮	Source: DAR 2003	Unknown
Benfluralin	Increased relative thyroid weight, follicular cell hyperplasia, adenoma, carcinoma	5.4	136	2-year rat ▮ ▮	Source: DAR 2006 EFSA (2008)	Unknown
Benthiavalicarb	Follicular cell hyperplasia	9.9	249	2-year rat ▮ ▮	Source: DAR 2004 EFSA (2007)	Unknown
Bitertanol	Increased relative thyroid weight	100	300	28-day rat ▮	Source: DAR 2005	Liver enzyme induction (hypothesised) (Chan et al., 2006)
Bixafen	Increased relative thyroid weight	2.8	17.4	2-year rat ▮ ▮	Source: DAR 2011 EFSA Scientific Committee (2012)	Liver enzyme induction (hypothesised) (US EPA, 2018)
Boscalid	Increased relative thyroid weight	22	57	1-year dog ▮	Source: DAR 2002 Supported by 2-year rat (NOAEL 22 mg/kg bw for increased relative thyroid weight, follicular cell hypertrophy, hyperplasia and adenoma ▮	Liver enzyme induction (hypothesised) (EC Review report for the active substance boscalid, 2008)
Bromide ion	Increased relative thyroid weight	12		90-day rat ▮ ▮	Source: JMPR 1988 JMPR 1988: ADI bromide ion: 0.1 mg/kg bw/d. The evaluations include the establishment of a NOAEL based upon all available data on the effects on the thyroid at 300 ppm sodium bromide (240 ppm bromide), equivalent to 12 mg bromide/kg bw/day.	NIS inhibition (Pavelka, 2004; Velický et al., 2004)

Active substance	Indicator of specific effect	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Study	Remark	MoA
Bromopropylate	Increased relative thyroid weight	3.7	26	119-week rat ▉	Source: JMPR 1993 JMPR evaluations on bromopropylate (1993) considered.	Unknown
Bromoxynil	follicular cell hyperplasia	4.4	39.4	90-day rat ▉ ▉	Source: DAR 2000 EFSA Scientific Committee (2017)	Interference with transthyretin (van den Berg, 1991)
Bromuconazole	Increased relative thyroid weight	6.48	87.2	24-month rat ▉	Source: DAR 2009	Unknown
Bupirimate	Increased relative thyroid weight, follicular cell adenoma	24.6	156	2-year rat ▉	Source: DAR 2009 EFSA (2010) Slight changes in thyroid weights with NOAEL at 3 mg/kg bw in 90-day dog ▉, not considered adverse because low control values and not associated to histopathological findings.	Liver enzyme induction (hypothesised) (Lemaire et al., 2006)
Buprofezin	Follicular cell hypertrophy/ hyperplasia	0.9	8.7	2-year rat ▉	Source: DAR 2005 EFSA (2010)	Unknown
Carbaryl	Follicular cell hypertrophy, follicular cell adenoma	60.2	484.6	2-year rat ▉	Source: DAR 2004 EFSA (2006)	Antagonism of T3, T4, receptors (hypothesised) (Sun et al., 2008)
Carbetamide	Follicular cell hypertrophy	20.1	150.3	2-year mouse ▉	Source: DAR 2005	Liver enzyme induction (hypothesised) (ECHA, 2015)
Carbosulfan	Increased relative thyroid weight	3.1	71.9	2-year rat ▉	Source: DAR 2004	Unknown
Chlordane	Follicular cell neoplasia	0.6	6	18-month rat ▉	Source: EHC (Environmental Health Criteria) 1984 Source: EHC 1984 (Environmental Health Criteria 34) 0.6 is not a true NOAEL, but the LOAEL divided by 10.	Liver enzyme induction (hypothesised) (US EPA, 1997; Patrick, 2009)

Active substance	Indicator of specific effect	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Study	Remark	MoA
Chlorpropham	Increased relative thyroid weight, follicular cell hypertrophy/ hyperplasia	5	50	1-year dog ▮▮ ▮▮	Source: DAR 1999 EFSA Scientific Committee (2017)	Liver enzyme induction (hypothesised) (EFSA Scientific Committee, 2017)
Clethodim	Increased relative thyroid weight	62	250	1-year dog ▮ ▮	Source: DAR 2005	Unknown
Clodinafop	Follicular cell hypertrophy	11.3	29.5	2-year rat ▮▮ ▮	Source: DAR 2003	Unknown
Clofentezine	Follicular cell adenoma	1.72	17.3	2-year rat ▮▮	Source: DAR 2005 EFSA (2009) 2-year rat ▮▮ and 28-day rat ▮▮ combined. Relationship to treatment for the incidence of follicular tumours in males in the 2-year rat study considered equivocal	Liver enzyme induction (hypothesised) (Hurley, 1998; Stoker and Kavlock, 2010)
Cyantraniliprole	follicular cell hypertrophy	7	27	90-day rat ▮▮	Source: DAR 2014	Liver enzyme induction (hypothesised) (JMPR, 2013)
Cycloxydim	Increased relative thyroid weight	50	250	3-month dog ▮▮	Source: DAR 2006	Unknown
Cyflufenamid	Increased relative thyroid weight, follicular cell hypertrophy, follicular cell adenoma, follicular cell carcinoma	20	220	2-year rat ▮ ▮	Source: DAR 2006 EFSA (2009)	Liver enzyme induction (hypothesised) (APVMA, 2012).
Cyproconazole	Follicular cell hypertrophy	24.7	52.8	13-week rat ▮▮	Source: DAR 2004	Liver enzyme induction (hypothesised) (Peffer et al., 2007)
Cyprodinil	Follicular cell hypertrophy	3.14	19	90-day rat ▮▮	Source: DAR 2004 EFSA (2006)	Liver enzyme induction (hypothesised) (Waechter, 2010)

Active substance	Indicator of specific effect	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Study	Remark	MoA
Dazomet	Increased relative thyroid weight	1	3.1	1-year dog ▮▮▮▮▮	Source: DAR 2009	Interaction between this fumigant and molecular iodine in the thyroid gland (Buxeraud et al., 1992). Wide distribution, affinity to the thyroid (EFSA 2010 Conclusion on the peer review).
Desmedipham	Follicular cell hyperplasia	3.2	15.7	2-year rat ▮▮▮ ▮▮	Source: DAR 2000 EFSA Scientific Committee (2017)	Unknown
Diclofop	Follicular cell adenoma	2.25	22.5	2-year rat ▮▮▮ ▮	Source: DAR 2007	Unknown
Dicloran	Follicular cell hypertrophy	7.5	75	90-day rat ▮▮ ▮	Source: DAR 2005 Not true NOAEL (LOAEL divided by 10).	Unknown
Diethofencarb	Follicular cell adenoma/follicular cell carcinoma	42.7	220.3	2-year rat ▮▮ ▮	Source: DAR 2007 EFSA (2010)	Liver enzyme induction (hypothesised) (Hosokawa et al., 1992)
Dinocap	Follicular cell hypertrophy	11	121	1-year rat ▮▮▮▮▮	Source: DAR 2000	Unknown
Dithianon	Increased relative thyroid weight	7.9	37.1	1-year dog study ▮▮▮	Source: DAR 2010	Liver enzyme induction (hypothesised) (Colnot and Dekant, 2017)
Etofenprox	Increased relative thyroid weight	25.5	186.7	2-year rat study ▮▮▮▮	Source: DAR 2005 EFSA (2009) 90-day rat ▮▮▮▮ and 2-year rat ▮▮▮▮ studies combined.	Liver enzyme induction (hypothesised) (ECHA, 2012a), antagonism of T3, T4 receptors (Du et al., 2010)
Etridiazole	Follicular cell adenoma/carcinoma	5	30	104-week rat ▮▮▮	Source: DAR 2007 EFSA (2010)	Liver enzyme induction (hypothesised) (ECHA, 2012b)
ETU (Ethylenethiourea)	Follicular cell hyperplasia	0.37	9.25	2-year rat ▮▮▮ ▮	Source: JMPR 1993 Metabolite of maneb, mancozeb, metiram and zineb	TPO inhibition
Fenamidone	Follicular cell hypertrophy/ hyperplasia	3.6	7.1	2-year rat ▮▮ ▮	Source: DAR 2000 EFSA PPR Panel (2016)	Unknown
Fenamirol	Increased relative thyroid weight	19	83	90-day rat ▮▮▮▮	Source: DAR 1996	Liver enzyme induction (hypothesised) (Lemaire et al., 2006)

Active substance	Indicator of specific effect	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Study	Remark	MoA
Fenbuconazole	Increased relative thyroid weight, follicular cell hyperplasia, follicular cell adenoma/ carcinoma	3	31	2-year rat study	Source: DAR 2005 EFSA (2010) 13-week rat ▮▮▮▮ and 2-year rat study ▮▮▮▮ combined	Liver enzyme induction (hypothesised) (Hurley, 1998)
Fenoxycarb	Follicular cell hypertrophy	10.1	49.6	13-week rat ▮▮▮	Source: DAR 2009	Unknown
Fipronil	Increased relative thyroid weight	0.06	1.3	104-week rat ▮▮▮	Source: DAR 2004 EFSA (2006)	Liver enzyme induction (hypothesised) (Roques et al., 2013)
Fenpyrazamine	Follicular cell hypertrophy	4.25	12.7	2-year rat ▮▮▮	Source: DAR 2011 EFSA Scientific Committee (2012)	Liver enzyme induction (hypothesised) (ECHA, 2012c)
Flazasulfuron	Increased relative thyroid weight	50	250	13-week dog ▮▮▮	Source: DAR 1999	Unknown
Flubendiamide	Follicular cell hypertrophy	2.2	44	2-year rat ▮▮▮	Source: DAR 2008, JMPR 2010 EFSA (2013) 1-year and 2-year rat studies ▮▮▮ combined.	Liver enzyme induction (hypothesised) (EFSA, 2013)
Flufenacet	Increased relative thyroid weight	1.2	19	2-year rat ▮▮▮	Source: DAR 1998	Liver enzyme induction (hypothesised) (Christenson et al., 1996)
Flumioxazin	Increased (relative) thyroid weight	19.3	90	90-day rat ▮▮▮	Source: DAR 1998	Unknown
Fluopicolide	Follicular cell hypertrophy/ hyperplasia	32	109	2-year rat ▮▮▮	Source: DAR 2005	Liver enzyme induction (hypothesised) (EFSA, 2009)
Fluopyram	Follicular cell hypertrophy	1.2	6	2-year rat ▮▮▮	Source: DAR 2012, JMPR 2010 EFSA (2013)	Liver enzyme induction (hypothesised) (Rouquié et al., 2014)
Fluoxastrobin	Increased relative thyroid weight	0.7	1.5	1-year dog ▮▮▮	Source: DAR 2003 EFSA (2005)	Liver enzyme induction (hypothesised) (PubChem Compound Database, 2019)
Fluquinconazole	Follicular cell hypertrophy/ hyperplasia, adenocarcinoma	0.44	4.77	104-week rat ▮▮▮	Source: DAR 2005 EFSA (2011)	Liver enzyme induction (hypothesised) (EFSA, 2011)

Active substance	Indicator of specific effect	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Study	Remark	MoA
Flutolanil	Increased relative thyroid weight	37	299	90-day rat	Source: DAR 2005	Altered gene expression (Teng et al., 2018)
Fluxapyroxad	Increased relative thyroid weight	2.7	14	1-year study rat	Source: DAR 2011, JMPR 2012 EFSA Scientific Committee (2012)	Liver enzyme induction (hypothesised) (US EPA, 2012)
Folpet	Follicular cell hyperplasia	68.4	228	2-year rat	Source: DAR 2003	Unknown
Fuberidazole	Increased relative thyroid weight	3.6	18	1-year dog	Source: DAR 2005 EFSA (2008)	Unknown
Haloxyfop-P (Haloxyfop-R)	Follicular cell hypertrophy/increased relative thyroid weight	2	5	13-week dog	Source: DAR 2004 EFSA (2009)	Unknown
Heptachlor	Follicular cell neoplasia	1.3	2.6	18-month rat	Source: EHC 1984 JMPR evaluations 1991 considered.	TPO inhibition (Song et al., 2012)
Hymexazol	Follicular cell hyperplasia	98	292	13-week dog	Source: DAR 2007 EFSA (2010)	Unknown
Imazosulfuron	Follicular cell hypertrophy	75	150	1-year dog	Source: DAR 1998 EFSA (2007)	Unknown
Ioxynil	Follicular cell adenoma	0.5	1.5	2-year rat	Source: DAR 2000 EC review report (2004) considered	Interference with transthyretin and with cellular TH-signalling pathway (Akiyoshi et al., 2012). Interfere with T3-induced activation of T3-response genes (Otsuka et al., 2014)
Isoxaflutole	Follicular cell hyperplasia/follicular cell adenoma	2	20	2-year rat	Source: DAR 1997, SCP opinion 1999	Liver enzyme induction (hypothesised) (JMPR, 2013)
Lufenuron	Follicular cell hypertrophy/ hyperplasia	0.4	4	1-year dog	Source: DAR 2006 EFSA (2009) Not true NOAEL, but LOAEL divided by 10.	Unknown
Maleic hydrazide	Follicular cell hypertrophy/ hyperplasia	63	625	1-year dog	Source: DAR 1997	Unknown

Active substance	Indicator of specific effect	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Study	Remark	MoA
Mancozeb	Follicular cell adenoma and carcinoma	4.8	37.5	2-year rat ██████	Source: DAR 2000 JMPR 1993 evaluations for mancozeb considered	TPO inhibition by metabolite ETU (Hurley, 1998). Decreased iodine uptake (Axelstad et al., 2011)
Maneb	Follicular cell hyperplasia	5	24	90-day rat ██████	Source: DAR 2000 JMPR evaluations on maneb (1993) considered	TPO inhibition by metabolite ETU (US EPA, 2001)
MCPA (metabolite of MCPB)	Increased thyroid weight, follicular cell hyperplasia	0.75	3.75	1-year dog ██████	Source: DAR 2001	Unknown
Meptyldinocap	Follicular cell hypertrophy	11.4	44.3	1-year rat ██████████	Source: DAR 2012 EFSA (2014)	Unknown
Metam (incl. -potassium and -sodium)	Increased relative thyroid weight	13.03	29.03	2-year mouse ████████	Source: DAR 2007	Unknown
Metiram	Follicular cell hyperplasia	2.6	29.9	1-year dog ██████	Source: DAR 2000 JMPR evaluations on metiram (1993) considered	TPO inhibition
Metribuzin	follicular cell hyperplasia	1.3	13.8	2-year rat ████████	Source: DAR 2004 EFSA (2006)	Liver enzyme induction (hypothesised) (Syngenta, 2009)
Myclobutanil	Increased relative thyroid weight	15	51.5	90-day rat ██████	Source: DAR 2005	Liver enzyme induction (hypothesised) (Martin et al., 2007)
Orthosulfamuron	Increased relative thyroid weight/ follicular cell adenoma	5	500	2-year rat ██████	Source: DAR 2011 EFSA (2014)	Liver enzyme induction (hypothesised) (EFSA, 2014)
Oryzalin	Follicular cell hyperplasia/adenoma/ carcinoma	36	111	24-month rat ████████	Source: DAR 2007 EFSA (2010)	Unknown
Oxadiazon	Follicular cell hypertrophy	17.8	62.1	90-day rat ██████████	Source: DAR 2006 EFSA (2010)	Unknown
Oxyfluorfen	Increased relative thyroid weight, follicular cell adenoma	1.94	57	104-week rat ████████	Source: DAR 2006	Unknown

Active substance	Indicator of specific effect	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Study	Remark	MoA
Pendimethalin	Increased relative thyroid weight, follicular cell hypertrophy	43	88	2-year rat ███ ███	Source: DAR 1998 EFSA PPR Panel (2016) 90-day rat ███████ and 2-year rat ███████████ studies combined	Liver enzyme induction (hypothesised) (Hurley, 1998)
Penflufen	Follicular cell hypertrophy	38	425	1-year dog ███ ███	Source: DAR 2011 EFSA Scientific Committee (2012)	Liver enzyme induction (hypothesised) (ECHA, 2017)
Penthiopyrad	Follicular cell hypertrophy	60	200	18-month mouse █████████	Source: DAR 2012 EFSA (2013) Combination of all rat studies leads to a NOAEL of 83 mg/kg bw for thyroid effects in rats	Liver enzyme induction (hypothesised) (JMPR, 2011)
Pethoxamid	Follicular cell hyperplasia/follicular cell adenoma	17	70	2-year rat █████████	Source: DAR 2002 EFSA Scientific Committee (2017)	Liver enzyme induction (hypothesised) (EFSA Scientific Committee, 2017)
Phoxim	Increased relative thyroid weight	4	12	90-day rat █████ █████	Source: JECFA 1999	Unknown
Picolinafen	Increased relative thyroid weight, follicular cell hypertrophy/hyperplasia	3.75	37.5	1-year dog ████ ████	Source: DAR 2000 All dog studies combined.	Unknown
Propaquizafop	Follicular cell hypertrophy	50	75	2-year rat ████ ████	Source: DAR 2005	Unknown
Propineb	Increased thyroid weight	0.74	4.5	9-week rat █████████	Source: DAR 1996 EFSA PPR Panel (2016) and JMPR evaluations (1993) considered Supported by the NOAEL of 0.5 mg/kg for increased thyroid weight from the 2-year study rat	TPO inhibition by the metabolite PTU (EFSA PPR Panel, 2016)
Propyzamide	Follicular cell hyperplasia and adenoma	8.5	42.6	2-year rat study ███████	Source: DAR 1998 EFSA PPR Panel (2016) 15-week rat ██████████ and 2-year rat studies ███████ combined.	Liver enzyme induction (hypothesised) (US EPA, 2016)

Active substance	Indicator of specific effect	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Study	Remark	MoA
Proquinazid	Follicular hypertrophy/ follicular hyperplasia	1.2	12	2-year rat ▮▮ ▮▮	Source: DAR 2006 EFSA (2009)	Liver enzyme induction (hypothesised) (Norwegian Food Safety Authority, 2012)
Prothioconazole	Uncharacterised because only changes to thyroid hormone levels were observed				Source: DAR 2004	Liver enzyme induction (hypothesised) (EFSA, 2007)
PTU (propylenethiourea)		0.5	5	Long-term thyroid function study ▮▮▮▮	Source: JMPR 1993 Metabolite of propineb	TPO inhibition
Pymetrozine	Follicular cell hyperplasia	3.7	39.3	2-year rat ▮▮▮▮	Source: DAR 1998 EFSA (2014)	Liver enzyme induction (hypothesised) (EFSA, 2014)
Pyrethrins	Follicular cell hyperplasia and adenoma	4.37	42.9	2-year rat ▮▮▮▮	Source: DAR 2002 EFSA Scientific Committee (2012)	Liver enzyme induction (hypothesised) (JMPR, 2003)
Pyridalyl	Increased relative thyroid weight	47.4	133	90-day rat ▮▮ ▮	Source: DAR 2012	Unknown
Pyridate	Uncharacterised because only changes to thyroid hormone levels were observed				Source: DAR 1996	Unknown
Pyrimethanil	Follicular cell hypertrophy/ hyperplasia	17	221	2-year rat ▮▮ ▮	Source: DAR 2004 EFSA (2006)	Liver enzyme induction (hypothesised) (JMPR, 2007)
Pyriofenone	Increased relative thyroid weight	185	321	1-year rat ▮▮	Source: DAR 2012	Liver enzyme induction (hypothesised) (ECHA, 2018)
Quinmerac	Increased relative thyroid weight, inflammation	95	417	1-year dog ▮▮▮	Source: DAR 2007	Liver enzyme induction (hypothesised) (JMPR, 2015)
Quinoclamine	Increased relative thyroid weight	2.9	38.3	104-week rat ▮▮▮	Source: DAR 2005	Unknown
Quintozene	Follicular cell hypertrophy and hyperplasia, follicular cell adenoma	1	150	2-year rat ▮▮▮	Source: DAR 1997 Not reviewed by EFSA. JMPR evaluation on quintozene (1995) considered.	Liver enzyme induction (hypothesised) (JMPR, 1995)
Quizalofop-P-tefuryl	Follicular cell hypertrophy	1.3	39.5	2-year rat ▮▮▮	Source: DAR 2007 EFSA (2008)	Liver enzyme induction (hypothesised) (EFSA, 2008)

Active substance	Indicator of specific effect	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Study	Remark	MoA
Sedaxane	Follicular cell hypertrophy	11	67	2-year rat ▮ ▮	Source: DAR 2012 EFSA (2013)	Liver enzyme induction (hypothesised) (ECHA, 2018)
Silthiofam	Follicular cell hyperplasia/adenoma/ carcinoma	50.5	149.8	2-year rat ▮ ▮	Source: DAR 2000 EFSA PPR Panel (2016)	Liver enzyme induction (hypothesised) (EFSA, 2016)
Spinosad	Increased relative thyroid weight	2.7	8.2	1-year dog ▮	Source: DAR 2001 EFSA Scientific Committee (2018)	Unknown
Spirodiclofen	Follicular cell hyperplasia	19.88	110.14	108-week rat ▮	Source: DAR 2004	Unknown
Spiromesifen	Follicular cell hypertrophy	6.5	15.9	1-year rat ▮ ▮	Source: DAR 2004 EFSA (2007)	Liver enzyme induction (hypothesised) (JMPR, 2016)
Sulfoxaflor	Increased relative thyroid weight	79.4	155	28-day rat ▮ ▮	Source: JMPR 2011	Liver enzyme induction (hypothesised) (JMPR, 2011)
Tembotrione	Follicular cell hyperplasia	0.79	8.3	2-year rat ▮ ▮	Source: DAR 2009 EFSA (2013) 90-day rat ▮ and 2-year rat ▮ studies combined	Unknown. Liver enzyme induction (hypothesised) (Antonenko et al., 2015)
Tepraloxydim	Increased relative thyroid weight	14	66	90-day dog ▮	Source: DAR 2002	Unknown
Terbuthylazine	Follicular cell hyperplasia	6.97	41.5	24-month rat ▮	Source: DAR 2007	Liver enzyme induction (hypothesised) (Ghisari et al., 2015)
Tetraconazole	Follicular cell hypertrophy	1	8.3	28-day rat ▮ ▮	Source: DAR 2005 EFSA (2008)	Liver enzyme induction (hypothesised) (EFSA, 2008)
Thiabendazole	Follicular cell hypertrophy/ hyperplasia, follicular cell adenoma	10	30	90-day rat ▮ 2-year rat ▮	Source: DAR 1996 EFSA (2014)	Liver enzyme induction (hypothesised) (EMEA, 2004)
Thiacloprid	Follicular cell hypertrophy	1.2	2.5	2-year rat ▮	Source: DAR 2000	Liver enzyme induction (hypothesised) (JMPR, 2006)
Thiamethoxam	Follicular cell hypertrophy	198.6	710.6	28-day rat ▮	Source: DAR 2002	Liver enzyme induction (hypothesised) (Rose, 2012)

Active substance	Indicator of specific effect	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Study	Remark	MoA
Thiencarbazone	Increased relative thyroid weight	149	335	90-day dog	Source: DAR 2012	Unknown
Thiophanate-methyl	Increased relative thyroid weight, follicular cell hypertrophy/ hyperplasia	8	40	1-year dog , 2-year rat	Source: DAR 1997 EFSA Scientific Committee (2018)	Liver enzyme induction (hypothesised) (JMPR, 2017), TPO inhibition (hypothesised) (EFSA Scientific Committee, 2018)
Tolylfluanid	Increased relative thyroid weight	33	93	90-day dog	Source: DAR 2003 EFSA (2005)	TPO inhibition (US EPA, 2002)
Topramezone	Follicular cell hypertrophy/ hyperplasia, follicular cell adenoma	0.4	3.6	2-year rat	Source: DAR 2009 EFSA (2014)	Liver enzyme induction (hypothesised) (EFSA, 2014)
Triadimefon	follicular cell adenoma	16.4	114	2-year rat	Source: JMPR 2004	Liver enzyme induction (hypothesised) (Wolf et al., 2006)
Trifluralin	Increased relative thyroid weight	3.0	30	2-year rat	Source: DAR 2003 The NOAEL is not a true NOAEL, but the LOAEL divided by 10	Liver enzyme induction (hypothesised) (Saghir et al., 2008)
Tritosulfuron	Increased relative thyroid weight	92	287	90-day dog	Source: DAR 2002	Unknown
Valifenalate (formerly valiphenal)	Increased relative thyroid weight	7	50	1-year dog	Source: DAR 2012 EFSA (2013)	Unknown
Vinclozolin	Increased relative thyroid weight	4.9	48.7	1-year dog	Source: DAR 1997	Liver enzyme induction (hypothesised) (Matsuura et al., 2005)
Ziram	Follicular cell hypertrophy	0.56	5.5	2-year rat	Source: DAR 1998, addendum 2003 EC review report on ziram (2004) considered.	TPO inhibition (Marinovich et al., 1997)
Zoxamide	Increased relative thyroid weight	278	994	1-year dog	Source: DAR 2001	Unknown

CAG: cumulative assessment group; AS: active substance; NOAEL: no observed adverse effect level; LOAEL: lowest observed adverse effect level; bw: body weight; TPO: thyroperoxidase; NIS: sodium iodide symporter; TTR: transthyretin; DIO: iodothyronine deiodinase; TR: thyroid receptor; ETU: ethylenethiourea.

Table B.2: CAG on C-cell hypertrophy, hyperplasia and neoplasia: toxicological characterisation of ASs to be considered in long-term cumulative exposure/risk assessments

Active substance	Indicator of specific effect	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Study	Remark	MoA
2,4-D	C-cell hyperplasia	75	150	2-year rat ▮▮▮▮▮	Source: DAR 1997 and addendum	Unknown
2,4-DB	C-cell hyperplasia, C-cell carcinoma	30	90	2-year rat ▮▮▮▮▮	Source: DAR 1996	Unknown
Amitrole	C-cell hyperplasia	0.3	13	1-year dog, ▮▮▮▮	Source: DAR 1996	Unknown
Buprofezin	C-cell hyperplasia	8.7	89.5	2-year rat ▮▮▮▮	Source: DAR 2005	Unknown
Desmedipham	C-cell hyperplasia	3.18	15.71	2-year rat ▮▮▮	Source: DAR 2004	Unknown
Fenamidone	C-cell hyperplasia	2.8	7.1	2-year rat ▮▮▮	Source: DAR 2000	Unknown
Fenbuconazole	C-cell adenoma	3	30	24-month rat ▮▮▮ and 104-rat ▮▮▮	Source: DAR 2005	Unknown
Flazasulfuron	C-cell hyperplasia	2	19	2-month dog ▮▮▮▮	Source: DAR 1999	Unknown
Folpet	C-cell hyperplasia	1800	4000	90-day dog ▮▮▮	Source: DAR 2003	Unknown
Hexythiazox	C-cell adenoma	29.3	207	24-month rat ▮▮▮	Source: DAR 2006 EFSA	Unknown
Imidacloprid	C-cell hyperplasia	51.3	102.6	24-month rat ▮▮▮	Source: DAR 2005	Unknown
Ioxynil	C-cell neoplasia	0.6	1.8	2-year rat ▮▮▮	Source: DAR 2000	Unknown
Ipconazole	C-cell hyperplasia	1.5	5	1-year dog ▮▮▮	Source: DAR 2011	Unknown
Oryzalin	C-cell adenoma	12	36	24-month rat ▮▮▮	Source: DAR 2007	Unknown
Oxyfluorfen	C-cell adenoma and carcinoma	1.94	57	2-year rat ▮▮▮▮	Source: DAR 2006	Unknown
Thiram	C-cell hyperplasia	1.5	7.5	2-year rat ▮▮ ▮▮	Source: DAR 1997 EFSA Scientific Committee (2017)	Unknown
Ziram	C-cell hyperplasia	7.7	23.7	2-year rat ▮▮▮	Source: DAR 1998 JMPR evaluation of Ziram (1996) considered	Unknown

CAG: cumulative assessment group; AS: active substance; NOAEL: no observed adverse effect level; LOAEL: lowest observed adverse effect level; bw: body weight.

Appendix C – Hypothyroidism: uncertainty question 1

Appendix C can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2019.5801