Establishment of cumulative assessment groups of pesticides for their effects on the nervous system

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Abstract
Cumulative assessment groups of pesticides have been established for five effects on the nervous system: brain and/or erythrocyte acetylcholinesterase inhibition, functional alterations of the motor, sensory and autonomic divisions, and histological neuropathological changes in neural tissue. 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 nervous system, 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 nervous system, five 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 PPR Panel, 2013a). These specific effects were brain and/or erythrocyte acetylcholinesterase (AChE) inhibition, functional alterations of three divisions of the nervous system (motor, sensory and autonomic functions) and histological neuropathological changes in neural tissues. There was insufficient information to address the combined effects of pesticides with respect to developmental neurotoxicity and cognitive effects.

A cumulative assessment group (CAG) was established for each of the five specific effects and more than 400 active substances (AS) were screened for potential inclusion in these CAGs. Any AS possessing a chemical structure associated to a mode of action (MoA) of direct relevance for the effect or exhibiting selected indicators (toxicological endpoints) reflecting the specific effect in regulatory toxicological studies was included in the respective CAG.

In total, 47 ASs were included in the CAG for brain and/or erythrocyte AChE inhibition. They were 119 to be included in the CAG for functional alterations of the motor division, while the CAGs for functional alterations of the sensory and autonomic divisions both contained 101 ASs. The CAG for histological neuropathological changes in neural tissues contained 19 ASs only. All ASs included in the CAGs were characterised by no observed adverse effect levels (NOAELs) for short- and 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, it would be sufficient to perform CRAs with the CAGs for brain and/or erythrocyte AChE inhibition and for functional alterations of the motor division to cover the combined effects of pesticides associated with all five CAGs.

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 intraspecies differences in toxicological sensitivity.

With respect to the composition of the CAGs, the uncertainty about the total number of ASs in the CAG for functional alterations of the motor division 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 104 was derived for the number of ASs actually causing functional alteration of the motor division. A similar exercise was not conducted with the CAG for brain and/or erythrocyte AChE inhibition because the association between the chemical structure and the MoA is obvious for organophosphorus and N-methyl carbamate insecticides.

A mechanism for periodic update of the CAGs established in the present report should be put in place by EFSA in order to make use of relevant new information. It is also recommended to deploy a testing and assessment strategy to provide enough information supporting the establishment of CAGs covering developmental toxicity and, 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 cumulative dietary exposure assessment to pesticides that have acute effects on the nervous system using SAS® software (EFSA, 2019a), the RIVM scientific report on cumulative dietary exposure assessment of pesticides that have acute effects on the nervous system using MCRA software (van Klaveren et al., 2019) and the EFSA scientific report on the cumulative dietary risk characterisation of pesticides that have acute effects on the nervous system (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 provides 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 provides 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 the Authority 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 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 cumulative risk assessment (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 nervous system.

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 nervous system, using the results of official controls conducted by Member States under the annual monitoring programmes foreseen by Regulation (EC) No 396/2005.

These assessments will use the CAGs established in the present report and will be presented in a separate EFSA scientific report which will deal with the following assessment questions:

- What is the acute cumulative risk of brain and/or erythrocyte acetylcholinesterase (AChE) inhibition resulting from combined dietary exposure to pesticide residues?
- What is the acute cumulative risk of functional alteration of the motor division of the nervous system (e.g. locomotor activity, muscle strength, coordination and equilibrium) resulting from combined dietary exposure to pesticide residues?

These CRAs will be conducted under the assumption of dose addition (EFSA, 2008). In 2015, European Commission 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 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 element 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, as the forthcoming CRAs will be performed using exclusively the active substances (ASs) included in the CAGs and following the dose-addition model, an uncertainty analysis will be conducted in order to appreciate how using the CAGs as established in this report may under- or overestimate the actual risk to consumers, as formulated in the above assessment questions. To prepare for this, this report will consider the following question:
How sure is it that the CAG contains all the ASs causing the specific effect and only ASs causing this effect?

How sure is it 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

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

The first of these data collections (RIVM, ICPS, ANSES, 2013) was outsourced to a consortium of the Dutch 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 and Safety (ANSES). It covered the ASs approved until 31 May 2009 and identified as having effects on the nervous system by the Danish Technical University (DTU), under an earlier grant awarded by EFSA (Nielsen et al., 2012), and all ASs approved between 1 June 2009 and 31 December 2011. This data collection was used by the PPR Panel to establish a first proposals of CAGs in 2013 (EFSA PPR Panel, 2013a). The sources of this data collection were official documents produced during the approval of ASs under Directive 91/414/EEC and Regulation (EC) No 1107/2009: Draft Assessment Reports (DARs), Renewal Assessment Reports (RARs) as well as the respective Addenda, peer review experts’ meeting reports, EFSA conclusions and European Commission review reports. Original study reports submitted by applicants during the peer review process were also occasionally consulted when the sources of information were insufficiently detailed. Additionally, Joint Meeting on Pesticide Residues (JMPR) evaluation reports or open literature (e.g. PubMed) were also searched for additional information on modes of action (MoAs). All available acute and repeated dose in vivo toxicological studies performed by oral administration in mammals were considered. In vitro studies were used when they provided information on known or presumed neurotoxic MoAs. For each AS covered by this data collection, the main principles followed by the contractor were as follows:

- Both acute and repeated dose effects were reported.
- For each endpoint related to neurotoxicity, only the no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) observed in the most sensitive species and most sensitive sex were collected. In case the NOAELs in two different species were almost identical, the NOAELs and LOAELs for both species were recorded. Higher NOAELs and LOAELs observed in additional studies were not recorded. Comparable studies with respect to study design and strain of animals were combined to derive overall NOAELs and LOAELs.
- Observations indicative of neurotoxicity observed at (near) lethal doses were not collected when the reviewer attributed these effects to general toxicity.
- In case in a single study several critical endpoints were observed at the same NOAEL/LOAEL, they were recorded separately.
- Human data were always collected, even if the NOAELs/LOAELs for a certain endpoint were higher than those obtained in animal studies.
- Effects on AChE were reported when the inhibition was statistically significant (p < 0.05) and reached at least 20% decrease of the control level.
- Collected NOAELs which were lower than the NOAELs that formed the basis for the acceptable daily intake (ADI) or acute reference dose (ARfD) were flagged.

The second data collection was outsourced to the same consortium (RIVM, ICPS, ANSES, 2016). It covered all ASs approved after 1 January 2012 and until 31 May 2013, a number of new ASs not yet approved but pending approval at that time, and an additional list of non-approved ASs present in the European Union (EU) consumer’s diet as evidenced 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). The sources of this data collection were official documents produced during the approval of ASs under Regulation (EC) No 1107/2009: DARs, RARs as well as the respective addenda, peer review expert meeting reports, EFSA conclusions and European Commission review reports. If necessary, original study reports were consulted for more details. When a European evaluation was not available or was outdated, assessment reports from recognised international bodies (e.g. JMPR, United
States Environmental Protection Agency (US-EPA) 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 MoAs. In contrast with the first data collection, the
second data collection was organised in accordance with the specific effects identified for the nervous
system 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.
- NOAELs/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
  indicator(s) has 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 (ADI/ARfD).
- Cases where age-related changes were not clearly separated from treatment-related effects
  were flagged.
- Information on statistical significance tests regarding observations in the studies was collected.
- Any limitation which could have had an impact on the acceptability of the study and the
  evaluation/occurrence of the specific effect was flagged.

In addition, EFSA conducted an internal complementary data collection to consolidate the
information regarding 24 ASs, following comparable principles.

All the details of the data collections can be found in the respective external scientific report (RIVM,
ICPS, ANSES, 2013, 2016) and the resulting data collection spreadsheets. The collected information
slightly evolved over time based on the growing experience about the exact information needed to
establish CAGs. It is acknowledged that the most recent data collection was performed with higher quality
standards and that some relevant information might have been omitted in the previous data collections.

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, 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, this step consisted
in identifying those which should be considered in CRA. Such effects, which can result from a
combined action of pesticides, were 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, were
  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 were therefore also not considered as relevant for CRA. In
discriminating between an adverse and a non-adverse effect, consideration was given to its
adaptive nature, its transient or persistent nature, its magnitude, its association with other
alterations, whether it was 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 were not relevant for cumulative risk assessment.

Evaluating the unambiguous nature of the effect: A specific effect needed 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 descriptors/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 on the basis of 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. If this was not the case, this had to be clearly highlighted.

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 enough 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 3.3.3.

However, it needs to be highlighted that any of the ASs of a CAG can be used as an 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 MOET:

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

$$\frac{1}{\text{MOET}} = \frac{1}{\text{MOE}_1} + \frac{1}{\text{MOE}_2} + \frac{1}{\text{MOE}_3} + \ldots + \frac{1}{\text{MOE}_n},$$

where \( \text{MOE}_i \) is the margin of exposure for the \( i \)th chemical,

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

Indirectly, 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) for each chemical.

\( \text{RPF}_i = \frac{\text{RF}_{IC}}{\text{RF}_i} \), where \( \text{RF}_{IC} \) and \( \text{RF}_i \) are the reference points for the IC and chemical \( i \),

\( \text{MOET} = \sum E_i \times \text{RPF}_i \), where the denominator sums over all chemicals including the IC.

The present report was elaborated in such a way to make both options possible. In particular, it includes 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:

$$\frac{1}{\text{MOET}} = \sum E_i \times \frac{\text{RF}_{IC}}{\text{RF}_i},$$

inverting the previous equation and substituting for \( \text{RF}_i \)

$$\frac{1}{\text{MOET}} = \sum E_i \times \frac{\text{RF}_{IC}}{\text{RF}_i},$$

cancelling out \( \text{RF}_{IC} \) in numerator and denominator

So: $$\frac{1}{\text{MOET}} = \sum E_i \times \frac{\text{RF}_i}{\text{MOE}_i} = \frac{1}{\text{MOE}_1} + \frac{1}{\text{MOE}_2} + \frac{1}{\text{MOE}_3} + \ldots + \frac{1}{\text{MOE}_n},$$
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 $R_{fPIC}$, e.g. through choosing a different IC or errors in the $R_{fP}$ 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 et al., 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 causing the specific effect, the results of the assessment will tend to underestimate the risk. If, in contrast, it includes ASs not causing 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 to assess the cumulative effects of pesticides eliciting the same adverse effect by different MoAs. Similarly, the ESFA Scientific Committee recommends adoption of the mixture assessment concept of dose addition as a pragmatic and precautionary default assumption, unless there are indications that the alternative concept of response addition is more appropriate (EFSA Scientific Committee, 2019).

For the CAGs subject to a CRA as indicated in Section 1.2 (effects on motor division and brain and/or erythrocyte AChE inhibition), Question 1 was addressed in the present report using a combination of weight of evidence and expert knowledge elicitation (EKE) techniques, described in the following section. With respect to Question 2, this report reviewed the available information regarding the MoAs leading to these effects, but a full assessment, relying on expert judgement, was only possible during the respective CRAs (EFSA, 2019b) after identification of the ASs driving the risks.

For the other CAGs (functional alteration of the sensory and autonomic functions, histological neuropathological changes in neural tissue), a similar exercise was not done, because these effects are less critical than functional effects on the motor division in terms of cumulative risks (see Section 3.3.3).

### 2.2.5. Weight of evidence and expert knowledge elicitation technique

The amount, reliability, relevance and consistency of evidence for causing effects on the nervous system vary between ASs. This makes it uncertain which substances should be included in a given CAG, with some substances being more likely to belong to a certain CAG 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 causes the effect. This was done by developing a structured procedure which combines techniques for weight of evidence assessment (EFSA Scientific Committee, 2017) and EKE (EFSA, 2014). This procedure comprised the following sequence of tasks:
1) Defining in precise terms the specific effect that is to be assessed.

2) Identifying lines of evidence that were important for assessing whether the AS causes the effect: lines of evidence typically included the indicators as defined in Section 2.2.2 but were not necessarily restricted to these indicators. Depending on the specific effect, additional factors contributing to the evidence could be defined.

3) Rating the weight of each line of evidence: the lines of evidence were assessed with respect to their reliability and relevance to the assessment question. This assessment was conducted by expert discussion and resulted in the allocation of a coefficient or weight to each line of evidence, varying from 1 to 10 and which was a relative measure of the contribution that positive findings for each line of evidence results in an increase of 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 gave 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 (see Annex A), 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 EKE procedure, using a modified version of the ‘Sheffield’ EKE protocol described by EFSA (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, adjust.

8) When developing the consensus distribution for some of the more diverse subgroups, the experts found it helpful first to divide the subgroup into subsets of substances for which the probability of causing functional alterations of the motor division was thought to be similar, express those probabilities on an approximate scale, and then use this to inform their collective judgement on the consensus distribution for the subgroup as a whole.

9) 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 substances 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.

The results of this procedure comprised (a) a probability distribution for the number of substances in each subgroup that cause the specific effect, each with accompanying rationale, and (b) two probability distributions for the total number of substances 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 (EFSA, 2019b).
3. Assessment

3.1. Identification of the specific effects

On the basis of the results of the project commissioned by EFSA to DTU (Nielsen et al., 2012) and of the first data collection performed by the consortium RIVM/ICPS/ANSES (RIVM, ICPS, ANSES, 2013), the PPR Panel identified five specific effects of pesticides on the nervous system (EFSA PPR Panel, 2013a), which were confirmed as follows:

- Functional alteration of the motor division of the nervous system (e.g. locomotor activity, muscle strength, coordination and equilibrium).
- Functional alteration of the sensory division of the nervous system (e.g. reflex action, sensory motor responses).
- Functional alteration of the autonomic division of the nervous system (i.e. modulation of the autonomic activity by cholinergic neurons).
- Brain and/or erythrocyte AChE inhibition (neurochemical effect).
- Histological neuropathological changes in neural tissue (e.g. axonal degeneration and demyelination).

The rationale behind the identification of these effects by the PPR Panel was given in details in the Scientific Opinion. It considered the high complexity of the nervous system with respect to its anatomic organisation and variety of physiological functions.

With respect to the criteria listed in Section 2.2.1, all specific effects result from systemic exposure, are adverse, relevant for humans, specific and can be observed as primary effects.

The functional alterations of the motor, sensory and autonomic divisions of the nervous system are phenomenological effects considered as relevant for CRA, because, although of variable nature at biochemical level, they concern very specific and specialised functions of the organism.

AChE inhibition is quantified by objective instrumental measurements, directly related to the MoA. Therefore, the unambiguosity of the nature of this effect is strongly demonstrated.

AChE inhibition and functional alterations of the motor, sensory and autonomic divisions of the nervous system, by their nature, can be triggered by acute and chronic exposures.

Neuropathological effects are unambiguously associated to histopathological observations, which can also be of variable nature, although consisting in most cases of axonal and myelin degeneration. As they commonly result in the alteration of the specialised function of nervous cells, they are also considered as specific effects justifying the establishment of a CAG. Owing to its nature, this specific effect is triggered by chronic exposures only.

Interdependencies

There are interdependencies between these specific effects because they are associated to common MoAs. For instance, some pesticide targets (e.g. neurotransmitter receptors, ion channels) are expressed in some but not all neurons. These targets can be found in the central nervous system or in the peripheral nervous system, or in both. However, given the tremendous complexity of the nervous system and the huge number of different tasks it performs, neurons play different roles, and thus they can be divided into three classes: sensory neurons, motor neurons, and interneurons (which connect one neuron to another). Interneurons are thus involved in processing information, both in simple reflex circuits (consisting of only a few neurons) and in more complex circuits in the brain (which involve more complex neuronal networks, i.e. integrated nervous pathways). For these reasons, the major pesticide classes with known MoA can impair the motor, sensory and autonomic function despite interacting with different neuronal targets. Hence, pesticides targeting gamma-aminobutyric acid (GABA) receptors in interneurons can induce either motor or sensory signs of toxicity because of the physiological role of these neurons in the nervous system. This is reflected in the data which show that many ASs with different MoAs affect all 3 functional divisions of the nervous system: motor, sensory and autonomic (see Section 3.3.1).

AChE inhibition is obviously an important mechanism of neurotoxicity leading to functional alterations observed in the motor, sensory and autonomic divisions of the nervous system. This interdependency should however not suggest that some assessments might be seen as refinements of other assessments in the usual sense of this word when used in regulatory pesticide risk assessment. Indeed, the risk assessments which will be conducted with respect to these effects will each address a specific assessment question, e.g. 'What is the risk of functional alteration of the motor division of the...
nervous system resulting from the exposure to pesticide residues? or ‘What is the risk of cholinesterase inhibition resulting from the exposure to pesticide residues?’ These questions are different and need to be addressed through individual assessments.

**Effects not leading to CAGs**

Based on the information collected by DTU (Nielsen et al., 2012), a number of reported effects of pesticides on the nervous system were not considered as relevant for CRA, because they were either not adverse, observed at doses above the maximum tolerated dose (MTD) (e.g. vacuoles in brain) or not statistically significant and within the historical control range (i.e. induction of neoplasm, particularly observed astrocytoma).

Neurochemical effects of pesticides on the nervous system, other than AChE inhibition, were not proposed as specific effects because they are not directly measured in regulatory studies.

Owing to the lack of specific requirements for developmental neurotoxicity (DNT) testing of pesticides in EU at the time of the Scientific Opinion of the Panel (EFSA PPR Panel, 2013a), the number of studies of this type available to the panel was not enough to propose specific effects and CAGs with respect to this type of toxicity. Therefore, DNT was not addressed in this report.

Similarly, behavioural tests assessing the effects of pesticides on the cognitive function (e.g., learning and memory) are often used as a higher tier of neurotoxicity evaluation. Because of the scarcity of information related to this type of test in the data collection, the effects of pesticides on this function were not addressed in this report.

### 3.2. Characterisation of the specific effects

All indicators of effects of pesticides on the nervous system were reviewed in view of characterising the five specific effects. In this process, several observations (such as prostration, opisthotonus, laboured breathing, tachypnoea, dyspnoea, exophthalmos, lethargy, coma, hypothermia, emesis and alopecia) were found as often occurring secondary to general systemic toxicity after high doses. Accordingly, they were not deemed appropriate observations to characterise any of the five specific effects.

#### 3.2.1. Functional alteration of the motor division of the nervous system

The specific indicators of toxicity observable in toxicological studies contributing to the evidence that an AS causes a functional alteration of the motor division of the nervous system were classified in four categories:

- Reduced motor activity: hypoactivity, recumbency (if not observed in isolation), etc.
- Increased motor activity: tremor, choreoathetosis, hyperactivity, convulsions, etc.
- Alteration of muscle strength: reduced grip strength, increased or decreased muscle tone, muscle fasciculation, weakness, ptosis, inability to stand, paresis, paralysis, etc.
- Coordination: ataxia, abnormal gait, landing foot splay, etc.

In this list of indicators, ‘etc.’ is to be understood as covering synonyms of the indicators listed, but not as an indication that additional indicators were envisaged. For instance, ‘hunched position/posture’, ‘lateral posture’ and ‘curved body position’ are considered as synonyms of ‘recumbency’.

Recumbency was considered as an indicator of functional alteration of the motor division when other indicators were also present, or, when this was not the case, if the AS had a chemical structure known to be capable to induce this specific effect.

Reduced and increased motor activity, although looking at first sight as opposite effects, were considered as two equally valid manifestations of the specific effect. These two types of effects are indeed regularly observed with the same AS.

#### 3.2.2. Functional alteration of the sensory division of the nervous system

The specific indicators of toxicity observable in toxicological studies contributing to the evidence that an AS causes a functional alteration of the sensory division of the nervous system were classified in three categories:

- Decreased reactivity: hyporeactivity, righting reflex (air drop), touch response (handling reactivity), approach response, pupil response, tail pinch response, analgesic reflex (nociception response), patellar reflex, etc.
- Increased reactivity: hyperreactivity, exaggerated auditory response (startle reflex), etc.
• Proprioception and sensory deficits: proprioception deficit, paraesthesia (except for pyrethrins and pyrethroids, where it can be considered as a local effect in studies with administration through the diet), hyperaesthesia, etc.

In this list of indicators, ‘etc.’ is to be understood as covering synonyms of the indicators given, but not as an indication that additional indicators are envisaged.

3.2.3. Functional alteration of the autonomic division of the nervous system

The specific indicators of toxicity observable in toxicological studies contributing to the evidence that an AS causes a functional alteration of the autonomic division of the nervous system were:

• miosis
• mydriasis
• increased salivation
• lacrimation
• piloerection
• urination

or any synonym of these indicators.

The combination of two or more autonomic signs of toxicity provides a stronger support for a specific effect on the autonomic division. Salivation is considered an indicator of alteration of autonomic division, as a result of systemic exposure, and not a local effect, when other indicators are also present, or, when this is not the case, if the AS has a chemical structure known to be capable to induce this type of neurotoxicity.

Similarly, piloerection is considered as an indicator of functional alteration of the autonomic division when other indicators are also present, or, when this is not the case, if the AS has a chemical structure known to be capable to induce this specific effect.

3.2.4. Brain and/or erythrocyte AChE inhibition

This neurochemical effect is directly defined by its indicator. It was however considered relevant only when the inhibition leads to a statistically significant (p < 0.05) decrease of the AChE activity of 20% or more compared to concurrent control groups (JMPR, 1999; US Environmental Protection Agency, 2000).

3.2.5. Neuropathological effects

The specific indicators of toxicity observable in toxicological studies contributing to the evidence that an AS causes histologic neuropathological effects were:

• axonal degeneration (such as sciatic nerve axonopathy)
• myelin degeneration
• neuronal degeneration/necrosis.

It is known that the presence of certain artefacts in microscopic sections of tissues can result in misinterpretations leading to diagnostic pitfalls. For this reason, special care needs to be dedicated to the interpretation of histopathological findings by paying due consideration to observations in control animals and dose–response relationship.

Sciatic nerve axonopathy, without concurrent changes in motor neurons or spinal tracts, may be consistent with an increase of age-related effects due to systemic toxicity and diminished repair capacity of the nerve. Therefore, this indicator is rather considered to be of confirmatory nature when other evidence is available.

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

3.3.1. General provisions

Establishment of CAGs:

Based on the three data collections (RIVM, ICPS, ANSES, 2013, 2016; EFSA internal data collection) referred to in Section 2.1 and indicators listed in Section 3.2, CAGs were elaborated for the five specific effects of pesticides on the nervous system.
An AS was included in a CAG if it has a known MOA capable to induce directly the specific effect or if at least one of the respective indicators was observed at a statistically significant and/or biologically relevant level in at least one toxicological study with this AS and the study was assessed as ‘acceptable’ in the DAR, RAR or equivalent document, unless:

- This observation was age related or occurred at or above the MTD, or,
- Consideration of the dose-response relationship showed that the observation was not treatment related.¹

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. Data from reproductive toxicity studies were not considered for the establishment of CAGs since toxicological endpoints were not always clearly reported as pertaining to dams or pups. Teratogenicity studies were also disregarded as they do not adequately examine toxic effects on the nervous system.

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.

With respect to the functional alterations of the motor, sensory and autonomic divisions, it was considered appropriate to include ASs in CAGs on the sole basis of a known relevant MoA, even in the absence of factual observation of indicators of a specific effect in the available toxicological studies. This is because, if a pesticide affects a target site which is critical to nervous system function, then it can be expected that effects may occur in all three functional divisions due to the interdependencies discussed in Section 3.1. This is supported by the available evidence for most of the known neurotoxic MoA listed in Section 3.3.2. Appendix C shows the observed indicators of functional alterations of the three divisions of the nervous system for N-methyl carbamate insecticides, macrocyclic lactone insecticides, neonicotinoid insecticides, organophosphorus pesticides, organochlorine insecticides, phenylpyrazole insecticides, pyrethrin and pyrethroids ester insecticides, chlormequat, mepiquat, sulfoxaflor, indoxacarb and amitraz. Except for amitraz for which effects on the autonomic function are not observed, effects are always seen at varying intensities in the motor, sensory and autonomic divisions of the nervous system for all these chemical classes. Therefore, while assuming functional effect on the sole basis of a demonstrated MoA is a reasonable default approach, it may overestimate the risk in a few cases.

**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. NOAELs for short- and long-term cumulative exposure/risk assessments were derived for each AS from the most sensitive indicator, using all available information across studies, species and sexes.

All indicators listed in Section 3.2 were equally valid for the setting of NOAELs for specific effects.

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

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, was set at 10 in all cases.

Human studies reported in the spreadsheets 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.

For ASs included in the CAGs related to AChE inhibition and functional alterations of the motor, sensory and autonomic divisions, both acute and chronic NOAELs were established, as these effects can be triggered by both acute and chronic exposures.

In order to establish NOAELs for ASs with a known MoA regarding functional alterations of the motor, sensory and autonomic divisions, preference was given to neurotoxicity studies, unless dog or mouse was more sensitive than rats for these effects and studies in these species resulted in lower

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¹ In other words, dose-response relationship was established when the effect was observed in at least two 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.
NOAELs. When indicators of functional alteration of the nervous system were not observed at statistically significant level in neurotoxicity studies, the highest tested dose in these studies was defined as the NOAEL for the respective division. In the absence of acute neurotoxicity studies, available 28-day or 90-day neurotoxicity studies were used to set NOAELs for acute exposure/risk assessments. In the absence of 28-day or 90-day neurotoxicity studies, several options were considered to set NOAELs for chronic exposure/risk assessment, including the use of acute neurotoxicity studies (N-methyl carbamates), the use of other regulatory studies, the use of the NOAELs for AChE inhibition or the use of the NOAEL leading to the ADI. Similar options were considered to set NOAELs for acute exposure/risk assessment when neurotoxicity studies were totally missing. The impact of missing neurotoxicity studies will be addressed in the context of the overall uncertainty analysis (Second bullet point of Section 4.5 (overall uncertainty analysis)).

EFSA conclusions on 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 nervous system and to ensure consistency of the NOAELs proposed for the specific effect with the ADI and ARfD of the AS at the time of the preparation of the present report. 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. JMPR evaluations).

Selection of Index Compounds:

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 the 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

Two ICs were selected when a CAG is to be used for both acute and chronic exposure/risk assessments. This is the case for the CAGs related to the brain and/or erythrocyte AChE inhibition and to the functional alterations of the motor, sensory and autonomic divisions of the nervous system.

In subsequent CRAs using ICs, RPFs will 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. Chemical classes with known or presumed neurotoxic MoA

Based on knowledge of toxicological properties of pesticides and scientific peer-reviewed open literature, the following chemical classes and ASs were considered as having a known neurotoxic MoA in mammals:

a) N-methyl carbamate insecticides (reversible AChE inhibitors):
   - Benzofuranyl methylcarbamate: benfuracarb, carbofuran, carbosulfan;
   - Carbamate: carbaryl;
   - Dimethyl carbamate: pirimicarb;
   - Formamidine: formetanate (also agonist of the octopamine receptor in insects which is equivalent to the alpha-2 adrenergic receptor in mammals);
   - Oxime carbamate: aldicarb, methomyl, oxamyl, thiodicarb;
   - Phenyl methylcarbamate: methiocarb.

b) Macrocyclic lactone insecticides (GABA-gated chloride channel agonist):
   - Avermectins: abamectin, emamectin benzoate;
   - Milbemycin: milbemectin.

c) Neonicotinoid insecticides (agonist of nicotinic acetylcholine receptor (nAChR)): acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, thiamethoxam.

d) Organophosphorus pesticides (irreversible AChE inhibitors):
   - Organophosphates: chlorfenvinphos, dichlorvos, monocrotophos;
• Organothiophosphates: azinphos-ethyl, azinphos-methyl, cadusafos, chlorpyrifos, chlorpyrifos-methyl, diazinon, dimethoate, ethion, ethoprophos, fenitrothion, fenthion, fosfiazate, malathion, methidathion, omethoate, oxydemeton-methyl, parathion, parathion-methyl, phenthoate, phosalone, phosmet, phoxim, pirimiphos-methyl, profenofos, pyrazophos, triazophos;
• Phosphonates: trichlorfon;
• Phosphonothioates: fonofos;
• Phosphoramidates: fenamiphos;
• Phosphoramidothioates: acephate, methamidophos;
• Phosphonic acids: ethephon (growth regulator);

Although considered as a weak AChE inhibitor, the fungicide tolclofos-methyl has also a typical organophosphorus structure and is metabolically activated to the oxon moiety.

e) Organochlorine insecticides (GABA-gated chloride channel blockers):
• Cycloalkanes: lindane;
• Cycloalkenes: dieldrin, endrin, endosulfan (allocated to subgroup 5 in the EKE process), heptachlor.

f) Phenylpyrazole insecticides (GABA-gated chloride channel blockers): fipronil.

g) Pyrethrins and pyrethroid ester insecticides (bind to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting) state): acrinathrin, alpha-cypermethrin, beta-cyfluthrin, beta-cypermethrin, bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, fenvalerate, lambda-cyhalothrin, permethrin, tau-fluvalinate, tefluthrin, tetramethrin, zeta-cypermethrin.

h) Chloromequat and mepiquat are alkyl quaternary ammonium plant growth regulators. They are partial agonists of the nAChR. Chloromequat chloride showed in vitro a weak agonistic activity on muscarinic receptors, with potency 10^5 lower than for atropine, and also showed to be a partial agonist of the nAChR, with a potency of about 1% of that of acetylcholine (EFSA, 2009).

i) Sulfoxaflor is a sulfonamide insecticide. Toxicity and mechanistic studies in rats, rabbits, dogs and mice indicate that sulfoxaflor is an activator of the mammalian nAChR, but to a much lesser degree than in insects and in a species-specific manner.2

j) Indoxacarb belongs to a relatively new class of sodium channel blocker insecticides (SCBIs) with a MoA distinct from all other sodium channel-targeting insecticides, including pyrethroids, but similar to that of cationic local anaesthetics (Zhang et al., 2016).

k) Amitraz: formamidine acaricide, octopamine receptor agonist in insects which is equivalent to alpha-2 adrenergic receptor in mammals. Several effects of amitraz in mammals are mediated by its interaction with alpha-2 adrenoceptors (Costa et al., 1989).

All ASs falling under items a) to k) above have MoAs directly relevant for the functional alterations of the motor, sensory and autonomic divisions of the nervous system and are included in the respective CAGs (see Section 3.1). With respect to brain and/or erythrocyte AChE inhibition, only groups a) and d) are relevant.

Less convincing (e.g. being scarce or lack of consistency) mechanistic evidence is available for the following ASs, which were considered as having a presumed mode of neurotoxic action in mammals:

l) Metaldehyde is a molluscicide. It leads to an increase in monoamine oxidase (MAO) activity and to a decrease in GABA, norepinephrine (NE) and 5-hydroxytryptamine (5-HT) concentrations which leads to neuronal excitation (GABA) and decreased seizure threshold (NE, 5-HT), both of which can result in convulsions (Yas-Natan et al., 2007).

m) Triadimefon and triadimenol are triazole fungicides. Triadimefon and triadimenol are inhibitors of dopamine transporter, leading to increased synaptic concentrations of dopamine (Walker and Mailman, 1996).

n) Dithiocarbamates (mancozeb, maneb, metiram, propineb, thiram and ziram): although the molecular mechanisms underlying the neurotoxicity of dithiocarbamate fungicides in general are not well understood, the generation of CS2 in vivo has been shown to cause Lys-Lys thiourea cross-linking of neurofilament proteins resulting in the accumulation of neurofilaments in the axon, which causes the characteristic lesion of axonal swelling (Valentine et al., 1997). This is

2 https://toxnet.nlm.nih.gov/cgi-bin/sis/search/a?dbs+hsdb:@term+@DOCNO+8245
presumed to cause peripheral neuropathy and subsequent motor and some sensory symptoms under chronic exposure.

o) Molinate is a thiocarbamate herbicide. The neurotoxic effects observed may be a consequence of its metabolite molinate sulfone, which inhibits aldehyde dehydrogenase (ALDH) by covalently binding to the active-site Cys residue. Inhibition of ALDH, particularly the ALDH2 isoenzyme, in the brain results in accumulation of endogenous neurotoxins, such as 3,4-dihydroxyphenylacetaldehyde (DOPAL), a dopamine metabolite, or 4-hydroxy-2-nonenal (4HNE), a product of lipid peroxidation, which may account for the observed neurotoxicity (Allen et al., 2010). As ALDH2 is widely expressed in the frontal and temporal cortex, hippocampus, mid-brain, basal ganglia and cerebellum, it plays a crucial function in protecting neurons in the brain and the spinal cord (Chen et al., 2016).

p) Pymetrozine, a pyridine azomethine insecticide, acts by overstimulating and eventually silencing vanilloid-type transient receptor potential (TRPV) channels in insect chordotonal stretch receptor neurons. However, there is no indication of cross-reactivity of TRPV channels in mammals. In this regard, afidopyropen, an insecticide with a similar MoA than pymetrozine that stimulated TRPV channels from two different insect species, did not affect the function of the mammalian TRPV channel TRPV4 (Kandasamy et al., 2017).

q) Dicofol is believed to act by at least four mechanisms, possibly all functioning simultaneously. It may reduce potassium transport across the membrane. Dicofol also alters the porous channels through which sodium ions pass. These channels activate (open) normally but are inactivated (closed) slowly, thus interfering with the active transport of sodium out of the nerve axon during repolarisation. Dicofol inhibits neuronal adenosine triphosphatases (ATPases), particularly Na\(^+\) K\(^+-\)ATPase, and Ca\(^{2+}\)-ATPase which play vital roles in neuronal repolarisation. Dicofol also inhibits the ability of calmodulin, a calcium mediator in nerves, to transport calcium ions that are essential for the release of neurotransmitters.\(^3\)

These ASs (items l to q) are included in CAGs only in case of observation of relevant indicators in toxicological studies.

This is also the case for the following ASs for which no information is available on the mode of neurotoxic action:

- 2,4-D (phenoxyacetic herbicide), bromide ion (comes from methyl bromide, fumigant), carbetamide (carbanilate herbicide), chlorpropham (carbanilate herbicide, growth regulator), desmedipham (carbanilate herbicide), dicamba (benzoic acid herbicide), dicofol (bridge diphenyl acaricide), ethephon (growth regulator), fenpropidin (unclassified fungicide), fenpropimorph (morpholine fungicide), flufenacet (anilide herbicide), fluquinconazole, tebuconazole and tetraconazole (triazole fungicides), glufosinate (organophosphorus herbicide), isoxaflutole (oxazole herbicides), lufenuron (benzoylphenylurea), metribuzin (triazinone herbicide), triallate (thiocarbamate herbicide), oxasulfuron (sulfonylurea), penflufen (anilide herbicide), pyriproxyfen (pyridazine herbicide), spirotétam (tetramic acid insecticide), tebuconazole (benzimidazole, carbamate fungicide).

In this section, only the most well-established neurotoxic MoAs were described. However, other MoAs could occur simultaneously for example in the case of organochlorine insecticides (e.g. endosulfan and lindane) acting mainly as GABA-A receptor antagonists, but also inhibiting Ca- and Mg-ATPase (Jayaraj et al., 2016). Hence, there is some uncertainty regarding allocating specific pesticides to CAGs according to pesticide classes.

3.3.3. Cumulative assessment groups

This section presents the CAGs proposed to be used for future CRAs. They differ to some extent from those initially elaborated by the PPR Panel and published in the Scientific Opinion of 2013 (EFSA PPR Panel, 2013a), because only one (RIVM, ICPS, ANSES, 2013) of the three data collections used in this report was available to the Panel when it adopted its opinion and comments submitted during the public consultation on a draft of the present report were considered (EFSA, 2019c).

Functional alteration of the motor, sensory and autonomic divisions:

In total, 85 ASs have a known MoA of direct relevance for functional alterations of the motor, sensory and autonomic divisions and are included in the three CAGs: abamectin, acephate, acetamiprid, acrinathrin, aldicarb, alpha-cypermethrin, amitraz, azinphos-ethyl, azinphos-methyl, benfuracarb, beta-
cyfluthrin, beta-cypermethrin, bifenthrin, cadusaphos, carbaryl, carbofuran, carbosulfan, chlorfenvinophos, chloromequat, chlorpyriphos, chlorpyriphos-methyl, clothianidin, cyfluthrin, cypermethrin, deltamethrin, diazinon, dichlorvos, dieldrin, dimethoate, dinofuran, emamectin, endosulfan, endrin, esfenvalerate, ethephon, ethion, ethoprophos, fenamiphos, fenitrothion, fenpropathrin, fenthion, fenvalerate, fipronil, fonofos, formetanate, fosthiazate, heptachlor, imidacloprid, indexacarb, lambda-cyhalothrin, lindane, malathion, mep Quit, metamidophos, methidathion, methomyl, milbemectin, monocrotophos, oxamyl, oxydemeton-methyl, parathion, parathion-methyl, permethrin, phenthoate, phosphalom, phoxim, pirimicarb, pirimiphos-methyl, profenofos, pyrazophos, pyrethrins, sulfoxaflor, tau-fluvalinate, tefluthrin, tetramethrin, thiacloprid, thiamethoxam, thiodicarb, toclofos-methyl, triazophos, trichlorfon, zeta-cypermethrin.

The chemical classes of these ASs and their MoAs are described in Section 3.3.2, points a) to k).

Based on factual observations of relevant indicators, additional ASs were included in these CAGs as follows:

- **CAG on the functional alteration of the motor division (Tables B.1 and B.2):** 2,4-D, bromide ion, carbetamide, chlorpropham, desmedipham, dicamba, dicofol, fenpropidin, fenpropimorph, flufenacet, fluquinconazole, glufosinate, isoxaflutole, lufenuron, mancozeb, maneb, metaldehyde, metiram, molinate, oxasulfuron, penflufen, propineb, pymetrozine, pyridate, spirotetramat, tebuconazole, tembotrione, tetraconazole, thiophanate-methyl, thiram, tri-allate, triadimefon, triadimenol, ziram. These additional 34 ASs lead to a total of 119 ASs included in this CAG.

- **CAG on the functional alteration of the sensory division (Tables B.2 and B.6):** cymoxanil, dicamba, dicofol, fenpropimorph, flufenacet, glufosinate, halosulfuron-methyl, metaldehyde, molinate, oxasulfuron, propineb, sulcotrione, tebuconazole, tembotrione, thiram, tri-allate. These additional 16 ASs lead to a total of 101 ASs included in this CAG.

- **CAG on the functional alteration of the autonomic division (Tables B.3 and B.7):** 2,4-D, carbetamide, chlorpropham, dicamba, dicofol, flufenacet, fluquinconazole, glufosinate, metaldehyde, metiram, oxasulfuron, propineb, pyridate, tebuconazole, thiram, tri-allate. These additional 16 ASs lead to a total of 101 ASs included in this CAG.

In the CAG on the functional alteration of the motor division, the ICs for the acute and chronic risk assessments are proposed as oxamyl and emamectin benzoate, respectively. Oxamyl was selected based on the robustness of data and the number of endpoints affected in the acute neurotoxicity study. Emamectin benzoate was selected based on the robustness of data and consistency of effects across studies and animal species (rat, dog and mouse).

In the CAG on the functional alteration of the sensory division, the ICs for the acute and chronic risk assessments are proposed as oxamyl and endrin, respectively. Oxamyl was selected on the basis of the robustness of findings (dose relationship in mid and high dose males and statistical significance). Endrin was selected based on the consistency of findings observed in two strains of rats.

In the CAG on the functional alteration of the autonomic division, the ICs for the acute and chronic risk assessments are proposed as oxamyl and methamidophos, respectively. Both were selected based on the robustness of data.

Furthermore, all proposed ICs have a known MoA.

In the NOAEL-setting process, it was noted that for 19 ASs belonging to chemical classes with a known MoA (amitraz, azinphos-ethyl, chlorfenvinophos, chloromequat, dichlorvos, dieldrin, endrin, ethion, fosthiazate, methiocarb, monocrotophos, oxamyl, oxydemeton-methyl, parathion, parathion-methyl, phenthoate, phoxim, pyrazophos, tetramethrin and triazophos) neurotoxicity studies are not available. For an additional set of 14 ASs (carbetamide, chlorpropham, desmedipham, fenpropidin, fluquinconazole, isoxaflutole, mancozeb, maneb, metiram, oxasulfuron, propineb, pyridate, tetraconazole, thiophanate-methyl), functional alterations of the motor division were observed in single and/or repeated dose studies, but specific neurotoxicity studies were not available. This lack of neurotoxicity studies is a source of uncertainty affecting the setting of NOAELs for functional alterations of the motor, sensory and autonomic divisions. This will need to be considered in the interpretation of the cumulative risk assessments when they will be performed.

For 50 ASs belonging to chemical classes with a known MoA, the acute NOAELs characterising their effects on the three functional divisions of the nervous system were derived from the same neurotoxicity study. The comparison of the NOAELs for the three divisions indicates that the motor division is the most sensitive to the effects of chemicals with neurotoxic MoA. Indeed:
The comparison between the NOAELs of individual ASs in the motor and sensory divisions showed that the NOAELs are lower in the motor division in 28 cases and lower in the sensory division in two cases. In 20 cases, the NOAELs were the same.

The comparison between the NOAELs of individual ASs in the motor and autonomic divisions showed that the NOAELs are lower in the motor division in 23 cases and lower in the autonomic division in three cases. In 24 cases, the NOAELs were the same.

In view of this comparison, it is concluded that the cumulative risk assessments for the effects of pesticides on the motor division might be enough to cover the cumulative risks on all functional divisions.

Brain and/or erythrocyte AChE inhibition:

This effect is defined at biochemical level and is always associated with organophosphorous and N-methyl carbamate insecticides, and only ASs of these chemical classes of pesticides are included in the CAG. Since erythrocyte AChE is a membrane bound enzyme, oxidative stress induced by other chemical classes may also lead indirectly to a decrease in erythrocyte AChE activity (Banerjee et al., 1999; El-Demerdash, 2011). ASs acting via this indirect pathway are however not included in the CAG as they do not meet the precise definition of the specific effect.

In total, 47 ASs are included in the CAG for the brain and/or erythrocyte AChE inhibition (Tables B.4 and B.8). These ASs are: acephate, aldicarb, azinphos-ethyl, azinphos-methyl, benfuracarb, cadusaphos, carbaryl, carbofuran, carbosulfan, chlorfenviphos, chlorpyriphos, chlorpyriphos-methyl, diazinon, dichlorvos, dimethoate, ethephon, ethion, ethoprophos, fenamiphos, fenitrothion, fenthion, fonofos, formetanate, fosfathiazate, malathion, methamidophos, methidathion, methiocarb, methomyl, monocrotophos, omethoate, oxydemeton-methyl, parathion, parathion-methyl, phosalone, phosmet, phoxim, pirimicarb, pirimiphos-methyl, profenofos, pyrazophos, thiodicarb, tolclofos-methyl, triazophos, trichlorfon.

The ICs for the acute and chronic risk assessments are proposed as oxamyl and omethoate, respectively. Oxamyl was selected based on the robustness of data. Omethoate was selected based on the consistency of findings across studies and animal species (rat, dog, mouse and rabbit).

Generally, the NOAELs related to AChE inhibition are notably lower than the NOAELs of the respective ASs for their effects on the motor, sensory and autonomic divisions of the nervous system as well as for their neuropathological effects.

Neuropathological effects:

With respect to this specific effect, no chemical class or MoA linking a biochemical mechanism and the observed histopathological changes has been identified. For a few ASs, only presumed MoAs have been proposed. Therefore, ASs are included in this CAG based on factual observations only.

In total, 19 ASs are included in the CAG for neuropathological effects (Table B.9). These ASs are: chlorfenapyr, cymoxanil, cypermethrin, emamectin benzoate, fenpropidin, flufenacet, indoxacarb, isoxaflutole, lindane, mancozeb, molinate, oxasulfuron, quinoxalone, tau-fluvalinate, tembotrione, thiram, tri-allate, trichlorfon, ziram.

The IC is proposed as emamectine, which was selected based on the consistency of findings across studies and animal species (rat, dog and mouse).

A comparison between this CAG and the CAG for functional alterations of the motor division showed that:

- The CAG for neuropathological effects contains about 6 times less ASs than the CAG for the motor division. This is explained by the fact that functional and biochemical mechanisms, not necessarily associated to neuropathological effects, are major causal factors of alteration of the motor function.
- Three out of the 19 ASs included in the CAG for neuropathological effects are not in the CAG for the motor division. These ASs are chlorfenapyr, cymoxanil and quinoxalone.
- The number of ASs in the CAG for motor division with NOAELs for chronic effects exceeds by about 60 the number of ASs in the CAG for neuropathological effects. Four ASs (chlorfenapyr, cymoxanil, quinoxalmine and ziram) have NOAELs for neuropathological effects, but not NOAEL for chronic effects in the motor division.
- From the ASs included in the CAG for neuropathological effects and having a NOAEL for chronic effects in the motor division, five have lower NOAELs for neuropathological effects (lindane, mancozeb, thiram, tri-allate and trichlorfon), three have lower NOAELs in the motor division and seven have the same NOAELs for both effects.
In view of this comparison, it is anticipated that the cumulative risks for the neuropathological effects will be covered by cumulative risk assessments for the effects on the motor division and therefore may not require separate assessment.

Nine tables (see Appendix B) were prepared to support all possible acute and chronic exposure/risk assessments that could be conducted using the five CAGs. 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 the information (e.g. DAR, 2011; JMPR, 1997), the EFSA conclusions considered (e.g. EFSA (2008)), some additional elements of the assessment 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 are not shown in the study reference details.

3.3.4. Use of the CAGs to assess consumer safety

As indicated in Section 3.3.3, it is expected that CRA conducted with the CAG for the effects on the motor division will show higher risks than those carried out with the CAGs for the effects on the sensory and autonomic divisions and for the neuropathological effects. Therefore, in order to assess the combined effects of pesticide residues present in consumer diet on the nervous system, it would be enough to perform CRA with the CAGs for AChE inhibition and for the functional alterations of the motor division, if similar protection goals would apply to all these effects.

In conducting these CRA, 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. effects 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 which is consistent with the 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).

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 properly when these regulatory documents were drafted, as their main purpose is to establish the reference values of the ASs. 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.

In addition, for several ASs, especially for ASs which are not approved anymore in EU, the quality of the database 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.

A particular source of uncertainties with respect to the effects of pesticides on the nervous system stems from the fact that a neurotoxicity study is not always available, despite the fact that Commission Regulation (EU) No 283/2013 setting out the data requirements for ASs provides that such study should be performed for ASs with structures that are similar or close to those capable of inducing neurotoxicity. This absence of a neurotoxicity study may result in overestimated NOAELs for some ASs (and thus underestimating the actual risk) as information on some indicators is missing in this case.

Specific sources of uncertainties related to the CAGs for functional alterations of the motor division, brain and/or erythrocyte AChE inhibition and other specific effects are addressed in Sections 4.2–4.4. In Section 4.5, 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 the functional alterations of the motor division

4.2.1. Question 1: Does the CAG for the functional alteration of the motor division contain all ASs contributing to this effect and only ASs causing 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 4.5 (overall uncertainty analysis). The second one is whether ASs not causing the effect are included in the CAG. For the CAG on motor division, the possibility of including ASs not contributing to the effect has been addressed by Weight of Evidence and EKE techniques 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, in such a way that the answer to the question is potentially observable, at least in principle (EFSA, 2014). The question of interest for CRA is, for each AS, ‘Does this chemical cause any functional alteration of the motor division of the nervous system (locomotor activity, muscular strength and coordination)?’ In regulatory practice, causation of toxic effects is determined by established standard procedures for the conduct, reporting and interpretation of toxicity studies. The elicitation question was therefore defined as follows: ‘If the required set of studies (including neurotoxicity studies if relevant) was performed and reported perfectly, and the results were analysed and interpreted according to the standard procedure, would this chemical be assessed as positive for effects on the motor division of the nervous system?’ For the purpose of risk assessment, these two framings of the question are equivalent.

b) The lines of evidence and their respective weights are:

- Belonging of the AS to one of the following chemical classes: organophosphates, N-methyl carbamates, organochlorines, macrocyclic lactones, pyrethroids, neonicotinoids or phenylpyrazoles: 32,000. This exaggerated weight was applied in order to ensure that ASs with and without relevant chemical structures are separated completely in the ranking process, and placed in different subgroups, to increase the homogeneity of evidence in each subgroup and make it easier for experts to judge the probability of CAG membership.
- AS for which a MoA is presumed (rather than known) in the data collection tables: 3.
- Observation of decreased motor activity: 4 if hypoactivity is reported; 2 if any indicator of reduced activity, but not hypoactivity, is reported. From the indicators of reduced motor activity, hypoactivity is the one which is the most closely related to the adverse effect of a chemical. The other indicators must be considered more carefully and are rather considered as confirmatory information supporting other observations.
- Observation of increased motor activity: 3 if only one indicator is reported; 4 if more than one indicator is reported.
- Observation of effects on the muscular strength: 3 if only one indicator is reported or in the absence of any neurotoxicity study: 4; 4 if more than one indicator is reported.
- Observation of effects on the motor coordination: 3 if only one indicator is reported or in the absence of any neurotoxicity study: 4; 4 if more than one indicator is reported.
- Concomitant observation of indicators of functional alteration of the sensory function: 3 in the absence of any neurotoxicity study: 4; 4 if any indicator is reported.
- Observation of indicators in more than one species: 3.
- Observation of a dose-response relationship for the most sensitive indicator: 4.

In case of observations of decreased and increased activity for a same AS, the combined weight is however limited to an upper limit of 8.

Neurotoxicity studies are essential to observe indicators related to motor coordination, muscular strength and sensory function. In the absence of such studies for certain ASs, it was conservatively assumed that these ASs would show these indicators if these studies were conducted. These cases were highlighted in the tabulated evidence and the effect of this assumption was considered by the experts when making their judgements.
neurochemical effects were not scored and treated separately as a seventh subgroup of ASs. The compositions of these seven subgroups are as follows:

- **Subgroup 1 (18 ASs):** acetamiprid, aldicarb, carbofuran, clothianidin, cypermethrin, deltamethrin, emamectin, ethoprophos, fenpropatrin, fenvalerate, fipronil, formetanate, metamidophos, milbemectin, omethoate, oxydemeton-methyl, pyrethrins and thiamethoxam.

- **Subgroup 2 (20 ASs):** abamectin, acrinathrin, azinphos-methyl, beta-cyfluthrin, betacypermethrin, bifenthrin, cadusapon, carbaryl, dichlorvos, esfenvalerate, fenitrothion, fosfathiazate, imidacloprid, oxamyl, permethrin, tau-fluvalinate, tefluthrin, thiacloprid, trichlorfon and zeta-cypermethrin.

- **Subgroup 3 (14 ASs):** alpha-cypermethrin, benfuracarb, cyfluthrin, diazinon, dimethoate, fenamiphos, fenthion, lambda-cyhalothrin, lindane, methidathion, monocrotophos, pirimicarb, pyrazophos and thiodicarb.

- **Subgroup 4 (20 ASs):** acephate, carbosulfan, chlorpyrifos, chlorpyrifos-methyl, dieldrin, dinoterfuran, endrin, fonofos, heptachlor, malathion, methiocarb, methomyl, parathion, parathion-methyl, phosalone, phoxim, pirimiphos-methyl, tetramethrin and triazophos.

- **Subgroup 5 (19 ASs):** bromide ion, carbetamide, chlormequat, dicamba, dicofol, endosulfan, flufenacet, flufenconazole, indoxacarb, meipquat, metaldehyde, metribuzin, molinate, oxasulfuron, pyridate, sulfoxaflor, tebuconazole, thiram and triallate.

- **Subgroup 6 (22 ASs):** 2,4-D, amitraz, chlorpropham, desmedipham, fenpropidin, fenpropimorph, glufosinate, isoxaflutole, lufenuro, mancozeb, maneb, metiram, penfluhen, propineb, pymetrozine, tembotrione, tetraconazole, thiophanate-methyl, triadimefon, triadimenol, spirotetramat and ziram.

- **Subgroup 7 (7 ASs):** azinphos-ethyl, chlorfenvinphos, ethephon, ethion, phenthoate, profenofos and tolclofos-methyl.

d) The probability estimations for subgroups 1 to 7 concluded that:

**In subgroup 1 (18 ASs):**
- All ASs in this group have relevant structure and mechanism and show clear evidence of dose-response relationship and effects on two or more species.
- All show both reduced and increased motor activity.
- All showed effect on coordination, muscle strength, and on the sensory division of the nervous system, except one AS for which no neurotoxicity study was available, but in this case muscle strength effects were seen in another study.
- Overall, it was judged almost certain (99–100%) that all substances cause effects on the motor division.

**In subgroup 2 (20 ASs):**
- All ASs have relevant structure or mechanism.
- All ASs show dose-response relationship.
- All ASs show several indicators of effects on the motor division.
- For each ASs, one of the lines of evidence was missing (missing indicators, missing observations in the sensory division or missing observation in a second species).
- Overall, it was judged almost certain (99–100%) that all 20 substances cause motor division effect.

**In subgroup 3 (14 ASs):**
- All ASs have relevant structure and mechanism and substantial evidence of effects (indicators).
- For four ASs, the available data do not show a dose response, but this is either mitigated by the observations available for another mixture of the same isomers (alpha-cypermethrin), a metabolite (dimethoate) and/or possibly due to the limitations of the studies (e.g. no neurotoxicity study available for monocrotophos). It is also unlikely that the effects on the top dose were observed on doses above the MTD as such cases would have been excluded by the data collection process.
- Overall, it was judged almost certain (99–100%) that all ASs cause effects on the motor division of the nervous system.
In subgroup 4 (20 ASs):

- All ASs have relevant structure or mechanism.
- All ASs have few positive indicators of effects on the motor division.
- However, data available for most substances is thought to be old and less complete/robust. For six ASs, a neurotoxicity study is not available.
- For some substances there is positive evidence from human/medical data of the effects on the motor function, which was not captured by the data collection.
- Overall, it was judged almost certain (99–100%) that all 20 substances cause motor division effect.

In subgroup 5 (19 ASs):

- Only two ASs have known structural alerts of neurotoxicity.
- Five ASs (chlorimequat, endosulfan, indoxacarb, mepipquat and sulfoxaflor) have a known neurotoxic MoA. In addition, dicofol, being structurally related to DDT is believed to act by at least four mechanisms. Three ASs (oxasulfuron, thiram and triallate) cause neuropathology.
- All ASs have several types of motor division effects, all show a dose–response, and all but three have effects in two or more species.
- Sixteen ASs have effects on the sensory division.
- 5–6 ASs were judged extremely likely (95–99%) to cause motor division effects, 1–2 ASs were judged unlikely (10–33%), 1–2 as likely as not (33–66%) and most ASs likely (66–90%) or very likely (90–95%).
- Overall, it was judged that the number of ASs causing motor division effects is likely (66–90%) to be between 15 and 17, with a plausible range from 12 to 19.

In subgroup 6 (22 ASs):

- Six ASs have known or presumed MoA (amitraz, mancozeb, maneb, metiram, propineb and ziram).
- For all AS show at least one indicator, and many two or more.
- Neurotoxicity studies were available for 12 of the 22 ASs, detecting motor division effects in most but not all cases.
- A dose–response was reported for nine ASs only.
- In seven cases, effects were seen in two or more species.
- Five ASs were judged extremely likely (95–99%) to cause motor division effects; four were judged likely (66–90%); five as likely as not (33–66%); two unlikely (10–33%); three very unlikely (5–10%) and three extremely unlikely (1–5%).
- Overall, it was judged that the number of ASs that cause motor division effects is likely (60–90%) to be between 10 and 12, with plausible range between 8 and 15.

In subgroup 7 (7 ASs):

- Five out of the seven ASs have clearly relevant structure/mechanism, for the other two this is questionable.
- Four of the seven ASs inhibit brain AChE, one (ethephon) inhibits erythrocytes AChE but not brain, and for two ASs information on brain AChE inhibition is missing.
- For three ASs, a neurotoxicity study is available. None of these studies show effects on the motor division, but two of these studies are old and not likely to detect such effects.
- The group judged that it is almost certain (99–100%) that chlorfenvinfos, ethion, profenofos, azinphos-ethyl and penthoate cause effects on the motor division, but that it is extremely unlikely (1–5%) that either ethephon or tolclofos-methyl cause the effects.
- Overall it was judged extremely likely (95–99%) that five ASs cause motor division effects extremely unlikely (1–5%) that 6 cause the effects.

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

Appendix D provides details on the evidence collected for each AS, its score and the subgroup it belongs to. After the EKE session, the available technical information was further reviewed and this resulted in the identification of a few inaccuracies in the information reported in Appendix D, e.g. regarding the availability of neurotoxicity studies. However, these inaccuracies are impactless on the result of the EKE session.
e) Assessing the total number of ASs causing motor division effects:

- It is nearly certain that all the ASs of subgroups 1, 2, 3 and 4 cause alterations on the motor function. The same is true for five of the seven substances in subgroup 7.
- The number of ASs of subgroup 5 causing alterations on the motor function is most likely between 15 and 17, with plausible range from 12 to 19.
- The number of ASs of subgroup 6 causing alterations on the motor function is most likely between 10 and 11, with plausible range from 8 to 15.
- The elicited distributions for the seven subgroups are plotted together in Figure 1. The number of substances considered differs between subgroups so, to facilitate comparison, the elicited distributions were rescaled to percentage of substances.
- 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 alterations of motor function. The median estimate was 104 ASs, with a 90% confidence interval of 100 to 107 (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 alterations of motor function. The median estimate was again 104 ASs but with a 90% confidence interval of 97–109 (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 the cumulative risk assessment is performed.

**Figure 1:** Distributions quantifying uncertainty about the percentage of substances in each subgroup that cause motor division effects. The vertical axis (probability density) quantifies the experts’ judgement of the likelihood of different proportions of substances causing motor division effects within each subgroup.
Figure 2: Distribution quantifying uncertainty about the total number of substances from subgroups 1–7 that cause motor division effects, 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 1–7 that cause motor division effects, obtained using Monte Carlo simulation assuming perfect positive dependence between the elicited distributions for the seven subgroups.
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 functional alterations of the motor division, on the basis of the observed risk-drivers (EFSA, 2019b). This was facilitated by the information given in Section 3.3.2 about 11 MoAs identified to be of direct relevance for functional alterations of the motor division, and, in turn, defining 11 clusters of ASs within which dose addition is virtually certain for the respective effect.

Between AS not belonging to the same cluster, the uncertainty about how closely combined effects conform to those predicted by dose addition was addressed in EFSA (2019b) considering the chemical structures and 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 extent to which individual consumers have simultaneous exposures to multiple ASs with different MoAs.

4.3. CAG for the erythrocyte and/or brain AChE inhibition

4.3.1. Question 1: Does the CAG for the brain and/or erythrocyte AChE inhibition contain all ASs contributing to this effect and only ASs causing 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 4.5 (overall uncertainty analysis).

As to whether ASs not causing the effect are included in the CAG, the chance of including ASs not contributing to the effect is here virtually non-existent because all ASs but 2 are organophosphorous or N-methyl carbamate insecticides acting biologically via AChE inhibition. The two exceptions are tolclofos-methyl and ethephon, which have other biological actions, but have shown weak, but however significant, inhibition of AChE in experimental studies.

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 brain and/or erythrocyte AChE inhibition was performed in the light of the observed risk drivers (EFSA, 2019b) and their MoAs (All ASs in the CAGs for AChE inhibition belong to the chemical classes a) or d) listed in Section 3.3.2).

4.4. CAGs for the functional alterations of the sensory and autonomic divisions of the nervous system, and for the neuropathological effects

As indicated in Section 3.3.4, it is suggested not to perform CRAs for these CAGs, as those carried out with the CAG for the alterations of the motor division are expected to provide more critical results. Therefore, uncertainty is not considered further for these CAGs.

It is, however, noted that MoAs leading to neuropathological effects are not the same as those leading to the other specific effects. For this reason, the assessment of the modes of neurotoxic action conducted in Section 4.2.2 cannot be used to support uncertainty analyses related to CRA performed for neuropathological effects. For these effects, information on relevant MoAs is scarce, but where available, more details can be found in Table B.9 of Appendix B.

4.5. 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 by the application of the 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 the functional alteration of the motor division) and Section 4.3.1. (CAG for brain and/or erythrocyte AChE inhibition) and of the individual contribution of each AS to the risk. For the CAGs related to the functional alterations of the sensory and autonomic division and to histological neuropathological changes in neural tissues, this source of uncertainty should be addressed based on appropriate lines of evidence.

- Uncertainty related to the characterisation of ASs included in the CAG.
  - Are all ASs in the CAG characterised with acute and/or chronic NOAELs?
  - Can these acute and/or chronic NOAELs be either under- or overestimated?

These questions need 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 indicator), the quality of the toxicological dossiers of the ASs included in the CAG (availability of the ad hoc studies for the hazard characterisation, e.g. neurotoxicity studies) and the hazard characterisation principles defined for each CAG (including the eventual measures taken to establishing surrogate NOAELs, e.g. from LOAELs or from chronic studies when acute studies are not available, etc.).

- Uncertainty regarding relative contribution of ASs to the cumulative risk resulting from the use of NOAELs rather than BMDLs.
- Uncertainty regarding the slope and the shape 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. 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 nervous system.
- 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 MoA in Section 3.3.2 is useful in the evaluation of this source of uncertainty. It must, however, be kept in mind that this distribution is based on the best known mechanisms and that the use of this distribution implies a simplification of the inherent complexity of the functioning of the nervous system.
- Uncertainties about the inter- and intraspecies variability in toxicological sensitivity. This source of uncertainty concerns the adequacy for human risk assessment of the toxicological characterisation of ASs based on animal data.
- Uncertainties resulting from the use of acute and chronic exposure calculation models not necessarily reflecting the actual time course of toxicokinetic and toxicodynamic processes. For instance, being exposed to a N-methylcarbamate insecticide at breakfast and to an organophosphate at dinner may not lead to the same risk as being exposed to the same compounds in the reverse order. This is because although both organophosphorus and N-methylcarbamate insecticides bind to AChE, inhibition by the latter is spontaneously reversible whereas that by organophosphorus insecticides is considered irreversible. Functional recovery
following exposure to organophosphorus insecticides requires the synthesis of new enzyme before AChE activity returns to normal values. The cumulative exposure calculation model, which sums up all doses ingested within a 24-hour period, does not take these toxicokinetic and toxicodynamic properties into account and does not produce different results for different sequences of exposure to chemicals.

- If the type of administration (diet or gavage) of the test substance in toxicological studies might be of importance, then this should be flagged in the data tables and considered in the uncertainty analysis.

5. Conclusions

Cumulative assessment groups for the effects of pesticides on the nervous system were previously established by the PPR Panel in 2013. The five specific effects of pesticides on this system that are of relevance for CRA have been confirmed: functional alterations of the motor, sensory and autonomic divisions, histological neuropathological changes in neural tissue, and brain and/or erythrocyte AChE inhibition. The CAGs have been updated on the basis of 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 nervous system should be focussed on their specific effects on the motor division and on brain and/or erythrocyte AChE inhibition because the highest risks are expected to be observed for these effects.

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

Due to the current scarcity of data with respect of DNT of pesticides, it is currently premature to evaluate if specific effects of pesticides in this area deserve the establishment of CAGs and the performance of CRAs. Therefore, a testing and assessment methodology should be developed and applied on a consistent basis to provide enough information supporting the establishment of CAGs covering DNT if appropriate.

If the outcome of CRAs conducted with the CAGs established in this report exceeds regulatory thresholds of acceptance, empirical research is needed on how ASs driving the risk combine their effects at the anticipated dietary exposure levels, especially if they act according to dissimilar MoAs, and on the extent to which this combination of effects deviates from dose addition.

If the outcome of CRAs conducted with these CAGs, as currently characterised by NOAELs, exceeds the 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 reflect 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 a sensitivity analysis, starting with all subgroups of substances included and removing them one at a time in order of increasing probability of CAG membership, and use the results of this to inform expert judgement of the contribution of CAG membership uncertainty to overall uncertainty. Though less rigorous, this would avoid the need for probabilistic calculations.

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. This would be the opportunity to include additional non-approved ASs found in food commodities with relevant MoA in the respective CAGs. This would solve a source of underestimation of cumulative risks (EFSA, 2019b).
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Abbreviations

4HNE 4-hydroxy-2-nonenal
5-HT 5-hydroxytryptamine
ACHE acetylcholinesterase
ADI acceptable daily intake
ALDH aldehyde dehydrogenase
ANSES French Agency for Food, Environmental and Occupational Health and Safety
ARfD acute reference dose
AS active substance
ATPase adenosine triphosphatase
BMDL lower confidence limit of the benchmark dose
CAG cumulative assessment group
CRA cumulative Risk Assessment
| Abbreviation | Full Form |
|--------------|-----------|
| DAR          | Draft Assessment Report |
| DNT          | developmental neurotoxicity |
| DOPAL        | 3,4-dihydroxyphenylacetaldehyde |
| DTU          | Danish Technical University |
| EKE          | expert knowledge elicitation |
| EHC          | Environmental Health Criteria Monograph |
| GABA         | gamma-aminobutyric acid |
| IC           | Index Compound |
| ICPS         | International Centre for Pesticides and Health Risk Prevention |
| JMPR         | Joint Meeting on Pesticides Residues |
| LOAEL        | lowest observed adverse effect level |
| LOEL         | lowest observed effect level |
| MAO          | monoamine oxidase |
| MoA          | mode of action |
| MOE          | margin of exposure |
| MOET         | total margin of exposure |
| MRL          | maximum residue level |
| MTD          | maximum tolerated dose |
| nAChR        | nicotinic acetylcholine receptor |
| NE           | norepinephrine |
| NOAEL        | no observed adverse effect level |
| NOEL         | no observed effect level |
| NTE          | neuropathy target esterase |
| PAFF         | Committee Standing Committee on Plants, Animals, Food and Feed |
| PPR          | EFSA Panel on Plant Protection Products and their Residues |
| RAR          | Renewal Assessment Report |
| RfP          | reference point |
| RIVM         | National Institute for Public Health and the Environment |
| RPF          | relative potency factor |
| SCBI         | sodium channel blocker insecticide |
| TRPV         | vanilloid-type transient receptor potential |
| UF           | uncertainty factor |
| US-EPA       | United States Environmental Protection Agency |
| VGSC         | voltage-gated sodium channel |
Appendix A – List of active substances considered in view of establishing CAGs for effects of pesticides on the nervous system

Active substances covered by the first outsourced data collection (RIVM, ICPS, ANSES, 2013):

| Active Substance | Active Substance | Active Substance | Active Substance |
|------------------|------------------|------------------|------------------|
| 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 | Fluufenacet (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-tetfuril |
| Abamectin (aka avermectin) | Cypermethrin | Fluoxastrobine | Methiocarb (aka mercaptodimethur) | Rimisulfuron (aka renidurin) |
| Acetamiprid | Cyproconazole | Flupyr-sulfuron-methyl (DPX KE 459) | Methomyl | Siltiorfam |
| Acibenzolar-S-methyl (benzothiadiazole) | Cyprodinil | Fluquinconazole | Methoxyfenozide | Sintofen (aka Cintofen) |
| Aclonifen | Cyromazine | Fluorochloridone | Metiram | S-Metolachlor |
| Alpha-Cypermethrin (aka alphamethrin) | Daminozide | Fluoxypyr | Metosulam | Sodium 5-nitroguaiacolate |
| Aluminium ammonium sulfate | Dazomet | Flurtamone | Metrafenone | Sodium hypochlorite |
| Aluminium phosphate | 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 | Niclosulfuron | Sulfofungin |
| Benfluralin | Diflubenzuron | Fosetyl | Omethoate | Sulfuryl fluoride |
| Bensulfuron | Diflufenican | Fosthiazate | Oryzalin | Tau-Fluvalinate |
| Bentazon | Dimethachlor | Fuberidazole | Oxadiargyl | Tebuconazole |
| Benthiavincarb | Dimethenamid-P | Gibberellin | Oxadiazon | Tebufluconazole |
| Benzoic acid | Dimethoate | Glufosinate | Oxamyl | Tebupenrypyl |
| Beta-Cyfluthrin | Dimethomorph | Glyphosate (incl. trimethium aka sulfofate) | Oxsulfuron | Teflubenzuron |
| Bifenazate | Dimoxystrobin | Haloxyfop-P/R | Oxyfluorfen | Tefluthrin |
| Bifentrazone | Dinoflazic | Hexythiazox | Paclotental | Tepraloxydim |
| Bispyribac | Diquat (dibromide) | Hymexazol | Penconazole | Terbutylazine |
| Boscalid | Dithianon | Imazalil (aka eniconazole) | Penicycuron | Tetraconazole |
| Bromadiolone | Diuron | Imazamox | Pendimethalin | Thiabendazole |

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| Active substances covered by the second 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 | Chlor dane | Fenitrothion | Metam (incl. - potassium and - sodium) | Pyroxsulam |
| Aldicarb | Chlorfenapyr | Fenpropethrin | Methamidophos | Quintozone |
| Aluminium sulfate | Chlorfenvinphos | Fenpyrazamine | Methidathion | Resmethrin |
| Ametocrotoin | Chlorobenzilate | Fenthion | Methoxychlor | Sediexane |
| 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          | Tecazene        |
| Azinphos-ethyl   | Dichlofluanid            | Flupyrad             | Oxydemeton-methyl | Tembotrione     |
| Azinphos-methyl  | Dichlorvos               | Fluxapyroxad         | Parathion         | Tetradoxon      |
| Benalaxyl-M      | Dicloran                 | Fonofos              | Parathion-methyl  | Tetramethrin    |
| Benfuracarb      | Dicofol                  | Halosulfuron methyl  | Penflufen         | Thiencarbazone  |
| Benomyl          | Dicropthos               | HCH                  | Penthiopyrad      | Thiodicarb      |
| Benzalkonium chloride | Didecyldimethylammonium chloride | Heptachlor         | Permethrin        | Tolfenpyrad     |
| Beta-cypermethrin| Dieldrin                 | Hexachlorobenzene    | Phenthoate        | Topramezone     |
| Bifenthrin       | Dinofuran                | Hexaconazole         | Phosalone         | Triadimefon     |
| Bitertanol       | Diphenylamine            | Indolylbutyric acid  | Phosphone         | Triazophos      |
| Bixafen          | Dithiocarbamates         | Ipconazole           | Phoxim            | Trichlorfon     |
| Bromide ion      | Emamectin benzoate       | Iron sulfate         | Pinoxaden         | Trifluralin     |
| Bromopropylate   | Endosulfan               | Isoprocarb           | Procymidone       | Valifenolate    |
| Cadusafos (aka ebufos) | Endrin                  | Isopyrazam           | Pefenofos         | Vinlozolin      |
| Camphchlor       | EPN                      | Lindane              | Propargite        |                 |

**Active substances covered by the EFSA data collection:**

| Aluminium phosphide | Etoxazole | Pymetrozine |
|--------------------|-----------|-------------|
| Benthiavalicarb    | Fenpyroximate | Pyriproxyfen |
| Bifenazate         | Iprovalicarb  | Tebuconazole |
| Copper compounds   | Lufenuron  | Tebufenpyrad |
| Cyromazine         | Magnesium phosphide | Tetraconazole |
| Difenoconazole     | Metamitron  | Thiamethoxam |
| Diflubenzuron      | Metribuzin  | Thiophanate-methyl |
| Etofenprox         | Milbemectin  | Tolylfluanid  |
### Appendix B – Tables supporting Cumulative Risk Assessments using the CAGs for effects of pesticides on the nervous system

Note 1: In 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 functional effects on motor division: toxicological characterisation of ASs to be considered in acute 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            | Abnormal gait, uncoordinated movements and reduced motor activity| 75               | 250              | Acute neurotoxicity rat       | Source: RAR 2013 EFSA conclusions on 2,4-D (2014) considered           | Unknown                   |
| Abamectin        | Ataxia                                                           | 1.5              | 6                | Acute neurotoxicity rat       | Source: DAR 2005, addendum 2007 EFSA conclusions on abamectin (2008) considered | GABA-gated chloride channel agonist |
| Acephate         |                                                                  | 74.2             | –                | 49-day neurotoxicity study    | Source: JMPR 2002 AChE inhibitor                                       | AChE inhibitor             |
| Acetamiprid      | Reduced motor activity, tremor                                  | 10               | 30               | Acute neurotoxicity rat       | Source: DAR 2001 Agonist of nAChR                                      |                            |
| Acrinathrin      | Abnormal gait                                                   | 1                | 3                | Acute neurotoxicity rat       | Source: DAR 2007 EFSA conclusions on aacinathrin (2013) considered     | Binding to VGSC            |
| Aldicarb         | Decreased motor activity, tremor, ataxic gait, decreased hindlimb strength | 0.1             | 0.5              | Acute neurotoxicity rat       | Source: DAR 1996 AChE inhibitor                                       | AChE inhibitor             |
| Alpha-Cypermethrin | Ataxia                                                       | 2.3             | 6.8              | 90-day dog                    | Source: DAR 2000 Binding to VGSC                                      |                            |
| Amitraz          | Hypoactivity                                                    | 1                | 4                | 90-day dog                    | Source: DAR Neurotoxicity studies not available                         | Partial agonist of presynaptic \(\alpha\)-adrenergic receptor |
| Active substance  | Indicator of specific effect                                      | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                  | Remark                                                                                     | MoA                  |
|------------------|-----------------------------------------------------------------|------------------|-----------------|------------------------|-------------------------------------------------------------------------------------------|----------------------|
| Azinphos-ethyl   | AChE inhibition (erythrocyte)                                   | 0.0125           | 0.025           | 90-day dog             | Administration via diet                                                                  | AChE inhibitor       |
|                  |                                                                 |                  |                 |                        | Source: JMPR 1973 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate |                      |
| Azinphos-methyl  | Reduced motor activity, reduced grip strength, abnormal gait    | 2                | 6               | Acute neurotoxicity rat| Administration via gavage                                                                | AChE inhibitor       |
|                  |                                                                 |                  |                 |                        | Source: DAR 1996, Addendum 6, 2000 (neurotoxicity)                                       |                      |
| Benfuracarb      | Increased motor activity, tremor, hyperactivity                 | 2                | 20              | 28-day rat             | Administration via gavage                                                                | AChE inhibitor       |
|                  |                                                                 |                  |                 |                        | Source: DAR 2004 Acute neurotoxicity study not available                                |                      |
| Beta-Cyfluthrin  | Reduced motor activity, tremor, uncoordinated gait              | 2                | 10              | Acute neurotoxicity rat| Administration via gavage                                                                | Binding to VGSC      |
|                  |                                                                 |                  |                 |                        | Source: JMPR 2006, DAR 1996                                                             |                      |
| Beta-cypermethrin| Abnormal gait, tremor                                          | 1                | 10              | 90-day dog             | Administration via capsule                                                              | BINDING to VGSC      |
|                  |                                                                 |                  |                 |                        | Source: DAR 2013                                                                         |                      |
| Bifenthrin       | Increased motor activity, tremor, convulsion, abnormal gait     | 35               | 75              | Acute neurotoxicity rat| Administration via diet                                                                | Binding to VGSC      |
|                  |                                                                 |                  |                 |                        | Source: DAR                                                                             |                      |
| Cadusafos        | Decreased hindlimb strength                                     | 25               | 40              | Acute neurotoxicity rat| Administration via diet                                                                | AChE inhibitor       |
|                  |                                                                 |                  |                 |                        | Source: DAR, addendum 2005                                                                |                      |
| Carbaryl         | Tremors, decreased motor activity, ataxic gait                  | 10               | 50              | Acute neurotoxicity rat| Administration via gavage                                                                | AChE inhibitor       |
|                  |                                                                 |                  |                 |                        | Source: DAR 2004                                                                         |                      |
| Carbofuran       |                                                                 | 0.3              | –               | Acute neurotoxicity rat| Administration via gavage, Highest tested dose                                           | AChE inhibition      |
|                  |                                                                 |                  |                 |                        | Source: Revised DAR 2008                                                                  |                      |
| Carbosulfan      | Reduced motor activity                                          | 5                | 30              | Acute neurotoxicity rat| Administration via gavage                                                                | AChE inhibitor       |
|                  |                                                                 |                  |                 |                        | Source: Revised DAR 2009                                                                  |                      |
| Active substance   | Indicator of specific effect                                      | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                        | Remark                                                                                     | MoA                          |
|-------------------|------------------------------------------------------------------|------------------|------------------|------------------------------|--------------------------------------------------------------------------------------------|------------------------------|
| Chlorpropham      | Reduced motor activity                                           | 50               | 125              | Acute dog                    | Source: DAR 1999, JMPR 2005                                                              | Unknown                      |
| Chlorfenvinphos   | AChE inhibition (brain, erythrocyte)                             | 0.15             | 15               | 2-year rat                   | Source: JMPR 1994 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor               |
| Chlorimequat      | Ataxia                                                           | 10               | 32               | 1-year dog                   | Source: DAR 2007 Neurotoxicity studies not available ARFD: 0.09 mg/kg bw                  | Partial agonist of muscarinic and nicotinic acetylcholine receptor |
| Chlorpyrifos      | Decreased motor activity, decreased grip performance             | 10               | 50               | Acute neurotoxicity rat      | Source: DAR 1999                                                                     | AChE inhibitor               |
| Chlorpyrifos-methyl | --                                                                | 75               | --               | Acute neurotoxicity rat      | Source: RAR 2017                                                                     | AChE inhibition               |
| Clothianidin      | Decreased activity, tremor                                      | 200              | 400              | Acute neurotoxicity rat      | Source: DAR 2003                                                                     | Agonist of nAChR             |
| Cyfluthrin        | Choreoathetosis                                                  | 1                | 2.5              | Acute neurotoxicity rat      | Source: JMPR 2006                                                                     | Binding to VGSC              |
| Cypermethrin      | Decreased activity, decreased grip strength, splayed legs, landing foot splay, abnormal locomotion, lateral head movements | 20               | 60               | Acute neurotoxicity rat      | Source: DAR EC review report on cypermethrin (2005) considered.                        | Binding to VGSC              |
| Deltamethrin      | Ataxia, landing-foot splay, tremor                              | 1                | 10               | 1-year dog                   | Source: DAR 1998 EC review report on deltamethrin (2002) considered.                    | Binding to VGSC              |
| Diazinon          | Ataxic gait                                                      | 2.5              | 150              | Acute neurotoxicity rat      | Source: DAR 2004 EFSA conclusions on diazinon (2006) considered                      | AChE inhibitor               |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|------------------|------------------|-------|--------|-----|
| Dichlorvos       | Ataxia                      | 8                | 16               | 28-day rat | Administration via diet | Source: DAR 2003 Neurotoxicity studies not available | AChE inhibition |
| Dicofol          | Ataxia                      | 15               | 75               | Acute neurotoxicity rat | Administration via gavage | Source: DAR 2006 4 presumed MoA (Section 3.3.2) |
| Dieldrin         | Increased motor activity, convulsions, tremors | 1 | 5 | 3-days rat-mechanistic study | Administration via gavage | Source: PPR panel, 2007 Neurotoxicity studies not available | GABA-gated chlorine channel antagonist |
| Dimethoate       | Ataxia, convulsions, reduced grip strength, reduced motor activity, tremor | 20 | 200 | Acute neurotoxicity rat | Administration via gavage | Source: DAR | AChE inhibitor |
| Dinotefuran      | Reduced motor activity      | 100              | 300              | Acute single dose rabbit | Administration via gavage | Source: JMPR 2012 | Agonist of nAChR |
| Emamectin benzoate | Tremor                  | 5 (1)            | 10               | Acute neurotoxicity rat | Administration via gavage | Source: DAR 2008 EFSA conclusions on emamectin benzoate (2012) considered Additional UF of 5 due to small dose spacing and steep dose response curve, and possible acute effects in dogs at 5 mg/kg bw | GABA-gated chloride channel agonist |
| Endosulfan       | Increased motor activity, tremor, reduced motor activity, convulsions, abnormal gait | 3 | 6 | Acute neurotoxicity rat | Administration via gavage | Source: DAR 2001 and addendum | GABA-gated chloride channel blocker |
| Endrin           | Convulsions                | 0.025            | 0.05             | 2-year dog | Administration via diet | Source: EHC 130, 1992 Neurotoxicity studies not available ADI: 0.0002 (JMPR 1994) | GABA-gated chloride channel antagonist |
| Esfenvalerate    | Reduced grip strength      | 3.2              | 6.4              | 90-day neurotoxicity rat | Administration via diet | Source: JMPR 2002 | Binding to VGSC |
| Active substance | Indicator of specific effect                                      | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                              | Remark                                                                 | MoA                     |
|------------------|------------------------------------------------------------------|------------------|------------------|------------------------------------|----------------------------------------------------------------------|-------------------------|
| Ethephon         | Reduced motor activity                                           | 500              | 1,000            | Acute neurotoxicity rat Administration via gavage | Source: DAR 2004                                                   | AChE inhibitor          |
| Ethion           | AChE inhibition (brain)                                          | 0.06             | 0.71             | 90-day dog Administration via diet | Source: JMPR 1990 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor          |
| Ethoprophos      | Reduced motor activity, abnormal posture and gait                | 5                | 25               | Acute neurotoxicity rat Administration via gavage | Source: DAR 2004                                                   | AChE inhibitor          |
| Fenamiphos       | Ataxia, muscle fascication                                       | 0.37             | 1.52             | Acute neurotoxicity rat Administration via gavage | Source: DAR 2003                                                   | AChE inhibitor          |
| Fenitrothion     | Increased motor activity, tremor, abnormal gait, hypoactivity, reduced grip strength | 12.5             | 50               | Acute neurotoxicity rat Administration via gavage | Source: DAR 2003                                                   | AChE inhibitor          |
| Fenpropathrin    | Increased motor activity, tremor                                 | 15               | 30               | Acute neurotoxicity rat Administration via gavage | Source: JMPR 2012                                                  | Binding to VGSC         |
| Fenthion         | Reduced motor activity                                           | 1.04             | 49.05            | Acute neurotoxicity rat Administration via gavage | Source: DAR 1996, Addendum Tox 2001                              | AChE inhibitor          |
| Fenvalerate      | Ataxia, tremor                                                   | 20               | 80               | Acute neurotoxicity rat Administration via gavage | Source: JMPR 2012                                                  | Binding to VGSC         |
| Fipronil         | Landing-foot splay                                               | 2.5              | 7.5              | Acute neurotoxicity rat Administration via gavage | Source: DAR 2004                                                   | GABA-gated chloride channel blocker |
| Flufenacet       | Ataxia, reduced motor activity                                   | 7.5              | 75               | Acute neurotoxicity rat Administration via gavage | Source: DAR 2004                                                   | Unknown                 |
| Fonofos          | Increased motor activity, abnormal gait                          | 4                | 7                | Acute neurotoxicity rat (author not reported) Administration via gavage | Source: US EPA 1999 No reference values available | AChE inhibitor          |
| Active substance  | Indicator of specific effect                                      | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                      | Remark                                      | MoA                                                      |
|-------------------|------------------------------------------------------------------|------------------|------------------|--------------------------------------------|---------------------------------------------|----------------------------------------------------------|
| Formetanate       | Ataxia, tremor, reduced motor activity                            | 1                | 10               | Acute neurotoxicity rat                    | Source: DAR 2004                            | AChE inhibitor                                           |
|                   |                                                                  |                  |                  | Administration via gavage                 |                                             |                                                          |
| Fosthiazate       | Ataxia, uncoordinated movements                                  | 5.4              | 26.8             | 28-day dog                                 | Source: DAR 1998                            | AChE inhibitor                                           |
|                   |                                                                  |                  |                  | Administration via capsule                | Neurotoxicity studies not available         |                                                          |
| Glufosinate       | Hunched posture                                                  | 100              | 500              | Acute neurotoxicity rat                    | EFSA 2005                                   | Unknown                                                  |
|                   |                                                                  |                  |                  | Administration via gavage                 |                                             |                                                          |
| Heptachlor        | Increased motor activity, convulsions                            | 0.5              | 5                | 6-month rat                                | Source: JMPR 1970, CICADS 2006              | GABA-gated chloride channel antagonist                  |
|                   |                                                                  |                  |                  | Administration via gavage                 | Not true NOAEL, but LAOEL divided by 10.   |                                                          |
|                   |                                                                  |                  |                  |                                             | ADI: 0.0001 mg/kg (JMPR 1994)              |                                                          |
|                   |                                                                  |                  |                  |                                             | 14-day neurotoxicity rat                    |                                                          |
|                   |                                                                  |                  |                  |                                             | : No effect at 69 mg/kg bw per day (highest tested dose) |                                                          |
| Imidacloprid      | Tremor                                                           | 23.5             | 45.4             | 90-day dog                                 | Source: DAR 2005                            | Agonist of nAChR                                        |
|                   |                                                                  |                  |                  | Administration via diet                    |                                             |                                                          |
| Indoxacarb        | Decreased motor activity                                         | 50               | 100              | Acute neurotoxicity rat                    | Source: DAR 2000                            | Voltage-dependent sodium channel blocker                |
|                   |                                                                  |                  |                  | Administration via gavage                 |                                             |                                                          |
| lambda-Cyhalothrin| Ataxia, tremor, convulsion                                       | 0.5              | 3.5              | 1-year dog                                 | Source: DAR 1996, JMPR 2007                | Binding to VGSC                                          |
|                   |                                                                  |                  |                  | Administration via capsule                | EFSA conclusions on lambda-cyhalothrin (2014) considered |                                                          |
|                    |                                                                  |                  |                  | Acute neurotoxicity rat                    |                                             |                                                          |
|                    |                                                                  |                  |                  | Administration via gavage                 |                                             |                                                          |
| Lindane           | Increased forelimb grip strength                                 | 6                | 20               | Acute neurotoxicity rat                    | Source: JMPR 2002                            | GABA-gated chloride channel antagonist                  |
|                   |                                                                  |                  |                  | Administration via gavage                 | JMPR evaluation of lindane (2002)          |                                                          |
| Malathion         | Reduced ambulatory activity and total motor activities, reduced  | 1,000            | 2,000            | Acute neurotoxicity rats                   | Source: DAR                                 | AChE inhibitor                                           |
|                   | hindlimb resistance                                              |                  |                  | Administration via gavage                 |                                             |                                                          |

Source: DAR 2004, DAR 2005, DAR 2000, DAR 1996, DAR 2004, JMPR 1970, CICADS 2006, EFSA 2005, EFSA 2005.
| Active substance       | Indicator of specific effect                          | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                        | Remark                                                                 | MoA                                      |
|-----------------------|------------------------------------------------------|------------------|------------------|------------------------------|-------------------------------------------------------------------------|------------------------------------------|
| Mepiquat              | Lateral position, spasm                              | 32               | 95               | 3-month dogs                 | Activation of nicotinic and muscarinic acetylcholine receptors          | Source: DAR 2005 EFSA conclusions on mepiquat (2008) considered          |
| Metaldehyde           | Ataxia, reduced motility                             | 30               | 75               | 1-year dog                   | GABA inhibitor (presumed)                                              | Source: DAR 2009 EFSA conclusions on metaldehyde (2010) considered.     |
| Methamidophos         | Increased or decreased muscle tone, abnormal gait, tremor, hypoactivity | 1                | 3                | Acute neurotoxicity rat      | AChE inhibitor                                                          | Source: DAR 2000                                                               |
| Methidathion          | Tremor, ataxia                                       | 4                | 8                | Acute neurotoxicity rat      | AChE inhibitor                                                          | Source: JMPR 1997 addendum                                                        |
| Methiocarb            | Tremor                                               | 0.5              | 1.5              | Developmental study rat      | AChE inhibitor                                                          | Source: DAR 2004 EFSA (2018) Neurotoxicity studies not available.         |
| Methomyl              | Tremor                                               | 0.75             | 2                | Acute neurotoxicity rat      | AChE inhibitor                                                          | Source: DAR 2000                                                               |
| Milbemectin           | Staggering gait, tremor                              | 10               | 30               | 13-week dog                  | Glutamate-gated chloride (GluCl) allosteric modulator                  | Source: DAR 2001, RAR, 2017                                                 |
| Monocrotophos         | Tremor                                               | 0.4              | 2.5              | 2-year dog                   | AChE inhibitor                                                          | Source: JMPR 1972 Neurotoxicity studies not available.                     |
| Omethoate (metabolite of dimethoate) | Increased motor activity, tremor, abnormal gait, reduced grip strength | 0.35             | 5                | Acute neurotoxicity rat      | AChE inhibitor                                                          | Source: DAR 2004 EFSA (dimethoate)                                         |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|----------------------------|-----------------|-----------------|-------|--------|-----|
| Oxamyl (IC)      | Ataxia, hunched posture, landing-foot splay, reduced motor activity, tremor | 0.1             | 0.75            | Acute neurotoxicity | Administration via gavage | Source: DAR 2003 EFSA conclusions on oxamyl (2006) considered | AChE inhibitor |
| Oxydemeton-methyl | Increased or decreased muscle tone | 1.88            | 3.75            | 90-day dog | Administration via diet | Source: DAR 2004 Neurotoxicity studies not available NOAEL for AChE inhibition: 0.2 mg/kg bw | AChE inhibitor |
| Parathion        | Tremor                      | 1.75            | 5.6             | 90-day rat | Administration via diet | Source: ECCO 2001, JMPR 1995 Neurotoxicity studies not available NOAEL for AChE inhibition: 0.2 mg/kg bw | AChE inhibitor |
| Parathion-methyl | Tremor                      | 1.69            | 5.9             | 90-day rat | Administration via diet | Source: DAR 2001 Neurotoxicity studies not available NOAEL for AChE inhibition: 0.25 mg/kg bw | AChE inhibitor |
| Penflufen        | Reduced motor activity      | 50              | 100             | Acute neurotoxicity rat | | EFSA (2012) Unknown | Unknown |
| Permethrin       | Abnormal gait, tremor       | 150             | 300             | Single dose rat | Administration via gavage | Source: DAR 1998, JMPR 1999 Binding to VGSC | |
| Phenthoate       | AChE inhibition (erythrocytes) | 0.29            | 0.87            | 2-year dog | Administration via diet | Source: JMPR 1980 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate. | AChE inhibitor |
| Phosalone        | Tremor, hypoactivity        | 25              | 60              | Acute neurotoxicity rat | Administration via gavage | Source: DAR 2004 EFSA (2006) | AChE inhibitor |
| Phosmet          | Ataxia, tremor              | 22.5            | –               | Acute neurotoxicity rat | Administration via gavage | Source: DAR 2004 EFSA (2006) | AChE inhibitor |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|------------------|------------------|-------|--------|-----|
| Phoxim           | Convulsions                 | 5                | 25               | 90-day dog Administration via diet | Source: JECFA 1999 Neurotoxicity studies not available NOAEL for AChE inhibition: 1.3 mg/kg bw | AChE inhibitor |
| Pirimicarb       | Hunched posture             | 10               | 40               | Acute neurotoxicity rat Administration via gavage | Source: DAR 2003 EFSA (2005) | AChE inhibitor |
| Pirimiphos-methyl| Convulsions, reduced grip strength | 150             | 1,500            | Acute neurotoxicity rat Administration via gavage | Source: DAR 2003 | AChE inhibitor |
| Profenofos       | Ataxia, abnormal gait, tremors, altered posture | 190             | 380              | Acute neurotoxicity rat Administration via gavage | Source: JMPR 2007 | AChE inhibitor |
| Pymetrozine      | Decreased motor activity    | 12.5             | 125              | Acute neurotoxicity rat Administration via gavage | Source: DAR 1998 (and addendum 1999) Not true NOAEL, but LOAEL divided by 10 | Chordotonal organ TRPV channel modulator (presumed) |
| Pyrazophos       | Increased or decreased muscle tone, weakness, abnormal gait | 0.45            | 8                | 6-month dog Administration via diet | Source: DAR 1998 Neurotoxicity studies not available NOAEL for AChE inhibition: 0.05 mg/kg bw | AChE inhibitor |
| Pyrethrins       | Tremor                      | 20               | 63               | Acute neurotoxicity rat Administration via gavage | Source: DAR 2007, 2012 EFSA (2013) | Binding to VGSC |
| Pyridate         | Prostration, tremor, hunched posture, hypoactivity | 40             | 80               | 90-day dog Administration via gavage | Source: RAR 2013 | Unknown |
| Spirotetramat    | Reduced motor activity      | 100              | 200              | Acute neurotoxicity rat Administration via gavage | Source: DAR 2012 EFSA (2013) | Unknown |
| Sulfoxadiazin    | Reduced motor activity      | 25               | 75               | Acute neurotoxicity rat Administration via gavage | Source: DAR 2012 EFSA (2014) | Nicotinergic AChR partial agonist |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                  | Remark                                                                 | MoA                  |
|------------------|----------------------------|------------------|------------------|------------------------|------------------------------------------------------------------------|----------------------|
| Tau-fluvalinate  | Altered posture           | 10               | 60               | Acute neurotoxicity rat Administration via gavage | Source: DAR 2007 Binding to VGSC |                      |
| Tefluthrin       | Tremor                    | 0.5              | 1.5              | 90-day dog  | Administration via capsule | Source: DAR 2006 EFSA (2010) Binding to VGSC |                      |
| Tembotrione      | Reduced motor activity    | 200              | 500              | Acute neurotoxicity rat Administration via capsule | Source: DAR 2012 EFSA (2013) Unknown |                      |
| Tetramethrin     | Increased motor activity: tremor | 31          | 63               | 6-month dog  | Administration via diet | Source: WHO 1990 Neurotoxicity studies not available No reference values available Binding to VGSC |                      |
| Thiacloprid      | Reduced motor activity    | 3.1              | 11               | Acute neurotoxicity rat Administration via gavage | Source: DAR 2000, 2017 EC review report on thiacloprid (2004) considered agonist of nAChR |                      |
| Thiamethoxam     | Impairment of gait, increased forelimb grip strength, hypoactivity | 100              | 500              | Acute neurotoxicity rat Administration via gavage | Source: DAR 2002 EC review report on thiamethoxam (2007) considered agonist of nAChR |                      |
| Thiodicarb       | Reduced motor activity, ataxia, tremor | 5               | 20               | Acute neurotoxicity rat Administration via gavage | Source: DAR 2003 AChE inhibitor |                      |
| Thiram           | Reduced grip strength     | 5                | 150              | Acute neurotoxicity rat Administration via gavage | Source: DAR 2003 Neurotoxic effect might be due to the metabolite CS₂ (presumed) |                      |
| Tolclofos-methyl | Decreased motor activity  | 200              | 700              | Acute neurotoxicity rat Administration via gavage | Source: RAR 2016 AChE inhibitor |                      |
| Triadimefon      | Increased motor activity, abnormal gait | 2               | 35               | Acute neurotoxicity rat Administration via gavage | Source: JMPR 2004 JMPR evaluations on triadimefon (2004) considered Inhibition of dopamine transporter (presumed) |                      |
### Table: Active Substance and Indicators of Specific Effect

| Active Substance | Indicator of Specific Effect                                      | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                  | Remark                                                                                     | MoA               |
|------------------|-----------------------------------------------------------------|------------------|------------------|------------------------|--------------------------------------------------------------------------------------------|-------------------|
| Tri-allate       | Flat footed appearance/decreased motor activity                 | 60               | 300              | Acute neurotoxicity   | Source: DAR 2007 EFSA conclusions on tri-allate (2009) considered                           | Unknown          |
|                  |                                                                 |                  |                  |                        | Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate            | AChE inhibitor    |
| Triazophos       | AChE inhibition (erythrocytes)                                  | 0.012            | 0.13             | 1-year dog            | Source: JMPR 2002 AChE inhibitor                                                          |                  |
| Trichlorfon      | Increased or decreased muscle tone                              | 10               | 50               | Acute neurotoxicity   | Source: DAR 2004 and addendum 2005 EFSA conclusions on zeta-cypermethrin (2008) considered| AChE inhibitor    |
| zeta-Cypermethrin | Ataxia, tremor, convulsions, staggered gait, splayed hindlimbs | 10               | 50               | Acute neurotoxicity   | Source: DAR 1998 Neurotoxic effect might be due to the metabolite CS2 (presumed)          | Binding to VGSC   |
| Ziram            | Ataxia, hunched posture                                         | 15               | 300              | Acute neurotoxicity   | Source: DAR 1998 Neurotoxic effect might be due to the metabolite CS2 (presumed)          |                  |

CAG: cumulative assessment group; AS: active substance; NOEL: no observed effect level; LOEL: lowest observed effect level; NOAEL: no observed adverse effect level; LOAEL: lowest observed adverse effect level; bw: body weight; GABA: gamma-aminobutyric acid; AChE: acetylcholinesterase; VGSC: voltage-gated sodium channel; nAChR: nicotinic acetylcholine receptor; MoA: mode of action.
### Table B.2: CAG on functional effects on sensory division: toxicological characterisation of ASs to be considered in acute exposure/risk assessments

| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|------------------------------|------------------|------------------|-------|--------|-----|
| Abamectin        | Reduced splay reflex         | 0.5              | 1.5              | Acute neurotoxicity rat Administration via gavage | Source: DAR 2005, addendum 2007 EFSA conclusions on abamectin (2008) considered | GABA-gated chloride channel agonist |
| Acephate         | –                            | 74.2             | –                | 49-day neurotoxicity rat Administration via diet Highest tested dose | Source: JMPR 2002 | AChE inhibitor |
| Acetamiprid      | –                            | 100              | –                | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2001 | Agonist of nAChR |
| Acrinathrin      | –                            | 37.5             | –                | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2007 | Binding to VGSC |
| Aldicarb         | Decreased reactivity: tail pinch response | 0.1              | 0.5              | Acute neurotoxicity rat Administration via gavage | Source: DAR 1996 | AChE inhibitor |
| alpha-cypermethrin | Righting reflex            | 20               | 40               | Acute neurotoxicity rat Administration via gavage | Source: DAR 2000 | Binding to VGSC |
| Amitraz          | Hyperreactivity             | 12               | 50               | 90-day rat Administration via gavage | Source: DAR Neurotoxicity studies not available | Partial agonist of presynaptic α2-adrenergic receptor |
| Azinphos-ethyl   | AChE inhibition (erythrocyte) | 0.0125           | 0.025            | 90-day dog Administration via diet | Source: JMPR 1973 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor |
| Azinphos-methyl  | Decreased reactivity: righting reflex (air drop) | 2                | 6                | Acute neurotoxicity rat Administration via gavage | Source: DAR 1996, Addendum 6, 2000 (neurotoxicity) | AChE inhibitor |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|------------------------------|------------------|------------------|-------|--------|-----|
| Benfuracarb      | Decreased reactivity: analgesic reflex (nociception response) | 2                | 20               | 28-day rat, Administration via gavage | Source: DAR 2004 Acute neurotoxicity study not available | Known AChE inhibitor |
| beta-Cyfluthrin  | Decreased touch responses, tail pinch response and impaired righting. | 2                | 10               | Acute neurotoxicity rat, Administration via gavage | Source: JMPR 2006, DAR 1996 | Binding to VGSC |
| beta-cypermethrin| Decreased reactivity: hypoactivity, tail pinch response | 100              | 500              | Acute neurotoxicity rat | Source: DAR 2013 | Known Binding to VGSC |
| Bifenthrin       | –                            | 75               | –                | Acute neurotoxicity rat, Administration via diet | Source: DAR | Known Binding to VGSC |
| Cadusafos        | –                            | 40               | –                | Acute neurotoxicity rat, Administration via gavage | Source: DAR, addendum 2005 | AChE inhibitor |
| Carbaryl         | Impaired toe pinch           | 10               | 50               | Acute neurotoxicity rat, Administration via gavage | Source: DAR 2004 | AChE inhibitor |
| Carbofuran       | –                            | 0.3              | –                | Acute neurotoxicity rat, Administration via gavage | Source: Revised DAR 2008 | AChE inhibition |
| Carbosulfan      | Slot tail pinch response     | 5                | 30               | Acute neurotoxicity rat, Administration via gavage | Source: Revised DAR 2009 | AChE inhibitor |
| Chlorfenvinphos  | AChE inhibition (brain, erythrocyte) | 0.15           | 15               | 2-year rat (author not reported, JMPR 1994), NOAEL for AChE inhibition used as surrogate | Source: JMPR 1994 | AChE inhibitor |
## CAGs of Pesticides for Effects on Nervous System

| Active Substance     | Indicator of Specific Effect                                                                 | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                                                 | Remark                                                                | MoA                                                                 |
|----------------------|---------------------------------------------------------------------------------------------|------------------|-----------------|----------------------------------------------------------------------|-----------------------------------------------------------------------|----------------------------------------------------------------------|
| Chlormequat          | Decreased reflex response                                                                  | 50               | 62.5            | 90-day dog Administration via det                                    | Source: DAR 2007                                                      | Partial agonist of muscarinic and nicotinic acetylcholine receptor    |
| Chlorpyrifos         | —                                                                                           | 100              | —               | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 1999                                                      | AChE inhibitor                                                       |
| Chlorpyrifos-methyl  | —                                                                                           | 75               | —               | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: RAR 2017                                                      | AChE inhibition                                                      |
| Clothianidin         | Pin-point constriction                                                                      | 200              | 400             | Acute neurotoxicity rat Administration via gavage                    | Source: DAR 2003                                                      | Agonist of nAChR                                                     |
| Cyfluthrin           | —                                                                                           | 7.5              | —               | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: JMPR 2006                                                     | Binding to VGSC                                                      |
| Cypermethrin         | —                                                                                           | 60               | —               | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR                                                           | Binding to VGSC                                                      |
| Deltamethrin         | Absent approach response, absent touch response, absent startle and tail pinch responses, altered air righting reflex | 15               | 50              | Acute neurotoxicity rat Administration via gavage                    | Source: Source: DAR 1998, addendum                                   | Binding to VGSC                                                      |
| Diazinon             | Decreased tail pinch response, impaired righting reflex                                     | 300              | 600             | Acute neurotoxicity rat Administration via gavage                    | Source: DAR 2004                                                      | AChE inhibitor                                                       |
| Dicamba              | Abnormal righting reflex. Increased tail flick latency time                                 | 30               | 300             | Acute neurotoxicity rat Administration via gavage                    | Source: revised DAR                                                   | Not true NOAEL, but LOAEL divided by 10                               | Unknown                                                             |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|----------------------------|------------------|------------------|-------|--------|-----|
| Dichlorvos       | AChE inhibition (erythrocytes) | 0.1              | 1.5              | 13-week rat | Source: DAR 2003 (addendum) Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor |
| Dicofol          | Decreased reactivity- hypoactivity | 75               | 350              | Acute neurotoxicity rat | Source: DAR 2006 | 4 presumed MoA (Section 3.3.2) |
| Dieldrin         | Absence of pupil response   | 0.3              | 2                | Virtual NOAEL corresponding to the ARfD of 0.003 mg/kg bw/d derived by the PPR Panel and considered as protective for all endpoints. | Source: PPR panel, 2007 Neurotoxicity studies not available | GABA-gated chlorine channel antagonist |
| Dimethoate       | Absence of pupil response   | 2                | 20               | Acute neurotoxicity rat | Source: DAR | AChE inhibitor |
| Dinotefuran      | –                           | 1,500            | –                | Acute neurotoxicity rat | Source: JMPR 2012 | Agonist of nAChR |
| Emamectin benzoate | –                         | 82.2 (16.4)      | –                | Acute neurotoxicity rat | Source: DAR 2008 EFSA (2012) Additional UF of 5 due to small dose spacing and steep dose response curve, and possible acute effects in dogs at 5 mg/kg bw | GABA-gated chloride channel agonist |
| Endosulfan       | Hyperreactivity             | 3                | 6                | Acute neurotoxicity rat | Source: DAR 2001 and addendum | GABA-gated chloride channel blocker |
| Endrin (IC)      | Hyperreactivity             | 0.05             | 1.0              | 2-year rat | Source: EHC 130, 1992 Neurotoxicity studies not available ADI: 0.0002 (JMPR 1994) | GABA-gated chloride channel antagonist |
| Active substance | Indicator of specific effect                                      | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                      | Remark                                                                 | MoA                  |
|------------------|-----------------------------------------------------------------|------------------|------------------|-------------------------------------------|------------------------------------------------------------------------|----------------------|
| Esfenvalerate    | Increased reaction to touch                                     | 20               | 80               | Acute neurotoxicity rat                   | Source: JMPR 2002 Approved.                                            | Binding to VGSC      |
| Ethephon         | –                                                               | 2,000            | –                | Acute neurotoxicity rat                   | Source: DAR 2004 AChE inhibitor                                        |                      |
| Ethion           | AChE inhibition (brain)                                         | 0.06             | 0.71             | Acute neurotoxicity rat                   | Source: JMPR 1990 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor      |
| Ethoprophos      | –                                                               | 25               | –                | Acute neurotoxicity rat                   | Source: DAR 2004 AChE inhibitor                                        |                      |
| Fenamiphos       | –                                                               | 2.31             | –                | Acute neurotoxicity rat                   | Source: DAR 2003 AChE inhibitor                                        |                      |
| Fenitrothion     | Decreased reactivity: tail pinch response, righting reflex (air drop) | 12.5             | 50               | Acute neurotoxicity rat                   | Source: DAR 2003 AChE inhibitor                                        |                      |
| Fenpropathrin    | –                                                               | 30               | –                | Acute neurotoxicity rat                   | Source: JMPR 2012 Binding to VGSC                                     |                      |
| Fenthion         | Impaired righting reflex, increased touch response              | 1.04             | 49.05            | Acute neurotoxicity rat                   | Source: DAR 1996, Addendum Tox 2001 AChE inhibitor                     |                      |
| Fenvalerate      | –                                                               | 360              | –                | Acute neurotoxicity rat                   | Source: JMPR 2012 Binding to VGSC                                     |                      |
| Active substance       | Indicator of specific effect                                                                 | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                      | Remark                                                                 | MoA                                         |
|------------------------|-----------------------------------------------------------------------------------------------|------------------|------------------|-----------------------------|------------------------------------------------------------------------|---------------------------------------------|
| Fipronil               | Approach response, tail pinch response, air righting reflex                                   | 5                | 50               | Acute neurotoxicity rat     | Source: DAR 2004                                                       | GABA-gated chloride channel blocker        |
| Fonofos                | Decreased reactivity: patellar reflex                                                          | 4                | 7                | Acute neurotoxicity rat     | Source: US EPA 1999                                                    | AChE inhibitor                             |
| Formetanate            | Diminished reaction to tail pinch test, abnormal response to visual placing test, auditory starle response | 1                | 10               | Acute neurotoxicity rat     | Source: DAR 2004                                                       | AChE inhibitor                             |
| Fosthiazate            | AChE inhibition (brain)                                                                       | 0.54             | 5.4              | 90-day dog                 | Source: DAR 1998                                                       | AChE inhibition                            |
| Halosulfuron methyl    | Decreased reactivity: righting reflex (air drop)                                              | 600              | 2,000            | Acute neurotoxicity rat     | Source: DAR 2007                                                       | Unknown                                    |
| Heptachlor             | Hyperreactivity                                                                               | 2                | 7                | 14-day neurotoxicity rat    | Source: JMPR 1970, CICADS 2006 ADI: 0.0001 mg/kg (JMPR 1994)           | GABA-gated chloride channel antagonist     |
| Imidacloprid           | –                                                                                           | 307              | –                | Acute neurotoxicity rat     | Source: DAR 2005                                                       | Agonist of nAChR                          |
| Indoxacarb             | –                                                                                           | 100              | –                | Acute neurotoxicity rat     | Source: DAR 2000                                                       | Voltage-dependent sodium channel blocker   |
| Lambda-cyhalothrin     | More energetic response to touch                                                              | 1                | 10               | Acute neurotoxicity rat     | Source: DAR                                                            | Binding to VGSC                            |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|------------------|------------------|-------|--------|-----|
| Lindane          |                             | 6                |                  |       |        |     |
| Malathion        |                             | 2,000            |                  |       |        |     |
| Mepiquat         | Lack of pupillary reflex    | 300              | 1,200            |       |        |     |
| Metaldehyde      | Reduced righting reflex, reduced toe/tail pinch response | 150 | 250 |       |        |     |
| Methamidophos    |                             | 9                |                  |       |        |     |
| Methidathion     | Decreased reactivity: analgesic reflex (nociception response) | 4 | 5 |       |        |     |
| Methiocarb       | AChE inhibition (erythrocytes) | 0.5 | 2 |       |        |     |
| Methomyl         |                             | 2                |                  |       |        |     |

- Neurotoxicity studies not available
- NOAEL of 6 mg/kg bw/d used by JMPR (2002) to establish the ARfD used as surrogate
- Source: DAR
- AChE inhibitor
- Source: DAR 2005
- Activation of nicotinic and muscarinic acetylcholine receptors
- Source: DAR 2000
- Paused GABA inhibitor
- Source: JMPR 1997 addendum
- AChE inhibitor
- Source: DAR 2004
- Neurotoxicity studies not available
- NOAEL for AChE inhibition used as surrogate
- Source: DAR
- AChE inhibition
- Source: DAR
| Active substance          | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                          | Remark                                                                 | MoA                                                  |
|--------------------------|------------------------------|------------------|------------------|-------------------------------|------------------------------------------------------------------------|------------------------------------------------------|
| Milbemectin              |                              | 160              | –                | Acute neurotoxicity rat       | Source: RAR 2017                                                      | GABA-gated chloride channel agonist                  |
| Monocrotophos            | AChE inhibition (brain, erythrocytes) | 0.1              | 0.3              | Single dose rat               | Source: JMPR 1995, addendum Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition                                      |
| Omethoate (metabolite of dimethoate) | Decreased reactivity: pupil response | 0.35             | 5                | Acute neurotoxicity rat       | Source: DAR 2004 (dimethoate)                                         | AChE inhibitor                                       |
| Oxamyl (IC)              | Righting reflex, tail pinch | 0.1              | 0.751            | Acute neurotoxicity rat       | Source: DAR 2003                                                      | AChE inhibitor                                       |
| Oxydemeton-methyl        | AChE inhibition (brain, erythrocytes) | 0.2              | 0.6              | 14-day rat                    | Source: DAR 2004 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition                                      |
| Parathion                | AChE inhibition (brain)      | 0.25             | 2.5              | 2-year rat                    | Source: JMPR 1995 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition                                      |
| Parathion-methyl         | AChE inhibition (erythrocytes) | 0.25             | 2.5              | 2-year rat                    | Source: DAR 2001 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition                                      |
| Permethrin               | Hyperreactivity              | 150              | 300              | Single dose rat               | Source: JMPR 1999                                                      | Binding to VGSC                                       |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|------------------|------------------|-------|-------|-----|
| Phenthoate       | AChE inhibition (erythrocytes) | 0.29             | 0.87             | 2-year dog Administration via diet | Source: JMPR 1980 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition |
| Phosalone        | –                           | 60               | –                | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2004 | AChE inhibitor |
| Phosmet          | –                           | 22.5             | –                | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2004 | AChE inhibitor |
| Phoxim           | AChE inhibition (erythrocytes) | 1.3              | 5                | 90-day dog Administration via diet | Source: JECFA 1999 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition |
| Pirimicarb       | –                           | 110              | –                | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2003 | AChE inhibitor |
| Pirimiphos-methyl | –                           | 1,500            | –                | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2003 | AChE inhibitor |
| Profenofos       | –                           | 380              | –                | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: JMPR 2007 | AChE inhibitor |
| Pyrazophos       | AChE inhibition (erythrocytes) | 0.05             | 0.125            | 2-year dog Administration via diet | Source: JMPR 1992 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|-----------------|-----------------|-------|--------|-----|
| Pyrethrins       |                             | 200             | –               | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2007, 2012 | Binding to VGSC |
| Sulfoxadiazin    | Decreased reactivity: touch response (handling reactivity) | 75               | 750             | Acute neurotoxicity rat Administration via gavage | Source: DAR 2012 | Nicotinergic AChR partial agonist |
| Tau-fluvalinate  |                             | 100             | –               | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2007 | Binding to VGSC |
| Tebuconazole     | Poor reflexes               | 10§             | 100             | Acute mouse Administration via gavage | Source: DAR 2008 | Not true NOAEL, but LOAEL divided by 10 |
| Tefluthrin       |                             | 10              | –               | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2006 | Binding to VGSC |
| Tembotrione      | Decreased reactivity: approach response | 500             | 2,000           | Acute neurotoxicity rat Administration via gavage | Source: DAR 2012 | Unknown |
| Tetramethrin     |                             | –               | –               | –                 | Information insufficient | Binding to VGSC |
| Thiacloprid      |                             | 109             | –               | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2000, RAR, 2017 | Agonist of nAChR |
| Thiodicarb       | Decreased reactivity: tail pinch response | 5               | 20              | Acute neurotoxicity rat Administration via gavage | Source: DAR 2003 | AChE inhibitor |
| Thiacloprid      | Uncoordinated landing in the righting reflex | 100             | 500             | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2002 | Agonist of nAChR |
| Thiamethoxam     |                             | 109             | –               | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2000, RAR, 2017 | Agonist of nAChR |
| Active substance       | Indicator of specific effect                                      | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                        | Remark                                                                 | MoA                  |
|------------------------|------------------------------------------------------------------|------------------|-----------------|------------------------------|------------------------------------------------------------------------|----------------------|
| Thiram                 | Handling reactivity, approach response, startle response, air    | 5                | 150             | Acute neurotoxicity rat      | Source: DAR 2003                                                        | Neurotoxic effect    |
|                        | righting                                                          |                  |                 |                              | might be due to the metabolite CS₂ (presumed)                         |                      |
| Trichlorfon            | Decreased reactivity: righting reflex (air drop)                  | 10               | 50              | Acute neurotoxicity rat      | Source: JMPR 2002                                                        | AChE inhibitor       |
|                        |                                                                  |                  |                 | Administration via gavage    |                                                                       |                      |
| Tolclofos-methyl       | –                                                                | 2,000            | –               | Acute neurotoxicity rat      | Source: RAR 2016                                                        | AChE inhibitor       |
|                        |                                                                  |                  |                 | Administration via gavage    |                                                                       |                      |
|                        |                                                                  |                  |                 | Highest tested dose          |                                                                       |                      |
| Triazophos             | AChE inhibition (erythrocytes)                                    | 0.012            | 0.13            | 1-year dog                  | Source: JMPR 2002                                                        | AChE inhibitor       |
|                        |                                                                  |                  |                 | Administration via diet      | Neurotoxicity studies not available NOAEL for AChE inhibition          |                      |
|                        |                                                                  |                  |                 |                              | used as surrogate                                                       |                      |
| zeta-Cypermethrin      | Righting reflex                                                   | 50               | 250             | Acute neurotoxicity rat      | Source: DAR 2004 and addendum 2005                                     | Binding to VGSC      |
|                        |                                                                  |                  |                 | Administration via gavage    |                                                                       |                      |

CAG: cumulative assessment group; AS: active substance; NOEL: no observed effect level; LOEL: lowest observed effect level; NOAEL: no observed adverse effect level; LOAEL: lowest observed adverse effect level; bw: body weight; GABA: gamma-aminobutyric acid; AChE: acetylcholinesterase; VGSC: voltage-gated sodium channel; nAChR: nicotinic acetylcholine receptor; MoA: mode of action.
Table B.3: CAG on functional effects on autonomic division: toxicological characterisation of ASs to be considered in acute exposure/risk assessments.

| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|------------------|------------------|--------|--------|-----|
| Abamectin        |                            | 6                |                  | Acute neurotoxicity rat | Administration via gavage | Source: DAR 2005, addendum 2007 | GABA-gated chloride channel agonist |
|                  |                            |                  |                  | 49-day neurotoxicity study | Administration via diet | Source: JMPR 2002 | AChE inhibitor |
| Acetamiprid      | Urination                   | 10               | 30               | Acute neurotoxicity rat | Administration via gavage | Source: DAR 2001 | Agonist of nAChR |
| Acrinathrin      | Salivation                  | 10               | 37.5             | Acute neurotoxicity rat | Administration via gavage | Source: DAR 2007 | Known Binding to VGSC |
| Aldicarb         | Salivation, lacrimation     | 0.1              | 0.5              | Acute neurotoxicity rat | Administration via gavage | Source: DAR 1996 | Known AChE inhibitor |
| Alpha-Cypermethrin| Salivation                  | 4                | 10               | Acute neurotoxicity rat | Administration via gavage | Source: DAR 2000 | Known Binding to VGSC |
| Amitraz          | Hypoactivity                | 1                | 4                | 90-day dog | Administration via diet | Source: DAR | Neurotoxicity studies not available, therefore the source of the ARfD (0.01 mg/kg bw/d, SCoFCAH 2003) is used as surrogate | Partial agonist of presynaptic α2-adrenergic receptor |
| Azinphos-ethyl   | AChE inhibition (erythrocyte)| 0.0125           | 0.025            | 90-day dog | Administration via diet | Source: JMPR 1973 | Neurotoxicity studies not available NOAEL for AchE inhibition used as surrogate | AChE inhibitor |
| Azinphos-methyl  | Salivation, lacrimation     | 2                | 6                | Acute neurotoxicity rat | Administration via gavage | Source: DAR 1996, Addendum 6, 2000 (neurotoxicity) | AChE inhibitor |
| Active substance         | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                        | Remark                                                                 | MoA                      |
|-------------------------|------------------------------|------------------|------------------|------------------------------|------------------------------------------------------------------------|--------------------------|
| Benfuracarb             | Lacrimation                  | 2                | 20               | 28-day rat Administration via gavage | Source: DAR 2004 Acute neurotoxicity study not available               | AChE inhibitor           |
| Beta-Cyfluthrin         | Salivation                   | 2                | 10               | Acute neurotoxicity rat Administration via gavage | Source: JMPR 2006, DAR 1996                                              | Binding to VGSC          |
| Beta-cypermethrin       | Salivation                   | 20               | 100              | Acute neurotoxicity rat Administration via gavage | Source: DAR 2013 Binding to VGSC                                      | Binding to VGSC          |
| Bifenthrin              | –                             | 75               | –                | Acute neurotoxicity rat Administration via diet Highest tested dose | Source: DAR                                                          | Binding to VGSC          |
| Cadusafos               | Urination                    | 25               | 40               | Acute neurotoxicity rat Administration via gavage | Source: DAR, addendum 2005                                             | AChE inhibitor           |
| Carbaryl                | Salivation, urination        | 10               | 50               | Acute neurotoxicity rat Administration via gavage | Source: DAR 2004 AChE inhibitor                                       | AChE inhibitor           |
| Carbofuran              | –                             | 0.3              | –                | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: Revised DAR 2008 AChE inhibition                               | AChE inhibition          |
| Carbosulfan             | –                             | 30               | –                | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: Revised DAR 2009 AChE inhibition                               | AChE inhibition          |
| Chlorfenvinphos         | AChE inhibition (brain, erythrocyte) | 0.15          | 15               | 2-year rat (author not reported, JMPR, 1994) Administration via gavage | Source: JMPR 1994 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor          |
| Chlormequat             | salivation                   | 5                | 10               | 1-year dog Administration via diet | Source: DAR 2007 Neurotoxicity studies not available                  | Partial agonist of muscarinic and nicotinic acetylcholine receptor |
| Active substance       | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                      | Remark                                                | MoA         |
|------------------------|-------------------------------|-----------------|-----------------|-------------------------------------------|-------------------------------------------------------|-------------|
| Chlorpropham           | Salivation (accompanied by vomiting and retching) | 125             | 625             | 14-day dog Administration via capsule     | Source: DAR 1999                                      | Unknown     |
| Chlorpyrifos           | –                             | 100             | –               | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 1999                                      | AChE inhibitor |
| Chlorpyrifos-methyl    | –                             | 75              | –               | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: RAR 2017                                      | AChE inhibition |
| Clothianidin           | –                             | 400             | –               | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2003                                      | Agonist of nAChR |
| Cyfluthrin             | Salivation                    | 2.5             | 7.5             | Acute neurotoxicity rat Administration via gavage | Source: JMPR 2006                                      | Binding to VGSC |
| Cypermethrin           | Urination                     | 20              | 60              | Acute neurotoxicity rat Administration via gavage | Source: DAR                                           | Binding to VGSC |
| Deltamethrin           | Mydriasis                     | 1               | 2.5             | 90-day dog Administration via capsule     | Source: EC Review report on deltamethrin (2002) considered | Binding to VGSC |
| Diazinon               | Lacrimation, salivation       | 300             | 600             | Acute neurotoxicity rat Administration via gavage | Source: DAR 2004                                      | AChE inhibitor |
| Dichlorvos             | AChE inhibition (erythrocytes) | 0.1             | 1.5             | 13-week rat                              | Source: DAR 2003 (addendum) Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|------------------|------------------|-------|--------|-----|
| Dicofol          | Lacrimation, salivation     | 25               | 250              | Acute neurotoxicity rat Administration via gavage | Source: DAR 2006 Not true NOAEL, but LOAEL divided by 10 | 4 presumed MoA (Section 3.3.2) |
| Dieldrin         |                             | 0.3              |                  | Virtual NOAEL corresponding to the ARfD of 0.003 mg/kg bw/d derived by the PPR Panel and considered as protective for all endpoints. | Source: PPR panel, 2007 Neurotoxicity studies not available | GABA-gated chlorine channel antagonist |
| Dimethoate       | Lacrimation, salivation     | 20               | 200              | Acute neurotoxicity rat Administration via gavage | Source: DAR | AChE inhibitor |
| Dinotefuran      |                             | 1,500            | –                | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: JMPR 2012 | Agonist of nAChR |
| Emamectin benzoate | Salivation                 | 2.74             | 27.4             | Acute neurotoxicity rat Administration via gavage | Source: DAR 2008 Not true NOAEL, but LOAEL divided by 10 | GABA-gated chloride channel agonist |
| Endosulfan       | Salivation                  | 3                | 6                | Acute neurotoxicity rat Administration via gavage | Source: DAR 2001 and addendum | GABA-gated chloride channel blocker |
| Endrin           | Convulsions                 | 0.025            | 0.05             | 2-year dog Administration via diet | Source: EHC 130, 1992 Neurotoxicity studies not available NOAEL for functional alteration of the motor division used as surrogate ADI: 0.0002 (JMPR 1994) | GABA-gated chloride channel antagonist |
| Esfenvalerate    | Salivation                  | 20               | 80               | Acute neurotoxicity rat Administration via gavage | Source: JMPR 2002 | Binding to VGSC |
| Ethephon         | Miosis                      | 50               | 500              | Acute neurotoxicity rat Administration via gavage | Source: DAR 2004 Not true NOAEL, but LOAEL divided by 10 | AChE inhibitor |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|-----------------|----------------------------|-----------------|-----------------|-------|--------|-----|
| Ethion          | AChE inhibition (brain)     | 0.06            | 0.71            | 90-day dog Administration via diet | Source: JMPR 1990 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor |
| Ethoprophos     | –                          | 25              | –               | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2004 | AChE inhibitor |
| Fenamiphos      | Miosis, piloerection       | 1.52            | 2.31            | Acute neurotoxicity rat Administration via gavage | Source: DAR 2003 | AChE inhibitor |
| Fenitrothion     | Miosis, salivation         | 12.5            | 50              | Acute neurotoxicity rat Administration via gavage | Source: DAR 2003 | AChE inhibitor |
| Fenpropathrin   | –                          | 30              | –               | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: JMPR 2012 | Binding to VGSC |
| Fenthion        | Autonomic signs (not specified) | 1.04            | 49.05           | Acute neurotoxicity rat Administration via gavage | Source: DAR 1996, Addendum Tox 2001 | AChE inhibitor |
| Fenvalerate     | –                          | 360             | –               | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: JMPR 2012 | Binding to VGSC |
| Fipronil        | Miosis                     | 5               | 50              | Acute neurotoxicity rat Administration via gavage | Source: DAR 2004 | GABA-gated chloride channel blocker |
| Flufenacet      | Urination                  | 7.5             | 75              | Acute neurotoxicity rat Administration via gavage | Source: DAR 1998 Not true NOAEL, but LOAEL divided by 10 | unknown |
| Fonofos         | Urination                  | 4               | 7               | Acute neurotoxicity rat (author not reported) Administration via gavage | Source: US EPA 1999 No reference values available | AChE inhibitor |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|------------------|------------------|-------|--------|-----|
| Formetanate      | Miosis                      | 1                | 10               | Acute neurotoxicity rat Administration via gavage | Source: DAR 2004 | AChE inhibitor |
| Fosthiazate      | AChE inhibition (brain)     | 0.54             | 5.4              | 90-day dog Administration via capsule | Source: DAR 1998 | AChE inhibition |
| Heptachlor       | –                           | 69               | –                | 14-day neurotoxicity rat Administration via gavage | Source: CICADS, 2006 | GABA-gated chloride channel antagonist |
| Imidacloprid     | –                           | 307              | –                | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2005 | Agonist of nAChR |
| Indoxacarb       | –                           | 100              | –                | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2000 | Voltage-dependent sodium channel blocker |
| Lambda-cyhalothrin | Salivation, lacrimation  | 1                | 10               | Acute neurotoxicity rat Administration via gavage | Source: DAR Not true NOAEL, but LOAEL divided by 10 | Binding to VGSC |
| Lindane          | –                           | 6                | –                | – | Neurotoxicity studies not available NOAEL of 6 mg/kg bw/d used by JMPR (2002) to establish the ARfD used as surrogate |
| Malathion        | Salivation                  | 500              | 1,000            | Acute neurotoxicity rat Administration via gavage | Source: DAR | AChE inhibitor |
| Mepiquat         | –                           | 1,200            | –                | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2005 | Activation of nicotinic and muscarinic acetylcholine receptors |
| Active substance                  | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                        | Remark                                                                 | MoA                      |
|----------------------------------|-------------------------------|------------------|------------------|------------------------------|------------------------------------------------------------------------|--------------------------|
| Metaldehyde                      | Mydriasis                     | 7.5              | 75               | 28-day dog                   | Administration via diet                                                | Presumed GABA inhibitor  |
| Methamidophos                    | Urination, lacrimation        | 1                | 3                | Acute neurotoxicity rat      | Administration via gavage                                              | AChE inhibitor           |
| Methidathion                     | Lacrimation, salivation       | 4                | 5                | Acute neurotoxicity rat      | Administration via gavage                                              | AChE inhibitor           |
| Methiocarb                       | AChE inhibition (erythrocytes) | 0.5              | 2                | 28-day rat                   | Administration via gavage                                              | AChE inhibition           |
| Methomyl                         | Lacrimation, salivation       | 0.75             | 2                | Acute neurotoxicity rat      | Administration via gavage                                              | AChE inhibitor           |
| Milbemectin                      | Salivation (accompanied by vomiting) | 3              | 10               | 13-week dog                  | Administration via capsule                                              | Glutamate-gated chloride (GluCl) allosteric modulator |
| Monocrotophos                    | AChE inhibition (brain, erythrocytes) | 0.1            | 0.3              | Single dose rat              | Administration via gavage                                              | AChE inhibition           |
| Omethoate (metabolite of dimethoate) | —                            | 5                | —                | Acute neurotoxicity rat      | Administration via gavage                                              | AChE inhibitor           |
| Oxamyl (1C)                      | Salivation, urination         | 0.1              | 0.75             | Acute neurotoxicity rat      | Administration via gavage                                              | AChE inhibitor           |
| Oxydemeton-methyl                | Salivation                    | 1.88             | 3.75             | 90-day dog                   | Administration via diet                                                | AChE inhibitor           |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|------------------|------------------|-------|--------|-----|
| Parathion        | AChE inhibition (brain)     | 0.25             | 2.5              | 2-year rat Administration via diet | Source: JMPR 1995 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition |
| Parathion-methyl | AChE inhibition (erythrocytes) | 0.25             | 2.5              | 2-year rat Administration via diet | Source: DAR 2001 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition |
| Permethrin       | Urination                   | 250              | 500              | 28-day neurotoxicity rat Administration via diet | Source: JMPR 1999 | |
| Phenthoate       | AChE inhibition (erythrocytes) | 0.29             | 0.87             | 2-year dog Administration via diet | Source: JMPR 1980 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition |
| Phosalone        | –                           | 60               | –                | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2004 | AChE inhibitor |
| Phosmet          | Salivation                  | 9                | 36               | Acute neurotoxicity rat Administration via gavage | Source: DAR 2004 | AChE inhibitor |
| Phoxim           | Salivation                  | 5                | 25               | 90-day dog Administration via gavage | Source: JECFA 1999 Neurotoxicity studies not available NOAEL for AChE inhibition: 1.3 mg/kg bw/d | AChE inhibitor |
| Pirimicarb       | –                           | 110              | –                | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2003 | AChE inhibitor |
| Active substance       | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                                                                                                                                                                                 | Remark                                                                 | MoA                          |
|------------------------|------------------------------|------------------|------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|------------------------------|
| Pirimiphos-methyl      |                              | 1,500            |                  | Acute neurotoxicity rat Administration via gavage Highest tested dose                                                                                                                                 | Source: DAR 2003                                                      | AChE inhibitor                |
| Profenofos             | Lacrimation, miosis         | 190              | 380              | Acute neurotoxicity rat Administration via gavage                                                                                                                                                      | Source: JMPR 2007                                                    | AChE inhibitor                |
| Pyrazophos             | AChE inhibition (erythrocytes) | 0.05            | 0.125            | 2-year dog Administration via diet                                                                                                                                                                      | Source: JMPR 1992 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition               |
| Pyrethrins             |                              | 200              |                  | Acute neurotoxicity rat Administration via gavage Highest tested dose                                                                                                                                 | Source: DAR 2007, 2012                                               | Binding to VGSC               |
| Pyridate               | Mydriasis, salivation       | 60               | 80               | 90-day dog Administration via capsule                                                                                                                                                                  | Source: DAR                                                          | Unknown                       |
| Sulfoxaflor            | Lacrimation                 | 75               | 750              | Acute neurotoxicity rat Administration via gavage                                                                                                                                                      | Source: DAR 2012                                                    | Nicotinicergic AChR partial agonist |
| Tau-fluvalinate        | Salivation                  | 10               | 60               | Acute neurotoxicity rat Administration via gavage                                                                                                                                                      | Source: DAR 2007                                                    | Binding to VGSC               |
| Tebuconazole           | Salivation                  | 250              | 500              | Acute neurotoxicity rat Administration via gavage                                                                                                                                                      | Source: DAR 2006                                                    | Unknown                       |
| Tefluthrin             |                              | 10               |                  | Acute neurotoxicity rat Administration via gavage Highest tested dose                                                                                                                                 | Source: DAR 2006                                                    | Binding to VGSC               |
| Tetramethrin           |                              |                  |                  |                                                                                                                                                                                                          | Information insufficient No reference values available             | Binding to VGSC               |
| Active substance       | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                                                 | Remark                                                                 | MoA                                                                 |
|------------------------|------------------------------|-----------------|-----------------|----------------------------------------------------------------------|------------------------------------------------------------------------|----------------------------------------------------------------------|
| Thiacloprid            | Mydriasis                    | 2.2             | 22              | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2000, RAR, 2017                                            | Agonist of nAChR                                                     |
| Thiametoxam            | Lacrimation                  | 500             | 1,500           | Acute neurotoxicity rat Administration via gavage                   | Source: DAR 2002                                                      | Agonist of nAChR                                                     |
| Thiodicarb             | Salivation                   | 5               | 20              | Acute neurotoxicity rat Administration via gavage                   | Source: DAR 2003                                                      | AChE inhibitor                                                       |
| Thiram                 | Urination                    | 5               | 150             | Acute neurotoxicity rat Administration via gavage                   | Source: DAR 2003                                                      | Neurotoxic effect might be due to the metabolite CS₂ (presumed)      |
| Tolclofos-methyl       | –                            | 2,000           | –               | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: RAR 2016                                                      | AChE inhibitor                                                       |
| Tri-allate             | Lacrimation, salivation      | 400             | 500             | Acute neurotoxicity rat Administration via gavage Highest tested dose | Source: DAR 2007                                                      | Unknown                                                              |
| Triazophos             | AChE inhibition (erythrocytes)| 0.012           | 0.13            | 1-year dog Administration via diet                                  | Source: JMPR 2002 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor                                                       |
| Trichlorfon            | Salivation                   | 50              | 200             | Acute neurotoxicity rat Administration via gavage                   | Source: JMPR 2002                                                      | AChE inhibitor                                                       |
| Zeta-cypermethrin      | Lacrimation, salivation      | 50              | 250             | Acute neurotoxicity rat Administration via gavage                   | Source: DAR 2004 and addendum 2005                                   | Binding to VGSC                                                      |

CAG: cumulative assessment group; AS: active substance; NOEL: no observed effect level; LOEL: lowest observed effect level; NOAEL: no observed adverse effect level; LOAEL: lowest observed adverse effect level; bw: body weight; GABA: gamma-aminobutyric acid; AChE: acetylcholinesterase; VGSC: voltage-gated sodium channel; nAChR: nicotinic acetylcholine receptor; MoA: mode of action.
| Active substance         | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                      | Remark                                                                 | MoA                  |
|-------------------------|------------------------------|------------------|------------------|--------------------------------------------|-----------------------------------------------------------------------|----------------------|
| Acephate                | AChE inhibition (brain)      | 2.5              | 5                | Acute single dose rat via gavage           | Source: JMPR 2002                                                    | AChE inhibition      |
| Aldicarb                | AChE inhibition (erythrocytes) | 0.05             | 0.1              | Acute neurotoxicity rat via gavage         | Source: DAR 1996                                                    | AChE inhibition      |
| Azinphos-ethyl          | AChE inhibition (erythrocytes) | 0.0125           | 0.025            | 90-day neurotoxicity dog via diet          | Source: JMPR 1973                                                   | AChE inhibition      |
| Azinphos-methyl         | AChE inhibition (erythrocytes) | 1                | 2                | acute neurotoxicity rat via gavage         | Source: DAR 1996; Addendum 6, 2000; ScoFCAH meeting March 2006       | AChE inhibition      |
| Benfuracarb             | AChE inhibition (brain, erythrocytes) | 1.81            | 9.4              | 28-day neurotoxicity rat via diet          | Source: DAR 2004 EFSA (2009)                                        | AChE inhibition      |
| Cadusafos               | AChE inhibition (erythrocytes) | 0.23             | 0.46             | 28-day rat via diet                        | Source: DAR 2004 EFSA (2009)                                        | AChE inhibition      |
| Carbaryl                | AChE inhibition (brain, erythrocytes) | 1               | 10               | 90-day neurotoxicity rat via gavage        | Source: DAR 2004 EFSA (2006)                                        | AChE inhibition      |
| Carbofuran              | AChE inhibition (brain)      | 0.015            | 0.03             | Acute neurotoxicity rat via gavage         | Source: revised DAR 2008                                            | AChE inhibition      |
| Carbosulfan             | AChE inhibition (brain, erythrocytes) | 0.5             | 5                | Acute neurotoxicity rat via gavage         | Source: Revised DAR 2009 EFSA (2009)                                 | AChE inhibition      |
| Chlorfenvinphos         | AChE inhibition (brain, erythrocytes) | 0.15            | 15               | 2-year rat (author not reported, JMPR 1994) Administration via diet | Source: JMPR 1994 Long term NOAEL used as surrogate acute NOAEL. Supported by NOAEL of 0.18 mg/kg bw/d for brain AChE inhibition in the 28-day mouse (Tennekes, 1991) | AChE inhibition      |
| Active substance          | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                                                                       | Remark                                                                 | MoA           |
|--------------------------|------------------------------|------------------|------------------|--------------------------------------------------------------------------------------------|------------------------------------------------------------------------|---------------|
| Chlorpyrifos             | AChE inhibition (erythrocytes) | 0.5              | 2.5              | Comparative cholinesterase assay rat Administration via gavage                              | Source: EFSA (2014)                                                   | AChE inhibition|
| Chlorpyrifos-methyl      | AChE inhibition (erythrocytes) | 7.5 (10)         | 75               | Acute neurotoxicity rat Administration via gavage                                            | Source: RAR 2017 EC review report 2005 considered                      | AChE inhibition|
| Diazinon                 | AChE inhibition (erythrocytes) | 2.5              | 25               | Acute neurotoxicity rat Administration via gavage                                            | Source: DAR 2004 EFSA (2006)                                          | AChE inhibition|
| Dichlorvos               | AChE inhibition (erythrocytes) | 0.1              | 1.5              | 13-week rat Administration via gavage                                                       | Source: DAR 2003 (addendum) EFSA (2006)                               | AChE inhibition|
| Dimethoate               | AChE inhibition (erythrocytes) | 1                | 2                | Acute neurotoxicity rat Administration via diet                                             | Source: DAR 2004 EFSA (2006, 2013)                                   | AChE inhibition|
| Ethephon                 | AChE inhibition (erythrocytes) | 6                | 14               | 28-day cholinesterase inhibition study in dogs Administration via diet                       | Source: DAR 2004 EFSA conclusions on ethephon (2008) considered       | AChE inhibition|
| Ethion                   | AChE inhibition (brain)       | 0.06             | 0.71             | 90-day dog Administration via diet                                                          | Source: JMPR 1990 Long term NOAEL used as surrogate acute NOAEL.     | AChE inhibition|
| Ethoprophos              | AChE inhibition (erythrocyte)  | 0.95             | 2                | Single dose study Administration via diet                                                    | Source: DAR 2004, addendum EFSA (2006)                                | AChE inhibition|
| Fenamiphos               | AChE inhibition (erythrocytes) | 0.25             | 0.5              | Acute oral dog Administration via gavage                                                    | Source: DAR 2003 EFSA (2006)                                          | AChE inhibition|
| Fenitrothion             | AChE inhibition (brain, erythrocytes) | 1.32             | 3.99             | 90-day neurotoxicity rat Administration via diet                                             | Source: DAR 2003 EFSA (2006)                                          | AChE inhibition|
| Fenthion                 | AChE inhibition (erythrocytes) | 1                | 50               | Acute single dose rat study Administration via diet                                          | Source: JMPR 1997 JMPR evaluations on fenthion (1997) considered     | AChE inhibition|
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|------------------------------|------------------|------------------|-------|--------|-----|
| Fonofos          | AChE inhibition (erythrocytes) | 0.2              | 1                | 1-year dog Administration via capsule | Source: EPA 1999 US evaluation not completed because voluntary withdrawal in 1999 Long term NOAEL used as surrogate acute NOAEL | AChE inhibition |
| Formetanate      | AChE inhibition (brain, erythrocytes) | 0.5              | 2                | Acute oral cholinesterase activity study in female rats Administration via gavage | Source: DAR 2004 EFSA (2006) | AChE inhibition |
| Fosthiazate      | AChE inhibition               | 0.54             | 5.4              | 90-day dog Administration via capsule | Source: DAR 1998 EC review report 2003 considered | AChE inhibition |
| Malathion        | AChE inhibition (brain)       | 34               | 340              | 90-day rat Administration via diet | Source: DAR | AChE inhibition |
| Methamidophos    | AChE inhibition (brain, erythrocytes) | 0.3              | 0.7              | Acute neurotoxicity rat Administration via gavage | Source: DAR 2000 EC review report on methamidophos (2006) considered | AChE inhibition |
| Methidathion     | AChE inhibition (brain, erythrocytes) | 1                | 4                | Acute neurotoxicity, rat Administration via gavage | Source: JMPR 1997 addendum | AChE inhibition |
| Methiocarb       | AChE inhibition (erythrocytes) | 0.5              | 2                | 28-day rat Administration via gavage | Source: DAR 2004 EFSA (2018) | AChE inhibition |
| Methomyl         | AChE inhibition (brain, erythrocytes) | 0.25             | 0.5              | Acute neurotoxicity rat Administration via gavage | Source: DAR EFSA (2006 and 2009) | AChE inhibition |
| Monocrotophos    | AChE inhibition (brain, erythrocytes) | 0.1              | 0.3              | Single dose rat Administration via gavage | Source: JMPR 1995, addendum | AChE inhibition |
| Omethoate (metabolite of dimethoate) | AChE inhibition (brain) | 0.25             | 0.35             | Acute neurotoxicity rat Administration via gavage | Source: DAR 2004 (dimethoate) | AChE inhibition |
| Oxamyl (IC)      | AChE inhibition (brain, erythrocytes) | 0.1              | 0.75             | Acute neurotoxicity rat Administration via gavage | Source: DAR 2003 EFSA (2005) | AChE inhibition |
| Oxydemeton-methyl | AChE inhibition (brain, erythrocytes) | 0.2              | 0.6              | 14-day rat Administration via diet | Source: DAR 2004 EFSA (2006) | AChE inhibition |
| Active substance       | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                      | Remark                                                                 | MoA                  |
|------------------------|------------------------------|------------------|------------------|--------------------------------------------|----------------------------------------------------------------------|----------------------|
| Parathion              | AChE inhibition (brain)      | 0.25             | 2.5              | 2-year rat                                 | Administration via diet                                              | AChE inhibition      |
|                        |                              |                  |                  | Source: JMPR 1995                          | Long term NOAEL used as surrogate acute NOAEL                        |                      |
| Parathion-methyl       | AChE inhibition (erythrocytes)| 0.25             | 2.5              | 2-year rat                                 | Administration via diet                                              | AChE inhibition      |
|                        |                              |                  |                  | Source: DAR 2001                           | Long term NOAEL used as surrogate acute NOAEL                        |                      |
| Phenthoate             | AChE inhibition (erythrocytes)| 0.29             | 0.87             | 2-year dog                                 | Administration via diet                                              | AChE inhibition      |
|                        |                              |                  |                  | Source: JMPR 1980                           | Long term NOAEL used as surrogate acute NOAEL                        |                      |
| Phosalone              | AChE inhibition (brain, erythrocytes) | 25               | 60               | Acute neurotoxicity rat                    | Administration via gavage                                            | AChE inhibition      |
|                        |                              |                  |                  | Source: DAR 2004                           | EFSA (2006)                                                          |                      |
| Phosmet                | AChE inhibition (brain, erythrocytes) | 4.5              | 22.5             | Acute neurotoxicity rat                    | Administration via gavage                                            | AChE inhibition      |
|                        |                              |                  |                  | Source: DAR 2004                           | EFSA (2006)                                                          |                      |
| Phoxim                 | AChE inhibition (erythrocytes) | 1.3              | 5                | 90-day dog                                 | Administration via diet                                              | AChE inhibition      |
|                        |                              |                  |                  | Source: JECFA 1999                         |                                                                     |                      |
| Pirimicarb             | AChE inhibition (brain, erythrocyte) | 10               | 25               | 1-year dog                                 | Administration via capsule                                          | AChE inhibition      |
|                        |                              |                  |                  | Source: DAR 2003                           |                                                                     |                      |
| Pirimiphos-methyl      | AChE inhibition (brain, erythrocytes) | 15               | 150              | Acute neurotoxicity rat                    | Administration via gavage                                            | AChE inhibition      |
|                        |                              |                  |                  | Source: DAR 2003                           | EFSA (2005)                                                          |                      |
| Profenofos             | AChE inhibition (erythrocytes) | 0.5              | 25               | Acute neurotoxicity rat                    | Administration via gavage                                            | AChE inhibition      |
|                        |                              |                  |                  | Source: JMPR 1997                          |                                                                     |                      |
| Pyrazophos             | AChE inhibition (erythrocytes) | 0.05             | 0.125            | 2-year dog                                 | Administration via gavage                                            | AChE inhibition      |
|                        |                              |                  |                  | Source: JMPR 1992                          | Long term NOAEL used as surrogate acute NOAEL                        |                      |
| Thiodicarb             | AChE inhibition (brain, erythrocytes) | 0.5              | 5                | Acute neurotoxicity rat                    | Administration via gavage                                            | AChE inhibition      |
|                        |                              |                  |                  | Source: 2003 EFSA (2005)                   | NOAEL derived from the LOAEL with an UF of 10                        |                      |
| Tolclofos-methyl       | AChE inhibition (erythrocytes) | 14               | 564              | 9-month mouse                              | Administration via diet                                              | AChE inhibition      |
|                        |                              |                  |                  | Source: DAR 2003                           | EFSA (2017)                                                          |                      |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|------------------|------------------|-------|--------|-----|
| Triazophos       | AChE inhibition (erythrocytes) | 0.012            | 0.13             | 1-year dog Administration via diet | Source: JMPR 2002 Long term NOAEL used as surrogate acute NOAEL | AChE inhibition |
| Trichlorfon      | AChE inhibition (erythrocytes) | 10               | 50               | Acute neurotoxicity rat Administration via gavage | Source: DAR 1998 | AChE inhibition |
| Abamectin        | Ataxia, tremor              | 0.25             | 0.5              | 18-week dog Administration via gavage | Source: DAR 2005 EFSA (2008) | GABA-gated chloride channel agonist |
| Acephate         | –                           | 74.2             | –                | 49-day neurotoxicity study Administration via diet Highest tested dose | Source: JMPR 2002 | AChE inhibitor |
| Acetamiprid      | Hunched posture             | 7.1              | 17.5             | 2-year rat Administration via diet | Source: DAR 2001 | Agonist of nAChR |
| Acrinathrin      | Reduced grip strength       | 0.24             | 2.4              | 90-day neurotoxicity rat Administration via diet | Source: DAR 2007 Not true NOAEL, but LOAEL divided by 10 EFSA (2013) | Binding to VGSC |
| Aldicarb         | Decreased motor activity, tremor, ataxic gait, decreased hindlimb strength | 0.1 | 0.5 | Acute neurotoxicity rat Administration via gavage | Source: DAR 1996 | AChE inhibitor |
| Alpha-cypermethrin | Ataxia                     | 2.3              | 6.8              | 90-day dog Administration via diet | Source: DAR 2000 Chronic neurotoxicity studies not available | Binding to VGSC |

CAG: cumulative assessment group; AS: active substance; NOEL: no observed effect level; LOEL: lowest observed effect level; NOAEL: no observed adverse effect level; LOAEL: lowest observed adverse effect level; bw: body weight; AChE: acetylcholinesterase; MoA: mode of action.

Table B.5: CAG on functional effects on motor division: toxicological characterisation of ASs to be considered in chronic exposure/risk assessments
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|------------------|------------------|-------|--------|-----|
| Amitraz          | Reduced motor activity      | 0.25             | 1                | 2-year dog | Administration via diet | Source: DAR Neurotoxicity studies not available | Partial agonist of presynaptic α2-adrenergic receptor |
| Azinphos-ethyl   | AChE inhibition (erythrocyte) | 0.0125           | 0.025            | 90-day dog | Administration via diet | Source: JMPR 1973 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor |
| Azinphos-methyl  | Increased reactivity, tremor, decreased forelimb grip strength | 3.23             | 6.99             | 90-day neurotoxicity rat | Administration via diet | Source: DAR 1996, Addendum 6, 2000 (neurotoxicity) | AChE inhibition |
| Benfuracarb      | Increased motor activity, convulsions; ataxia | 2.5              | 5                | 2-year dog | Administration via capsule | Source: DAR 2004 | AChE inhibition |
| Beta-Cyfluthrin  | Fore- and hindlimb strength | 7.99             | 26.81            | 90-day neurotoxicity rat | Administration via diet | Source: JMPR 2006, DAR 1996 Binding to VGSC |
| Beta-cypermethrin| Ataxia, tremor, convulsions | 1                | 3                | 1-year dog | Administration via capsule | Source: DAR 2013 | Binding to VGSC |
| Bifenthrin       | Increased motor activity, tremor | 1.5              | 3.0              | 1-year dog | Administration via capsule | Source: DAR | Binding to VGSC |
| Bromide ion      | Muscle strength: increased or decreased muscle tone | 7.76             | 77.6             | 90-day rat | Administration via diet | Source: JMPR 1988 Not true NOAEL, but LOAEL divided by 10 | Unknown |
| Cadusafos        | AChE inhibition (erythrocytes) | 0.045            | 0.22             | 2-year rat | Administration via diet | Source: DAR 2004 The NOAEL of 0.031 mg/kg bw/d in the 90-day neurotoxicity rat (Watt and Freeman, 2001) was disregarded due to the inappropriate dose spacing and incompatibility with the ARFD | AChE inhibitor |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|-----------------|-----------------|-------|--------|-----|
| Carbaryl         | Tremor, erythrocyte and brain AChE inhibition | 1 | 10 | 90-day neurotoxicity study Administration via gavage | Source: DAR 2004 EFSA (2006) | AChE inhibition |
| Carbofuran       | Reduced grip strength, landing foot splay | 34 | 67.5 | 90-day neurotoxicity rat Administration via diet | Source: Revised DAR 2008 | AChE inhibition |
| Carbosulfan      | Decreased motor activity | 1.4 | 78.9 | 90-day neurotoxicity rat Administration via diet | Source: Revised DAR 2009 | AChE inhibition |
| Carbetamide      | Ataxia | 30 | 300 | 90-day dog Administration via capsule | Source: DAR 2005 | Unknown |
| Chlormequat      | Ataxia | 10 | 32 | 1-year dog Administration via diet | Source: DAR 2007 | Neurotoxicity studies not available Partial agonist of muscarinic and nicotinic acetylcholine receptor |
| Chlorpyrifos     | Decreased motor activity | 5 | 15 | 90-day neurotoxicity rat Administration via diet | Source: DAR 1999 | AChE inhibitor |
| Chlorpyrifos-methyl | Muscle weakness | 10 | 50 | 90-day dog Administration via diet | Source: DAR 1997 | AChE inhibition |
| Clothianidin     | Reduced motor activity | 35.8 | 52.3 | 30-day dog Administration via diet | Source: DAR 2003 | agonist of nAChR |
| Cyfluthrin       | Ataxia | 2.4 | 11 | 1-year dog Administration via diet | Source: DAR 1996; addendum Chronic neurotoxicity studies not available | Binding to VGSC |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|------------------|------------------|-------|--------|-----|
| Cypermethrin     | Ataxia, tremor              | 5                | 15               | 1-year dog Administration via capsule | Source: DAR Chronic neurotoxicity studies not available ADI: 0.05 mg/kg bw per day based on the 2-year rat study (EC Review Report 2005) | Binding to VGSC |
| Deltamethrin     | Ataxia, landing-foot splay, tremor | 1                | 10               | 1-year dog Administration via capsule | Source: DAR EC review report on deltamethrin (2002) considered | Binding to VGSC |
| Desmedipham      | Ataxia, reduced motor activity, tremor | 52.5             | 168              | 1-year dog Administration via diet | Source: DAR 2000 | Unknown |
| Diazinon         | Reduced forelimb and hindlimb grip strength | 17               | 177              | 90-day neurotoxicity rat Administration via diet | Source: DAR 2004 | ACHE inhibition |
| Dicamba          | Reduced motor activity, tremor | 50               | 300              | 13-week dog Administration via diet | Source: Revised DAR | Unknown |
| Dichlorvos       | AChE inhibition (erythrocytes) | 0.008            | 0.08             | 2-year dog Administration via capsule | Source: DAR 2003 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor |
| Dicofol          | Reduced grip strength       | 0.3              | 5.6              | 90-day rat Administration via gavage | Source: DAR 2006 | 4 presumed MoA (Section 3.3.2) |
| Dieldrin         |                             | 0.025            |                  |                   | Source: JMPR 1977 Neurotoxicity studies not available NOAEL in dog and rat which served as basis to the JMPR ADI used as surrogate | GABA-gated chlorine channel antagonist |
| Dimethoate       |                             | 11.25            |                  | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR | AChE inhibitor |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|------------------------------|------------------|------------------|-------|--------|-----|
| Dinotefuran      | Hypoactivity                 | 400              | 3,806            | 90-day neurotoxicity rat | Source: JMPR 2012 | agonist of nAChR |
| Emamectin benzoate (IC) | Tremor                    | 0.25 (0.05)      | 0.5              | 1-year dog | Additional UF of 5 due to small dose spacing and steep dose response curve |
| Endosulfan       | Tremor                       | 0.57             | 2.3              | 1-year dog | GABA-gated chloride channel blocker |
| Endrin           | Convulsions                  | 0.025            | 0.05             | 2-year dog | GABA-gated chloride channel blocker |
| Esfenvalerate    | Reduced grip strength        | 3.2              | 6.4              | 90-day neurotoxicity rat | Source: JMPR 2002 | Binding to VGSC |
| Ethephon         | –                            | 400              | –                | 90-day neurotoxicity rat | Source: DAR 2004 | AChE inhibitor |
| Ethion           | AChE inhibition (brain)      | 0.06             | 0.71             | 90-day dog | AChE inhibitor |
| Ethoprophos      | Reduced grip strength, reduced motor activity, tremor | 2.65 | 27.11 | 90-day neurotoxicity rat | Source: DAR 2004 | AChE inhibition |
| Fenamiphos       | Tremor                       | 0.56             | 1.7              | 2-year rat | AChE inhibitor |
### CAGs of pesticides for effects on nervous system

| Active substance | Indicator of specific effect                        | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                      | Remark                                                                 | MoA                          |
|------------------|----------------------------------------------------|------------------|------------------|-------------------------------------------|------------------------------------------------------------------------|------------------------------|
| Fenitrothion     | Tremor, reduced grip strength                      | 4.85             | 17.6             | 90-day neurotoxicity rat **Administration via diet** | Source: DAR 2003 AChE inhibitor                                          |                             |
| Fenpropathrin    | Increased motor activity, tremor                   | 3.1              | 7.7              | 1-year dog **Administration via diet**     | Source: JMPR 2012 Binding to VGSC                                       |                             |
| Fenpropidin      | Ataxia, paresis limbs, reduced motor activity      | 5                | 20               | 1-year dog **Administration via capsule**  | Source: DAR Unknown                                                    |                             |
| Fenpropimorph    | Landing-foot splay                                 | 0.8              | 8.5              | 3-month neurotoxicity rat **Administration via diet** | Source: DAR Unknown                                                    |                             |
| Fenvalerate      | Increased motor activity                           | 37.5             | 75               | 6-month dog **Administration via diet**    | Not approved. ADI: 0.0125 mg/kg bw per day (EMEA); ADI: 0.02 mg/kg bw per day (JMPR, 1986). Binding to VGSC |                             |
| Fenthion         | Decreased activity                                | 1.63             | 8.5              | 90-day neurotoxicity rat **Administration via diet** | Source: JMPR 1995; Addendum Tox 2001 AChE inhibitor |                             |
| Fipronil         | Convulsions                                        | 0.019            | 0.059            | 2-year rat **Administration via capsule**  | Source: EFSA conclusions on fipronil (2006) considered GABA-gated chloride channel blocker |                             |
| Flufenacet       | Deficits in stride width                          | 1.14             | 27               | 1-year dog **Administration via capsule**  | Source: DAR 1998 unknown                                               |                             |
| Fluquinconazole  | Ataxia, hunched posture, tremor                    | 0.44             | 4.77             | 2-year rat **Administration via diet**     | Source: DAR Unknown                                                    |                             |
| Fonofos          | Tremor, abnormal gait                              | 2.5              | 6.75             | 90-day neurotoxicity rat (author not reported, US EPA 1999) **Administration via diet** | Source: US EPA 1999 No reference values available AChE inhibitor |                             |
| Formetanate      | Ataxia, head shaking                               | 1.8              | 18               | 29-day dog **Administration via diet**     | Source: DAR 2004 AChE inhibitor                                          |                             |
| Fosthiazate      | Ataxia                                             | 0.54             | 5.4              | 28-day dog **Administration via capsule**  | Source: DAR Neurotoxicity studies not available AChE inhibition          |                             |
| Active substance       | Indicator of specific effect                                      | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                          | Remark                                                                 | MoA                                           |
|------------------------|------------------------------------------------------------------|------------------|------------------|-------------------------------|------------------------------------------------------------------------|-----------------------------------------------|
| Glufosinate            | Ataxia, convulsions, Hyperactivity followed by hypoactivity, tremor | 4.5              | 8.4              | 1-year dog                    | Administration via diet                                                | Source: DAR                                  | Unknown                                      |
| Heptachlor             | Increased motor activity, convulsions                            | 0.5              | 5                | 6-month rat                   | Administration via gavage                                              | Source: JMPR 1970                            | GABA-gated chloride channel antagonist      |
|                        |                                                                   |                  |                  |                               | Not true NOAEL, but LAOEL divided by 10.                                |                                               |                                              |
|                        |                                                                   |                  |                  |                               | ADI: 0.0001 mg/kg (JMPR 1994)                                          |                                               |                                              |
|                        |                                                                   |                  |                  |                               | 14-day neurotoxicity rat                                                |                                               |                                              |
|                        |                                                                   |                  |                  |                               | No effect at 69 mg/kg bw/d (highest tested dose)                       |                                               |                                              |
|                        |                                                                   |                  |                  |                               | 90-day neurotoxicity study not available                               |                                               |                                              |
| Imidacloprid           | Tremor                                                           | 23.5             | 45.4             | 90-day dog                    | Administration via diet                                                | Source: DAR 2005                             | Agonist of nAChR                            |
| Indoxacarb             | Ataxia, hunched posture                                          | 2.6              | 14               | 18-month mouse                | Administration via diet                                                | Source: DAR 2000                             | Voltage-dependent sodium channel blocker   |
| Isoxaflutole           | Limited use of hindlimbs                                         | 20               | 500              | 2-year rat                    | Administration via diet                                                | Source: DAR 1997                             | Unknown                                     |
| Lambda-Cyhalothrin     | Ataxia, convulsions, tremor                                      | 0.5              | 3.5              | 1-year dog                    | Administration via capsule                                             | Source: DAR 1996                             | Binding to VGSC                             |
|                        |                                                                   |                  |                  |                               | Chronic neurotoxicity studies not available                           |                                               |                                              |
| Lindane                | Increased motor activity, convulsions                            | 6                | 24.3             | 2-year rat                    | Administration via diet                                                | Source: JMPR 2002                             | GABA-gated chloride channel antagonist      |
|                        |                                                                   |                  |                  |                               | Chronic neurotoxicity studies not available                           |                                               |                                              |
| Lufenuron              | Convulsions                                                      | 1.9              | 20               | 2-year rat                    | Administration via diet                                                | Source: DAR 2006                             | Unknown                                     |
| Malathion              |                                                                   | 1,486            |                  | 90-day neurotoxicity are      | Administration via diet                                                | Source: DAR                                  | AChE inhibitor                              |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-------------------------------|------------------|-----------------|-------|--------|-----|
| Mancozeb        | Paralysis                     | 49               | 328             | 3-month rat | Source: DAR 2000 | presumed Neurotoxic effect might be due to the metabolite CS$_2$ |
| Maneb           | Paresis limbs                 | 75               | 200             | 1-year dog | Source: DAR 2000 | presumed Neurotoxic effect might be due to the metabolite CS$_2$ |
| Mepiquat        | Convulsions, lateral position | 32               | 95              | 3-month dog | Source: DAR | Activation of nicotinic and muscarinic acetylcholine receptors |
| Methamidophos   | AChE inhibition (brain, erythrocytes) | 0.1             | 0.22            | 2-year rat | Source: DAR 2000 | AChE inhibitor |
| Methidathion    | Increased motor activity: tremor, hyperactivity | 0.16            | 0.8             | 2-year rat | Source: JMPR 1992 | AChE inhibitor |
| Methiocarb      | Muscle weakness, tremor        | 2.2              | 8.6             | 2-year dog | Source: DAR 2004 | AChE inhibitor |
| Methomyl        | Reduced fore- and hindlimb grip strength | 9               | 95              | 90-day neurotoxicity rat | Source: DAR | AChE inhibitor |
### CAGs of pesticides for effects on nervous system

| Active substance     | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                | Remark                                                        | MoA                        |
|----------------------|-------------------------------|------------------|------------------|----------------------|---------------------------------------------------------------|-----------------------------|
| Metiram              | Reduced grip strength         | 25.4             | 81.4             | 3-month rat          | Source: DAR 2000                                              | Presumed Neurotoxic effect might be due to the metabolite CS₂ |
| Milbemectin          | –                             | 59               | –                | 90-day neurotoxic rat| Source: RAR 2017                                              | Glutamate-gated chloride (GluCl) allosteric modulator |
| Molinate             | Ataxia                        | 1.8              | 13               | 2-year rat           | Source: DAR                                                   | Presumed Inhibition of ALDH by molinate sulfone |
| Monocrotophos        | AChE inhibition (brain, erythrocytes) | 0.005         | 0.05             | 2-year rat           | Source: JMPR 1991 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor |
| Omethoate (metabolite of dimethoate) | Increased motor activity: tremor | 0.3             | 2.9              | 2-year rat           | Source: DAR 2004 Chronic neurotoxicity studies not available | AChE inhibitor |
| Oxamyl               | Ataxia, hunched posture, ptosis | 1.69            | 15.3             | 90-day neurotoxic rat| Source: DAR 2003                                             | AChE inhibitor |
| Oxsulfuron           | Ataxia                        | 1.3              | 11               | 1-year dog           | Source: DAR                                                   | Unknown                     |
| Oxydemeton-methyl    | AChE inhibition (brain, erythrocytes) | 0.027         | 0.224            | 2-year rat           | Source: DAR 2004 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor |
| Parathion            | Tremor, abnormal gait         | 0.25             | 2.5              | 2-year rat           | Source: ECCO 2001, JMPR 1995 Neurotoxicity studies not available NOAEL for AChE inhibition: 0.25 mg/kg bw/d | AChE inhibitor |
| Active substance   | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                      | Remark                                                                                       | MoA          |
|-------------------|------------------------------|------------------|------------------|--------------------------------------------|-----------------------------------------------------------------------------------------------|--------------|
| Parathion-methyl  | Tremor, abnormal gait        | 0.25             | 2.5              | 2-year rat                                 | Source: DAR 2001 Neurotoxicity studies not available NOAEL for AChE inhibition: 0.25 mg/kg bw/d | AChE inhibitor|
| Permethrin        | Increased motor activity, tremor | 40              | 100              | 2-year rat                                 | Source: JMPR 1999 Binding to VGSC                                                          |              |
| Phenthoate        | AChE inhibition (erythrocytes) | 0.29             | 0.87             | 2-year dog                                 | Source: JMPR 1980 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor|
| Phosalone         | Reduced grip strength        | 11.5             | 45.9             | 90-day neurotoxicity rat                   | Source: DAR 2004 AChE inhibitor                                                            |              |
| Phosmet           |                              | 9.4              | –                | 90-day neurotoxicity rat                   | Source: DAR 2004 AChE inhibitor                                                            |              |
| Phoxim            | AChE inhibition (erythrocytes) | 0.1              | 0.38             | 2-year dog                                 | Source: JECFA 1999 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition|
| Pirimicarb        | Tremor                       | 3.5              | 10               | 1-year dog                                 | Source: DAR EFSA conclusions in pirimicarb (2005) considered                              | AChE inhibitor|
| Pirimiphos-methyl | Hunched posture              | 9                | 36               | 2-year mouse                               | Source: DAR AChE inhibitor                                                                |              |
| Profenofos        |                              | –                | 36               | 90-day neurotoxicity rat                   | Source: JMPR 2007 AChE inhibitor                                                            |              |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|------------------|------------------|-------|--------|-----|
| Propineb        | Ataxia (Hind-limb wheelbarrowing) | 4.3              | 41               | 90-day dog Administration via diet | Source: DAR 1996 | Presumed Neurotoxic effect might be due to the metabolite CS2 |
| Pyrazophos      | Muscle strength: increased or decreased muscle tone, weakness; coordination: abnormal gait | 0.45             | 8                | 6-month dog Administration via diet | Source: DAR 1998 | Neurotoxic studies not available NOAEL for AChE inhibition: 0.05 mg/kg bw/d AChE inhibitor |
| Pyrethrins      | Ataxia, paresis limbs, tremor | 30               | 86               | 56-day dog Administration via diet | Source: DAR 2007 | Chronic neurotoxicity studies not available Binding to VGSC |
| Sulfoxaflor     | –                            | 94.9             | –                | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR 2012 | Nicotinergic AChR partial agonist |
| tau-Fluvalinate | Choreoathetosis (Ruffling of body, pawing), transient hyperactivity followed by hypoactivity | 0.5              | 1                | 2-year rat Administration via gavage | Source: DAR EFSA (2018) | Chronic neurotoxicity studies not available Binding to VGSC |
| Tebuconazole    | Reduced motor activity       | 100              | 300              | 4-week rat Administration via gavage | Source: DAR 2006 | Unknown |
| Tefluthrin      | ataxia                       | 0.5              | 2                | 1-year dog Administration via capsule | Source: DAR EFSA (2010) | Binding to VGSC |
| Tembotrione     | Reduced motor activity: hypoactivity | 26.7             | 111              | 90-day dog Administration via diet | Source: DAR 2012 | Unknown |
| Tetraconazole   | Hunched posture              | 17               | 65               | 28-day rat Administration via diet | Source: DAR 2005 | Unknown |
| Tetramethrin    | Increased motor activity: tremor | 31               | 63               | 6-month dog Administration via diet | Source: WHO 1990 Neurotoxicity studies not available No reference values available | Binding to VGSC |
| Active substance                  | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                                                 | Remark                                                                 | MoA                        |
|----------------------------------|-----------------------------|------------------|------------------|----------------------------------------------------------------------|------------------------------------------------------------------------|----------------------------|
| Thiacloprid                      | --                          | 101              | --               | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR 2017                                                      | Agonist of nAChR           |
| Thiametoxam                      | --                          | 95.4             | --               | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR 2002                                                      | Agonist of nAChR           |
| Thiodicarb                       | Increased motor activity    | 12.8             | 38.3             | 1-year dog Administration via diet                                  | Source: DAR 2003                                                      | AChE inhibitor             |
| Thiram                           | Dragging of hind feet and tail, paralysis | 5.3             | 20               | 18-month rat Administration via diet                                | Source: DAR 1997 and addenda                                         | Presumed Neurotoxic effect might be due to the metabolite CS₂ |
| Tolclofos-methyl                 | --                          | 735.7            | --               | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: RAR 2016                                                      | AChE inhibitor             |
| Triadimefon                      | Increased motor activity: hyperactivity | 3.4             | 54.6             | 90-day neurotoxicity rat Administration via diet                   | Source: JMPR 2004                                                    | Inhibition of dopamine transporter (presumed) |
| Triadimenol (a metabolite of Triadimefon) | Increased motor activity | 3.4             | 45               | 3-month neurotoxicity rat Administration via diet                 | Source: DAR                                                            | Inhibition of dopamine transporter (presumed) |
| Tri-allate                       | Landing-foot splay, reduced grip strength | 32.9            | 128.8            | 3-month neurotoxicity rat Administration via diet                 | Source: DAR                                                            | Unknown                    |
| Triazophos                       | AChE inhibition (erythrocytes) | 0.012           | 0.13             | 1-year dog Administration via diet                                | Source: JMPR 2002 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor             |
### Table B.6: CAG on functional effects on sensory division: toxicological characterisation of ASs to be considered in chronic exposure/risk assessments

| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-------------------------------|-------------------|-------------------|-------|--------|-----|
| Trichlorfon      | –                             | 168               | –                 | 90-day neurotoxicity rat | Administration via diet | Source: DAR addendum, 2005 | AChE inhibitor |
| Zeta-Cypermethrin| Landing-foot splay, reduced motor activity | 5                  | 26                | 90-day neurotoxicity rat | Administration via diet | Source: DAR | Known Binding to VGSC |
| Abamectin        | Decreased pupil reactivity    | 0.25              | 0.5               | 1-year dog | Administration via diet | Source: DAR 2005 EFSA (2008) | GABA-gated chloride channel agonist |
| Acephate         | –                             | 74.2              | –                 | 49-day neurotoxicity study | Administration via diet | Source: JMPR 2002 | AChE inhibitor |
| Acetamiprid      | –                             | 118               | –                 | 90-day neurotoxicity study | Administration via diet | Source: DAR 2001 | Agonist of nAChR |
| Acrinathrin      | –                             | 62.6              | –                 | 90-day neurotoxicity rat | Administration via diet | Source: DAR 2007 | Binding to VGSC |
| Aldicarb         | Decreased tail pinch response | 0.1               | 0.5               | Acute neurotoxicity rat | Administration via gavage | Source: DAR 1996 | AChE inhibitor |

CAG: cumulative assessment group; AS: active substance; NOEL: no observed effect level; LOEL: lowest observed effect level; NOAEL: no observed adverse effect level; LOAEL: lowest observed adverse effect level; bw: body weight; GABA: gamma-aminobutyric acid; AChE: acetylcholinesterase; VGSC: voltage-gated sodium channel; nAChR: nicotinic acetylcholine receptor; MoA: mode of action.
| Active substance        | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                      | Remark                                                                 | MoA                        |
|------------------------|------------------------------|------------------|------------------|-------------------------------------------|------------------------------------------------------------------------|---------------------------|
| Alpha-cypermethrin     |                              | 1.5              |                  | Chronic neurotoxicity studies not available NOAEL used to derive the ADI (1-year dog, EC review report on alpha-cypermethrin 2004) used as surrogate | Binding to VGSC            |
| Amitraz               | Hyperreactivity              | 12               | 50               | 90-day rat Administration via gavage      | Source: DAR Neurotoxicity studies not available | Partial agonist of presynaptic α2-adrenergic receptor                  |
| Azinphos-ethyl         | AChE inhibition (erythrocyte) | 0.0125           | 0.025            | 90-day dog Administration via diet        | Source: JMPR 1973 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor            |
| Benfuracarb           | Abnormal righting reflex     | 3.23             | 6.99             | 90-day neurotoxicity rat                   | Source: DAR 1996, Addendum 6, 2000 (neurotoxicity)                  | AChE inhibition            |
| Beta-Cyfluthrin        |                              | 26.81            |                  | 90-day neurotoxicity rat                   | Source: JMPR 2006, DAR 1996                                         | Binding to VGSC            |
| Beta-cypermethrin      | Hyperreactivity              | 0.82             | 8.2              | 90-day rat Administration via diet         | Source: DAR 2013 Not true NOAEL, but LOAEL divided by 10              | Binding to VGSC            |
| Bifenthrin             |                              | 11.8             |                  | 90-day neurotoxicity rat                   | Source: DAR                                                            | Binding to VGSC            |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|------------------|------------------|-------|--------|-----|
| Cadusafos        |                             | 27               | –                | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR 2004 | AChE inhibition |
| Carbaryl         | Decreased pupil size        | 10               | 30               | 90-day neurotoxicity study Administration via gavage | Source: DAR 2004 | AChE inhibition |
| Carbofuran       |                             | 67.5             | –                | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: Revised DAR 2008 | AChE inhibition |
| Carbosulfan      |                             | 130.7            | –                | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: Revised DAR 2009 | AChE inhibition |
| Chlormequat      | Diminished reflex response  | 50               | 62.5             | 90-day dog Administration via diet | Source: DAR 2007 Neurotoxicity studies not available | Known Partial agonist of muscarinic and nicotinic acetylcholine receptor |
| Chlorpyrifos     |                             | 15               | –                | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR 1999 | AChE inhibition |
| Chlorpyrifos-methyl |                       | 250             | –                | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR 1997 | AChE inhibition |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|--------------------------------|-----------------|-----------------|-------|--------|-----|
| Clothianidin     |                                | 177             | –               | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR 2003 | agonist of nAChR |
| Cyfluthrin       |                                | 0.3             |                 | Chronic neurotoxicity studies not available NOAEL used to derive the ADI (pharmacological study in mice, EC review report on cyfluthrin 2002) used as surrogate | Binding to VGSC |
| Cymoxanil        | Hyperreactivity                | 30              | 90              | 2-year rat Administration via diet | Source: DAR | Unknown |
| Cypermethrin     | Hypersensitivity to noise      | 5               | 15              | 1-year dog Administration via capsule | Source: DAR Chronic neurotoxicity studies not available ADI: 0.05 mg/kg bw per day based on the 2-year rat study (EC Review Report 2005) | Binding to VGSC |
| Deltamethrin     | Hyperreactivity to noise       | 4               | 14              | 90-day neurotoxicity rat Administration via diet | Source: DAR addendum Binding to VGSC |
| Diazinon         |                                | 177             | –               | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR 2004 | AChE inhibition |
| Dichlorvos       | AChE inhibition (erythrocytes) | 0.008           | 0.08            | 2-year dog Administration via diet Highest tested dose | Source: DAR 2003 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor |
| Dieldrin         | Hyperreactivity                | 0.05            | 0.25            | 2-year rat Administration via diet | Source: JMPR 1977 ADI (PTDI): 0.0001 mg/kg bw per day (JMPR 1994). GABA-gated chlorine channel antagonist |
| Dimethoate       |                                | 11.25           | –               | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR | AChE inhibitor |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|-----------------|-----------------|-------|--------|-----|
| Dinotefuran      |                             | 3,413           | 90-day neurotoxicity rat | Administration via diet Highest tested dose | Source: JMPR 2012 | Agonist of nAChR |
| Emamectin benzoate | Hyperreactivity         | 0.5 (0.1)       | 0.75            | 1-year dog | Administration via gavage | Source: DAR 2008 EFSA conclusions on emamectin benzoate (2012) considered Additional UF of 5 due to small dose spacing and steep dose response curve | GABA-gated chloride channel agonist |
| Endosulfan       | Exaggerated auditory reflex (startle reflex) | 0.57            | 2.3             | 1-year dog | Administration via diet | Source: DAR 2001 and addendum Chronic neurotoxicity study not available | GABA-gated chloride channel blocker |
| **Endrin (IC)**  | Hyperreactivity          | 0.05            | 1               | 2-year rat | Administration via diet | Source: EHC 130, 1992 Neurotoxicity studies not available ADI (PTDI): 0.0002 (JMPR 1994) | GABA-gated chloride channel antagonist |
| Esfenvalerate    |                             | 20.1            | 90-day neurotoxicity rat | Administration via diet Highest tested dose | Source: JMPR 2002 | Binding to VGSC |
| Ethephon         |                             | 400             | 90-day neurotoxicity rat | Administration via gavage Highest tested dose | Source: DAR 2004 | AChE inhibitor |
| Ethion           | AChE inhibition (brain)   | 0.06            | 0.71            | 90-day dog | Administration via diet | Source: JMPR 1990 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor |
| Ethoprophos      | Negative air drop, pupillary responses, decreased analgesic reflex | 2.65            | 27.11           | 90-day neurotoxicity rat | Administration via diet | Source: DAR 2004 | AChE inhibition |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|------------------|------------------|-------|--------|-----|
| Fenamiphos      | --                          | 3.1              | --               | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR 2003 | AChE inhibition |
| Fenitrothion     | --                          | 13.8             | --               | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR 2003 | AChE inhibitor |
| Fenpropathrin    | Uncoordinated righting reflex | 15               | 50               | 90-day neurotoxicity rat Administration via diet | Source: JMPR 2012 | Binding to VGSC |
| Fenpropimorph   | Retarded pupillary reflex    | 7.1              | 71               | 90-day neurotoxicity rat Administration via diet | Source: DAR | Unknown |
| Fenthion        | --                          | 8.5              | --               | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: JMPR 1995; Addendum Tox 2001 | AChE inhibitor |
| Fenvalerate     | Hyperreactivity              | 15               | 50               | 18-month mouse Administration via diet | Source: JMPR 2012 | Binding to VGSC |
| Fipronil        | Hyperreactivity              | 0.2              | 2                | 1-year dog Administration via capsule | Source: DAR 2004 | GABA-gated chloride channel blocker |
| Flufenacet      | Hypo-reactivity, reduced reaction to movement and sound, hyperreactivity | 27               | 59               | 1-year dog Administration via diet | Source: DAR 1998 | Unknown |
| Fonofos         | Decreased reactivity patellar reflex | 2.5             | 6.75             | 90-day neurotoxicity rat (author not reported, US EPA 1999) Administration via diet | Source: US EPA 1999 No reference values available | AChE inhibitor |
| Formetanate     | --                          | 18.4             | --               | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR 2017 | AChE inhibition |
| Active    | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                     | Remark                                                                 | MoA                      |
|-----------|------------------------------|------------------|------------------|---------------------------|------------------------------------------------------------------------|--------------------------|
| Fosthiazate | AChE inhibition              | 0.42             | 2.36             | 2-year rat                | Source: DAR Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor           |
| Glufosinate | Decrease in alertness and/or startle response | 52.1             | 521              | 90-day neurotoxicity rat  | Source: DAR Not true NOAEL, but LOAEL divided by 10                   | Unknown                  |
| Heptachlor | Hyperreactivity              | 2                | 7                | 14-day neurotoxicity rat  | Source: CICADS, 2006 90-day neurotoxicity study not available. Poor data. ADI (PTDI): 0.0001 mg/kg bw/d (JMPR 1994). | GABA-gated chloride channel antagonist |
| Imidacloprid | –                            | 196              | –                | 90-day neurotoxicity rat  | Source: DAR 2005 Agonist of nAChR                                     | Voltage-dependent sodium channel blocker |
| Indoxacarb | Hyperreactivity              | 2.6              | 14               | 18-month mouse            | Source: DAR 2000                                                     | Voltage-dependent sodium channel blocker |
| Lambda-cyhalothrin | Ataxia, convulsions, tremor | 0.5              | –                | 1-year dog                | Chronic neurotoxicity studies not available NOAEL for functional alteration of the motor division used as surrogate ADI: 0.0025 mg/kg bw/d, based on multigeneration rat study (EFSA 2014) | Binding to VGSC           |
| Lindane    |                               | 0.47             | –                |                           | Chronic neurotoxicity studies not available NOAEL used to derive the ADI (2-year rat, JMPR 2002) used as surrogate | GABA-gated chloride channel antagonist |
| Active substance     | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA                  |
|----------------------|-------------------------------|------------------|------------------|-------|--------|----------------------|
| Malathion            |                               | 1,486            |                  | 90-day neurotoxicity are administration via diet highest tested dose | Source: DAR | AChE inhibitor        |
| Metaldehyde          | No reaction to noise          | 30               | 90               | 1-year dog administration via diet | Source: DAR | Presumed GABA inhibitor |
| Methamidophos        |                               | 4.26             |                  | 90-day neurotoxicity rat administration via diet highest tested dose | Source: DAR 2000 | AChE inhibitor        |
| Mepiquat             |                               | 517              |                  | 90-day neurotoxicity rat administration via diet highest tested dose | Source: DAR | Activation of nicotinic and muscarinic acetylcholine receptors |
| Methidathion         | Hyperreactivity               | 0.16             | 1.72             | 2-year rat administration via diet | Source: JMPR 1992 Chronic neurotoxicity studies not available | AChE inhibition |
| Methiocarb           | AChE inhibition (erythrocytes)| 1.32             | 6.46             | 90-day dog administration via diet | Source: DAR 2004 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition |
| Methomyl             |                               | 95               |                  | 90-day neurotoxicity rat administration via diet highest tested dose | Source: DAR | AChE inhibition        |
| Milbemectin          |                               | 59               |                  | 90-day neurotoxicity rat administration via diet highest tested dose | Source: RAR 2017 Glutamate-gated chloride (GluCl) allosteric modulator |                 |
| Molinate             | Patellar hyperreflexia, paraesthesia, proprioception deficit | 10               | 50               | 1-year dog administration via capsule | Source: DAR | Presumed Inhibition of ALDH by molinate sulfone |
| Active substance       | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                          | Remark                                                                 | MoA                        |
|-----------------------|------------------------------|------------------|------------------|-----------------------------------------------|------------------------------------------------------------------------|----------------------------|
| Monocrotophos         | AChE inhibition (brain, erythrocytes) | 0.005            | 0.05             | 2-year rat <br> Administration via diet      | Source: JMPR 1991 <br> Neurotoxicity studies not available <br> NOAEL for AChE inhibition used as surrogate | AChE inhibitor             |
| Omethoate             | AChE inhibition (erythrocytes)   | 0.027            | 0.04             | 2-year rat <br> supplementary 32-week rat    | Source: DAR 2004 (dimethoate) <br> Chronic neurotoxicity studies not available <br> NOAEL for AChE inhibition used as surrogate | AChE inhibitor             |
| Omethoate (metabolite of dimethoate) | AChE inhibition (erythrocytes) | 0.027            | 0.04             | 2-year rat <br> supplementary 32-week rat    | Source: DAR 2004 (dimethoate) <br> Chronic neurotoxicity studies not available <br> NOAEL for AChE inhibition used as surrogate | AChE inhibitor             |
| Oxamyl                | Hyperreactivity, absent pupillary response | 1.69             | 15.3             | 90-day neurotoxicity rat <br> Administration via diet | Source: DAR 2003 | AChE inhibitor |
| Oxasulfuron           | Hindlimb flexor reflex         | 83               | 425              | 2-year carcinogenicity rat <br> Administration via diet | Source: DAR | Unknown |
| Oxydemeton-methyl     | AChE inhibition (brain, erythrocytes) | 0.027            | 0.224            | 2-year rat <br> Administration via diet      | Source: DAR 2004 <br> Neurotoxicity studies not available <br> NOAEL for AChE inhibition used as surrogate | AChE inhibitor             |
| Parathion             | AChE inhibition (brain)         | 0.25             | 2.5              | 2-year rat <br> Administration via diet      | Source: JMPR 1995 <br> Neurotoxicity studies not available <br> NOAEL for AChE inhibition used as surrogate | AChE inhibition             |
| Parathion-methyl      | AChE inhibition (erythrocytes)  | 0.25             | 2.5              | 2-year rat <br> Administration via diet      | Source: DAR 2001 <br> Neurotoxicity studies not available <br> NOAEL for AChE inhibition used as surrogate | AChE inhibition             |
| Permethrin            | Hypersensitivity               | 100              | 250              | 28-day neurotoxicity rat <br> Administration via diet | Source: JMPR 1999 | Binding to VGSC |
| Active substance       | Indicator of specific effect                     | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                                                 | Remark                                                                                           | MoA                  |
|-----------------------|-------------------------------------------------|------------------|------------------|----------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|---------------------|
| Phenthoate            | AChE inhibition (erythrocytes)                  | 0.29             | 0.87             | 2-year dog Administration via diet                                    | Source: JMPR 1980 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor      |
| Phosalone             | –                                               | 45.9             | –                | 90-day neurotoxicity rat Administration via diet Highest tested dose  | Source: DAR 2004                                                                                   | AChE inhibitor      |
| Phosmet               | –                                               | 9.4              | –                | 90-day neurotoxicity rat Administration via diet Highest tested dose  | Source: DAR 2004                                                                                   | AChE inhibitor      |
| Phoxim                | AChE inhibition (erythrocytes)                  | 0.1              | 0.38             | 2-year dog Administration via diet                                    | Source: JECFA 1999 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition      |
| Pirimicarb            | –                                               | 77.1             | –                | 90-day neurotoxicity rat Administration via diet Highest tested dose  | Source: DAR                                                                                      | AChE inhibition      |
| Pirimiphos-methyl     | –                                               | 21.1             | –                | 90-day neurotoxicity rat Administration via diet Highest tested dose  | Source: DAR                                                                                      | AChE inhibition      |
| Profenofos            | –                                               | 36               | –                | 90-day neurotoxicity rat Administration via diet Highest tested dose  | Source: JMPR 2007                                                                                | AChE inhibitor      |
| Propineb              | Sensory changes (proprioceptive deficit)        | 4.3              | 41.4             | 90-day dog Administration via diet                                    | Source: DAR 1996 Presumed Neurotoxic effect might be due to the metabolite CS2                    | Presumed AChE inhibitor |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|------------------|------------------|-------|--------|-----|
| Pyrazophos       | Decreased pupil reactivity  | 0.45             | 8                | 6-month dog | Source: DAR 1998 Neurotoxicity studies not available NOAEL for AChE inhibition: 0.05 mg/kg bw/d AChE inhibitor |
| Pyrethrins       | 4                           |                  |                  | Chronic neurotoxicity studies not available NOAEL used to derive the ADI (2-year rat, EFSA 2013) used as surrogate Binding to VGSC |
| Sulcotrione      | Decrease in proprioception, increased patellar reflex | 300             | 600              | 16-week dog; 90-day dog | Source: DAR Unknown |
| Sulfoxaflor      | –                           | 94.9             | –                | 90-day neurotoxicity rat | Source: DAR 2012 Nicotinergic AChR partial agonist |
| tau-Fluvalinate  | Decreased responsiveness to sensory stimuli, increase in click response | 2               | 6                | 8-day neurotoxicity rat | Source: DAR Chronic neurotoxicity studies not available ADI: 0.005 mg/kg bw/d, 2-year rat (EFSA, 2018) Binding to VGSC |
| Tefluthrin       | Increased response to sound | 1.5             | 5.9              | 2-year carcinogenicity rat | Source: DAR Binding to VGSC |
| Tetramethrin     |                             |                  |                  |                   | Neurotoxicity studies not available No reference values available Binding to VGSC |
| Thiacloprid      | –                           | 101              | –                | 90-day neurotoxicity rat | Source: DAR 2017 Agonist of nAChR |
| Thiametoxam      | –                           | 95.4             | –                | 90-day neurotoxicity rat | Source: DAR 2002 Agonist of nAChR |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|-----------------|-----------------|-------|--------|-----|
| Thiodicarb       |                             | 46              |                 | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR 2003 | AChE inhibition |
| Tolclofos-methyl |                             | 735.7           |                 | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: RAR 2016 | AChE inhibitor |
| Tri-allate       | Increased alertness, impaired righting reflex | 33              | 129             | 3-month neurotoxicity rat Administration via diet | Source: DAR | Unknown |
| Triazophos       | AChE inhibition (erythrocytes) | 0.012           | 0.13            | 1-year dog Administration via diet | Source: JMPR 2002 | AChE inhibitor |
|                  |                             |                 |                 | **Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate** | | |
| Trichlorfon      |                             | 168             |                 | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR addendum, 2005 | AChE inhibitor |
| Zetacypermethrin |                             | 47              |                 | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR | Known Binding to VGSC |

CAG: cumulative assessment group; AS: active substance; NOEL: no observed effect level; LOEL: lowest observed effect level; NOAEL: no observed adverse effect level; LOAEL: lowest observed adverse effect level; bw: body weight; GABA: gamma-aminobutyric acid; AChE: acetylcholinesterase; VGSC: voltage-gated sodium channel; nAChR: nicotinic acetylcholine receptor; MoA: mode of action.
Table B.7: CAG on functional effects on autonomic division: toxicological characterisation of ASs to be considered in chronic 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            | Urination                     | 75               | 150             | 1-year neurotoxicity rat Administration via diet | Source: DAR 1997 | Unknown |
| Abamectin        | Salivation, mydriasis         | 0.25             | 0.5             | 18-week dog Administration via gavage | Source: DAR 2005 EFSA conclusions on abamectin (2008) considered | GABA-gated chloride channel agonist |
| Acephate         | –                             | 74.2             | –               | 49-day neurotoxicity study Administration via diet Highest tested dose | Source: JMPR 2002 | AChE inhibitor |
| Acetamiprid      | –                             | 118              | –               | 90-day neurotoxicity study Administration via diet Highest tested dose | Source: DAR 2001 | Agonist of nAChR |
| Acrinathrin      | Salivation                    | 5                | 10              | 28-day rat Administration via gavage | Source: DAR 2007 | Binding to VGSC |
| Aldicarb         | Salivation, lacrimation       | 0.1              | 0.5             | Acute neurotoxicity rat Administration via gavage | Source: DAR 1996 | AChE inhibitor |
| Alpha-cypermethrin|                              | 1.5              |                 | Chronic neurotoxicity studies not available NOAEL used to derive the ADI (1-year dog, EC review report on alpha-cypermethrin 2004) used as surrogate | | Binding to VGSC |
| Amitraz          |                               | 0.3              |                 | Neurotoxicity studies not available NOAEL used to derive the ADI (SCOFC, 2003) used as surrogate | | Partial agonist of presynaptic α2-adrenergic receptor |
| Active substance          | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                      | Remark                                                                 | MoA                           |
|--------------------------|------------------------------|------------------|------------------|-------------------------------------------|----------------------------------------------------------------------|-------------------------------|
| Azinphos-ethyl           | AChE inhibition (erythrocyte) | 0.0125           | 0.025            | 90-day dog                                | Administration via diet                                               | AChE inhibitor                 |
|                          |                              |                  |                  | Source: JMPR 1973 Neurotoxicity studies not available NOAEL for AchE inhibition used as surrogate |
| Azinphos-methyl          |                              | 6.99             | –                | 90-day neurotoxicity rat                  | Administration via diet                                               | AChE inhibition                 |
|                          |                              |                  |                  | Source: DAR 1996, Addendum 6, 2000 (neurotoxicity) |
| Benfuracarb              | Salivation                   | 2.5              | 5                | 2-year dog                                | Administration via capsule                                            | AChE inhibition                 |
| Beta-cyfluthrin          |                              | 26.81            | –                | 90-day neurotoxicity rat                  | Administration via diet                                               | Binding to VGSC                |
|                          |                              |                  |                  | Source: JMPR 2006, DAR 1996               |
| Beta-cypermethrin        | Salivation                   | 20               | 100              | Acute neurotoxicity rat                   | Administration via gavage                                              | Binding to VGSC                |
|                          |                              |                  |                  | Source: DAR 2013 Chronic neurotoxicity studies not available |
| Bifenthrin               |                              | 11.8             | –                | 90-day neurotoxicity rat                  | Administration via diet                                               | Binding to VGSC                |
| Cadusafos                |                              | 27               | –                | 90-day neurotoxicity rat                  | Administration via diet                                               | AChE inhibition                 |
| Carbaryl                 | Salivation                   | 10               | 30               | 90-day neurotoxicity study               | Administration via gavage                                              | AChE inhibition                 |
| Carbetamide              | Salivation                   | 150              | 300              | 28-day dog                                | Administration via capsule                                            | Unknown                        |
| Carbofuran               |                              | 67.5             | –                | 90-day neurotoxicity rat                  | Administration via diet                                               | AChE inhibition                 |
|                          |                              |                  |                  | Source: Revised DAR 2008                  |                                                                     |
| Active substance      | Indicator of specific effect          | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                    | Source | Remark                                                                 | MoA                        |
|-----------------------|---------------------------------------|------------------|------------------|------------------------------------------|--------|------------------------------------------------------------------------|----------------------------|
| Carbosulfan           |                                       | 130.7            | --               | 90-day neurotoxicity rat administration via diet Highest tested dose | Source: Revised DAR 2009 | AChE inhibition                                                        |                            |
| Chlorfenvinphos       | AChE inhibition (brain, erythrocytes)  | 0.15             | 15               | 2-year rat (author not reported, JMPR 1994) Administration via diet | Source: JMPR 1994 | Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor             |
| Chloromequat          | Salivation                            | 5                | 10               | 1-year dog administration via diet       | Source: DAR 2007 | Neurotoxicity studies not available                                     | Partial agonist of muscarinic and nicotinic acetylcholine receptor |
| Chlorpyrifos          |                                       | 15               | --               | 90-day neurotoxicity rat administration via diet Highest tested dose | Source: DAR 1999 | AChE inhibition                                                        |                            |
| Chlorpyrifos-methyl   |                                       | 250              | --               | 90-day neurotoxicity rat administration via diet Highest tested dose | Source: DAR 1997 | AChE inhibition                                                        |                            |
| Clothianidin          | Salivation                            | 19.3             | 40.9             | 90-day dog administration via diet       | Source: DAR 2003 |                                                                 | Agonist of nAChR          |
| Cyfluthrin            |                                       | 0.3              | --               |                                                                 |        | Chronic neurotoxicity studies not available NOAEL used to derive the ADI (pharmacological study in mice, EC review report on cyfluthrin 2002) used as surrogate | Binding to VGSC           |
| Cypermethrin          | Salivation                            | 6                | 20               | 1-year dog administration via diet       | Source: DAR zeta-cypermethrin | Chronic neurotoxicity studies not available ADI: 0.05 mg/kg bw per day based on the 2-year rat study (EC Review Report 2005) | Binding to VGSC           |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|------------------|------------------|-------|--------|-----|
| Deltamethrin     | Mydriasis                   | 1                | 2.5              | 90-day dog Administration via diet | Source: DAR EC review report on deltamethrin considered | Binding to VGSC |
| Diazinon         | --                          | 177              | --              | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR 2004 | AChE inhibition |
| Dicamba          | Salivation                  | 50               | 300             | 13-week dog Administration via capsule | Source: Revised DAR year | Unknown |
| Dichlorvos       | AChE inhibition (erythrocytes) | 0.008            | 0.08            | 2-year dog | Source: DAR 2003 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor |
| Dicofol          | Salivation                  | 3.31             | 9.78            | 90-day dog Administration via diet | Source: DAR 2006 | Unknown |
| Dieldrin         |                             | 0.025            |                 | | Source: JMPR 1977 Neurotoxicity studies not available NOAEL in dog and rat which served as basis to the JMPR ADI used as surrogate | GABA-gated chlorine channel antagonist |
| Dimethoate       | --                          | 11.25            | --              | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR | AChE inhibitor |
| Dinotefuran      | --                          | 3,413            | --              | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: JMPR 2012 | Agonist of nAChR |
| Active substance         | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                          | Remark                                                                                     | MoA                                    |
|-------------------------|------------------------------|------------------|------------------|-------------------------------|--------------------------------------------------------------------------------------------|----------------------------------------|
| Emamectin benzoate      | Mydriasis                    | 0.5 (0.1)        | 0.75             | 1-year dog                   | Administration via gavage                                                                   |                                        |
|                         |                              |                  |                  |                               | Additional UF of 5 due to small dose spacing and steep dose response curve                  | GABA-gated chloride channel agonist    |
| Endosulfan              |                              | 0.6              |                  |                               | Source: JMPR 1998 Chronic neurotoxicity study not available NOAEL in 2-year rat and 1-year dog which served as basis to the JMPR ADI used as surrogate |                                        |
| Endrin                  |                              | 0.02             |                  |                               | Neurotoxicity studies not available NOAEL based on the JMPR ADI (PTDI) of 0.0002 mg/kg bw/d (JMPR 1994) | GABA-gated chloride channel antagonist |
| Esfenvalerate           |                              | 20.1             | –                | 90-day neurotoxicity rat      | Administration via diet Highest tested dose                                                  | Binding to VGSC                         |
| Ethephon                |                              | 400              | –                | 90-day neurotoxicity rat      | Administration via gavage Highest tested dose                                                  |                                        |
| Ethion                  | AChE inhibition (brain)      | 0.06             | 0.71             | 90-day dog                   | Administration via diet                                                                       |                                        |
| Ethoprophos             | Salivation, lacrimation      | 2.65             | 27.11            | 90-day neurotoxicity rat      | Source: JMPR 1990 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition                         |
### CAGs of pesticides for effects on nervous system

| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|-----------------|-----------------|-------|--------|-----|
| Fenamiphos       |                             | 3.1             |                 | 90-day neurotoxicity rat | Source: DAR 2003 | AChE inhibition |
|                  |                             |                 | Administration via diet |       |        |     |
|                  |                             |                 | Highest tested dose |       |        |     |
| Fenitrothion     |                             | 12.5            |                 | 90-day neurotoxicity rat | Source: DAR 2003 | AChE inhibitor |
|                  |                             |                 | Administration via diet |       |        |     |
|                  |                             |                 | Highest tested dose |       |        |     |
| Fenpropathrin    |                             | 38              |                 | 90-day neurotoxicity rat | Source: JMPR 2012 | Binding to VGSC |
|                  |                             |                 | Administration via diet |       |        |     |
|                  |                             |                 | Highest tested dose |       |        |     |
| Fenthion         |                             | 8.5             |                 | 90-day neurotoxicity rat | Source: JMPR 1995; Addendum Tox 2001 | AChE inhibitor |
|                  |                             |                 | Administration via diet |       |        |     |
|                  |                             |                 | Highest tested dose |       |        |     |
| Fenvalerate      |                             | 300             |                 | 14-day neurotoxicity rat | Source: JMPR 2012 | Binding to VGSC |
|                  |                             |                 | Administration via diet |       |        |     |
|                  |                             |                 | Highest tested dose |       |        |     |
| Fipronil         |                             | 8.9             |                 | 90-day neurotoxicity rat | Source: DAR 2004 | GABA-gated chloride channel blocker |
|                  |                             |                 | Administration via diet |       |        |     |
| Fluquinconazole  | Piloerection                | 1.73            | 8.81            | 28-day rat | Source: DAR | Unknown |
| Fonofos          | Urination                   | 2.5             | 6.75            | 90-day neurotoxicity rat (author not reported, US EPA 1999) | Source: US EPA 1999 | AChE inhibitor |
|                  |                             |                 | Administration via diet |       |        |     |
| Formetanate      |                             | 18.4            |                 | 90-day neurotoxicity rat | Source: DAR 2017 | AChE inhibition |
|                  |                             |                 | Administration via diet |       |        |     |
|                  |                             |                 | Highest tested dose |       |        |     |
| Active substance     | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                          | Remark                                                                 | MoA                                      |
|----------------------|------------------------------|------------------|------------------|-------------------------------|------------------------------------------------------------------------|------------------------------------------|
| Fosthiazate          | AChE inhibition              | 0.42             | 2.36             | 2-year rat                    | Source: DAR Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor                          |
| Glufosinate          | Trismus salivation           | 4.5              | 8.4              | 1-year dog                    | Administration via diet                                                | Source: DAR Unknown                      |
| Heptachlor           |                              | 69               | –                | 14-day neurotoxicity rat      | GABA-gated chloride channel antagonist                                 | Administration via gavage               |
| Imidacloprid         |                              | 196              | –                | 90-day neurotoxicity rat      | Source: DAR 2005 agonist of nAChR                                      | Administration via diet Highest tested dose |
| Indoxacarb           | Urination                    | 2.6              | 14               | 18-month mouse                | Source: DAR 2000 Voltage-dependent sodium channel blocker             |                                          |
| Lambda-Cyhalothrin   | Piloerection                 | 1.8              | 9.2              | 2-year mouse                  | Source: DAR Chronic neurotoxicity studies not available ADI: 0.0025 mg/kg bw/d, based on multigeneration rat study (EFSA 2014) | Binding to VGSC                          |
| Lindane              |                              | 0.47             | –                |                               | Chronic neurotoxicity studies not available NOAEL used to derive the ADI (2-year rat, JMPR 2002) used as surrogate | GABA-gated chloride channel antagonist |
| Lufenuron            | Salivation                   | 7                | 30               | 1-year dog                    | 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 |
|------------------|-----------------------------|------------------|------------------|-------|--------|-----|
| Malathion        | --                          | 1,486            | --               | 90-day neurotoxicity are | Source: DAR | AChE inhibitor |
|                  |                             |                  |                  | Administration via diet | Highest tested dose | |
| Mepiquat         | --                          | 517              | --               | 90-day neurotoxicity rat | Source: DAR | Activation of nicotinic and muscarinic acetylcholine receptors |
|                  |                             |                  |                  | Administration via diet | Highest tested dose | |
| Metaldehyde      | Salivation                  | 30               | 90               | 1-year dog | Source: DAR | Presumed GABA inhibitor |
| Methamidophos    | Urination                   | 10               | 50               | 28-day rat | Source: DAR 2007 | Unknown |
| (IC)             | AChE inhibition (brain, erythrocytes) | 0.1            | 0.22             | 2-year rat | Source: DAR 2000 | AChE inhibitor |
|                  |                             |                  |                  | Administration via diet | EC review report on methamidophos (2006) considered | |
|                  |                             |                  |                  | 90-day neurotoxicity rat | 90-day neurotoxicity rat | |
|                  |                             |                  |                  | with NOAEL at 0.067 mg/kg bw disregarded | with NOAEL at 0.067 mg/kg bw disregarded | |
|                  |                             |                  |                  | for reason of compatibility with the ADI. | for reason of compatibility with the ADI. | |
| Methidathion     | AChE inhibition (brain, erythrocytes) | 0.16           | 0.8              | 2-year rat | Source: JMPR 1992 | AChE inhibitor |
|                  |                             |                  |                  | Administration via diet | Neurotoxicity studies not available | |
|                  |                             |                  |                  | | NOAEL for AChE inhibition used as surrogate | |
| Methiocarb       | AChE inhibition (erythrocytes) | 1.32            | 6.46             | 90-day dog | Source: DAR 2004 | AChE inhibition |
|                  |                             |                  |                  | Administration via diet | Neurotoxicity studies not available | |
|                  |                             |                  |                  | | NOAEL for AChE inhibition used as surrogate | |
| Methomyl         | --                          | 95               | --               | 90-day neurotoxicity rat | Source: DAR | AChE inhibition |
|                  |                             |                  |                  | Administration via diet | Highest tested dose | |
| Active substance       | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                      | Remark                                                                 | MoA                                      |
|------------------------|-----------------------------|------------------|------------------|-------------------------------------------|----------------------------------------------------------------------|------------------------------------------|
| Milbemectin            |                             | 59               | –                | 90-day neurotoxicity rat                  | Source: RAR 2017                                                     | Glutamate-gated chloride (GluCl) allosteric modulator | |
|                        |                             |                  |                  | Administration via diet Highest tested dose |                                                                      |                                          |
| Molinate               | Salivation                  | 1                | 10               | 1-year dog                                | Source: DAR                                                         | Presumed Inhibition of ALDH by molinate sulfone | |
| Monocrotophos          | Salivation                  | 0.4              | 2.5              | 2-year dog                                | Source: JMPR 1972                                                   | AChE inhibition                          |
|                        |                             |                  |                  | Administration via diet                   |                                                                      |                                          |
| Omothoate (metabolite of dimethoate) | AChE inhibition (erythrocytes) | 0.027          | 0.04             | 2-year rat; supplementary 32-week rat     | Source: DAR 2004 (dimethoate) Chronic neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor                          |
|                        |                             |                  |                  | Administration via diet                   |                                                                      |                                          |
| Oxamyl                 |                             | 15.3             | –                | 90-day neurotoxicity rat                  | Source: DAR 2003                                                     | AChE inhibitor                          |
|                        |                             |                  |                  | Administration via diet Highest tested dose |                                                                      |                                          |
| Oxydemeton-methyl      | AChE inhibition (brain, erythrocytes) | 0.027          | 0.224            | 2-year rat                                | Source: DAR 2004 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor                          |
|                        |                             |                  |                  | Administration via diet                   |                                                                      |                                          |
| Parathion              | AChE inhibition (brain)     | 0.25             | 2.5              | 2-year rat                                | Source: JMPR 1995 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition                          |
| Active substance      | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark                                                                 | MoA                          |
|----------------------|-----------------------------|------------------|------------------|-------|------------------------------------------------------------------------|------------------------------|
| Parathion-methyl     | AChE inhibition (erythrocytes) | 0.25             | 2.5              | 2-year rat Administration via diet                                     | Source: DAR 2001 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition              |
| Permethrin           | piloerection                 | 100              | 250              | 28-day neurotoxicity rat Administration via diet                        | Source: JMPR 1999 Neurotoxicity studies not available ADI: 0.05 mg/kg bw per day (JMPR 1999) | Binding to VGSC              |
| Phenthoate           | AChE inhibition (erythrocytes) | 0.29             | 0.87             | 2-year dog Administration via diet                                     | Source: JMPR 1980 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor               |
| Phosalone            | --                           | 45.9             | --               | 90-day neurotoxicity rat Administration via diet Highest tested dose    | Source: DAR 2004 AChE inhibitor                                    |                             |
| Phosmet              | --                           | 9.4              | --               | 90-day neurotoxicity rat Administration via diet Highest tested dose    | Source: DAR 2004 AChE inhibitor                                    |                             |
| Phoxim               | AChE inhibition (erythrocytes) | 0.1              | 0.38             | 2-year dog Administration via diet                                     | Source: JECFA 1999 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibition              |
| Pirimicarb           | Urination (1) salivation (2) | 10               | 25(1)/35(2)     | 90-day dog Administration via diet (1), 1-year dog (2)                | Source: DAR AChE inhibitor                                        |                             |
| Pirimifos-methyl     | --                           | 21.1             | --               | 90-day neurotoxicity rat Administration via diet Highest tested dose    | Source: DAR AChE inhibition                                        |                             |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|------------------------------|------------------|------------------|-------|--------|-----|
| Profenofos       |                              | 36               |                  | 90-day neurotoxicity rat via diet | Highest tested dose | Source: JMPR 2007 | AChE inhibitor |
| Pyrazophos       | AChE inhibition (erythrocytes) | 0.05             | 0.125            | 2-year dog via diet | | Source: JMPR 1992 | AChE inhibition |
| Pyrethrins       |                              | 4                |                  | Chronic neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | | Binding to VGSC |
| Sufloraflor      |                              | 94.9             |                  | 90-day neurotoxicity rat via diet | | Source: DAR 2012 | Nicotinergic AChR partial agonist |
| tau-Flvalinate   | Salivation, lacrimation      | 0.5              | 1                | 2-year rat via gavage | | Source: DAR | Binding to VGSC |
| Tefluthrin       |                              | 26.6             |                  | 90-day neurotoxicity rat via gavage | | Source: DAR | Binding to VGSC |
| Tetramethrin     |                              |                  |                  |                     | Neurotoxicity studies not available No reference values available | | Binding to VGSC |
| Thiacloprid      |                              | 101              |                  | 90-day neurotoxicity rat via diet | Highest tested dose | Source: RAR 2017 | Agonist of nAChR |
| Active substance                  | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                                    | Remark                                      | MoA                              |
|----------------------------------|------------------------------|------------------|------------------|------------------------------------------|---------------------------------------------|-----------------------------------|
| Thiametoxam                     |                              | 95.4             | –                | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR 2002                        | Agonist of nAChR                  |
| Thiodicarb                      |                              | 46               | –                | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR 2003                        | AChE inhibition                   |
| Tolclofos-methyl                |                              | 735.7            | –                | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: RAR 2016                        | AChE inhibitor                   |
| Triadimenol                     | Piloerection                 | 40               | 209              | 90-day rat Administration via diet Highest tested dose | Source DAR                                | Presumed Inhibition of dopamine transporter |
| Tri-allate                      | Lacrimation                  | 33               | 129              | 3-month neurotoxicity rat Administration via diet | Source: DAR                              | Unknown                           |
| Triazophos                      | AChE inhibition (erythrocytes) | 0.012           | 0.13             | 1-year dog Administration via diet | Source: JMPR 2002 Neurotoxicity studies not available NOAEL for AChE inhibition used as surrogate | AChE inhibitor                   |
| Trichlorfon                     |                              | 168              | –                | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR addendum, 2005                | AChE inhibitor                   |
| Zeta-cypermethrin               |                              | 47               | –                | 90-day neurotoxicity rat Administration via diet Highest tested dose | Source: DAR                              | Known Binding to VGSC          |

CAG: cumulative assessment group; AS: active substance; NOEL: no observed effect level; LOEL: lowest observed effect level; NOAEL: no observed adverse effect level; LOAEL: lowest observed adverse effect level; bw: body weight; GABA: gamma-aminobutyric acid; AChE: acetylcholinesterase; VGSC: voltage-gated sodium channel; nAChR: nicotinic acetylcholine receptor; MoA: mode of action.
## Table B.8: CAG on brain and/or erythrocyte acetylcholinesterase inhibition: toxicological characterisation of ASs to be considered in chronic exposure/risk assessments

| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA             |
|------------------|------------------------------|------------------|------------------|-------|--------|-----------------|
| Acephate         | AChE inhibition (brain, erythrocytes) | 0.25             | 2.5              | 2-year rat Administration via diet | Source: JMPR 2002 | AChE inhibitor   |
| Aldicarb         | AChE inhibition (erythrocyte) | 0.05             | 0.1              | Acute neurotoxicity rat Administration via gavage | Source: DAR 1996 | AChE inhibition  |
| Azinphos-ethyl   | AChE inhibition (erythrocyte) | 0.0125           | 0.25             | 90-day dog Administration via diet | Source: JMPR 1973 | AChE inhibitor   |
| Azinphos-methyl  | AChE inhibition (erythrocyte) | 0.16             | 0.74             | 1-year dog Administration via diet | Source: DAR 1996, Addendum 6, 2000 (neurotox) | AChE inhibitor   |
| Benfuracarb      | AChE inhibition (erythrocytes) | 1.81             | 9.4              | 28-day neurotoxicity rat Administration via diet | Source: DAR 2004 EFSA (2009) | AChE inhibition  |
| Cadusafos        | AChE inhibition (erythrocytes) | 0.045            | 0.22             | 2-year rat Administration via diet | Source: DAR 2004 EFSA (2009) | AChE inhibitor   |
| Carbaryl         | AChE inhibition (brain, erythrocytes) | 1                | 10               | 90-day neurotoxicity rat Administration via gavage | Source: DAR 2004 EFSA (2006) | AChE inhibition  |
| Carbofuran       | AChE inhibition (brain) | 0.015            | 0.03             | Acute neurotoxicity rat Administration via gavage | Source: revised DAR 2008 | AChE inhibition  |
| Carbosulfan      | AChE inhibition (brain, erythrocytes) | 0.5              | 5                | Acute neurotoxicity rat Administration via gavage | Source: Revised DAR 2009 EFSA conclusions on carbosulfan (2009) considered. | AChE inhibition  |
| Chlorfenvinphos  | AChE inhibition (brain, erythrocytes) | 0.15             | 15               | 2-year rat (author not reported, JMPR 1994) Administration via diet | Source: JMPR 1994 | AChE inhibitor   |
| Chlorpyrifos     | AChE inhibition (erythrocytes) | 0.1              | 1                | 2-year rat Administration via diet | Source: Addenda to the original Assessment Report (2013) EFSA conclusions on chlorpyrifos (2014) considered | AChE inhibition  |
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | Source | MoA |
|------------------|-----------------------------|------------------|------------------|-------|--------|--------|-----|
| Chlorpyrifos-methyl | AChE inhibition (brain, erythrocytes) | 1 | 50 | 2-year rat Administration via diet | | Source: DAR 1997 EC review report on chlorpyrifos-methyl (2005) considered | AChE inhibitor |
| Diazinon | AChE inhibition (brain, erythrocytes) | 0.02 | 5.6 | 90-day dog Administration via diet | | Not approved EFSA conclusions on diazinon (2006) considered | AChE inhibitor |
| Dichlorvos | AChE inhibition (erythrocytes) | 0.008 | 0.08 | 2-year dog Administration via diet | | Source: DAR 2003 EFSA conclusions on dichlorvos (2006) considered | AChE inhibitor |
| Dimethoate | AChE inhibition (brain, erythrocytes) | 0.1 | 0.2 | 2-year rat Administration via diet | | Source: DAR 2004 EFSA conclusions on dimethoate (2006, 2013) considered ADI 0.001 (EFSA 2013) Overall NOAEL, combining reproduction, neurotoxicity and developmental neurotoxicity studies | AChE inhibitor |
| Ethephon | AChE inhibition (erythrocytes) | 6 | 14 | 28-day cholinesterase inhibition study in dogs Administration via diet | | Source: DAR 2004 EFSA conclusions on ethephon (2008) considered | AChE inhibition |
| Ethion | AChE inhibition (brain) | 0.06 | 0.71 | 90-day dog Administration via diet | | Source: JMPR 1990 | AChE inhibition |
| Ethoprophos | AChE inhibition (brain) | 0.04 | 2.4 | 2-year rat Administration via diet | | Source: DAR 2004 EFSA conclusions on ethoprophos (2006) considered | AChE inhibition |
| Fenamiphos | AChE inhibition (brain, erythrocytes) | 0.083 | 0.35 | 1-year dog Administration via diet | | Source: DAR 2003 EFSA conclusion on fenamiphos (2006) considered | AChE inhibition |
| Fenitrothion | AChE inhibition (brain, erythrocytes) | 0.5 | 1.5 | 2-year rat Administration via diet | | Source: DAR 2003 EFSA conclusions on fenitrothion (2006) considered | AChE inhibition |
| Fenthion | AChE inhibition (erythrocytes) | 0.05 | 0.23 | 1-year dog Administration via diet | | Source: DAR 1996 | AChE inhibitor |
| Active substance       | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark                                                                 | MoA |
|-----------------------|------------------------------|------------------|------------------|-------|----------------------------------------------------------------------|-----|
| Fonofos               | AChE inhibition (erythrocytes) | 0.2              | 1                | 1-year dog Administration via capsule | Source: EPA 1999 US evaluation not completed because voluntary withdrawal in 1999 | AChE inhibition |
| Formetanate           | AChE inhibition (erythrocytes) | 0.37             | 1.75             | 1-year dog Administration via diet | Source: DAR 2004 EFSA conclusions on formetanate (2006) considered | AChE inhibition |
| Fosthiazate           | AChE inhibition              | 0.42             | 2.36             | 2-year rat Administration via diet | Source: DAR EC review report on fosthiazate (2003) considered. | AChE inhibitor |
| Malathion             | AChE inhibition (erythrocyte) | 17               | 35               | 2-year rat and 2-year rat combined Administration via diet | Source: DAR | AChE inhibitor |
| Methamidophos         | AChE inhibition (brain, erythrocytes) | 0.1              | 0.22             | 2-year rat Administration via diet | Source: DAR 2000 EC review report on methamidophos (2006) considered | AChE inhibitor |
| Methidathion          | AChE inhibition (brain, erythrocytes) | 0.16             | 0.8              | 2-year rat Administration via diet | Source: JMPR 1992 | AChE inhibitor |
| Methiocarb            | AChE inhibition (erythrocytes) | 1.32             | 6.46             | 90-day dog Administration via diet | Source: DAR 2004 EFSA conclusions on methiocarb (2006, 2018) considered | AChE inhibition |
| Methomyl              | AChE inhibition (brain, erythrocytes) | 0.25             | 0.5              | Acute neurotoxicity rat Administration via gavage | Source: DAR EFSA conclusions on methomyl (2006 and 2009) considered | AChE inhibition |
| Monocrotophos         | AChE inhibition (brain, erythrocytes) | 0.005            | 0.05             | 2-year rat Administration via diet | Source: JMPR 1991 | AChE inhibitor |
| Omethoate (IC)        | AChE inhibition (erythrocytes) | 0.027            | 0.04             | 2-year rat supplementary 32-week rat Administration via diet | Source: DAR 2004 (dimethoate) EFSA conclusions on dimethoate (2013, 2018) considered. | AChE inhibitor |
| Oxamyl (IC)           | AChE inhibition (brain, erythrocytes) | 0.1              | 0.75             | Acute neurotoxicity rat Administration via gavage | Source: DAR 2003 EFSA conclusions on oxamyl (2005) considered | AChE inhibition |
| Active substance          | Indicator of specific effect                  | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study                  | Remark                                                                 | MoA          |
|--------------------------|-----------------------------------------------|------------------|------------------|------------------------|------------------------------------------------------------------------|--------------|
| Oxydemeton-methyl        | AChE inhibition (brain, erythrocytes)         | 0.027            | 0.224            | 2-year rat Administration via diet | Source: DAR 2004 AChE inhibitor                                      | AChE inhibitor |
| Parathion                | AChE inhibition (brain)                       | 0.25             | 2.5              | 2-year rat Administration via diet | Source: JMPR 1995 AChE inhibition                                     | AChE inhibition |
| Parathion-methyl         | AChE inhibition (erythrocytes)                | 0.25             | 2.5              | 2-year rat Administration via diet | Source: DAR 2001 AChE inhibition                                     | AChE inhibition |
| Phenthoate               | AChE inhibition (erythrocytes)                | 0.29             | 0.87             | 2-year dog Administration via diet | Source: JMPR 1980 AChE inhibitor                                     | AChE inhibitor |
| Phosalone                | AChE inhibition (erythrocytes)                | 0.17             | 0.9              | 1-year dog Administration via diet | Source: DAR 2004 AChE inhibition                                      | AChE inhibition |
| Phosmet                  | AChE inhibition (brain)                       | 1.1              | 1.8              | 2-year rat Administration via diet | Source: DAR 2004 EFSA conclusions on phosmet (2006) considered AChE inhibition | AChE inhibition |
| Phoxim                   | AChE inhibition (erythrocytes)                | 0.1              | 0.38             | 2-year dog Administration via diet | Source: JECFA 1999 AChE inhibitor                                    | AChE inhibition |
| Pirimicarb               | AChE inhibition (brain, erythrocytes)         | 10               | 25               | 1-year dog Administration via capsule | Source: DAR 2003 AChE inhibition                                     | AChE inhibition |
| Pirimiphos-methyl        | AChE inhibition (brain)                       | 0.4              | 2.1              | 2-year rat Administration via diet | Source: DAR 2003 EFSA conclusions on pirimiphos-methyl (2005) considered. | AChE inhibition |
| Profenofos               | AChE inhibition (erythrocytes)                | 0.017            | 0.56             | 2-year rat Administration via diet | Source: JMPR 2007 AChE inhibitor                                     | AChE inhibitor |
| Pyrazophos               | AChE inhibition (erythrocytes)                | 0.05             | 0.125            | 2-year dog Administration via diet | Source: JMPR 1992 AChE inhibition                                     | AChE inhibition |
| Thiodicarb               | AChE inhibition (brain, erythrocytes)         | 0.5              | 5                | Acute neurotoxicity rat Administration via gavage | Source: DAR 2003 EFSA conclusions on thiodicarb (2005) considered. NOAEL derived from the LOAEL with an UF of 10. | AChE inhibition |
| Toluclfos-methyl         | AChE inhibition (brain, erythrocytes)         | 6.9              | 34               | 2-year mouse Administration via diet | Source: DAR 2003 EFSA conclusions on tolclofos-methyl (2017) considered | AChE inhibition |
| Triazophos               | AChE inhibition (erythrocytes)                | 0.012            | 0.13             | 1-year dog Administration via diet | Source: JMPR 2002 AChE inhibitor                                     | AChE inhibitor |
### Table B.9: CAG on functional effects on neuropathological end-points: toxicological characterisation of ASs to be considered in chronic exposure/risk assessments

| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|------------------------------|------------------|------------------|-------|--------|-----|
| Trichlorfon      | AChE inhibition (brain)      | 4.5              | 13.3             | 2-year rat Administration via diet | Source: DAR 2004 | AChE inhibitor |
| Chlorfenapyr     | Myelin degeneration          | 14.8             | 27.6             | 90-day mouse | Source: JMPR 1992 | Unknown |
| Cymoxanil        | Axonal degeneration, myelin degeneration | 5             | 38               | 2-year rat | Source: DAR | Unknown |
| Cypermethrin     | Axonal degeneration (degeneration of trigeminus and increased galactosidase activity) | 25             | 50               | 7-day rat | Source: DAR | Unknown |
| Emamectin benzoate (IC) | Axonal degeneration, myelin degeneration | 0.25             | 0.5              | 90-day dog; 1-year dog | Source: DAR 2008 | Unknown |
| Fenpropidin      | Myelin degeneration          | 5                | 20               | 1-year dog | Source: DAR | Unknown |
| Flufenacet       | Axonal degeneration          | 1.14             | 27               | 1-year dog | Source: DAR 1998 | Unknown |
| Indoxacarb       | Axonal degeneration, neuronal degeneration/ necrosis | 4                | 20               | 18-month mouse | Source: DAR | Unknown |
| Isoxaflutole     | Axonal degeneration, myelin degeneration | 20              | 500              | 2-year rat | Source: DAR 1997 | Unknown |
| Lindane          | Myelin degeneration          | 0.5              | 5                | 3-day rat | Source: DAR Not true NOAEL, but LOAEL divided by 10 | Unknown |
| Mancozeb         | Myelin degeneration (myelin damage and Schwann cell proliferation) | 8.2              | 49               | 3-month rat | Source: DAR 2000 | Unknown |

CAG: cumulative assessment group; AS: active substance; NOEL: no observed effect level; LOEL: lowest observed effect level; NOAEL: no observed adverse effect level; LOAEL: lowest observed adverse effect level; bw: body weight; AChE: acetylcholinesterase; MoA: mode of action.

Table B.9: CAG on functional effects on neuropathological end-points: toxicological characterisation of ASs to be considered in chronic exposure/risk assessments.
| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-----------------------------|-----------------|-----------------|-------|--------|-----|
| Molinate         | Axonal degeneration, myelin degeneration | 1.8 | 13 | 2-year rat | Source: DAR | Presumed Sulfoxide metabolites can react with sulfhydryl groups of amino acids and proteins |
| Oxasulfuron      | Axonal degeneration, myelin degeneration (secondary to axonal degeneration) | 1.5 | 99 | 18-month mouse | Source: DAR | Unknown |
| Quinoclamine     | Myelin degeneration         | 3.82 | 40.2 | 18-month mouse | Source: DAR | Unknown |
| tau-Fluvalinate  | Axonal degeneration, myelin degeneration | 1 | 10 | 7-day neurotoxicity rat | Source: DAR | Unknown |
| Tembotrione      | Neuronal degeneration/ necrosis | 26.7 | 111 | 90-day dog | Source: DAR 2012 | Unknown |
| Thiram           | Sciatic nerve lesions (not specified) | 1.4 | 14 | 2-year rat | Source: DAR 1997 + Addenda | Presumed Cross-linking of axonal proteins via reaction of the metabolite CS2 with axonal proteins |
| Tri-allate       | Axonal degeneration, myelin degeneration | 6.4 | 32 | 3-month neurotoxicity rat | Source: DAR | Unknown |
| Trichlorfon      | Myelin degeneration | 31.2 | 168 | 90-day neurotoxicity rat | Source: DAR addendum, 2005 | Presumed Inhibition of neuropathy target esterase (NTE) and increased intracellular calcium |
### CAGs of pesticides for effects on nervous system

| Active substance | Indicator of specific effect | NO(A)EL mg/kg bw | LO(A)EL mg/kg bw | Study | Remark | MoA |
|------------------|-------------------------------|------------------|------------------|-------|--------|-----|
| Ziram            | Axonal degeneration           | 9                | 27               | 2-year rat | Source: DAR 1998 | Presumed Cross-linking of axonal proteins via reaction of the metabolite CS2 with axonal proteins |

CAG: cumulative assessment group; AS: active substance; NOEL: no observed effect level; LOEL: lowest observed effect level; NOAEL: no observed adverse effect level; LOAEL: lowest observed adverse effect level; bw: body weight; MoA: mode of action.
Appendix C – Observed indicators of functional alterations of the motor, sensory and autonomic divisions of the nervous system in regulatory studies conducted with ASs belonging to chemical classes with a known neurotoxic MoA

| Chemical classes/Active substances | Motor division | Sensory division | Autonomic division |
|------------------------------------|----------------|-----------------|-------------------|
| N-methylcarbamate insecticides     | ↑ motor activity, tremor, hyperactivity, ↓ motor activity, ataxia, muscle fasciculation, hunched posture, Landing-foot splay, convulsions, muscle weakness, ptosis | ↓ reactivity (tail pinch response, analgesic reflex (nociception response), righting reflex (air drop)), abnormal response to visual placing test, auditory startle response, ↓ Pupil response, hyperreactivity | Salivation, lacrimation, miosis, urination |
| Macro cyclic lactone insecticides  | ataxia, tremor, ↑ motor activity, ↓ motor activity | ↓ splay reflex, ↓ pupil reactivity, hyperreactivity | Salivation, mydriasis |
| Neonicotinoids insecticides        | Tremor, ↓ motor activity, hunched posture, ataxia, convulsions, ↑ grip strength, hypoactivity | ↑ reactivity, ↓ arousal, ↓ righting reflex | Urination, mydriasis, salivation |
| Organophosphorous insecticides     | ↓ motor activity, ↓ grip strength, hypoactivity, abnormal gait, ataxia, convulsions, tremor, ↑ motor activity, Hyperactivity, ↑ muscle strength, ↓ muscle strength, muscle weakness, hunched posture | ↓ reactivity (righting reflex (air drop), tail pinch), ↓ Pupil response, ↓ analgesic reflex, hyperreactivity | Salivation, lacrimation, Miosis, urination, piloerection |
| Organochlorine insecticides        | ↑ motor activity, Convulsions, tremor, ↓ motor activity, abnormal gait | ↑ reactivity, hyperreactivity | Salivation |
| Phenylpyrazole insecticides        | Landing-foot splay, convulsions | Approach response, ↓ tail pinch response, air righting reflex | Miosis |
| Pyrethrins and pyrethroid ester insecticides | ↑ motor activity, ↓ motor activity, abnormal gait, ↓ grip strength, ataxia, tremor, convulsions, choreoatethosis, Landing-foot splay, hunched posture, paresis limbs | ↓ reactivity, ↓ touch responses, ↓ tail pinch response and impaired righting, ↑ sensitivity to noise, ↓ startle response, ↑ startle response, righting reflex | Salivation, urination, mydriasis, piloerection |
| Amitraz                            | ↑ motor activity, convulsions | Hyperreactivity | |
| Chlormequat                        | ataxia | ↓ reflex response | Salivation |
| Indoxacarb                         | ataxia, hunched posture, Landing-foot splay, ↓ grip strength, ↓ motor activity | Hyperreactivity | Urination |
| Mepiquat                           | ↓ motor activity, lateral posture, convulsions | ↓ pupillary reflex | Salivation |
| Sulfoxafilor                        | ↓ motor activity | ↓ reactivity: touch response (handling reactivity) | Lacrimation |
Appendix D – Effects on the motor division Uncertainty question 1 – evidence collection and grouping of ASs P

Appendix D can be found in the online version of this output (‘Supporting information’ section): https://doi.org/10.2903/j.efsa.2019.5800