SCIENTIFIC REPORT

EFSA Journal

ADOPTED: DD Month YYYY

doi:10.2903/j.efsa.20YY.NNNN

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

European Food Safety Authority (EFSA)

Abstract

Under construction

Keywords: cumulative risk assessment, cumulative assessment groups, pesticides mixtures, nervous system, motor division, acetylcholinesterase inhibition

Requestor: EFSA

Question number: EFSA-Q-2017-00435

Correspondence: xxx@efsa.europa.eu

Acknowledgements: The [Panel or Scientific Committee or EFSA] wishes to thank the following for the support provided to this scientific output: [staff members or others who made a contribution but are not eligible as authors]. The Panel [Panel/Scientific Committee/EFSA] wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Suggested citation: [EFSA ACRONYM Panel (EFSA Panel name)] [or EFSA (European Food Safety Authority)] [or EFSA Scientific Committee], [add individual author names in the same order as it is on the first page, followed by a comma, in the format: Surname Initial(s), Surname Initial(s) and Surname Initial(s)], 20YY. [Full title, including output category]. EFSA Journal 20YY;volume(issue):NNNN, 89 pp. doi:10.2903/j.efsa.20YY.NNNN

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Cumulative assessment groups (CAG) for the effects of pesticides on the nervous system were already established in 2013 (EFSA, 2013a). These CAGs have been updated on the basis of additional information collected from more recent data collections. Five specific effects of pesticides on the nervous system have been confirmed: functional alterations of the motor, sensory and autonomic divisions, histological neuropathological effects on neurons as well as brain and/or erythrocyte acetylcholinesterase inhibition.

CAGs have been established for each of these effects. NOAELs following acute and chronic exposure have been defined to characterise the active substances (AS) included in each CAG for the respective specific effect. Index compounds (IC) have been selected and relative potency factors (RPF) were calculated to enable cumulative exposure and risk assessments.

For an efficient use of resources, it is recommended to focus the assessment of the combined risks of pesticides residues for the nervous system to their specific effects on the motor division and on brain and/or erythrocyte acetylcholinesterase 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 analysed in consistency with the anticipated assessment question which will govern cumulative risk assessments (CRA) conducted with these CAGs.

A mechanism of periodic update of the CAGs established in the present report will be put in place by EFSA in order to make use of all relevant information generated by pesticides manufacturers and not considered yet.

Table of contents

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.

EFSA and the PPR Panel have started in 2007 the development of the necessary methodologies to carry out cumulative risk assessment for MRL setting. This methodological development included a procedure to establish cumulative assessment groups (CAGs) of pesticides on the basis of their toxicological profile (EFSA, 2013a).

1.1. Background and Terms of Reference as provided by the requestor

In 2014, EFSA started a programme of activities aiming 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 has been requested by EFSA (EFSA, 2014a) to prepare a scientific report on CAGs of pesticides for their effects on the nervous system.

1.2. Interpretation of the Terms of Reference

The EFSA implementation plan of CRA also requests EFSA to carry out CRAs for the effects of pesticides on the nervous system. These assessments will be reported in a separate EFSA scientific report which will deal in particular with the following assessment questions:

What is the risk of neurochemical effects (i.e. brain and erythrocyte cholinesterase inhibition) resulting from combined dietary exposure to pesticide residues?

What is the 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?

In order to provide an appropriate basis to the CRA, this report will not be restricted to the elaboration of CAGs of pesticides for their effects on the nervous system, but will also include the selection of Index Compounds (ICs) and the establishment of Relative Potency Factors (RPFs).

Furthermore, 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 of consumers, as formulated in the above assessment questions. To prepare for this, for each of the CAGs established, this report will address the following question:

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

In the CRAs which will follow, it will be necessary to consider how sure it is that these ASs combine their individual toxicities according to the dose-addition model at their actual level in food. To prepare for this, the current report aso seeks to identify the toxicological mode of action in mammals of each AS within the CAG.

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 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 (ICPS) and the French Agency for Food, Environmental and Occupational Health & 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 at al., 2012), and all ASs approved between 1st June 2009 and 31 December 2011. 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), Draft Re-Assessment Reports (DRARs), as well as the respective Addenda, Experts' meeting reports, EFSA conclusions and 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, JMPR evaluation reports or open literature (e.g. PubMed) were also searched for additional information on mode/mechanisms of action (MoA). All available acute and repeated dose in vivo toxicological studies in mammals were considered. *In vitro* studies were used when they provided information on known or presumed neurotoxic MoAs. In this data collection, for each endpoint related to neurotoxicity, only the lowest NOAEL and LOAEL observed in the most sensitive species were collected. All details of the data collection can be found in the respective external scientific report (RIVM, ICPS, ANSES, 2013).

The second data collection was outsourced to the same consortium (RIVM, ICPS, ANSES, 2016). It covered all ASs approved after 1st January 2012 and until 31 May 2013, a number of new ASs not approved yet, but pending for approval at that time and an additional list of non-approved ASs present in 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, 2013b). The sources of this data collection were official documents produced during the approval of ASs under Regulation (EC) No 1107/2009: Draft Assessment Reports (DARs), Draft Re-Assessment Reports (DRARs), as well as the respective Addenda, evaluation and discussion tables, EFSA conclusions and Commission Review reports. If necessary, original study reports were consulted for more details. When European evaluation was not available or outdated, assessment reports from recognized international bodies (JMPR, EHC, US-EPA, ATSDR, PMRA…) were scrutinized. 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, this one was organised in consistency with the specific effects identified for the nervous system by the PPR Panel (EFSA, 2013a) and their respective indicators. In particular:

- All information regarding specific neurotoxic effects, from all animal species reported in the regulatory documents were collected (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 that were overlapping in two or more studies of the same duration in the same species for one specific effect were not combined;
- The lowest NOAEL/LOAEL for a specific effect observed in the most sensitive sex in the study has been reported;
- Considering that several experiments were carried out in different animal species, the lowest NOAEL and LOAEL were selected for each specific indicator based on combined data from two or more studies; therefore some NOAELs and LOAELs derive from different studies or reports;
- When several indicators have been observed in one study for one specific effect, the most sensitive indicator(s) has been indicated in the column "Indicator", and the others have been reported in the column "Remarks about the effect";

- Some NOAEL for acute effects were not derived from acute neurotoxicity studies, but were based on 14-, 28- or 90-day studies with observations being performed on the first day of dosing.

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

Human studies were included in these data collections, but not used for the establishment of CAG, as Commission Regulation (EU) No 283/2013 authorises their use only in case they are scientifically valid, ethically generated and lead to lower regulatory limit values compared to animal studies.

All the details of the data collections can be found in the respective external scientific report (RIVM, ICPS, ANSES, 2013 and 2016) and the 3 data collection spreadsheets in appendix A. The content of these tables slightly evolved over time on the basis of 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 annex A.

2.2. Methodologies

The establishment of CAGs follows 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 IC, the calculation of RPFs, an analysis of uncertainties about the adequacy of the CAG with respect to the specific effect, and finally the identification of the mode of action of each AS.

2.2.1. Identification of the specific effects

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

- Excluding local effects: Local effects, not being produced by the potentially absorbed dose, are excluded. Furthermore, they do not form the basis of reference values in regulatory dietary risk assessment.
- Excluding non-adverse effects: Non-adverse effects such as adaptive responses are not used as basis for setting a toxicological reference value and are therefore also not considered as relevant for cumulative risk assessment.
- Excluding effects not relevant to humans: Effects not considered as relevant for human are not relevant for cumulative risk assessment.
- Evaluating the unambiguous nature of the effect: A specific effect needs to be unambiguous and well-defined in terms of site and nature.
- Identifying and excluding non-specific effects and secondary effects: this is the case of effects caused by ageing or other physiological processes, or occurring as consequences of other primary adverse effects are considered as non-specific or secondary effects, e.g. effects due to general toxicity.

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

2.2.2. Characterisation of the specific effects

This step establishes the hazard characterization principles applicable to the identified specific effects. In practice, this means defining the descriptors/indicators (endpoints) observed in toxicological studies building evidence that an AS causes the specific effect and deciding how NOAELs are derived with respect to this effect.

2.2.3. Establishment of CAGs, selection of ICs and calculation of RPFs

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

The population of each CAG by the appropriate ASs is based on a critical analysis of the information collected as described in section 2.1. The criteria used to perform this critical analysis are described with sufficient details to enable an independent assessor to repeat it.

Once CAGs are populated, one of the ASs is selected as the IC and RPFs are calculated for the remaining ASs within that CAG. The approach used to select the IC and to calculate the RPFs is defined on ad-hoc basis for each specific effect. This approach should be such that it ensures an equal treatment of all ASs. If this is not the case, this needs to be clearly highlighted.

2.2.4. Analysis of uncertainties

The CAGs established in this report will be 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 2 questions have to be addressed. Question 1 will be addressed in the present report, while question 2 will be addressed in the forthcoming report dealing with the risk characterisation:

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 their actual level in food? Where possible, clusters of active substances for which dose-addition is virtually certain should be defined.

The rationale of using dose addition to perform cumulative risk assessment of pesticide residues is given in the Scientific Opinions of the PPR Panel on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile (EFSA PPR Panel, 2013a) and on the relevance of dissimilar mode of action and its appropriate application for cumulative risk assessment of pesticides residues in food (EFSA PPR Panel, 2013c).

Dose-addition is expected in principle when chemicals in a mixture act by the same MoA, and differ only in their potencies. However, in many cases, the CAGs elaborated following the methodology used in this report include ASs acting by unknown or different MoAs. As empirical information about the exact form of combined toxicity in such cases is missing, dose-addition remains an assumption. A public consultation conducted by EFSA indicated that this assumption was generally considered as leading to a possible overestimation of the actual risks because effects of compounds with different mode of action are summed (EFSA, 2014b).

For two selected CAGs (effects on motor division and neurochemical endpoints), Question 1 is addressed using a combination of weight of evidence and expert knowledge elicitation techniques,

described in the following section, while Question 2 is will be addressed later by expert judgement, as part of assessing overall uncertainty in the CRAs which will follow.

2.2.5. Weight of evidence and expert knowledge elicitation technique

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

- Defining in precise terms the specific effect that is to be assessed.
- Identifying lines of evidence that are important for assessing whether the active substance causes the effect: lines of evidence typically include the indicators as defined in section 2.2, but are not necessarily restricted to these indicators. Depending on the specific effect, additional factors contributing to the evidence may be defined.
- Rating the weight of each line of evidence: the lines of evidence are assessed with respect to their reliability and relevance to the assessment question. This assessment is conducted by expert discussion and results in the allocation of a coefficient or weight to each line of evidence, which is a relative measure of the contribution that positive findings for each line of evidence would make to increase the probability of a chemical causing the effect.
- Reviewing each active substance included in the CAG as a result of the process described in section 2.2.4 in order to identify which lines of evidence are positive.
- Integration of the lines of evidence by multiplying all coefficients corresponding to the lines of evidence for each active substance. This gives a score to each active substance which is proportionate to the number and strength of the lines of evidence and reflects the overall weight of evidence on whether the active substance is causing the effect.
- Clustering the active substances in different groups of similar weight of evidence on the basis of their score. This is done by ordering the active substances 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. This was done by expert discussion, balancing the need for a practical number of subgroups against the homogeneity of scores and lines of evidence within each subgroup.
- Assessing how many of the active substances in each subgroup actually cause the specific effect. This was done by a structured expert knowledge elicitation (EKE) procedure, using a modified version of the 'Sheffield' EKE protocol described by EFSA (2014c) to elicit a discrete probability distribution for the number of substances causing the effect. For each subgroup, experts first worked individually, reviewing the evidence and making their own judgements. 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. Finally, results for all the subgroups were displayed together for the experts to review and, where necessary, adjust.
- When developing the consensus distribution for some of the more diverse sub-groups, the experts found it helpful first to divide the sub-group into subsets of substances for which the probability of causing effects on 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 sub-group as a whole.

- The elicited distributions for the subgroups were combined by 1D Monte Carlo simulation (EFSA, 2018) to calculate a probability distribution for the total number of substances that actually cause the specific effect, assuming independence between subgroups.

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) a probability distribution for the total number of substances causing the effect, assuming independence between subgroups. Additional uncertainties, including possible deviations from independence, will be considered subsequently when assessing overall uncertainty.

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 5 specific effects of pesticides on the nervous system (EFSA, 2013a):

- 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. cholinergic modulation)
- Brain and/or erythrocyte acetylcholinesterase inhibition (neurochemical effect)
- Histological neuropathological effects on neurons (e.g. axonal degeneration and demyelination)

The rationale behind the identification of these effects by the PPR Panel is given in details in the Scientific Opinion. It considers 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 the five 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 cumulative risk assessment, because, although of variable nature at biochemical level, they concern very specific and specialised functions of the organism.

Neuropathological effects are unambiguously associated to histopathological observations, which can also be of variable nature, although consisting in most of the cases of axonal and myelin degeneration. As they commonly result in the alteration of the specialized function of nervous cells, they are also considered as specific effects justifying the establishment of a CAG.

Acetylcholinesterase (AChE) inhibition is objectified by direct measurements. Therefore the unambiguousness of the nature of the effect is higher for this neurochemical effect than for the other effects.

Although both organophosphorus and *N*-methylcarbamate insecticides bind to AChE, inhibition by the latter is, in most cases, spontaneously reversible whereas that by organophosphorous insecticides is relatively irreversible and functional recovery following exposure is the result of the synthesis of new enzyme and AChE levels will return to normal values if pesticide exposure is stopped. The inhibition of the enzyme activity results in accumulation of synaptic acetylcholine and consequent enhanced stimulation of postsynaptic cholinergic receptors (either muscarinic or nicotinic) in the central and/or peripheral nervous systems, as well as in the neuromuscular junction.

A given depression in peripheral and central AChE activity may, or may not, be accompanied by clinical signs or symptoms. Even a reduction in AChE activity not followed by clinical manifestations may impair the organism's ability to adapt to further exposures to anticholinesterase agents.

Accordingly, despite the toxic effects of anticholinesterase pesticides are considered as reversible, these effects should not be dismissed as they could be severe or even lethal.

Neurotoxic effects can be transient or persistent, reversible or irreversible, and latent, progressive or residual in nature. Clear irreversible effects on the nervous system are of greater concern than reversible effects and usually involve structural changes or long-lasting functional effects. However, the reversibility of neurotoxic effects must not be ignored for risk assessment but should be considered and interpreted cautiously.

In contrast to other tissues, neurons of the central nervous system show limited ability to repair and/or regenerate the neural damage. Once neuronal death has occurred, the lack of potential of these cells to achieve full recovery can result in a permanent damage. However, regeneration is still possible for neurons in the peripheral nervous system. In addition, neural plasticity can occur in the central nervous system as an adaptive response to changed environment or altered homeostatic conditions. This type of compensation may be suspected when a neurotoxic effect slowly resolves over the life-span.

On the other hand, the large reserve capacity of the nervous system may compensate for neural damage, although the resulting reduction in the reserve capacity should be considered as an adverse effect. As this capacity is limited, further exposures could eventually lead to future structural or functional manifestations of neurotoxicity because adaptive responses are gradually decreased, resulting in latent effects. Hence, clinical manifestations of neurotoxicity may become manifested with aging or following environmental exposures.

There are interdependencies between these specific effects because they may result from similar MoAs. The AChE inhibition is obviously an important mechanism leading to e.g. functional alterations of the motor division of the nervous system. These interdependencies should however not create the perception 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 assessment 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 with independent replies.

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, not statistically significant or unrelated to the treatment. This was the case for vacuoles in brain, changes in relative brain weight and neoplasms (particularly astrocytomas). Similarly, based on the first report of the RIVM/ICPS/ANSES consortium (RIVM/ICPS/ANSES, 2013), a number of effects (such as prostration, opisthotonus, laboured breathing, tachypnoea, dyspnoea, exophthalmos, lethargy, coma, hypothermia, vomiting and alopecia) were considered as effects often occurring secondary to general systemic toxicity after high doses. Accordingly, they are not deemed appropriate effects or observations to support the establishment of CAGs.

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, 2013a), the number of studies of this type available to the panel was not sufficient to propose specific effects and CAGs with respect to this type of toxicity. Therefore DNT is 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 data in the data collection, these tests were not addressed in this report.

3.2. Characterisation of the 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 an alteration of the motor function of the nervous system are classified in 4 categories:

- Reduced motor activity: hypoactivity, recumbency (if not observed in isolation), etc.
- Increased motor activity: tremor, choreo-athetosis, 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 are envisaged. For instance 'hunched position/posture', 'lateral posture' and 'curved body position' are considered as synonyms of 'recumbency'. Reduced and increased motor activity, although looking at first sight as opposite effects, are considered as 2 equally valid manifestations of the specific effect. These 2 types of effects are indeed regularly observed with the same AS.

This specific effect can be triggered by acute and chronic exposures.

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 an alteration of the sensory function of the nervous system are classified in 3 categories:

- Decreased reactivity: hyporeactivity, righting reflex (air drop), touch response (handling reactivity), approach response, pupil response, tail pinch response, analgesis reflex (nociception response), patellar reflex, etc.
- Increased reactivity: hyperreactivity, exaggerated auditory response (startle reflex), etc.
- Proprioception: proprioception deficit, paraesthesia, 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. Paraesthesia may be elicited by dermal exposure of either pyrethrins or pyrethroids, in which case it is considered as a local effect.

This specific effect can be triggered by acute and chronic exposures.

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 an alteration of the autonomic function of the nervous system are miosis, mydriasis, increased salivation, lacrimation, piloerection and urination, etc. The combination of 2 or more autonomic signs of toxicity provides a stronger support for a specific effect on the autonomic division.

This specific effect can be triggered by acute and chronic exposures.

3.2.4. Brain and/or erythrocyte acetylcholinesterase inhibition

This neurochemical effect is directly defined by its indicator. It is however considered relevant only when the inhibition leads to a statistically significant decrease of the acetylcholinesterase activity of 20% or more compared to control groups (Office of Pesticide Programs, 2000; JMPR, 1999).

This specific effect can be triggered by acute and chronic exposures.

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 are axonal degeneration (such as sciatic nerve axonopathy), myelin degeneration and/or 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, a special care needs to be dedicated to interpretation of histopathological findings by paying due consideration of 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.

This specific effect is triggered by chronic exposures only.

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

3.3.1. General provisions

Establishment of CAGs:

On the basis of the three data collections (RIVM, ICPS, ANSES, 2013 and 2016; EFSA internal data collection) referred to in section 2.1 and on the basis of the indicators listed in section 3.2, CAGs are elaborated for the 5 specific effects of pesticides on the nervous system.

An AS is in principle included in a CAG if at least one of the respective indicators is observed in a toxicological study with this AS, unless:

- It is considered, based on expert judgement, that the observation is age-related or secondary to a primary adverse effect;
- It is considered, based on expert judgement, that the observation is not treatment-related, e.g. not statistically significant, not supported by a dose-response relationship or not robust (e.g. only at top dose, inconsistent across studies, species and genders);
- The observation is made in a study which has only been considered supportive due to methodological weaknesses

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

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. Developmental studies were also disregarded as they do not adequately examine toxic effects on the nervous system. Few studies on developmental neurotoxicity were available at the time of the data collection, and for this reason were not considered because not providing a sufficient basis to establish separate CAGs for effects on the developing nervous system.

The above conditions may not apply to ASs possessing a specific feature which per se justifies its inclusion in the CAG (e.g. specific chemical structure, pesticidal mode of action of direct relevance for the specific effect). In such case, no further evidence is needed to include the AS in the CAG. This is particularly valid for the ASs of the following chemical classes: organophosphates, N-methyl carbamates, pyrethroids, mectins, neonicotinoids and phenylpyrazoles. These ASs have chemical structures known to be capable of inducing neurotoxicity and their respective MoAs are given in section 4.2.2.

Setting of NOAELs:

For each of AS included in a CAG, the lowest of all acute and chronic NOAELs related to the relevant indicators are defined as respective acute and chronic NOAELs for the specific effect. All indicators listed in section 3.2 are equally valid for the setting of NOAELs. In case only a LOAEL was available for a certain indicator, a default NOAEL was first determined from this LOAEL by applying an uncertainty factor (UF) of 10 to take into account variability (WHO, 2011).

When included in the CAGs related to the inhibition of acetylcholinesterase or to the functional alteration of the motor, sensory and autonomic divisions, ASs should in theory have an acute and a chronic NOAEL for the respective effect. This is however in practice not always the case because either the available information is not sufficient to derive both reference values, or the potency of the ASs in regard to the specific effect may be so low following acute or chronic exposure that the effect is not observed in the regulatory studies. In the latter case, the highest tested dose could have been used as NOAEL to enable the calculation of RPFs, but this was not done. Instead, this absence of certain NOAELs will be treated as a source of potential underestimation of the cumulative risks in the overall uncertainty analyses.

ASs included in the CAG related to the neuropathological effects are characterized by a chronic NOAEL only.

Selection of Index Compounds:

In view of exposure/risk assessments, an Index Compound (IC) is selected from the ASs included in the CAG. The IC is preferably selected between the 3 most potent ASs of the CAG on the basis of the following criteria:

- Quality of the study (study meeting the requirements of regulation (EC) No 1107/2009, considered acceptable, statistical analysis)
- Strength of the specific effect (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

Additional ASs may be considered in case none of the 3 most potent ones is found suitable.

Two ad-hoc ICs are 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 inhibition of acetylcholinesterase and to the functional alterations of the motor, sensory and autonomic divisions of the nervous system.

Calculation of RPFs:

RPFs were calculated to normalise the toxicity of all ASs in each CAG to the IC, by dividing the NOAEL of the IC by the NOAEL of the AS.

3.3.2. Cumulative assessment groups

This section presents the CAGs proposed to be used for future CRAs. They differ to some extent from those initially elaborated by the PPR Panel and published in the Scientific Opinion of 2013 (EFSA, 2013a), because only 1 (RIVM, ICPS, ANSES, 2013) of the 3 data collections used in this report was available to the Panel when it adopted its opinion. In addition, for the purpose of the present report original study reports were consulted on ad-hoc basis.

Functional alteration of the motor division:

In total, 120 ASs are included in the CAG on the alterations of the motor function of the nervous system. This includes 113 ASs showing relevant indicators of effects on the motor division in the data collections as described in section 3.3.1. It also includes 7 ASs (see sub-group 7 in section 4.2.1) for which no indicator of effects on the motor division was observed, but which were included in the CAG

for acetylcholinesterase inhibition and possessing an organophosphate or N-methyl carbamate structure. This is justified by the fact that these ASs, as they cause acetylcholinesterase inhibition, are intrinsically capable to induce functional alterations of the motor division.

The 120 ASs are: abamectin, acetamiprid, acephate, acrinathrin, aldicarb, alpha-cypermethrin, amitraz, azinphos-ethyl, azinphos-methyl, benfuracarb, beta-cyfluthrin, beta-cypermethrin, bifenthrin, bromide ion, cadusaphos, carbaryl, carbetamide, carbofuran, carbosulfan, chlorfenvinphos, chlormequat, chlorpropham, chlorpyriphos, chlorpyriphos-methyl, clothianidin, cyfluthrin, cypermethrin, 2,4-D, deltamehrin, desmedipham, diazinon, dicamba, dichlorvos, dicofol, dieldrin, dimethoate, dinotefuran, emamectin, endosulfan, endrin, esfenvalerate, ethephon, ethion, ethoprophos, fenamiphos, fenitrothion, fenpropathrin, fenpropidin, fenpropimorph, fenthion, fenvalerate, fipronil, flufenacet, fluquinconazole, fonofos, formetanate, fosthiazate, glufosinate, heptachlor, imidacloprid, indoxacarb, isoxaflutole, lambda-cyhalothrin, lindane, lufenuron, malathion, mancozeb, maneb, mepiquat, metaldehyde, metamidophos, methidathion, methiocarb, methomyl, metiram, metribuzin, milbemectin, monilate, monocrothophos, omethoate, oxamyl, oxasulfuron, oxydemeton-methyl, parathion, parathion-methyl, penflufen, penthoate, permethrin, phosalone, phosmet, phoxim, pirimicarb, piripmiphos-methyl, profenofos, propineb, pymetrozine, pyrazophos, pyrethrins, pyridate, spirotetramat, sulfoxaflor, tau-fluvalinate, tebuconazole, tefluthrin, tembotrione, tetraconazole, tetramethrin, thiacloprid, thiamethoxam, thiodicarb, thiophanate-methyl, thiram, tolclofos-methyl, triadimefon, triadimenol, triallate, triazophos, trichlorfon, zeta-cypermethrin, ziram.

In this CAG, 73 ASs have a NOAEL for acute effects and 75 a NOAEL for chronic effects.

The ICs for the acute and chronic exposure/risk assessments are oxamyl and emamectin benzoate, respectively. Oxamyl was preferred to methiocarb (aka mercaptodimethur) and aldicarb on the basis of the robustness of data and the number of endpoints affected in the acute neurotoxicity study. Emamectin benzoate was preferred to abamectin (aka avermectin), endrin, metamidophos, methidathion and fipronil on the basis of the robustness of data and consistency of effects across studies and animal species (rat, dog and mouse).

For a number of ASs (cadusafos, carbetamide, clhlorfenvinphos, chlormequat, chlorpropham, chlorpyriphos-methyl, desmedipham, dichlorvos, endosulfan, ethion, fenpropidin, fenthion, fluquinconazole, fosthiazate, isoxaflutole, mancozeb, maneb, metiram, monocrotophos, oxasulfuron, oxydemeton-methyl, parathion, parathion-methyl, penthoate, phoxim, propineb, pyrazophos, pyridate, tetraconazole, tetramethrin, thiophanate-methyl, tolclofos-methyl, triazophos), effects on the motor division were observed in single and/or repeated dose studies, but specific neurotoxicity studies were not available. This can be a source of underestimation of their RPFs, and consequently of their contribution to the cumulative risk. This will need to be considered in the interpretation of the cumulative risk assessments when they will be performed.

Functional alterations of the sensory division:

In total, 55 ASs are included in the CAG for the alterations of the sensory division of the nervous system. Only ASs showing at least one relevant indicator of functional alterations of the sensory division were included in the CAG. Other ASs possessing a chemical structure capable of inducing neurotoxicity were not added.

The 55 ASs are: 2,4-D, abamectin, aldicarb, azinphos-methyl, benfuracarb, beta-cyfluthrin, beta-cypermethrin, bifenthrin, chlormequat, clothianidin, cymoxanil, cypermethrin, deltamethrin, dicamba, dicofol, dieldrin, dimethoate, emamectin benzoate, endosulfan, endrin, esfenvalerate, ethoprophos, fenitrothion, fenpropimorph, fenvalerate, fipronil, flufenacet, formetanate, glufosinate, halosulfuron-methyl, heptachlor, imidacloprid, indoxacarb, mepiquat, metaldehyde, methamidophos, methomyl, molinate, oxamyl, oxasulfuron, propineb, pyrazophos, pyrethrins, sulcotrione, sulfoxaflor, tau-fluvalinate, tebuconazole, tefluthrin, tembotrione, thiamethoxam, thiodicarb, thiram, tri-allate, trichlorfon, zeta-cypermethrin.

In this CAG, 33 ASs have a NOAEL for acute effects and 32 a NOAEL for chronic effects.

The ICs for the acute and chronic exposure/risk assessments are oxamyl and endrin, respectively. Oxamyl was preferred to aldicarb and deltamethrin on the basis of the robustness of findings (dose-relationship in mid and high dose males, statistical significance and known mode of action). Endrin

was preferred to dieldrin, methamidophos and methidathion on the basis of the consistence of findings observed in two strains of rats.

Considering the similarity of MoAs intervening in alterations of the motor and sensory functions of the nervous system, a comparison between these 2 CAGs showed that:

- The CAG for sensory division contains about 2 times less ASs than the CAG for the motor division.
- 3 out of the 55 ASs included in the CAG for the sensory division are not in the CAG for the motor division. These ASs are cymoxanil, halosulfuron-methyl and sulcotrione and have low potencies.
- The number of ASs in the CAG for motor division with identified NOAELs for acute effects exceeds by 40 the number of ASs in the CAG for sensory division with identified NOAELs for acute effects. One AS (halosulfuron-methyl) has a NOAEL for acute effects in the sensory division, but not in the motor division.
- From the ASs having a NOAEL for acute effects in both the sensory and motor division, 5 (abamectin, clothianidin, deltamethrin, dimethoate, formetanate) have lower NOAELs for the sensory division, 14 lower NOAELs for the motor division and 13 same NOAELs in sensory and motor divisions.
- The number of ASs in the CAG for motor division with NOAELs for chronic effects exceeds by 49 the number of ASs in the CAG for sensory division with NOAELs for chronic effects. 6 ASs (2,4-D, beta-cypermethrin, cymoxanil, dieldrin, fenvalerate, metaldehyde and sulcotrione) have a NOAEL for chronic effects for the sensory division, but not for the motor division.
- From the ASs having a NOAEL for chronic effects in both the sensory and motor division, 1 (endrin) has lower NOAELs in the sensory division, 14 lower NOAELs in the motor division and 10 same NOAELs in sensory and motor divisions.

In view of this comparison, it is anticipated that the cumulative risks for the sensory division will be lower than that for the motor division and therefore may not require separate assessment (see section 3.3.4).

Functional alteration of the autonomic division:

In total, 59 ASs are included in the CAG for the alterations of the autonomic division of the nervous system. Only ASs showing at least one relevant indicator of functional alteration of the autonomic division were included in the CAG. Other ASs possessing a chemical structure capable of inducing neurotoxicity were not added.

The 59 ASs are: 2,4-D, abamectin, acetamiprid, acrinathrin, aldicarb, alpha-cypermethrin, benfuracarb, beta-cyfluthrin, beta-cypermethrin, carbetamide, chlormequat, chlorpropham, clothianidin, cyfluthrin, cypermethrin, deltamethrin, dicamba, dicofol, dimethoate, emamectin-benzoate, endosulfan, esfenvalerate, ethephon, ethoprophos, fenamiphos, fenitrothion, fipronil, flufenacet, fluquinconazole, formetanate, glufosinate, indoxacarb, lambda-cyhalothrin, lufenuron, mepiquat, metaldehyde, metamitron, methamidophos, methiocarb, methomyl, metribuzin, milbemectin, molinate, oxamyl, oxydemeton-methyl, phosmet, pirimicarb, pyrethrins, pyridate, sulfoxaflor, tau-fluvalinate, tebuconazole, tefluthrin, thiacloprid, thiodicarb, thiram, triadimenol, tri-allate, trichlorfon.

In this CAG, 40 ASs have a NOAEL for acute effects and 32 a NOAEL for chronic effects.

The ICs for the acute and chronic exposure/risk assessments are oxamyl and methamidophos, respectively. Oxamyl was preferred to methiocarb (aka mercaptodimethur) and aldicarb on the basis of the robustness of data and known mechanism of action. Methamidophos was preferred to methiocarb and abamectine (aka avermectine) on the basis of the data.

Considering the similarity of MoAs intervening in alterations of the motor and autonomic functions of the nervous system, a comparison between these 2 CAGs showed that:

- The CAG for autonomic division contains about 2 times less ASs than the CAG for the motor division.

- 1 out of the 59 ASs included in the CAG for the autonomic division is not in the CAG for the motor division. This AS is metamitron and has a low potency.
- The number of ASs in the CAG for motor division with NOAELs for acute effects exceeds by 34 the number of ASs in the CAG for autonomic division with NOAELs for acute effects. One AS (ethephon) has an NOAEL for acute effects in the autonomic division, but in for the motor division.
- From the ASs having a NOAEL for acute effects in both the autonomic and motor division, 1 (formetanate) has a lower NOAEL in the autonomic division, 18 lower NOAELs in the motor division and 20 same NOAELs in autonomic and motor divisions.
- The number of ASs in the CAG for motor division with NOAELs for chronic effects exceeds by 46 the number of ASs in the CAG for autonomic division with NOAELs for chronic effects. Three ASs (2,4-D, metaldehyde and metamitron) have an NOAEL for chronic effects in the autonomic division, but not for the motor division.
- From the ASs having a NOAEL for chronic effects in both the autonomic and motor division, 6 (acrinathrin, chlormequat, clothianidin, mepiquat, methiocarb, molinate) have lower NOAELs in the autonomic division, 11 lower NOAELs in the motor division and 12 same NOAELs in autonomic and motor divisions.

In view of this comparison, it is anticipated that the cumulative risks for the autonomic division will be lower than that for the motor division and therefore may not require separate assessment (see section 3.3.4).

Brain and/or erythrocyte acetylcholinesterase inhibition:

This is a biochemical effect always associated with organophosphorous and N-methyl carbamate insecticides and only ASs of these chemical classes are included in the CAG. Since erythrocyte acetylcholinesterase is a membrane bound enzyme, oxidative stress induced by other chemical classes may also lead indirectly to a decrease in erythrocyte acetylcholinesterase activity (Banerjee et al., 1999; El-Demerdash, 2011). In consistency with the criteria described in section 2.2.1, ASs acting via this indirect pathway are however not included in the CAG.

In total, 47 ASs are included in the CAG for the brain and/or erythrocyte acetylcholinesterase inhibition.

These ASs are: acephate, aldicarb, azinphos-ethyl, azinphos-methyl, benfuracarb, cadusaphos, carbaryl, carbofuran, carbosulfan, chlorfenvinphos, chlorpyriphos, chlorpyriphos-methyl, diazinon, dichlorvos, dimethoate, ethephon, ethion, ethoprophos, fenamiphos, fenitrothion, fenthion, fonofos, formetanate, fosthiazate, malathion, methamidophos, methidathion, methiocarb, methomyl, monocrotophos, omethoate, oxamyl, oxydemeton-methyl, parathion, parathion-methyl, phenthoate, phosalone, phosmet, phoxim, pirimicarb, pirimiphos-methyl, profenofos, pyrazophos, thiodicarb, toloclophos-methyl, triazophos, trichlorfon.

In this CAG, 23 ASs have a NOAEL for acute effects and all have a NOAEL for chronic effects.

The ICs for the acute and chronic exposure/risk assessments are oxamyl and omethoate, respectively. Oxamyl was preferred to formetanate, methiocarb and aldicarb on the basis of the robustness of data and known mechanism of action. Omethoate was preferred to aldicarb, monocrotophos, parathion and methiocarb on the basis of the consistency of findings across studies and animal species (rat, dog, mouse and rabbit).

Generally, the NOAELs related to acetylcholinesterase 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:

In total, 19 ASs are included in the CAG for neuropathological effects.

These ASs are: chlorfenapyr, cymoxanil, cypermethrin, emamectin benzoate, fenpropidin, flufenacet, indoxacarb, isoxaflutole, lindane, mancozeb, molinate, oxasulfuron, quinoclamine, tau-fluvalinate, tembotrione, thiram, tri-allate, trichorfon, ziram.

The IC is emamectine benzoate, which was preferred to tetraconazole, parathion-methyl, tembotrione and pyrazophos on the basis of the consistency of findings across studies and animal species (rat, dog and mouse).

Considering the relationship between neuropathological effects and functional alterations of the motor division of the nervous system, a comparison between these 2 CAGs 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 biochemical mechanisms, not necessarily associated to neuropathological effects, are major causal factors of alteration of the motor function.
- 3 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 quinoclamine.
- The number of ASs in the CAG for motor division with NOAELs for chronic effects exceeds by 60 the number of ASs in the CAG for neuropathological effects. 4 ASs (chlorfenapyr, cymoxanil, quinoclamine and ziram) have NOAELs for neuropathological effects, but no 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, 4 (lindane, mancozeb, thiram, tri-allate) have lower NOAELs for neuropathological effects, 3 lower NOAELs in the motor division and 8 same NOAELs for both effects.

In view of this comparison, it is anticipated that the cumulative risks for the neuropathological effects will be lower than that for the motor division and therefore may not require separate assessment (see section 3.3.4).

3.3.3. Summary tables

Nine tables (see Annex B) are prepared to support all possible acute and chronic exposure/risk assessments that could be conducted using the 5 CAGs. These tables include the following information: name of the active substance, indicator of specific effect, NOAEL, LOAEL, mode/mechanism of action, relative potency factor (RFP) and reference of the study from which the information was retrieved. In consistency with article 63 of Regulation (EC) No 1107/2009, the names of persons involved in these studies are confidential and not shown in the study reference details.

The information in these tables is restricted to the most sensitive indicator(s) observed for the respective AS, i.e. characterised with the lowest NOAEL and used for the calculation of RPFs. Additional information on the ASs and evidence supporting their inclusion in CAGs can be found in the excel tables compiled from the 3 data collections described in section 2.1 (Appendix A).

3.3.4. Use of the Cumulative Assessment Groups to assess consumer safety

Considering the comparisons of the compositions of these CAGs in section 3.3.2, it is expected that cumulative risk assessments conducted 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 may be sufficient to perform cumulative risk assessments with the CAGs for acetylcholinesterase inhibition and for the functional alterations of the motor division, assuming that similar protection goals would apply to all these effects.

In conducting these cumulative risk assessments, the potential contribution of metabolites and degradation products to the specific effects should be taken into account. In a refined assessment, it could be attempted to use the residue definition for risk assessment established with respect to the critical effect. If this is not appropriate, another residue definition could be considered on ad-hoc basis and 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, 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 obvious 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 summarised 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 largely exceeding the overall NOAEL for the respective study.

In addition, for a number of ASs, especially for ASs which are not approved anymore, the quality of the database is far from conforming to the current standards and causes an additional source of uncertainties. 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 active substances 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 CAG for the alterations of the motor function and to the CAG on acetylcholinesterase inhibitions are addressed in the section 4.2 and 4.3. 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?

The possibility of omitting ASs contributing to the effect is addressed later, in the assessment of overall uncertainty (see section 4.5).

For the CAG on motor division, the possibility of including ASs not contributing to the effect is addressed by the weight of evidence and EKE techniques described in section 2.4.2. The process was conducted as follows:

a) A key step in expert knowledge elicitation is specification of the question to be addressed in a well-defined manner and, if possible, such that the answer to the question is potentially observable, at least in principle (EFSA, 2014c). The question of interest for cumulative risk assessment is, for each active substance, 'Does this chemical cause any functional alteration of

the motor division of the nervous system (motor 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?' 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: organophosphate, N-methyl carbamates, mectins, pyrethroids, neonicotinoids, phenylpyrazoles or to any other chemical class if a MoA inducing neurotoxicity is known for this AS: 32000 if the AS belongs to one of these classes. 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 action 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 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 if more than one indicator is reported.
- Concomitant observation of effects on the sensory function: 3 in the absence of any neurotoxicity study**; 4 if any indicator is reported.
- Observation of effects in more than one species: 3.
- Observation of a dose-response relationship for the most sensitive effect: 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.

c) Based on the lines of evidence, scores were calculated for all ASs of the CAG. These scores were used to distribute the ASs into 6 sub-groups of decreasing scores. The first 4 sub-groups contained ASs with chemical structures associated to neurotoxicity. The next 2 sub-groups contained ASs of different chemical structures. The 7 ASs identified from the CAG on neurochemical effects were not scored and treated separately as a 7th group of ASs. The compositions of these 7 sub-groups are as follows:

- Sub-group 1 (18 ASs): acetamiprid, aldicarb, carbofuran, clothianidin, cypermethrin, deltamehrin, emamectin, ethoprophos, fenpropathrin, fenvalerate, fipronil, formetanate, metamidophos, milbemectin, omethoate, oxydemeton-methyl, pyrethrins, thiamethoxam.
- Sub-group 2 (20 ASs): abamectin, acrinathrin, azinphos-methyl, beta-cyfluthrin, beta-cypermethrin, cadusaphos, carbaryl, bifenthrin, dichlorvos, esfenvalerate, fenitrothion,

fosthiazate, imidacloprid, oxamyl, permethrin, tau-fluvalinate, tefluthrin, thiacloprid, trichlorfon, zeta-cypermethrin.

- Sub-group 3 (14 ASs): alpha-cypermethrin, benfuracarb, cyfluthrin, diazinon, dimethoate, fenamiphos, fenthion, lambda-cyhalothrin, lindane, methidathion, monocrothophos, pirimicarb, pyrazophos, thiodicarb.
- Sub-group 4 (20 ASs): acephate, carbosulfan, chlorpyrifos, chlorpyrifos-methyl, dieldrin, dinotefuran, endrin, heptachlor, methomyl, fonofos, malathion, methoocarb, parathion, parathion-methyl, phosalone, phosmet, phoxim, piripmiphos-methyl, tetramethrin, triazophos.
- Sub-group 5 (19 ASs): bromide ion, carbetamide, chlormequat, dicamba, dicofol, endosulfan, flufenacet, fluquinconazole, indoxacarb, mepiquat, metaldehyde, metribuzin, molinate, oxasulfuron, pyridate, sulfoxaflor, tebuconazole, thiram, triallate.
- Sub-group 6 (22 ASs): amitraz, chlorpropham, 2,4-D, desmedipham, fenpropidin, fenpropimorph, glufosinate, isoxaflutole, lufenuron, mancozeb, maneb, metiram, penflufen, propineb, pymetrozine, tembotrione, tetraconazole, thiophanate-methyl, triadimefon, triadimenol, spirotetramat, ziram.
- Sub-group 7 (7 ASs): azinphos-ethyl, chlorfenvinphos, ethephon, ethion, penthoate, profenofos, tolclofos-methyl.

d) The probability estimations for sub-groups 1-7 concluded that:

<u>In sub-group 1</u>:

- 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 nearly certain (more than 95% probability) that all substances cause effects on the motor division.

<u>In sub-group 2</u>:

- All ASs have relevant structure or mechanism.
- All ASs show dose-response relationship.
- All ASs show several or many 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 nearly certain (more than 95% probability) that all 20 substances cause motor division effect.

<u>In sub-group 3</u>:

- All ASs have relevant structure and mechanism and substantial evidence of effects (indicators).
- For four substances 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 nearly certain (more than 95% probability) that all ASs cause effects on the motor division of the nervous system

In sub-group 4:

- 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 6 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 nearly certain (more than 95% probability) that all 20 substances cause motor division effect.

In sub-group 5:

- Only two ASs have known structural alerts of neurotoxicity.
- None of the ASs have strong evidence for a relevant mode of action. Five ASs (endosulfan, flufenacet, indoxacarb, metaldehyde, sulfoxaflor) have a presumed mode of action and 2 ASs (oxasulfuron 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.
- 16 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 most likely to be between 15 and 17, with a plausible range from 12 to 19.

In sub-group 6:

- 6 ASs have some evidence of relevant structure or presumed mechanism (amitraz, mancozeb, maneb, metiram, propineb, 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 9 ASs only.
- In 7 cases, effects were seen in two or more species.
- 5 ASs were judged extremely likely (95-99%) to cause motor division effects; 4 were judged likely (66-90%); 5 as likely as not (33-66%); 2 unlikely (10-33%); 3 very unlikely (5-10%) and 3 extremely unlikely (1-5%).
- Overall, it was judged that the number of ASs that cause motor division effects is most likely to be 10 or 11, with plausible range between 8 and 15.

In sub-group 7:

- 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 acetylcholinesterase, one (ethephon) inhibits erythrocytes acetylcholinesterase but not brain, and for two ASs information on brain acetylcholinesterase 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 nearly certain (more than 95% probability) that chlorfenvinfos, ethion, profenofos, azinphos-ethyl and penthoate cause effects on the motor division, but

that it is extremely unlikely (probability ranging between 1 and 5%) that either ethephon or tolclophos-methyl cause the effects.

- Overall it was judged most likely that at least five ASs cause motor division effects with a very small probability that 6 cause the effects.

e) Assessing the total number of ASs causing motor division effects:

- It is nearly certain that all the ASs of sub-groups 1, 2, 3, and 4 cause alterations on the motor function. The same is true for 5 of the 7 substances in sub-group 7.
- The percentage of ASs of sub-group 5 causing alterations on the motor function is most likely between 15 and 17, with plausible range from 12 to 19.
- The percentage of ASs of sub-group 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 7 sub-groups are plotted together in Figure 1. The number of substances considered differs between sub-groups so, to facilitate comparison, the elicited distributions were rescaled to percentage of substances.
- The elicited distributions for the 7 sub-groups were combined by 1D Monte Carlo simulation, assuming independence between sub-groups. 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). The potential impact on this of dependencies between sub-groups will be considered as part of overall uncertainty analysis when the cumulative risk assessment is performed.

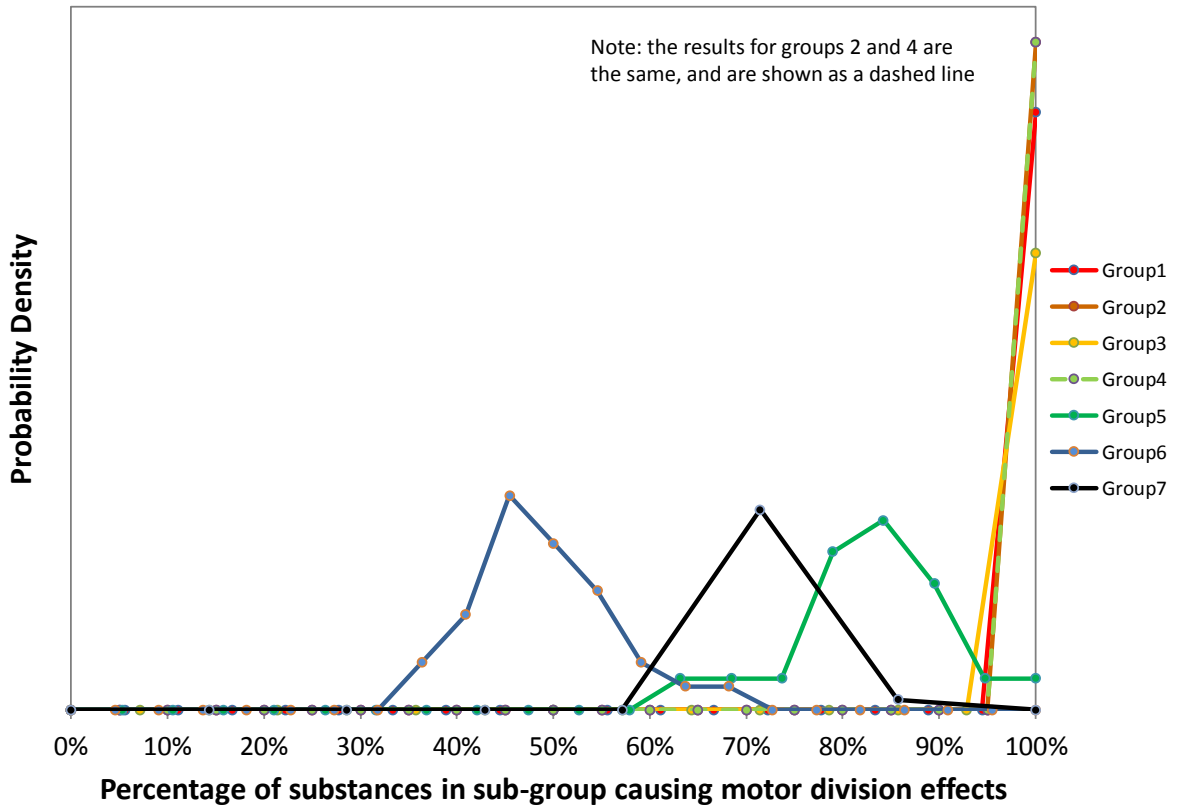

Figure 1. Distributions quantifying uncertainty about the percentage of substances in each sub-group that cause motor division effects. The vertical axis (probability density) quantifies the experts' judgement of the likelihood of different proportions of substances causing motor division effects within each sub-group.

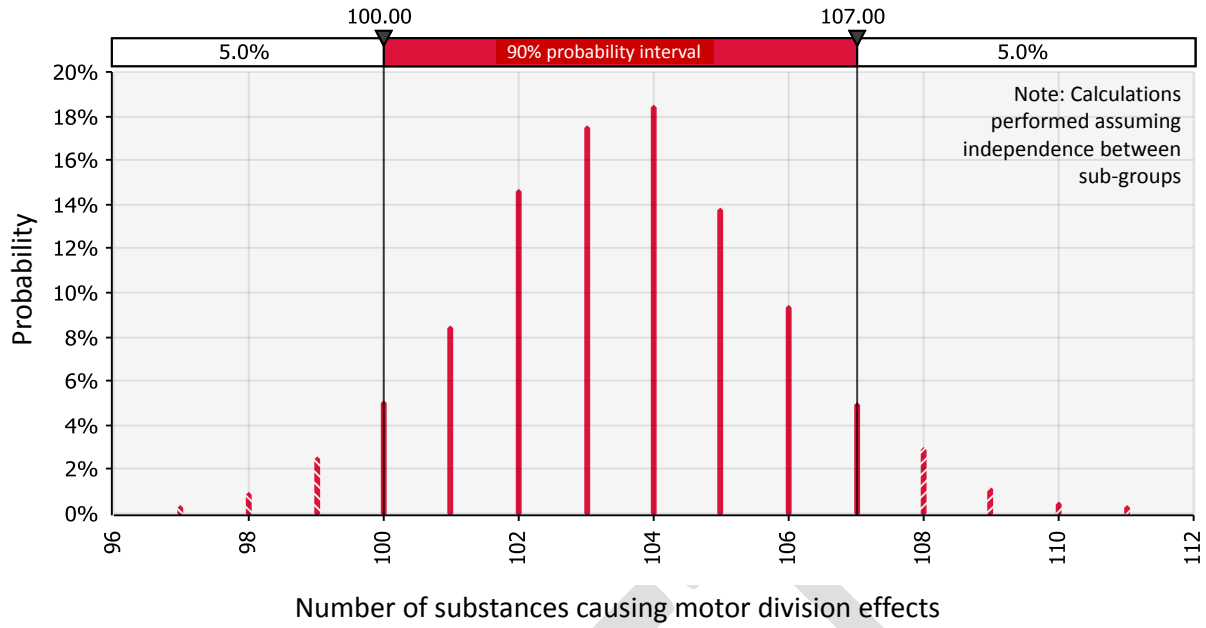

Figure 2. Distribution quantifying uncertainty about the total number of substances from sub-groups 1-7 that cause motor division effects, obtained using Monte Carlo simulation to combine the elicited distributions for the 7 sub-groups.

Appendix B provides more details on the elicitation process.

4.2.2. Assessment of the modes of neurotoxic action.

When cumulative risk assessments will be conducted using the CAG on functional alterations of the motor division the question 'How sure is it that these ASs combine their individual toxicities according to the dose addition model at their actual level in food?' will be addressed. If possible, clusters of ASs for which dose addition is virtually certain should also be defined in this context. The present section gives grounds to the risk assessor who will conduct these assessments by reviewing and sorting out the ASs in the CAG for the effects on the motor division according to their structure and mode of action.

A majority of ASs included in this CAG are acting according to a known MoA, which is associated to the chemical structure. This allows establishing sub-groups of ASs on the basis of their chemical structure and mode of action as follows:

Structures with known mode of neurotoxic action in mammals (subgroups 1 to 4 and 7)

a) N-methyl carbamate insecticides (Acetylcholinesterase (AChE) inhibitors):
   - benzofuranyl methylcarbamate insecticides : benfuracarb, carbofuran, carbosulfan
   - Carbamate insecticide : carbaryl
   - Dimethyl carbamate insecticides : pirimicarb
   - Formamidine insecticides: formetanate (also agonist of the octopamine receptor in insects which is equivalent to the alpha2-adrenoreceptor in mammals)
   - Oxime carbamate insectides : aldicarb, methomyl, oxamyl, thiodicarb
   - phenyl methylcarbamate insecticides: methiocarb

b) Macrocyclic lactone insecticides (GABA-gated chloride channel agonist):
   - Avermectin insecticides: abamectin, emamectin benzoate,
   - milbemycin insecticides: milbemectin

c) Neonicotinoids (agonist of nicotinic acethylcholine receptor (nAChR)): acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, thiamethoxam

d) Organophosphorous insectides (Acetylcholinesterase (AChE) inhibitors):

- Organophosphate: chlorfenvinphos, dichlorvos, monocrotophos
- Organothiophosphate: azinphos-ethyl, azinphos-methyl, cadusafos, chlorpyrifos, chlorpyrifos-methyl, diazinon, dimethoate, ethion, ethoprophos, fenitrothion, fenthion, malathion, methidathion, omethoate, oxydemeton-methyl, parathion, parathion-methyl, phenthoate, phosalone, phosmet, phoxim, profenofos, pyrazophos, pirimiphos-methyl, triazophos
- Organothiophosphate phosphate: fosthiazate (nematocide)
- Phosphonate: trichlorfon
- Phosphonothioate: fonofos
- Phosphoramidate: fenamiphos
- Phosphoramidothioate: acephate, methamidophos

e) Organochlorine insecticides (GABA-gated chloride channel antagonists):

- Lindane
- cyclodiene insecticides (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, betacyfluthrin, beta-cypermethrin, bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, fenvalerate, lambda-cyhalothrin, permethrin, tau-fluvalinate, tefluthrin, tetramethrin, zeta-cypermethrin

For the rest of ASs in the CAG having chemical structures other than those listed above and belonging to subgroups 5 and 6, the mode of action is either known, presumed, or, in the majority of cases, unknown.

Structures with known or presumed modes of neurotoxic action in mammals:

Amitraz (formamidine acaricide, octopamine receptor agonist in insects which is equivalent to alpha2 adrenergic receptor in mammals), chlormequat (growth retardant, partial agonist of muscarinic and nicotinic acetylcholine receptor), dithiocarbamate (mancozeb, maneb, metiram, propineb, thiram and ziram; neurotoxic effect might be due to the metabolite CS_2), indoxacarb (oxadiazin insecticide, voltage-dependent sodium channel blocker), mepiquat (growth inhibitor, activation of nicotinic and muscarinic acetylcholine receptors), metaldehyde (nematicide, GABA inhibitor), molinate (thiocarbamate herbicide, the metabolite molinate sulfone inhibits aldehyde dehydrogenase by covalently binding to the active-site Cys residue), pymetrozine (pyridine azomethine insecticide, chordotonal organ TRPV channel modulator), , sulfoxaflor (sulfoximine insecticide, nicotinergic acetylcholine receptor partial agonist), tolclophos-methyl (organophosphorus fungicide, acetylcholinesterase (AChE) inhibition), triadimefon and triadimenol (conazole fungicide, inhibition of dopamine transporter).

Structures with unknown modes of neurotoxic action in mammals:

Bromide ion (comes from methyl bromide, fumigant), carbetamide (carbanilate herbicide), chlorpropham (carbanilate herbicide, growth regulator), 2,4-D (phenoxyacetic herbicide), 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, tetraconazole, glufosinate (organophosphorus herbicide), isoxaflutole (oxazole herbicides), lufenuron (benzoylphenylurea), metribuzin (triazinone herbicide), triallate (thiocarbamate herbicide), oxasulfuron

(sulfonylurea), penflufen (anilide herbicide), pyridate (pyridazine herbicide), spirotetramat (tetramic acid insecticide), thiophanate-methyl (benzimidazole, carbamate fungicide) tembotrione (benzoylcyclohexanedione herbicides).

There is therefore a wide variety of chemical structures and MoAs known or presumed to be of relevance for the combined effects of pesticide ASs on the nervous system. EFSA (2013c) recommended that the cumulative risk from pesticides which produce common adverse outcomes on the same target organ/system should be assessed using the concept of dose addition, as a pragmatic and conservative default approach based on experimental evidence available up to that time. There is however uncertainty about how closely combined effects will conform to those predicted by dose addition, and this uncertainty is greater when considering chemicals with dissimilar modes of action. These uncertainties need to be taken into account as part of the risk characterisation for cumulative assessment. The information summarised above, regarding modes of action, will therefore be useful in subsequent stages of risk assessment following the present report. The extent to which these uncertainties impact the risk assessment will depend on various considerations, including the extent to which individual consumers have significant exposures to multiple active substances with different modes of action.

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

4.3.1. Question 1: Does the CAG for the brain and/or erythrocyte acetylcholinesterase inhibition contain all ASs contributing to this effect and only ASs causing this effect?

The possibility of omitting ASs contributing to the effect is addressed in the above general considerations.

The possibility of including ASs not contributing to the effect is virtually non-existent because all ASs but 2 are organophosphorous or N-methyl carbamate insecticides acting biologically via acetylcholinesterase inhibition.

One AS (tolclophos-methyl) is an organophosphorus fungicide and another one (ethephon) is a growth regulator not belonging to these chemical classes. However, both have shown weak but significant inhibition of acetylcholinesterase in experimental studies.

4.3.2. Assessment of the modes of neurotoxic action.

With the exception of ethephon, for which the mode of action is unknown, all ASs in the CAGs for acetylcholinesterase inhibition belong to the chemical structures a) or d) listed in 4.2.2.

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 recommended not to perform CRAs for these CAGs, as those carried out with the CAG for the alterations of the motor division is 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 cumulative risk assessments performed for neuropathological effects. For these effects, information on relevant MoAs is scarce, but can be found in table 9 of annex 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 CAG used and from the assumption that ASs in this CAG combine their effects by dose addition, it is recommended to consider systematically all relevant sources of uncertainties, including the following:

- Toxicological data collection: how certain is it that all information of relevance for the specific effect of interest was collected, based on the documentation consulted and on the collection criteria? The possibility that information of relevance in original studies is omitted or misreported in summary documents used as source of information will be considered. Also the criteria used by the data collector to extract information from the sources needs to be considered to identify potential bias for the establishment of CAGs.
- Quality and completeness of the toxicological data: how certain is it that all the toxicological data of relevance for the specific effect of interest have been generated (e.g. neurotoxicity studies)? The robustness of the original toxicological database needs to be considered.
- Composition of the CAG: 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 the individual contributions of ASs to the risk and of individual probabilities of CAG membership assessed in 4.2.2 and 4.3.2.
- Availability of NOAELs for all ASs in the CAG: As indicated in 3.3.1, NOAELs for acute or chronic effects may be missing for some ASs included in the CAG. In such case, the assessor should estimate the importance of the resulting potential underestimation of the risk assessment.
- 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 focus on ASs driving the risk and will consider whether they have similar or dissimilar MoAs. Eventual situations of antagonism in case of co-exposure to ASs with opposite MoA might also be considered. Empirical information available on their combined toxicity in peer-reviewed scientific literature will be considered if available.
- Uncertainties resulting from the fact that acute and chronic exposure calculation models do not necessarily reflect the real toxicokinetic and toxicodynamic processes. For instance, being exposed to a carbamate at breakfast and to an organophosphate at dinner is not leading to the same risk as being exposed to the same compounds in the reverse order. However the cumulative exposure calculation model does not produce different results for these different risks.

The assessor should ideally evaluate whether each of these sources of uncertainties tends to increase or decrease the outcome of the assessment question. The combined impact of these and other uncertainties should later be considered in the evaluation of the overall uncertainty associated to the reply given to the assessment question.

5. Recommendations

Due to the current scarcity of data with respect of developmental neurotoxicity 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 an appropriate testing and assessment methodology should be developed and applied on a consistent basis to provide sufficient information supporting the establishment of CAGs covering developmental neurotoxicity if appropriate.

If the outcome of CRAs conducted with these CAGs exceeds regulatory thresholds of acceptance, 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 exceeds the regulatory thresholds of acceptance established by risk managers, an alternative cumulative exposure/risk assessment should be considered with RPFs calculated using benchmark doses (BMD). This is not epected to change significantly the outcome of the assessment, but would make it independent from the dose selection in toxicological studies and better reflecting the actual relative potenties of ASs in the CAG.

The approaches developed in the present report to evaluate uncertainties should be integrated into the cumulative risk assessment 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 sub-groups 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.

References

Banerjee, B.D., Seth, V., Bhattacharya, A., Pasha, S.T., Chakraborty, A.K., 1999. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicol. Lett. 107, 33–47.

El-Demerdash F.M., 2011. Lipid peroxidation, oxidative stress and acetylcholinesterase in rat brain exposed to organophosphate and pyrethroid insecticides, Food Chem. Toxicol. 49 (2011) 1346–1352

EFSA (PPR Panel), 2013a. Scientific Opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile (2014 update). EFSA Journal 2013a; 11(7):3293, 131pp.

EFSA, 2013b. The 2010 European Union Report on Pesticide Residues in Food. EFSA Journal 2013; 11(3):3130.

EFSA (PPR Panel), 2013c. Scientific Opinion on relevance of dissimilar mode of action and its appropriate application for cumulative risk assessment of pesticides residues in food. EFSA Journal 2013; 11(12):3472, 40 pp.

EFSA (Scientific Evaluation of Regulated Product Department), 2014a. Project charter 'Implementation of cumulative risk assessment of pesticides'. EFSA Register of questions; 12 pp.

EFSA, 2014b. Outcome of the public consultation on the Scientific Opinion on the identification of pesticides to be included in cumulative assessment groups (CAGs) on the basis of their toxicological profile. EFSA supporting publication 2014:EN-538. 53 pp.

EFSA (Scientific Committee), 2014c. Guidance on Expert Knowledge Elicitation in Food and Feed Safety Risk Assessment. EFSA Journal 2014; 12(6):3734, 278 pp.

EFSA (PPR Panel), 2016. Guidance on the establishment of the residue definition for dietary risk assessment. EFSA Journal 2016;14(12):4549, 129 pp.

EFSA (Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Benfenati E, Chaudhry QM, Craig P, Frampton G, Greiner M, Hart A, Hogstrand C, Lambre C, Luttik R, Makowski D, Siani A, Wahlstroem H, Aguilera J, Dorne J-L, Fernandez Dumont A, Hempen M, Valtuena Martinez S, Martino L, Smeraldi C, Terron A, Georgiadis N and Younes M), 2017. Scientific Opinion on the guidance on the use of the weight of evidence approach in scientific assessments. EFSA Journal 2017;15(8):4971, 69 pp.

EFSA (Scientific Committee, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Younes M, Craig P, Hart A, Von Goetz N, Koutsoumanis K, Mortensen A, Ossendorp B, Germini A, Martino L, Merten C, Mosbach-Schulz O, Smith A and Hardy A), 2018. Scientific Opinion on the principles and methods behind EFSA's Guidance on Uncertainty Analysis in Scientific Assessment. EFSA Journal 2018;16(1):5122, 235 pp.

European Commission, 2011. The Rapid Alert System for Food and Feed, 2011 Annual Report. Available from: http://ec.europa.eu/food/food/rapidalert/docs/rasff_annual_report_2011_en.pdf

JMPR, 1999. Interpretation of Cholinesterase inhibition. Pesticide residues in food—1998. Report of the JMPR 1998. FAO Plant Production and Protection Paper, 148, FAO, Rome, pp. 17–21.

Nielsen E, Norhede P, Boberg J, Isling LK, Kroghsbo S, Hadrup N, Bredsdorff L, Mortensen A and Larsen JC, 2012. Identification of Cumulative assessment groups of pesticides. EFSA Supporting Publications 2012:EN-269, 303 pp.

Office of Pesticide Programs (US Environmental Protection Agency), 2000. Science policy on the use of data on cholinesterase inhibition for risk assessments of organophosphorous and carbamate pesticides. US EPA 2000, 51 pp.

RIVM, ICPS, ANSES, 2013. Toxicological data analysis to support grouping of pesticide active substances for cumulative risk assessment of effects on liver, on the nervous system and on reproduction and development. EFSA Supporting Publications 2013:EN-392, 88 pp.

RIVM, ICPS, ANSES, 2016. Toxicological data collection and analysis to support grouping of pesticide active substances for cumulative risk assessment of effects on the nervous system, liver, adrenal, eye, reproduction and development and thyroid system. EFSA supporting publication 2016:EN-999, 184 pp.

WHO (World Health Organisation), 2011. Guidelines for drinking-water quality – Fourth edition. (http://www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en/)

Glossary and Abbreviations

AChE	Acetylcholinesterase
ATSDR	Agency for Toxic Substances and Disease Registry
ANSES	French Agency for Food, Environmental and Occupational Health and Safety
AS	Active substance
BMD	Benchmark Dose
CAG	Cumulative assessment group
CRA	Cumulative Risk Assessment
DAR	Draft Assessment Report
DNT	Developmental Neurotoxicity
DRAR	Draft Re-Assessment Report
DTU	Technical University of Denmark
EFSA	European Food Safety Authority
EC	European Commission
EKE	Expert Knowledge Elicitation
EHC	Environmental Health Criteria Monograph
EU	European Union
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 Effect Level
MoA	Mode of Action
MRL	Maximum Residue Level
NOAEL	No Observed Effect Level
PMRA	Canadian Pest Management Regulatory Agency
PPR	EFSA Panel on Plant Protection Products and their Residues
RIVM	National Institute for Public Health and the Environment
RPF	Relative Potency Factor
TRPV	Transient Receptor Potential Vanilloid
UF	Uncertainty Factor
US-EPA	United States Environmental Protection Agency
VGSC	Voltage-gated Sodium Channel

Annex 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):

1-Methylcyclopropene	Cyazofamid	Fluazifop-P	Mesosulfuron	Pyridate
1-Naphthylacetamide (1-NAD)	Cyclanilide	Fluazinam	Mesotrione	Pyrimethanil
1-Naphthylacetic acid (1-NAA)	Cycloxydim	Fludioxonil	Metalaxyl-M	Pyriproxyfen
2,4-D	Cyflufenamid	Flufenacet (formerly fluthiamide)	Metaldehyde	Quinmerac
2,4-DB (metabolized to 2,4-D)	Cyfluthrin	Flumioxazin	Metamitron	Quinoclamine
2-Phenylphenol (incl. sodium salt orthophenyl phenol)	Cyhalofop-butyl	Fluometuron	Metazachlor	Quinoxyfen
6-Benzyladenine	Cymoxanil	Fluopicolide	Metconazole	Quizalofop-P-tefuryl
Abamectin (aka avermectin)	Cypermethrin	Fluoxastrobin	Methiocarb (aka mercaptodimethur)	Rimsulfuron (aka renriduron)
Acetamiprid	Cyproconazole	Flupyrsulfuron-methyl (DPX KE 459)	Methomyl	Silthiofam
Acibenzolar-S-methyl (benzothiadiazole)	Cyprodinil	Fluquinconazole	Methoxyfenozide	Sintofen (aka Cintofen)
Aclonifen	Cyromazine	Flurochloridone	Metiram	S-Metolachlor
Alpha-Cypermethrin (aka alphamethrin)	Daminozide	Fluroxypyr	Metosulam	Sodium 5-nitroguaiacolate
Aluminium ammonium sulphate	Dazomet	Flurtamone	Metrafenone	Sodium hypochlorite
Aluminium phosphide	Deltamethrin	Flusilazole	Metribuzin	Sodium o-nitrophenolate
Amidosulfuron	Desmedipham	Flutolanil	Metsulfuron-methyl	Sodium p-nitrophenolate
Amitrole (aminotriazole)	Dicamba	Flutriafol	Milbemectin	Spinosad
Azimsulfuron	Dichlorprop-P	Folpet	Molinate	Spirodiclofen
Azoxystrobin	Diclofop	Foramsulfuron	Myclobutanil	Spiroxamine
Beflubutamid	Diethofencarb	Forchlorfenuron	Napropamide	Sulcotrione
Benalaxyl	Difenoconazole	Formetanate	Nicosulfuron	Sulfosulfuron
Benfluralin	Diflubenzuron	Fosetyl	Omethoate	Sulfuryl fluoride
Bensulfuron	Diflufenican	Fosthiazate	Oryzalin	tau-Fluvalinate
Bentazone	Dimethachlor	Fuberidazole	Oxadiargyl	Tebuconazole
Benthiavalicarb	Dimethenamid-P	Gibberellin	Oxadiazon	Tebufenozide
Benzoic acid	Dimethoate	Glufosinate	Oxamyl	Tebufenpyrad
Beta-Cyfluthrin	Dimethomorph	Glyphosate (incl trimesium aka sulfosate)	Oxasulfuron	Teflubenzuron
Bifenazate	Dimoxystrobin	Haloxyfop-P/R	Oxyfluorfen	Tefluthrin

Bifenox	Dinocap	Hexythiazox	Paclobutrazol	Tepraloxydim
Bispyribac	Diquat (dibromide)	Hymexazol	Penconazole	Terbuthylazine
Boscalid	Dithianon	Imazalil (aka enilconazole)	Pencycuron	Tetraconazole
Bromadiolone	Diuron	Imazamox	Pendimethalin	Thiabendazole
Bromoxynil	Dodemorph	Imazaquin	Penoxsulam	Thiacloprid
Bromuconazole	Dodine	Imazosulfuron	Pethoxamid	Thiamethoxam
Bupirimate	Epoxiconazole	Imidacloprid	Phenmedipham	Thifensulfuron-methyl
Buprofezin	Esfenvalerate	Indoxacarb	Phosmet	Thiophanate-methyl
Calcium phosphide	Ethephon	Iodosulfuron	Picloram	Thiram
Captan	Ethofumesate	Ioxynil	Picolinafen	Tolclofos-methyl
Carbendazim	Ethoprophos	Iprodione	Picoxystrobin	Tolylfluanid
Carbetamide	Ethoxysulfuron	Iprovalicarb	Pirimicarb	Tralkoxydim
Carboxin	Etofenprox	Isoproturon	Pirimiphos-methyl	Triadimenol
Carfentrazone-ethyl	Etoxazole	Isoxaben	Prochloraz	Tri-allate
Carvone	Etridiazole	Isoxaflutole	Profoxydim (aka Clefoxydim)	Triasulfuron
Chloridazon (aka pyrazone)	Famoxadone	Kresoxim-methyl	Prohexadione (incl Prohexadione-calcium)	Triazoxide
Chlormequat (chloride)	Fenamidone	Lambda-Cyhalothrin	Propamocarb	Tribenuron (aka metometuron)
Chlorothalonil	Fenamiphos (aka phenamiphos)	Lenacil	Propaquizafop	Triclopyr
Chlorotoluron	Fenazaquin	Linuron	Propiconazole	Trifloxystrobin
Chlorpropham	Fenbuconazole	Lufenuron	Propineb	Triflumizole
Chlorpyrifos	Fenbutatin oxide	Magnesium phosphide	Propoxycarbazone	Triflumuron
Chlorpyrifos-methyl	Fenhexamid	Malathion	Propyzamide	Triflusulfuron
Chlorsulfuron	Fenoxaprop-P	Maleic hydrazide	Proquinazid	Trinexapac (aka cimetacarb ethyl)
Cinidon ethyl	Fenoxycarb	Mancozeb	Prosulfocarb	Triticonazole
Clethodim	Fenpropidin	Maneb	Prosulfuron	Tritosulfuron
Clodinafop	Fenpropimorph	MCPA	Prothioconazole	zeta-Cypermethrin
Clofentezine	Fenpyroximate	MCPB	Pymetrozine	Zinc phosphide
Clomazone	Fipronil	Mecoprop	Pyraclostrobin	Ziram (incl impurity TMTU)
Clopyralid	Flazasulfuron	Mecoprop-P	Pyraflufen-ethyl	Zoxamide
Clothianidin	Flonicamid (IKI-220)	Mepanipyrim	Pyrethrins	
Copper compounds	Florasulam	Mepiquat	Pyridaben	

Active substances covered by the 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	Chlordane	Fenitrothion	Metam (incl. -potassium and -sodium)	Pyroxsulam
Aldicarb	Chlorfenapyr	Fenpropathrin	Methamidophos	Quintozene
Aluminium sulphate	Chlorfenvinphos	Fenpyrazamine	Methidathion	Resmethrin
Ametoctradin	Chlorobenzilate	Fenthion	Methoxychlor	Sedaxane
Aminopyralid	Chromafenozide	Fenvalerate	Metobromuron	Spinetoram
Amisulbrom	Cyantraniliprole	Ferric phosphate	Monocrotophos	Spiromesifen
Amitraz	Cyflumetofen	Fluazifop	Nicotine	Spirotetramat
Anthraquinone	DDT	Flubendiamide	Orthosulfamuron	Sulfoxaflor
Azadirachtin	Diazinon	Flufenoxuron	Oxadixyl	Tecnazene
Azinphos-ethyl	Dichlofluanid	Fluopyram	Oxydemeton-methyl	Tembotrione
Azinphos-methyl	Dichlorvos	Fluxapyroxad	Parathion	Tetradifon
Benalaxyl-M	Dicloran	Fonofos	Parathion-methyl	Tetramethrin
Benfuracarb	Dicofol	Halosulfuron methyl	Penflufen	Thiencarbazone
Benomyl	Dicrotophos	HCH	Penthiopyrad	Thiodicarb
Benzalkonium chloride	Didecyldimethylammonium chloride	Heptachlor	Permethrin	Tolfenpyrad
Beta-cypermethrin	Dieldrin	Hexachlorobenzene	Phenthoate	Topramezone
Bifenthrin	Dinotefuran	Hexaconazole	Phosalone	Triadimefon
Bitertanol	Diphenylamine	Indolylbutyric acid	Phosphane	Triazophos
Bixafen	Dithiocarbamates	Ipconazole	Phoxim	Trichlorfon
Bromide ion	Emamectin benzoate	Iron sulphate	Pinoxaden	Trifluralin
Bromopropylate	Endosulfan	Isoprocarb	Procymidone	Valifenalate
Cadusafos (aka ebufos)	Endrin	Isopyrazam	Profenofos	Vinclozolin
Camphechlor	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

Annex B – Tables supporting Cumulative Risk Assessments using the CAGs for effects of pesticides on the nervous system

Table 1. CAG on functional effects on motor division: toxicological characterization of ASs to be considered in acute exposure/risk assessments.

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Ataxia	2,4-D	1.5	15	Unknown		0.067	Acute neurotoxicity rat ([redacted], 1994a)
Ataxia	Abamectin	1.5	6*	Known	GABA-gated chloride channel agonist	0.067	Acute neurotoxicity rat ([redacted], 2006a)
Reduced motor activity, tremor	Acetamiprid	10	30	Known	agonist of nicotinic acethylcholine receptor (nAChR)	0.010	Acute neurotoxicity rat ([redacted], 1997a)
Reduced grip strength	Acrinathrin	0.24	2.4	Known	binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.417	90-day neurotoxicity rat ([redacted], 2003)
Increased motor activity, tremor	Aldicarb	0.05	0.1	Known	Acetylcholinesterase (AChE) inhibitor	2.000	Acute neurotoxicity rat ([redacted], 1994b)
Ataxia	Alpha-Cypermethrin	2.3	6.8*	Known	binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.043	90-day dog ([redacted], 1984)
Reduced motor activity, reduced grip strength,	Azinphos-methyl	2	6	Known	Acetylcholinesterase (AChE) inhibitor	0.050	Acute neurotoxicity rat ([redacted], 1994)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
abnormal gait							
Increased motor activity, tremor, hyperactivity	Benfuracarb	2	20	Known	Acetylcholinesterase (AChE) inhibitor	0.050	28-day rat ([redacted], 1987a)
Reduced motor activity	Beta-Cyfluthrin	0.5	2	Known	binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.200	Acute neurotoxicity rat ([redacted], 1997)
Abnormal gait, tremor	Beta-cypermethrin	1	10	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.100	90-day neurotoxicity dog ([redacted], 1998f)
Increased motor activity, tremor, convulsion, abnormal gait	Bifenthrin	35	75*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.003	Acute neurotoxicity rat ([redacted], 1998)
Reduced motor activity	Carbosulfan	1.2	64.8	Known	Acetylcholinesterase (AChE) inhibitor	0.083	90-day neurotoxicity rat ([redacted], 1995)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Reduced motor activity	Chlorpropham	50	125	Unknown		0.002	Acute dog ([redacted], 2003)
Ataxia, reduced motor activity, convulsions	Clothianidin	60	177*	Known	agonist of nicotinic acethylcholine receptor (nAChR)	0.002	90-day neurotoxicity rat ([redacted], 2000b)
Choreoatetosis	Cyfluthrin	1	2.5	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.100	Acute neurotoxicity rat ([redacted], 1999)
Ataxia	Cypermethrin	7.5	75*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.013	2-year rat ([redacted], 1982)
Ataxia, landing-foot splay, tremor	Deltamethrin	1	10	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.100	1 year dog ([redacted], 1993)
Ataxia, reduced grip strength,	Dicamba	30	300	Unknown		0.003	Acute neurotoxicity rat ([redacted], 1993)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
reduced motor activity							
Ataxia	Dicofol	15	75	Unknown		0.007	Acute neurotoxicity rat ([redacted], 1992)
Increased motor activity, convulsions, tremors	Dieldrin	1	5	Known	GABA-gated chlorine channel antagonist	0.100	3-days rat-mechanistic study ([redacted], 1989)
Ataxia, convulsions, reduced grip strength, reduced motor activity, tremor	Dimethoate	20	200*	Known	Acetylcholinesterase (AChE) inhibitor	0.005	Acute neurotoxicity rat ([redacted], 1993b)
Reduced motor activity	Dinotefuran	100	300	Known	agonist of nicotinic acethylcholine receptor (nAChR)	0.001	Acute single dose rabbit ([redacted], 1998b)
Increased motor activity, tremor, reduced motor activity, convulsions, abnormal gait	Endosulfan	3	6	Known	GABA-gated chloride channel blocker	0.033	3-weeks rat ([redacted], 1997)
Ataxia, choreoatetosis, landing-foot splay, reduced grip strength, tremor	Esfenvalerate	1.8	1.9	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.056	Acute neurotoxicity rat ([redacted] 2000)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Reduced motor activity	Ethoprophos	5	25	Known	Acetylcholinesterase (AChE) inhibitor	0.020	Acute neurotoxicity rat ([redacted], 1994e)
Ataxia	Fenamiphos	1.52	2.31*	Known	Acetylcholinesterase (AChE) inhibitot	0.066	Acute neurotoxicity rat ([redacted], 1995)
Increased motor activity, tremor, abnormal gait, hypoactivity, reduced grip strenght	Fenitrothion	12.5	50	Known	Acetylcholinesterase (AChE) inhibitor	0.008	Acute neurotoxicity rat ([redacted], 1992)
Increased motor activity, tremor	Fenpropathrin	10	25	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.010	Acute neurotoxicity rat ([redacted], 1986a)
Reduced motor activity	Fenthion	1	50	Known	Acetylcholinesterase (AChE) inhibitor	0.100	Acute single dose rat ([redacted], 1997)
Increased motor activity, tremor	Fenvalerate	13.3	133	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.008	Acute neurotoxicity rat ([redacted], 1985)
Landing-foot splay	Fipronil	0.5	5	Known	GABA-gated chloride channel blocker	0.200	Acute neurotoxicity rat ([redacted], 1993a)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Ataxia, reduced motor activity	Flufenacet	7.5	75	Unknown		0.013	Acute neurotoxicity rat ([redacted], 1995)
Ataxia, tremor, reduced motor activity	Formetanate	5	10*	Known	Acetylcholinesterase (AChE) inhibitor	0.020	Acute neurotoxicity rat ([redacted], 2000)
Ataxia	Fosthiazate	5.4	26.8*	Known	Acetylcholinesterase (AChE) inhibitor	0.019	28-day dog ([redacted], 1989)
Hunched posture	Glufosinate	100	500*	Unknown	Organophosphorus herbicide, but no acetylcholinesterase activity observed	0.001	Acute neurotoxicity rat ([redacted], 1999)
Tremor	Imidacloprid	23.5	45.4*	Known	agonist of nicotinic acethylcholine receptor (nAChR)	0.004	90-day dog ([redacted], 1990)
Ataxia, hunched posture, landing-foot splay, reduced grip strength, reduced motor activity	Indoxacarb	50	100*	Known	Voltage-dependent sodium channel blocker	0.002	Acute neurotoxicity rat ([redacted], 1997b)
Reduced motor activity	Lambda-Cyhalothrin	0.52	1.3	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.192	Acute neurotoxicity rat ([redacted], 2006)
Convulsions	Lindane	3	30	Known	GABA-gated chloride channel antagonist	0.033	Acute neurotoxicity rat ([redacted], 1989)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Reduced motor activity	Mepiquat	58	174	Presumed	Activation of nicotinic and muscarinic acetylcholine receptors	0.002	Acute neurotoxicity rat ([redacted], 2002a; 2003b)
Tremor	Metaldehyde	7.5	75	Presumed	GABA inhibitor	0.013	28-day dog ([redacted], 2002)
Increased or decreased muscle tone, abnormal gait, tremor, hypoactivity	Methamidophos	1	3	Known	Acetylcholinesterase (AChE) inhibitor	0.100	Acute neurotoxicity rat ([redacted], 1993)
Muscle fasciculation, tremor	Methiocarb	0.25	2.5	Known	Acetylcholinesterase (AChE) inhibitor	0.400	Acute rat ([redacted], 1976b)
Tremor	Methomyl	0.75	2*	Known	Acetylcholinesterase (AChE) inhibitor	0.133	Acute neurotoxicity rat ([redacted], 1998a)
Reduced motor activity	Metribuzin	2	5	Unknown		0.050	Acute neurotoxicity rat ([redacted], 1999)
Decreased motor activity	Milbemectin	2	20	Known	Glutamate-gated chloride (GluCl) allosteric modulator	0.050	Acute neurotoxicity rat ([redacted], 1998a)
Increased motor activity, tremor, abnormal gait, reduced grip strength	Omethoate (metabolite of dimethoate)	0.35	5*	Known	Acetylcholinesterase (AChE) inhibitor	0.286	Acute neurotoxicity rat ([redacted], 2003)
Ataxia, hunched posture, landing-foot splay, reduced motor activity, tremor	**Oxamyl (IC)**	0.1	0.75	Known	cetylcholinesterase (AChE) inhibitor	1.000	Acute neurotoxicity ([redacted], 1997)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Increased or decreased muscle tone, tremor, hypoactivity	Oxydemeton-methyl	2	20	Known	Acetylcholinesterase (AChE) inhibitor	0.050	Acute single dose rat ([redacted], 1988)
Reduced motor activity	Penflufen	50	100	Unknown		0.002	Acute neurotoxicit rat ([redacted], 2009)
Abnormal gait, tremor	Permethrin	150	300*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.001	Acute single dose rat ([redacted], 1993a)
Ataxia, tremor	Phosmet	9	36*	Known	Acetylcholinesterase (AChE) inhibitor	0.011	Acute neurotoxicity rat ([redacted], 1998)
Hunched posture	Pirimicarb	10	40	Known	Acetylcholinesterase (AChE) inhibitor	0.010	Acute neurotoxicity rat ([redacted], 1996a)
Convulsions	Pirimiphos-methyl	150	1500*	Known	Acetylcholinesterase (AChE) inhibitor	0.001	Acute neurotoxicity rat ([redacted], 1995a)
Decreased motor activity	Pymetrozine	12.5	125	Known	Chordotonal organ TRPV channel modulator	0.008	Acute neurotoxicity rat ([redacted], 1997)
Tremor	Pyrethrins	20	63	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-	0.005	Acute neurotoxicity rat ([redacted], 1993)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
					conducting)		
Reduced motor activity	Pyridate	20	60	Unknown		0.005	90-day dog ([redacted], 1987)
Reduced motor activity	Spirotetramat	100	200	Unknown		0.001	Acute neurotoxicity rat ([redacted], 2005)
Reduced motor activity	Sulfoxaflor	7.5	25	Presumed	Nicotinergic AChR partial agonist	0.013	Acute neurotoxicity rat ([redacted], 2010)
Ataxia, mobility disturbances, recumbency	Tebuconazole	10	100	Unknown		0.010	Acute mouse ([redacted], 1983)
Ataxia, landing-foot splay, tremor	Tefluthrin	5	10*	known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.020	Acute neurotoxicity rat ([redacted], 2002)
Reduced motor activity	Tembotrione	200	500	Unknown		0.001	Acute neurotoxicity rat ([redacted], 2005)
Ataxia (poor coordination), hunched posture, recumbency	Tetraconazole	30	300	Unknown		0.003	Acute rat ([redacted], 2006)
Reduced motor activity	Thiacloprid	3.1	11*	Known	agonist of nicotinic acethylcholine receptor (nAChR)	0.032	Acute neurotoxicity rat ([redacted], 1998)
Higher grip strength	Thiamethoxam	100	500	Known	agonist of nicotinic acethylcholine receptor	0.001	Acute neurotoxicity rat ([redacted], 1997)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
					(nAChR)		
Reduced motor activity, ataxia, tremor	Thiodicarb	0.5	5	Known	Acetylcholinesterase (AChE) inhibitor	0.200	Acute neurotoxicity rat ([redacted], 2000a)
Convulsions, tremors	Thiophanate-methyl	39.3	393	Unknown		0.003	Acute toxicity rat ([redacted], 1970)
Reduced grip strength	Thiram	5	150	presumed	Neurotoxic effect might be due to the metabolite CS_2	0.020	Acute neurotoxicity rat ([redacted], 1993a)
Increased motor activity	Triadimefon	2	35	presumed	inhibition of dopamine transporter	0.050	Acute neurotoxicity rat ([redacted], 1996a)
Reduced motor activity	Tri-allate	36	72*	Unknown		0.003	8-week dog ([redacted], 1986)
Increased or decreased muscle tone	Trichlorfon	10	50	Known	cetylcholinesterase (AChE) inhibitor	0.010	Acute neurotoxicity rat ([redacted], 1996)
Ataxia, convulsions, hunched posture, landing-foot splay, reduced grip strength, tremor	zeta-Cypermethrin	10	50	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.010	Acute neurotoxicity rat ([redacted], 1998)
Ataxia, hunched posture	Ziram	1.5	15	Presumed	Neurotoxic effect might be due to the metabolite CS_2	0.067	Acute neurotoxicity rat ([redacted], 1994)

*highest dose tested

Table 2. CAG on functional effects on sensory division: toxicological characterization of ASs to be considered in acute exposure/risk assessments.

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Reduced splay reflex	Abamectin	0.5	1.5	Known	GABA-gated chloride channel agonist	0.200	Acute neurotoxicity rat ([redacted], 2006a)
Decreased reactivity: tail pinch response	Aldicarb	0.1	0.5*	Known	Acetylcholinesterase (AChE) inhibitor	1.000	Acute neurotoxicity rat ([redacted], 1994b)
Decreased reactivity: righting reflex (air drop)	Azinphos-methyl	2	6	Known	Acetylcholinesterase (AChE) inhibitor	0.050	Acute neurotoxicity rat ([redacted], 1994)
Decreased reactivity: analgesis reflex (nociception response)	Benfuracarb	2	20	Known	Acetylcholinesterase (AChE) inhibitor	0.050	28-day rat ([redacted], 1987a)
Decreased touch responses, tail pinch response and impaired righting.	Beta-Cyfluthrin	2	10*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.050	Acute neurotoxicity rat ([redacted], 1997)
Decreased reactivity: hypoactivity, tail pinch response	Beta-cypermethrin	100	500*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-	0.001	Acute neurotoxicity rat ([redacted], 1998d)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
					conducting)		
Decreased arousal	Clothianidin	10	100	Known	agonist of nicotinic acethylcholine receptor (nAChR)	0.010	Acute neurotoxicity rat ([redacted], 2000)
Hypersensitivity to noise	Cypermethrin	7.5	75*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.013	2-year rat ([redacted], 1982)
Decrease in acoustic startle response amplitude	Deltamethrin	0.1	1	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	1.000	Acute neurotoxicity rat ([redacted], 1994)
Abnormal righting reflex. Increased tail flick latency time	Dicamba	30	300	Unknown		0.003	Acute neurotoxicity rat ([redacted], 1993)
Decreased reactivity-hypoactivity	Dicofol	75	350*	Unknown		0.001	Acute neurotoxicity rat ([redacted], 1992)
Increased reactivity: hyperreactivity	Dieldrin	25	100*	Known	GABA-gated chlorine channel antagonist	0.004	Acute neurotoxicity hamster ([redacted], 1978)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Absence of pupil response	Dimethoate	2	20	Known	Acetylcholinesterase (AChE) inhibitor	0.050	Acute neurotoxicity rat ([redacted], 1993b)
Increased reactivity: hyperreactivity	Endosulfan	3	6	Known	GABA-gated chloride channel blocker	0.033	3-weeks rat ([redacted], 1997)
Increased reaction to touch, increased reaction to tail pinch	Esfenvalerate	1.8	1.9	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.056	Acute neurotoxicity rat ([redacted], 2000)
Decreased reactivity: tail pinch response, righting reflex (air drop)	Fenitrothion	12.5	50	Known	Acetylcholinesterase (AChE) inhibitor	0.008	Acute neurotoxicity rat ([redacted], 1992)
Approach response, tail pinch response, air righting reflex.	Fipronil	5	50*	Known	GABA-gated chloride channel blocker	0.020	Acute neurotoxicity rat ([redacted], 1997; [redacted], 1993a)
Diminished reaction to tail pinch test, abnormal response to visual placing test, auditory startle response	Formetanate	1	10*	Known	Acetylcholinesterase (AChE) inhibitor	0.100	Acute neurotoxicity rat ([redacted], 2000)
Decreased reactivity: righting reflex (air drop)	Halosulfuron methyl	600	2000*	Unknown		0.0002	Acute neurotoxicity rat ([redacted], 1994)
Increased reactivity	Imidacloprid	42	151	Known	agonist of nicotinic	0.002	Acute neurotoxicity

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
					acethylcholine receptor (nAChR)		rat ([redacted], 1994a)
Lack of pupillary reflex	Mepiquat	300	1200*	Presumed	Activation of nicotinic and muscarinic acetylcholine receptors	0.0003	Acute neurotoxicity rat ([redacted], 2002a; 2003b)
Reduced righting reflex, reduced toe/tail pinch response	Metaldehyde	150	250*	Presumed	GABA inhibitor	0.001	Acute neurotoxicity rat ([redacted], 2009)
No reaction to tail-pinch stimulus	Methomyl	1	1.9	Known	Acetylcholinesterase (AChE) inhibitor	0.100	Acute neurotoxicity rat ([redacted], 1996)
Righting reflex, tail pinch	**Oxamyl (IC)**	0.1	0.75	Known	Acetylcholinesterase (AChE) inhibitor	1.000	Acute neurotoxicity rat ([redacted], 1997)
Exaggerated startle response	Pyrethrins	63	200*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.002	Acute neurotoxicity rat ([redacted], 1993)
Decreased reactivity: touch response (handling reactivity)	Sulfoxaflor	75	750*	Presumed	Nicotinergic AChR partial agonist	0.001	Acute neurotoxicity rat ([redacted], 2010)
Poor reflexes	Tebuconazole	10	100	Unknown		0.010	Acute mouse ([redacted], 1983)
Decreased reactivity: approach response	Tembotrione	500	2000*	Unknown		0.0002	Acute neurotoxicity rat ([redacted], 2005)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Uncoordinated landing in the righting reflex	Thiamethoxam	100	500	Known	agonist of nicotinic acethylcholine receptor (nAChR)	0.001	Acute neurotoxicity rat ([redacted], 1997)
Decreased reactivity: tail pinch response	Thiodicarb	5	20	Known	Acetylcholinesterase (AChE) inhibitor	0.020	Acute neurotoxicity rat ([redacted], 2000d)
Handling reactivity, approach response, startle response, air righting	Thiram	5	150	presumed	Neurotoxic effect might be due to the metabolite CS_2	0.020	Acute neurotoxicity rat ([redacted], 1993a)
Decreased reactivity: righting reflex (air drop)	Thrichlorfon	10	50	Known	Acetylcholinesterase (AChE) inhibitor	0.010	Acute neurotoxicity rat ([redacted], [redacted], 1996)
Righting reflex	zeta-Cypermethrin	10	50	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.010	Acute neurotoxicity rat ([redacted], 1998)

*highest dose tested

Table 3. -CAG on functional effects on autonomic division: toxicological characterization of ASs to be considered in acute exposure/risk assessments.

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Urination	Acetamiprid	10	30	Known	agonist of nicotinic acethylcholine receptor (nAChR)	0.010	Acute neurotoxicity rat ([redacted], 1997a)
Salivation	Aldicarb	0.1	0.5*	Known	Acetylcholinesterase (AChE) inhibitor	1.000	Acute neurotoxicity rat ([redacted], 1994b)
Salivation	Alpha-Cypermethrin	4	10	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.025	Acute neurotoxicity rat ([redacted], 1993b)
Lacrimation	Benfuracarb	2	20	Known	Acetylcholinesterase (AChE) inhibitor	0.050	28-day rat ([redacted], 1987a)
Salivation	Beta-Cyfluthrin	2	10*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.050	Acute neurotoxicity rat ([redacted], 1997)
Salivation	Beta-cypermethrin	20	100	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an	0.005	Acute neurotoxicity rat ([redacted], 1998d)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
					activated (ion-conducting) to an inactivated (non-conducting)		
Salivation (accompanied by vomiting and retching)	Chlorpropham	125	625*	Unknown		0.001	14-day dog ([redacted], 1998)
Salivation	Cyfluthrin	2.5	7.5	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.040	Acute neurotoxicity rat ([redacted], 1999)
Urination	Cypermethrin	20	60	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.005	Acute neurotoxicity rat ([redacted], 1993)
Mydriasis	Deltamethrin	1	2.5	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-	0.100	90-day dog ([redacted], 1977)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
					conducting)		
Lacrimation, salivation	Dicofol	25	250	Unknown		0.004	Acute neurotoxicity rat ([redacted] 1985)
Lacrimation, salivation	Dimethoate	20	200*	Known	Acetylcholinesterase (AChE) inhibitor	0.005	Acute neurotoxicity rat ([redacted], 1993b)
Salivation	Endosulfan	3	6	Known	GABA-gated chloride channel blocker	0.033	3-week rat ([redacted], 1997)
Salivation	Esfenvalerate	1.8	1.9	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.056	Acute neurotoxicity rat ([redacted], 2000)
Miosis	Ethephon	50	500	Known	Inhibition of acetylcholinesterase (AChE)	0.002	Acute neurotoxicity rat ([redacted], 1996b)
Salivation	Ethoprophos	12	25	Known	Acetylcholinesterase (AChE) inhibitor	0.008	Acute mouse ([redacted], 1982)
Miosis, piloerection	Fenamiphos	1.52	2.31*	Known	Acetylcholinesterase (AChE) inhibitor	0.066	Acute neurotoxicity rat ([redacted], 1995)
Miosis, salivation	Fenitrothion	12.5	50	Known	Acetylcholinesterase (AChE) inhibition	0.008	Acute neurotoxicity rat ([redacted], 1992)
Miosis	Fipronil	5	50*	Known	GABA-gated chloride channel blocker	0.020	Acute neurotoxicity rat ([redacted], 1993a)
Urination	Flufenacet	7.5	75	unknown		0.013	Acute neurotoxicity rat ([redacted], 1995)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Miosis	Formetanate	1	10*	Known	Acetylcholinesterase (AChE) inhibitor	0.100	Acute neurotoxicity rat ([redacted], 2000)
Urination	Indoxacarb	50	100*	Known	Voltage-dependent sodium channel blocker	0.002	Acute neurotoxicity rat ([redacted], 1997b)
Mydriasis	Metaldehyde	7.5	75*	Presumed	GABA inhibitor	0.013	28-day dog ([redacted], 2002)
Urination, lacrimation	Methamidophos	1	3	Known	Acetylcholinesterase (AChE) inhibition	0.100	Acute neurotoxicity rat ([redacted], 1993)
Lacrimation, salivation	Methiocarb	0.25	2.5	Known	Acetylcholinesterase (AChE) inhibition	0.400	Acute rat ([redacted], 1976a)
Lacrimation, salivation	Methomyl	0.75	2*	Known	Acetylcholinesterase (AChE) inhibition	0.133	Acute neurotoxicity rat ([redacted], 1998a)
Salivation, miosis	Metribuzin	5	20	Unknown		0.020	Acute neurotoxicity rat ([redacted], 1999)
Salivation (accompanied by vomiting)	Milbemectin	3	10	Known	Glutamate-gated chloride (GluCl) allosteric modulator	0.033	13-week dog ([redacted], 1988)
Salivation, urination	**Oxamyl (IC)**	0.1	0.75	Known	Acetylcholinesterase (AChE) inhibition	1.000	Acute neurotoxicity rat ([redacted], 1997)
Salivation	Oxydemeton-methyl	2	20	Known	Acetylcholinesterase (AChE) inhibition	0.050	Acute single dose rat ([redacted], 1988)
Salivation	Phosmet	9	36*	Known	Acetylcholinesterase (AChE) inhibition	0.011	Acute neurotoxicity rat ([redacted], 1998)
Miosis	Pirimicarb	10	40	Known	Acetylcholinesterase (AChE) inhibition	0.010	Acute neurotoxicity rat ([redacted], 1996a)
Salivation, urination	Pyrethrins	63	200*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-	0.002	Acute neurotoxicity rat ([redacted], 1993)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
					conducting) to an inactivated (non-conducting)		
Salivation	Pyridate	30	80	Unknown		0.003	1-year dog ([redacted], 1989)
Lacrimation	Sulfoxaflor	75	750*	Presumed	Nicotinergic AChR partial agonist	0.001	Acute neurotoxicity rat ([redacted], 2010)
Salivation	Tebuconazole	250	500*	Unknown		0.0004	Acute neurotoxicity rat ([redacted], 1997)
Mydriasis, urination	Thiacloprid	53	109*	Known	agonist of nicotinic acethylcholine receptor (nAChR)	0.002	Acute neurotoxicity rat ([redacted], 1997)
Salivation	Thiodicarb	5	20	Known	Acetylcholinesterase (AChE) inhibitor	0.020	Acute neurotoxicity rat ([redacted], 2000d)
Urination	Thiram	5	150	presumed	Neurotoxic effect might be due to the metabolite CS_2	0.020	Acute neurotoxicity rat ([redacted], 1993a)
Lacrimation, salivation	Tri-allate	50	500	Unknown		0.002	Acute neurotoxicity rat ([redacted], 1984)

*highest dose tested

Table 4. CAG on brain and/or erythrocyte acetylcholinesterase inhibition: toxicological characterization of ASs to be considered in acute exposure/risk assessments.

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
AChE inhibition (brain)	Acephate	2.5	5*	Known	Acetylcholinesterase (AChE) inhibition	0.040	Acute single dose rat ([redacted], 1995)
AChE inhibition (erythrocytes)	Aldicarb	0.05	0.1	Known	Acetylcholinesterase (AChE) inhibition	2.000	Acute neurotoxicity rat ([redacted], 1994b)
AChE inhibition (brain, erythrocytes)	Azinphos-methyl	0.2	2	Known	Acetylcholinesterase (AChE) inhibition	0.500	28-day rat; acute neurotoxicity rat ([redacted], 1976; [redacted], 1994)
AChE inhibition (brain)	Carbofuran	0.015	0.03	Known	Acetylcholinesterase (AChE) inhibition	6.667	Acute neurotoxicity rat ([redacted], 2007c)
AChE inhibition (brain, erythrocytes)	Carbosulfan	0.5	5	Known	Acetylcholinesterase (AChE) inhibition	0.200	Acute neurotoxicity rat ([redacted], 1996, 1982b)
AChE inhibition (erythrocytes)	Chlorpyrifos	0.5	2	Known	Acetylcholinesterase (AChE) inhibition	0.200	Comparative cholinesterase assay ([redacted], 2010)
AChE inhibition (erythrocytes)	Chlorpyrifos-methyl	10	75*	Known	Acetylcholinesterase (AChE) inhibition	0.010	Acute oral neurobehavioural and cholinesterase inhibition study in rats ([redacted], 2013)
AChE inhibition (erythrocytes, brain)	Diazinon	2.5	25	Known	Acetylcholinesterase (AChE) inhibition	0.040	Acute neurotoxicity rat ([redacted], 1993, [redacted], 1994)
AChE inhibition (erythrocytes)	Dimethoate	1	2	Known	Acetylcholinesterase (AChE) inhibition	0.100	Acute neurotoxicity rat ([redacted], 1999)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
AChE inhibition (erythrocytes)	Ethoprophos	0.5	5	Known	Acetylcholinesterase (AChE) inhibition	0.200	Acute neurotoxicity rat ([redacted], 1994e)
AChE inhibition (erythrocytes)	Fenthion	0.1	1	Known	Acetylcholinesterase (AChE) inhibition	1.000	Acute single dose rat study ([redacted], [redacted], 1997)
AChE inhibition (brain, erythrocytes)	Formetanate	0.1	1	Known	Acetylcholinesterase (AChE) inhibition	1.000	Acute neurotoxicity rat ([redacted], 2000)
AChE inhibition (brain, erythrocytes)	Methamidophos	0.3	0.7	Known	Acetylcholinesterase (AChE) inhibition	0.333	Acute neurotoxicity rat ([redacted], 1993)
AChE inhibition (erythrocytes)	Methiocarb	0.05	0.5	Known	Acetylcholinesterase (AChE) inhibition	2.000	Acute neurotoxicity rat ([redacted], 1981)
AChE inhibition (brain, erythrocytes)	Methomyl	0.25	0.5	Known	Acetylcholinesterase (AChE) inhibition	0.400	Acute neurotoxicity rat ([redacted], 1998a)
AChE inhibition (brain)	Omethoate (metabolite of dimethoate)	0.25	0.35	Known	Acetylcholinesterase (AChE) inhibition	0.400	Acute neurotoxocity rat ([redacted], 2003)
AChE inhibition (brain, erythrocytes)	**Oxamyl (IC)**	0.1	0.75	Known	Acetylcholinesterase (AChE) inhibition	1.000	Acute neurotoxicity rat ([redacted], 1997)
AChE inhibition (brain)	Phosmet	4.5	22.5*	Known	Acetylcholinesterase (AChE) inhibition	0.022	Acute neurotoxicity rat ([redacted], 1998)
AChE inhibition (brain)	Pirimicarb	0.2	2	Known	Acetylcholinesterase (AChE) inhibition	0.500	Acute neurotoxicity rat ([redacted], 1979)
AChE inhibition (brain, erythrocytes)	Pirimiphos-methyl	15	150	Known	Acetylcholinesterase (AChE) inhibition	0.007	Acute neurotoxicity rat ([redacted], 1995a)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
AChE inhibition (erythrocytes)	Profenofos	0.5	25	Known	Acetylcholinesterase (AChE) inhibition	0.200	Acute neurotoxicity rat ([redacted], 1994)
AChE inhibition (brain, erythrocytes)	Thiodicarb	0.5	5	Known	Acetylcholinesterase (AChE) inhibition	0.200	Acute neurotoxicity rat ([redacted], 2000d)
AChE inhibition (erythrocytes)	Trichlorfon	10	50	Known	Acetylcholinesterase (AChE) inhibition	0.010	Acute neurotoxicity rat ([redacted], 1996)

*highest dose tested
^based on new data submitted in accordance with Article 21 and recent EFSA Conclusion (EFSA 2014;12(4):3640)
°based on the evaluation of new active substance data post approval (SANCO/10328/2004 rev.8) for the assessment of new data following inclusion of an active substance

Table 5. CAG on functional effects on motor division: toxicological characterization of ASs to be considered in chronic exposure/risk assessments.

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Ataxia, tremor	Abamectin	0.25	0.5	Known	GABA-gated chloride channel agonist	1.000	18-week dog ([redacted], 1976; [redacted], 1982)
Hunched posture	Acetamiprid	7.1	17.5	Known	agonist of nicotinic acethylcholine receptor (nAChR)	0.035	2-year rat ([redacted], 1999)
Reduced motor activity, lateral posture	Acrinathrin	10	25	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.025	28-day neurotoxicity rat ([redacted], 1987)
Ataxia, tremor	Alpha-Cypermethrin	2.3	6.8*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.109	90-day dog ([redacted], 1984)
Increased motor activity, convulsions	Amitraz	2.5	10*	Known	a2-adrenergic receptor agonist (closely related to the octopamine receptor in insects)	0.100	2-year rat ([redacted], 1973)
Reduced motor activity,	Azinphos-methyl	2.81	7.87*	Known	Acetylcholinesterase (AChE) inhibition	0.089	90-day neurotoxicity rat ([redacted], 1995)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
hypoactivity							
Increased motor activity, convulsions; ataxia	Benfuracarb	2.5	5	Known	Acetylcholinesterase (AChE) inhibition	0.100	2-year dog ([redacted], 1984)
Ataxia, choreoatetosis (Repetitive pawing), increased motor activity, reduced grip strenght	Beta-Cyfluthrin	2.02	7.99	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.124	90-day neurotoxicity rat ([redacted], 1997)
Increased motor activity, tremor	Bifenthrin	1.5	3.0	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.167	1-year dog ([redacted], 1985)
Muscle strength: increased or decreased muscle tone	Bromide ion	7.76	77.6	Unknown		0.032	90-day rat ([redacted], 2000)
Ataxia	Carbetamide	30	300*	Unknown		0.008	90-day dog ([redacted], 1985b)
Ataxia	Chlormequat	10	33	presumed	partial agonist of muscarinic and nicotinic acetylcholine receptor	0.025	1-year dog ([redacted], 1993)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Muscle weakness	Chlorpyrifos-methyl	10	50*	Known	Acetylcholinesterase (AChE) inhibition	0.025	90-day dog ([redacted], 1990)
Reduced motor activity	Clothianidin	35.8	52.3	Known	agonist of nicotinic acethylcholine receptor (nAChR)	0.007	30-day dog ([redacted], 2000)
Ataxia	Cyfluthrin	2.4	11	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.104	1-year dog ([redacted], 1997)
Ataxia, tremor	Cypermethrin	3.7	15	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.068	35-day dog ([redacted] 1976)
Ataxia, landing-foot splay, tremor	Deltamethrin	1	10	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.250	1-year dog ([redacted] 1993)
Ataxia, reduced motor activity,	Desmedipham	0.96	9.6	Unknown		0.260	1-year dog ([redacted], 1985)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
tremor							
Reduced motor activity, tremor	Dicamba	50	300*	Unknown		0.005	13-week dog ([redacted], 2003)
Ataxia	Dichlorvos	8	16*	Known	Acetylcholinesterase (AChE) inhibition	0.031	28-day rat ([redacted], 1982)
Reduced grip strenght	Dicofol	0.3	5.6	Unknown		0.833	90-day rat ([redacted], 1992)
Reduced motor activity: hypoactivity	Dinotefuran	400	3806*	Known	agonist of nicotinic acethylcholine receptor (nAChR)	0.001	90-day neurotoxicity rat ([redacted], 2001b)
Increased motor activity: tremor	**Emamectin benzoate (IC)**	0.25	0.5	Known	GABA-gated chloride channel agonist	1.000	1-year dog ([redacted], 1992)
Increased motor activity: tremor	Endosulfan	0.57	2.3*	Known	Glutamate-gated chloride channel (GluCl) allosteric modulator	0.439	1-year dog ([redacted] 1989, 1990)
Increased motor activity, tremor	Endrin	0.01	0.1	Known	GABA-gated chloride channel antagonist	25.000	2-year rat ([redacted], 1970a and [redacted], 1971)
Reduced grip strength	Esfenvalerate	3.2	6.4	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.078	90-day neurotoxicity rat ([redacted] 2000b)
Reduced grip strength, reduced	Ethoprophos	2.65	27.11*	Known	Acetylcholinesterase (AChE) inhibition	0.094	90-day neurotoxicity rat ([redacted], 1994a)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
motor activity, tremor							
Tremor	Fenamiphos	0.56	1.7*	Known	Acetylcholinesterase (AChE) inhibition	0.446	2-year rat ([redacted], 1972b)
Increased motor activity: tremor; muscle strength: reduced grip strenght	Fenitrothion	4.85	17.6*	Known	Acetylcholinesterase (AChE) inhibition	0.052	90-day neurotoxicity rat ([redacted], 1993)
Increased motor activity, tremor	Fenpropathrin	0.49	4.9	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.510	90-day mouse ([redacted], 1982c)
Ataxia, paresis limbs, reduced motor activity	Fenpropidin	5	20*	Unknown		0.050	1-year dog ([redacted], 1995)
Landing-foot splay	Fenpropimorph	0.8	8.5	Unknown		0.313	3-month neurotoxicity rat ([redacted], 1997a)
Convulsions	Fipronil	0.019	0.059	Known	GABA-gated chloride channel blocker	13.158	2-yar rat ([redacted], 1992 b)
Deficits in stride width	Flufenacet	1.14	27	unknown		0.219	1-year dog ([redacted], 1995b)
Ataxia, hunched posture, tremor	Fluquinconazole	0.44	4.77*	Unknown		0.568	2-year rat ([redacted], 1993)
Ataxia	Fosthiazate	0.54	5.4	Known	Acetylcholinesterase (AChE) inhibition	0.463	28-day dog ([redacted], 1989)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Ataxia, convulsions, Hyperactivity followed by hypoactivty, tremor	Glufosinate	4.5	8.4*	Unknown	Organophosphorus herbicide, but no acetylcholinesterase inhibition observed	0.056	1-year dog ([redacted], 1984a)
Increased motor activity, convulsions	Heptachlor	0.5	5	Known	GABA-gated chloride channel antagonist	0.500	6-month rat ([redacted], 1968)
Tremor	Imidacloprid	23.5	45.4*	Known	agonist of nicotinic acethylcholine receptor (nAChR)	0.011	90-day dog ([redacted], 1990)
Ataxia, hunched posture	Indoxacarb	2.6	14	Known	Voltage-dependent sodium channel blocker	0.096	18-month mouse ([redacted], 1997b)
Limited use of hindlilmbs	Isoxaflutole	20	500*	Unknown		0.013	2-year rat ([redacted], 1995a)
Ataxia, convulsions, tremor	Lambda-Cyhalothrin	0.5	3.5*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.500	1-year dog ([redacted], 1991)
Increased motor activity, convulsions	Lindane	6	12	Known	GABA-gated chloride channel antagonist	0.042	2-year rat ([redacted], 1990; [redacted], 1989)
Convulsions	Lufenuron	1.9	20	Unknown		0.132	2-year rat ([redacted], 1993a)
Paralysis	Mancozeb	49	328*	presumed	Neurotoxic effect might be due to the metabolite CS_2	0.005	3-month rat ([redacted], 1991)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Paresis limbs	Maneb	75	200*	presumed	Neurotoxic effect might be due to the metabolite CS_2	0.003	1-year dog ([redacted], 1957)
Convulsions, lateral position	Mepiquat	32	95*	Presumed	Activation of nicotinic and muscarinic acetylcholine receptors	0.008	3-month dog ([redacted], 1977b)
Muscle strength: increased or decreased muscle tone, reduced grip strenght; increased motor activity, tremor ; reduced motor activity, hypoactivity	Methamidophos	0.067	0.787	Known	Acetylcholinesterase (AChE) inhibitor	3.731	90-day neurotoxicity rat ([redacted], 1994)
Increased motor activity: tremor, hyperactivity	Methidathion	0.16	0.8	Known	Acetylcholinesterase (AChE) inhibitor	1.563	2-year rat ([redacted], 1986)
Muscle weakness, tremor	Methiocarb	2.2	8.6*	Known	Acetylcholinesterase (AChE) inhibitor	0.114	2-year dog ([redacted], 1980)
Reduced grip strength	Metiram	25.4	81.4*	presumed	Neurotoxic effect might be due to the metabolite CS_2	0.010	3-month rat ([redacted], 1992)
Reduced motor activity	Metribuzin	5	30	Unknown		0.050	4-week rat ([redacted], 1995)
Ataxia	Molinate	1.8	13	Presumed	the metabolite molinate sulfone inhibits aldehyde dehydrogenase by covalently binding to	0.139	2-year rat ([redacted], 1990)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
					the active-site Cys residue		
Increased motor activity: tremor	Omethoate (metabolite of dimethoate)	0.3	2.9*	Known	Acetylcholinesterase (AChE) inhibitor	0.833	2-year rat ([redacted], 1995)
Ataxia, hunched posture, ptosis	Oxamyl	1.69	15.3*	Known	Acetylcholinesterase (AChE) inhibitor	0.148	90-day neurotoxicity rat ([redacted], 1998)
Ataxia	Oxasulfuron	1.3	11	Unknown		0.192	1-year dog ([redacted], 1995)
Increased motor activity: tremor	Parathion	1.75	5.6*	Known	Acetylcholinesterase (AChE) inhibitor	0.143	90-day rat ([redacted] 1980)
Increased motor activity, tremor	Permethrin	40	100	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.005	2-year rat ([redacted], 1977)
Tremor	Pirimicarb	3.5	10	Known	Acetylcholinesterase (AChE) inhibitor	0.071	1-year dog ([redacted], 1998)
Hunched posture	Pirimiphos-methyl	9	36	Known	Acetylcholinesterase (AChE) inhibitor	0.028	2-year mouse ([redacted], 1996)
Ataxia (Hind-limb wheelbarrowing)	Propineb	4.3	41	presumed	Neurotoxic effect might be due to the metabolite CS2	0.058	90-day dog ([redacted], 1999)
Muscle strenght: increased or decreased muscle tone, weakness;	Pyrazophos	0.45	8*	Known	Acetylcholinesterase (AChE) inhibitor	0.556	6-month dog ([redacted], 1982)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
coordination: abnormal gait							
Ataxia, paresis limbs, tremor	Pyrethrins	30	86	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.008	56-day dog ([redacted], 1988c)
Choreoatetosis (Ruffling of body, pawing), transient hyperactivity followed by hypoactivity	tau-Fluvalinate	0.5	1	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.500	2-year rat ([redacted], 1984a)
Reduced motor activity	Tebuconazole	100	300*	Unkown		0.003	4-week rat ([redacted], 1987)
Tremor	Tefluthrin	0.5	1.5*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.500	90-day dog ([redacted], 1985)
Reduced motor activity: hypoactivity	Tembotrione	26.7	111*	Unknown		0.009	90-day dog ([redacted], 2004)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Hunched posture	Tetraconazole	17	65	Unknown		0.015	28-day rat ([redacted], 1988)
Increased motor activity: tremor	Tetramethrin	31	63	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.008	6-month dog ([redacted] 1981b)
Dragging of hind feet and tail, paralysis	Thiram	5	20	presumed	Neurotoxic effect might be due to the metabolite CS_2	0.050	18-month rat ([redacted], 1978)
Increased motor activity: hyperactivity	Triadimefon	3.4	54.6	Unknown		0.074	90-day neurotoxicity rat ([redacted], 1996b)
Increased motor activity	Triadimenol (a metabolite of Triadimefon)	3.4	45	Presumed	inhibition of dopamine transporter	0.074	3-month neurotoxicity rat ([redacted], 1996b)
Landing-foot splay, reduced grip strenght	Tri-allate	32.9	128.8*	Unknown		0.008	3-month neurotoxicity rat ([redacted], 1993)
Reduced motor activity: hypoactivity	Trichlorfon	31.2	168*	Known	Acetylcholinesterase (AChE) inhibitor	0.008	90-day neurotoxicity rat ([redacted], 1995)
Landing-foot splay, reduced motor activity	zeta-Cypermethrin	5	26	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an	0.050	90-day neurotoxicity rat ([redacted], 1999)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
					activated (ion-conducting) to an inactivated (non-conducting)		

*highest dose tested

Table 6. CAG on functional effects on sensory division: toxicological characterization of ASs to be considered in chronic exposure/risk assessments.

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Minimal reactivity to handling	2,4-D	0.5	5	Unknown		0.010	1-year neurotoxicity rat ([redacted], 1994b)
Decreased pupil reactivity	Abamectin	0.25	0.5	Known	GABA-gated chloride channel agonist	0.020	1-year dog ([redacted], 1984d)
Increased reactivity, exaggerated auditory response	Beta-Cyfluthrin	2	8.9	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.003	90-day neurotoxicity rat ([redacted], 1997)
Hyperreactivity	Beta-cypermethrin	0.82	8.2	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.006	90-day rat ([redacted], 1998c)
Exaggerated auditory response (startle reflex)	Bifenthrin	20	30*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an	0.0003	28-day neurotoxicity ([redacted], 1998b)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
					inactivated (non-conducting)		
Diminished reflex response	Chlormequat	50	62.5*	presumed	partial agonist of muscarinic and nicotinic acetylcholine receptor	0.0001	90-day dog ([redacted], 1977)
Hyperreactivity	Cymoxanil	30	90*	Unknown		0.0002	2-year rat ([redacted], 1994a)
Hypersensitivity to noise	Cypermethrin	5	15*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.001	1-year dog ([redacted] 1982)
Hypersensitivity to noise	Deltamethrin	4	14	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.001	13-week neurotoxicity rat ([redacted] 1998a)
Hyperreactivity	Dieldrin	0.05	0.25*	Known	GABA-gated chlorine channel antagonist	0.100	2-year rat ([redacted], 1977b)
Hyperreactivity	Emamectin benzoate	0.5	0.75	Known	GABA-gated chloride channel agonist	0.010	1-year dog ([redacted], 1992)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Exaggerated auditory response (startle reflex)	Endosulfan	0.57	2.3*	Known	Glutamate-gated chloride channel (GluCl) allosteric modulators	0.009	1-year dog ([redacted] 1989, 1990)
Hyperreactivity	**Endrin (IC)**	0.005	0.05	Known	GABA-gated chloride channel antagonist	1.000	90-day rat ([redacted],1956)
Hypersensitivity to noise	Esfenvalerate	15	25*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.0003	90-day rat ([redacted] 1984)
Negative air drop, pupillary responses, decreased analgesic reflex	Ethoprophos	2.65	27.11*	Known	Acetylcholinesterase (AChE) inhibitor	0.002	90-day neurotoxicity rat ([redacted], 1994a)
Retarded pupillary reflex	Fenpropimorph	7.1	71*	Unknown		0.001	3-month neurotoxicity rat ([redacted], 1997a)
Hyperreactivity	Fenvalerate	15	50	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.0003	18-month mouse ([redacted], 1976; [redacted], 1976a; [redacted], 1977b)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Hypo-reactivity, reduced reaction to movement and sound, hyperreactivity	Flufenacet	27	59*	Unknown		0.0002	1-year dog ([redacted], 1995b)
Decrease in alertness and/or startle response	Glufosinate	52.1	521	Unknown	Organophosphorus herbicide, but no acetylcholinesterase inhibition observed	0.0001	90-day neurotoxicity rat ([redacted], 1993a)
Hyperreactivity	Heptachlor	2	7	Known	GABA-gated chloride channel antagonist	0.003	14-day neurotoxicity rat ([redacted], 1995)
Hyperreactivity	Indoxacarb	2.6	14	Known	Voltage-dependent sodium channel blocker	0.002	18-month mouse ([redacted], 1997b)
No reaction to noise	Metaldehyde	30	90*	Presumed	GABA inhibitor	0.0002	1-year dog ([redacted], 2003)
Hyperreactivity	Methamidophos	0.074	0.899	Known	Acetylcholinesterase (AChE) inhibitor	0.068	90-day neurotoxicity rat ([redacted], 1994)
Hyperreflexic patellar reflexes, sensory changes (presthesia, proprioception deficit)	Molinate	10	50*	Presumed	the metabolite molinate sulfone inhibits aldehyde dehydrogenase by covalently binding to the active-site Cys residue	0.001	1-year dog ([redacted], 1991)
Hyperreactivity, absent pupillary response	Oxamyl	1.69	15.3*	Known	Acetylcholinesterase (AChE) inhibitor	0.003	90-day neurotoxicity rat ([redacted], 1998)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Hindlimb flexor reflex	Oxasulfuron	83	425	Unknown		0.0001	2-year carcinogenicity rat ([redacted], 1996)
Sensory changes (proprioceptive deficit)	Propineb	4.3	41.4	presumed	Neurotoxic effect might be due to the metabolite CS2	0.001	90-day dog ([redacted], 1999)
Decreased pupil reactivity	Pyrazophos	0.45	8*	Known	Acetylcholinesterase (AChE) inhibitor	0.011	6-months dog ([redacted], 1982)
Decrease in proprioception	Sulcotrione	300	600	Unknown		0.00002	16-week dog ([redacted], 1992)
Decreased responsiveness to sensory stimuli, increase in click response	tau-Fluvalinate	2	6	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.003	8-day neurotoxicity rat ([redacted] 1998b)
Increased response to sound	Tefluthrin	1.5	5.9	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.003	2-year carcinogenicity rat ([redacted], 1986)
Increased alertness, impaired righting reflex	Tri-allate	33	129*	Unknown		0.0002	3-month neurotoxicity rat ([redacted], 1993)

*highest dose tested

Table 7. CAG on functional effects on autonomic division: toxicological characterization of ASs to be considered in chronic exposure/risk assessments.

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Urination	2,4-D	75	150*	Unknown		0.001	1-year neurotoxicity rat ([redacted], 1994b)
Salivation, mydriasis	Abamectin	0.25	0.5	Known	GABA-gated chloride channel agonist	0.296	18-week dog ([redacted], 1983d); 1-year dog ([redacted], 1984d)
Salivation	Acrinathrin	5	10	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.015	28-day neurotoxicity rat ([redacted], 1987)
Salivation	Benfuracarb	2.5	5	Known	Acetylcholinesterase (AChE) inhibitor	0.030	2-year dog ([redacted], 1984)
Salivation	Carbetamide	150	300*	Unknown		0.0005	28-day dog ([redacted], 1985)
Salivation	Chlormequat	5	10	presumed	partial agonist of muscarinic and nicotinic acetylcholine receptor	0.015	1-year dog ([redacted], 1993)
Salivation	Clothianidin	19.3	40.9	Known	agonist of nicotinic acethylcholine receptor (nAChR)	0.004	90-day dog ([redacted], 2000a)
Salivation	Cypermethrin	6	20	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an	0.012	1-year dog ([redacted], 1995)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
					activated (ion-conducting) to an inactivated (non-conducting)		
Mydriasis	Deltamethrin	1	2.5	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.074	90-day dog ([redacted], 1979)
Salivation	Dicamba	50	300*	Unknown		0.001	13-week dog ([redacted], 2003)
Salivation	Dicofol	3.31	9.78	Unknown		0.022	90-day dog ([redacted], 1986)
Mydriasis	Emamectin benzoate	0.5	0.75	Known	GABA-gated chloride channel agonist	0.148	1-year dog ([redacted], 1992)
Hypersensitivity to sound	Esfenvalerate	15	25*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)		90 day rat ([redacted], 1984)
Salivation, lacrimation	Ethoprophos	2.65	27.11*	Known	Acetylcholinesterase (AChE) inhibitor	0.028	90-day neurotoxicity rat ([redacted], 1994a)
Piloerection	Fluquinconazole	1.73	8.81	Unknown		0.043	28-day rat ([redacted], 1992a)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Trismus salivation	Glufosinate	4.5	8.4*	unknown	Organophosphorus herbicide, but no acetylcholinesterase inhibition observed	0.016	1-year dog ([redacted], 1984a)
Urination	Indoxacarb	2.6	14	Known	Voltage-dependent sodium channel blocer	0.028	18-month mouse ([redacted], 1997b)
Piloerection	Lambda-Cyhalothrin (study performed with Cyhalothrin)	1.8	9.2	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.041	2-year mouse ([redacted], 1991)
Salivation	Lufenuron	3.64	29.8	Unknown		0.020	1-year dog ([redacted], 1992; [redacted], 1995)
Salivation	Mepiquat	16.6	166*	Presumed	Activation of nicotinic and muscarinic acetylcholine receptors	0.004	1-year dog ([redacted], 1994a)
Salivation	Metaldehyde	30	90*	Presumed	GABA inhibitor	0.002	1-year dog ([redacted], 2003)
Urination, lacrimation	**Methamidophos (IC)**	0.074	0.899	Known	Acetylcholinesterase (AChE) inhibitor	1.000	90-day neurotoxicity rat ([redacted], 1994)
Urination	Metamitron	10	50	Unknown		0.007	28-day rat ([redacted], 1990)
Salivation	Methiocarb	0.05	0.5*	Known	Acetylcholinesterase (AChE) inhibitor	1.480	29-day neurotoxicity dog ([redacted], 1981)
Salivation	Metribuzin	5	30	Unknown		0.015	4-week rat ([redacted], 1995)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Salivation	Molinate	1	10	Presumed	the metabolite molinate sulfone inhibits aldehyde dehydrogenase by covalently binding to the active-site Cys residue	0.074	1-year dog ([redacted], 1991)
Urination	Pirimicarb	10	25*	Known	Acetylcholinesterase (AChE) inhibitor	0.007	90-day dog ([redacted], 1968 & [redacted], 1995a)
Salivation, lacrimation	tau-Fluvalinate	0.5	1	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.148	2-year rat ([redacted], 1984a)
Piloerection	Tefluthrin	11.6	26.6*	Known	Binds to the voltage-gated sodium channel (VGSC) preventing its transition from an activated (ion-conducting) to an inactivated (non-conducting)	0.006	3-month neurotoxicity rat ([redacted], 2002)
Piloerection	Triadimenol (a metabolite of Triadimefon)	40	209*	Unknown	inhibition of dopamine transporter	0.002	90-day rat ([redacted], 1983)
Lacrimation	Tri-allate	33	129*	Unknown		0.002	3-month neurotoxicity rat ([redacted], 1993)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Urination	Trichlorfon	31.2	168*	Known	Acetylcholinesterase (AChE) inhibitor	0.002	90-day neurotoxicity rat ([redacted], 1995)

*highest dose tested

Table 8. CAG on brain and/or erythrocyte acetylcholinesterase inhibition: toxicological characterization of ASs to be considered in chronic exposure/risk assessments.

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
AChE inhibition (brain, erythrocytes)	Acephate	0.25	2.5	Known	Acetylcholinesterase (AChE) inhibitor	0.016	2-year rat ([redacted], 1981)
AChE inhibition (brain)	Aldicarb	0.0025	0.025	Known	Acetylcholinesterase (AChE) inhibitor	1.600	14-day dog ([redacted], 1985)
AChE inhibition (erythrocytes)	Azinphos-ethyl	0.0125	0.025	Known	Acetylcholinesterase (AChE) inhibitor	0.320	90-day neurotoxicity dog ([redacted], 1963)
AChE inhibition (brain, erythrocytes)	Azinphos-methyl	0.091	0.91	Known	Acetylcholinesterase (AChE) inhibitor	0.044	90-day neurotoxicity rat ([redacted], 1995)
AChE inhibition (erythrocytes)	Benfuracarb	1.81	9.4	Known	Acetylcholinesterase (AChE) inhibitor	0.002	28-day neurotoxicity rat ([redacted], 2003)
AChE inhibition (erythrocytes)	Cadusafos	0.045	0.22*	Known	Acetylcholinesterase (AChE) inhibitor	0.089	2-year rat ([redacted], 1986)
AChE inhibition (brain)	Carbaryl	0.373	3.73	Known	Inhibition of acetylcholinesterase (AChE) Acetylcholinesterase (AChE) inhibitor	0.011	1-year dog ([redacted] 1987)
AChE inhibition (brain, erythrocytes)	Carbofuran	0.041	0.41	Known	Acetylcholinesterase (AChE) inhibitor	0.100	90-day dog ([redacted], 1987b)
AChE inhibition (brain, erythrocytes)	Carbosulfan	1	10.5	Known	Acetylcholinesterase (AChE) inhibitor	0.004	2-year rat ([redacted], 1982a)
AChE inhibition (brain)	Chlorfenvinphos	0.018	0.18	Known	Acetylcholinesterase (AChE) inhibitor	0.222	28-day mouse (not reported)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
AChE inhibition (erythrocytes)	Chlorpyrifos	0.03	0.1	Known	Acetylcholinesterase (AChE) inhibitor	0.133	90-day rat ([redacted], 1968); 90-day dog ([redacted], 1968; [redacted], 1989b)
AChE inhibition (erythrocytes)	Chlorpyrifos-methyl	0.1	10	Known	Acetylcholinesterase (AChE) inhibitor	0.040	90-day dog ([redacted], 1990)
AChE inhibition (brain, erythrocytes)	Diazinon	0.015	4.5	Known	Acetylcholinesterase (AChE) inhibitor	0.267	1-year dog ([redacted], 1991)
AChE inhibition (erythrocytes)	Dichlorvos	0.23	2.3	Known	Acetylcholinesterase (AChE) inhibitor	0.017	2-year rat ([redacted], 1967)
AChE inhibition (erythrocytes)	Dimethoate	0.04	0.2	Known	Acetylcholinesterase (AChE) inhibitor	0.100	2-year rat ([redacted], 1986)
AChE inhibition (erythrocytes)	Ethephon	13	130	Known	Acetylcholinesterase (AChE) inhibitor	0.0003	2-year rat ([redacted], 1989)
AChE inhibition (brain)	Ethion	0.06	0.71	Known	Acetylcholinesterase (AChE) inhibitor	0.067	90-day dog ([redacted], 1988)
AChE inhibition (erythrocytes)	Ethoprophos	0.025	1	Known	Acetylcholinesterase (AChE) inhibitor	0.160	20-week dog ([redacted], 1990); 1-year dog ([redacted], 1986)
AChE inhibition (erythrocytes)	Fenamiphos	0.06	0.15	Known	Acetylcholinesterase (AChE) inhibitor	0.095	2-year dog ([redacted], 1972a)
AChE inhibition (brain, erythrocytes)	Fenitrothion	0.5	1.5	Known	Acetylcholinesterase (AChE) inhibitor	0.008	2-year rat ([redacted], 1974)
AChE inhibition (erythrocytes)	Fenthion	0.05	0.23	Known	Acetylcholinesterase (AChE) inhibitor	0.080	1-year dog ([redacted], 1990
AChE inhibition (erythrocytes)	Fonofos	0.04	0.4	Known	Acetylcholinesterase (AChE) inhibitor	0.100	2-year dog ([redacted], 1969)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
AChE inhibition (erythrocytes)	Formetanate	0.37	1.75	Known	Acetylcholinesterase (AChE) inhibitor	0.011	1-year dog ([redacted], 1986)
AChE inhibition (erythrocytes)	Fosthiazate	0.48	0.97	Known	Acetylcholinesterase (AChE) inhibitor	0.008	28-day rat ([redacted], 1989)
AChE inhibition (brain)	Malathion	4	35	Known	Acetylcholinesterase (AChE) inhibitor	0.001	90d-neurotoxicity rat ([redacted], 1994b)
AChE inhibition (brain, erythrocytes)	Methamidophos	0.03	0.06	Known	Acetylcholinesterase (AChE) inhibitor	0.133	90-day rat ([redacted], 1991)
AChE inhibition (brain, erythrocytes)	Methidathion	0.16	0.8	Known	Acetylcholinesterase (AChE) inhibitor	0.025	2-year rat ([redacted], 1986)
AChE inhibition (erythrocytes)	Methiocarb	0.005	0.05	Known	Acetylcholinesterase (AChE) inhibitor	0.800	90-day dog ([redacted], 1981)
AChE inhibition (brain)	Methomyl	9	95*	Known	Acetylcholinesterase (AChE) inhibitor	0.0004	90-day neurotoxicity rat ([redacted], 1998b)
AChE inhibition (brain, erythrocytes)	Monocrotophos	0.005	0.05	Known	Acetylcholinesterase (AChE) inhibitor	0.800	2-year rat ([redacted], 1967b); 2-year mouse ([redacted], 1982); 2-year rat ([redacted], 1983)
AChE inhibition (erythrocytes)	Omethoate (IC) (metabolite of dimethoate)	0.004	0.04	Known	Acetylcholinesterase (AChE) inhibitor	1.000	2-year rat ([redacted], 1995)
AChE inhibition (brain, erythrocytes)	Oxamyl	1.69	15.3*	Known	Acetylcholinesterase (AChE) inhibitor	0.002	90-day neurotoxicity rat ([redacted], 1998)
AChE inhibition (brain, erythrocytes)	Oxydemeton-methyl	0.027	0.224	Known	Acetylcholinesterase (AChE) inhibitor	0.148	2-year rat ([redacted], 1984)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
AChE inhibition (erythrocytes)	Parathion	0.001	0.01	Known	Acetylcholinesterase (AChE) inhibitor	4.000	1-year dog ([redacted],1981)
AChE inhibition (erythrocytes)	Parathion-methyl	0.1	0.5	Known	Acetylcholinesterase (AChE) inhibitor	0.040	2-year rat ([redacted], 1981)
AChE inhibition (erythrocytes)	Phenthoate	0.1	1	Known	Acetylcholinesterase (AChE) inhibitor	0.040	2-year rat ([redacted], 1984)
AChE inhibition (erythrocytes)	Phosalone	0.17	0.9	Known	Acetylcholinesterase (AChE) inhibitor	0.024	1-year dog ([redacted], 1992)
AChE inhibition (brain)	Phosmet	0.1	1	Known	Acetylcholinesterase (AChE) inhibitor	0.040	2-year mouse ([redacted].,1981)
AChE inhibition (erythrocytes)	Phoxim	0.1	0.38	Known	Acetylcholinesterase (AChE) inhibitor	0.040	2-year dog ([redacted], 1977)
AChE inhibition (brain, erythrocytes)	Pirimicarb	10	25*	Known	Acetylcholinesterase (AChE) inhibitor	0.0004	1-year dog ([redacted], 1998)
AChE inhibition (brain)	Pirimiphos-methyl	0.4	2.1	Known	Acetylcholinesterase (AChE) inhibitor	0.010	2-year rat ([redacted], 1974)
AChE inhibition (erythrocytes)	Profenofos	0.017	0.56	Known	Acetylcholinesterase (AChE) inhibitor	0.235	2-year rat ([redacted], 1981a)
AChE inhibition (brain, erythrocytes)	Pyrazophos	0.03	0.45	Known	Acetylcholinesterase (AChE) inhibitor	0.133	6-month dog ([redacted], 1982)
AChE inhibition (erythrocytes)	Thiodicarb	4.4	12.8	Known	Acetylcholinesterase (AChE) inhibitor	0.001	1-year dog ([redacted], 1986)
AChE inhibition (erythrocytes)	Tolclofos-methyl	3.8	12	Known	Acetylcholinesterase (AChE) inhibitor	0.001	9-month mouse ([redacted], 1978)
AChE inhibition (erythrocytes)	Triazophos	0.01	0.13	Known	Acetylcholinesterase (AChE) inhibitor	0.400	90-day dog ([redacted], 1988)
AChE inhibition (brain)	Trichlorfon	4.5	13.3	Known	Acetylcholinesterase (AChE) inhibitor	0.001	2-year rat ([redacted]. 1989)

*highest dose tested

Table 9. CAG on functional effects on neuropathological end-points: toxicological characterization of ASs to be considered in chronic exposure/risk assessments.

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
Myelin degeneration	Chlorfenapyr	14.8	27.6	Unknown		0.017	90-day mouse ([redacted], 1994)
Axonal degeneration, myelin degeneration	Cymoxanil	5	38	Unknown		0.050	2-year rat ([redacted], 1994a)
Axonal degeneration (degeneration of trigeminus and increased galactosidase activity)	Cypermethrin	25	50	Unknown		0.010	7-day rat ([redacted], 1978)
Axonal degeneration, myelin degeneration	**Emamectin benzoate (IC)**	0.25	0.5	Unknown		1.000	90-day dog ([redacted], 1994b); 1-year dog ([redacted], 1992)
Myelin degeneration	Fenpropidin	5	20*	Unknown		0.050	1-year dog ([redacted], 1995)
Axonal degeneration	Flufenacet	1.14	27	Unknown		0.219	1-year dog ([redacted], 1995b)
Axonal degeneration, neuronal degeneration/ necrosis	Indoxacarb	4	20	Unknown		0.063	18-month mouse ([redacted], 1997b)
Axonal degeneration,	Isoxaflutole	20	500*	Unknown		0.013	2-year rat ([redacted], 1995a)

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
myelin degeneration							
Myelin degeneration	Lindane	0.5	5	Unknown	Sequestration of lindane in the lipid-rich myelin sheath surrounding neurons?	0.500	3-day rat ([redacted], 1990)
Myelin degeneration (myelin damage and Schwann cell proliferation)	Mancozeb	8.2	49	Unknown		0.030	3-month rat ([redacted], 1991)
Axonal degeneration, myelin degeneration	Molinate	1.8	13*	Presumed	Sulfoxide metabolites can react with sulfhydryl groups of amino acids and proteins	0.139	2-yer rat ([redacted], 1990)
Axonal degeneration, myelin degeneration (secondary to axonal degeneration)	Oxasulfuron	1.5	99	Unknown		0.167	18-month mouse ([redacted], 1996)
Myelin degeneration	Quinoclamine	3.82	40.2*	Unknown		0.065	18-month mouse ([redacted], 1993)
Axonal degeneration, myelin degeneration	tau-Fluvalinate	1	10	Unknown		0.250	7-day neurotoxicity rat ([redacted], 1994)
Neuronal	Tembotrione	26.7	111*	Unknown		0.009	90-day dog ([redacted],

Indicator of specific effect	Active substance	NO(A)EL mg/kg bw	LO(A)EL mg/kg bw	Mode/mechanism of neurotoxic action		RPF	Study
degeneration/ necrosis							2004)
Sciatic nerve lesions (not specified)	Thiram	1.4	14*	presumed	Cross-linking of axonal proteins via reaction of the metabolite CS2 with axonal proteins	0.179	2-year rat ([redacted], 1991)
Axonal degeneration, myelin degeneration	Tri-allate	6.4	32	Unknown		0.039	3-month neurotoxicity rat ([redacted], 1993)
Myelin degeneration	Trichlorfon	31.2	168*	Presumed	Inhibition of neuropathy target esterase (NTE) and increased intracellular calcium	0.008	90-day neurotoxicity rat ([redacted], 1995)
Axonal degeneration	Ziram	9	27*	Presumed	Cross-linking of axonal proteins via reaction of the metabolite CS2 with axonal proteins	0.028	2-year rat ([redacted], 1994a)

*highest dose tested